Basic A1 Protein of the Myelin Membrane

THE COMPLETE AMINO ACID SEQUENCE*

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SUMMARY

Twenty-seven tryptic peptides were isolated from the A1 protein from bovine spinal cord. These, together with 16 peptic peptides, were utilized to establish the complete amino acid sequence of the 170 residues of the A1 protein. Peptide T, derived from the A1 protein by cleavage of the carboxyl of the single tryptophan residue, was useful in positioning the peptides which comprise the COOH-terminal end. Seven chymotryptic and nine tryptic peptides were isolated from Peptide T.

An unusual feature of the sequence is the methylated arginine residue at position 107 which is present as both the dimethyl and monomethyl derivatives. The methylated derivatives appear to be relatively resistant to tryptic hydrolysis. Located close to the methylated arginine residue is a Pro-Arg-Thr-Pro-Pro-Pro sequence, a structure which may be part of the Al protein, inducing the identical chemical and histological signs associated with EAE in guinea pigs. We report here the complete amino acid sequence of the 170 residues of the A1 protein from bovine spinal cord as established primarily with tryptic and peptic peptides. Preliminary reports on the sequence of both the bovine (15) and human A1 proteins have appeared (15, 16).

Although proteins are known which are either specific to the nervous system (17) or possible structural proteins of membranes (18), the A1 protein is the first protein in these categories to have its sequence defined. Such information should aid our understanding of the normal role of this protein in the myelin substructure and possibly in the process of myelinogenesis. In addition to its immunological relevance, knowledge of the sequence will be useful for comparative studies with Al proteins from other species, from peripheral nerve, and with other basic proteins from nervous tissue such as histones. The sequence has also proven invaluable in elucidating the regions of the Al protein responsible for inducing disease in guinea pigs (8, 13) and rabbits (19, 20), and thereby providing the model for peptide synthesis (14) which has led to definition of the essential residues: the three amino acids tryptophan, glutamine, and lysine. The sequence has also proven helpful in elucidating the site of glycosylation (21) by the polypeptide N-acetylgalactosaminyl transferase, thus defining the receptor sequence recognized by this enzyme (22), and suggesting a model for the sequence in mucins.

EXPERIMENTAL PROCEDURE AND RESULTS

Techniques—The materials and procedures of this study were essentially as described in earlier papers (4, 5, 10, 11). The bovine A1 protein from spinal cord was purified by gel filtration followed by Cellex-P chromatography (3). It appeared homogeneous (2, 5) by gel electrophoresis at pH 4.4 and 8.6, ultracentrifugation, and immunoelectrophoresis. When gel electrophoresis was followed by gel filtration, the A1 protein remained a single peak.

The abbreviations used are: EAE, experimental allergic encephalomyelitis; BNPS-skatole, a bromine adduct of 2-(3-nitrophenylsulfenyl)-3-methylindole.
phoresis at pH 10.5 was used, minor microheterogeneity was observed; 85 to 90% of the protein migrated in a single leading band toward the cathode followed by two faint bands.

In all cases hydrolysis with carboxypeptidase was carried out at 37° in 0.2 M triethylamine-bicarbonate buffer, pH 8.1, using a 50:1 ratio of peptide to enzyme (carboxypeptidases A and B together) unless otherwise noted (11). Hydrolysis with amino-peptidase M was usually carried out under the same conditions.

The hydrazinolysis procedure was used as described earlier (8, 10) to identify the COOH-terminal residue of some peptides. High voltage electrophoresis was performed at pH 4.7, 3500 volts, in buffer containing 2.5% pyridine and 2.5% acetic acid, unless otherwise noted. Ascending paper chromatography was also used for preparation and assessing purity of peptides. The system contained butanol-acetic acid-pyridine-water (122:38:189:151). The monomethyl- and dimethylarginine derivatives were identified as described previously (23).

The direct Edman procedure used was that in which the phenylthiohydantoin-amino acids were identified by thin layer chromatography as described by Blomback et al. (24). For the subtractive Edman procedure, the amino acid analysis of the peptide residue was performed after removal of the phenylthiohydantoin-amino acid. In some cases, the dansylation procedure was used to identify the NH2-terminal residue (25). All amino acid analyses were performed as previously described (3–5).

The peptide mapping of the tryptic peptides from the bovine A1 protein was performed as described previously (4, 9). For Peptide T, derived from the A1 protein by cleavage of the CH3COOH-tryptophanyl linkage with N-bromosuccinimide (26) or BNPS-skatole (27), the tryptic and chymotryptic peptides were similarly mapped.

Nomenclature-Tryptic peptides are designated by T, and peptic peptides by P. Tryptic and chymotryptic peptides derived from Peptide T are designated TT and TC, respectively. All peptides are numbered beginning with 1 for the NH2-terminal peptide and progressing to the COOH-terminal peptide. Since the complete amino acid sequence has been determined, each residue has been numbered in accord with the sequence of 170 residues. Reference to Fig. 6 will aid in following the description of each peptide.

Isolation of Peptides—Peptides were isolated from the peptic and tryptic digests of the bovine A1 protein by (a) resolution into fractions by ion exchange chromatography on Cellex-P or Dowex 50 resin or, for a few tryptic peptides, directly from a peptide map of the A1 protein or Peptide T; (b) resolution of the fractions into subfractions by gel filtration on Sephadex G-25, G-50, or G-75; and (c) isolation of peptides from the subfractions by preparative paper chromatography or high voltage electrophoresis or both. With these procedures, we were able to isolate most of the peptides, accounted for by the peptide mapping, in sufficient quantities for defining the sequence of the A1 protein. In no case were peptides found which did not fit the proposed sequence. Each peptide was judged pure when it gave a single band on both high voltage electrophoresis at pH 4.6 and paper chromatography, and gave amino acid residues in near integral ratios on amino acid analysis. If contaminating peptides were found, then preparative peptide mapping was used with 1 to 3 mg of material. This technique was adequate to purify all of the tryptic and peptic peptides, although, in some cases, electrophoresis for 4 to 8 hours was needed to resolve peptides of similar charge.

The yield of each peptide was estimated either from dry weight measurement or amino acid analysis. Recovery varied from 50 to 85% from paper electrophoresis and 30 to 70% from paper chromatography. It was observed that the cation exchange columns adsorbed a considerable portion of the peptides, particularly the very basic peptic peptides.

Tryptic Hydrolysis of Bovine A1 Protein and Purification of Tryptic Peptides—The digestion of 3.0 g of bovine A1 protein (170 amoles) was carried out for 4 hours at 37° with 90 mg of trypsin (treated with l-(tosylamide-2-phenyl)ethyl chloromethyl ketone) in 250 ml of 0.2 M triethylamine buffer, pH 8.1. After the solution was lyophilized, the peptide mixture was dissolved in 300 ml of H2O and applied to a column of Dowex AG-50X2 which had been equilibrated with 0.03 M ammonium acetate, pH 3.7. The peptide elution pattern is shown in Fig. 1. A linear gradient in pH and ionic strength was established at tube 147; at tube 900 the pH was 7.75, at which point 0.3 M ammonium hydroxide was applied directly to the column. Prior to elution of Peak I, the pH was 8.15; afterward, pH 10.45. Fractions A, B, C, etc. represent tubes which were combined and subsequently fractionated by gel filtration and paper electrophoresis. The isolated peptides are shown above the major peak from which they were derived.
Peptide T2 was also obtained from Peptide CB1 (11). Although Peptide T2 generally overlapped with Peptide T3, in the usual peptide map they were cleanly resolved with a chromatographic system lacking pyridine (11).

Peptide T3 was the major component of Fraction D, and was easily purified by electrophoresis (75% yield).

Peptide T4 was prepared from Fraction I. One of the largest tryptic peptides, a dodecapeptide, it was one of the last to elute from the Dowex column in tubes 942 to 952. As shown in Fig. 3, it could be separated from Peptides T8, T13, and T24 by electrophoresis. It was subsequently prepared in homogeneous form by paper chromatography which clearly separated it from traces of Peptide T13 and a small amount of faster moving peptide referred to as Peptide T6A. On peptide mapping, it sometimes partially overlapped Peptide T19.

Peptide T5 was not found in the eluate from the Dowex 50 column. It was prepared from the peptide map where it migrates rapidly because of charge +2, but exhibits a very high RF on chromatography (Fig. 3).

Peptide T6 was not found in the eluate from Dowex 50. It was easy to prepare, however, from the peptide map because it migrates faster on electrophoresis than any other peptide.

Peptide T6A was found in Fraction I; it was separated from Peptide T4 by paper chromatography as described under "Peptide T4." This peptide was not detected on the peptide map.

Peptide T7 was one of the first peptides to elute from the Dowex 50 column (Fraction B). It was also one of the most acidic of the peptides with charge −1 and was separated cleanly by peptide mapping as well. This peptide was purified from traces of Peptide T15 by paper electrophoresis.

Peptide T8 was prepared from Fraction I by paper electrophoresis which separated it from Peptides T12, T13, T17, and T24 (Fig. 3). It is the least basic of this group of peptides with charge 0, and as such migrates much more slowly than Peptides T17, T12, T13, and T24, the latter having the highest mobility of this group.

Peptide T9 was located in Fraction C from the Dowex 50 column, and was separated from other peptides on gel filtration (Fig. 2). It eluted mainly in the middle region (C2) which contained 62% Peptide T9, 30% T20, and 8% T15. On electrophoresis it migrated much faster than either Peptide T15 or T20 (the slowest). It is of interest that Peptide T9, a tetrapeptide, should elute earlier from the gel filtration than the Penta-peptide T20, which constituted 88% of the third region (C3). Peptide T15, an octapeptide, clearly eluted first and constituted 95% of the first region (C1).

Peptide T10 was derived from Fraction E of the Dowex 50 eluate. Resolution on gel filtration (Fig. 2/II) showed Peptide T10 primarily in Region E2. When Fraction E2 was subjected to electrophoresis, this peptide separated cleanly from Peptide T23 (much slower) and Peptide T3 (slower).

Peptide T11 was obtained from Fraction G following gel filtration, as shown in Fig. 2/I. Only Region G1 showed a significant yield of peptide (94% of material applied). Peptide T11 constituted 43% of the G1 region, and was purified by both electrophoresis and chromatography. On electrophoresis it migrated together with Peptide T22, just slower than Peptide T21 from which it was separated. Peptide T11 was subsequently separated by paper chromatography from Peptide T22. By this procedure, Peptide T11 appeared homogeneous when examined by peptide mapping.
Peptide T12 was found in Fraction 1 (tubes 953 to 962), and was isolated as described under "Peptide T12." It was also found in Fraction I (tubes 942 to 952) and separated by electrophoresis from Peptide T4 as shown in Fig. 3. It appeared homogeneous by peptide mapping.

Peptide T13 was located in Fraction C. On gel filtration, Fig. 21, it was the dominant peptide in the Region C1. It constituted 94% of the C1 fraction and was resolved well from Peptide T9 on electrophoresis.

Peptide T16, a dipeptide, was one of the last peptides to elute from the Dowex 50 column in Fraction I (tubes 963 to 972). It was separated from other peptides by paper chromatography and appeared homogeneous. It was not found on peptide mapping (Fig. 3).

Peptide T16A was not found in the Dowex 50 eluate, presumably because of the presence of 2 arginine residues, but was obtained in homogeneous form by preparative peptide mapping (Fig. 3).

Peptide T17 was eluted in low yield in Fraction I (tubes 963 to 972) and purified on paper chromatography. It also resolved well on peptide mapping, as shown in Fig. 3.

Peptide T18 was located in Fraction I (tubes 952 to 962). On electrophoresis it migrated just slightly faster than Peptide T8. Although it has zero net charge at pH 7, apparently at pH 4.7 the glutamic acid residue is partially titrated, giving the peptide a slightly basic character, barely more than Peptide T8 which contains aspartic acid and arginine.

Peptide T19 was not isolated from the Dowex 50 column. It is a large peptide (18 residues) containing 2 basic residues. On peptide mapping, where it was isolated for structural studies, it separated well from most peptides, but it was necessary to chromatograph for over 24 hours in order to resolve it from Peptide T4 which moves slightly slower.

Peptide T20 was located in Fraction C. On gel filtration it partially adsorbed to the Sephadex material and thus eluted in the last region, C3, where it constituted 92% of this fraction. It was prepared in homogeneous form from both Regions C2 and C3 by electrophoresis (Fig. 21).

Peptide T21 was found in Fraction G of the eluate from the Dowex 50 column. This peptide comprised 36% of Fraction G (see Fig. 2I1) and together with Peptides T11 and T22 constituted the bulk of this fraction. It was easily purified from Region G1 by electrophoresis because it migrated more rapidly than the other component peptides by virtue of a charge of +2.

Peptide T22, which constituted 21% of Fraction G1 (Fig. 2I1), was isolated following both electrophoresis and chromatography as described under "Peptide T11."

Peptide T23, a decapeptide, was one of the main components of the Al protein (Fig. 3) denoted in parentheses. Chymotryptic peptides are denoted by T.

Yield of Tryptic Peptides—From the Dowex 50 chromatography, 19 out of the 27 possible tryptic peptides, found on peptide
Table 1A

Amino acid composition of tryptic peptides from bovine A1 protein*  

| Amino Acid | T4 | T5 | T6 | T6A | T7 | T8 | T12 | T14 | T15 | T16 | T16A* |
|------------|----|----|----|-----|----|----|-----|-----|-----|-----|-------|
| Lysine     | 1.1(1) | 0.6(1) | 0.8(1) | 1.0(1) | 1.0(1) | 0.9(1) | 0.8(1) | 0.8(1) | 0.8(1) | 1.0(1) | 1.7(2) |
| Histidine  | 2.0(2) |
| Arginine   | 2.0(2) | 1.0(1) | 2.0(2) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.7(2) |
| Aspartic Acid | 2.0(2) | 1.0(1) | 2.0(2) | 0.9(1) | 1.8(2) | 1.1(1) |
| Threonine  | 1.0(1) | 0.9(1) | 0.9(1) | 0.9(1) | 0.8(1) |
| Serine     | 1.8(2) | 0.9(1) | 0.9(1) | 1.0(1) | 0.9(1) | 2.0(2) |
| Glutamic Acid | 2.0(2) | 1.0(1) |
| Proline    | 0.9(1) | 2.0(2) | 1.0(1) | 1.0(1) | 0.9(1) | 1.6(2) |
| Glycine    | 1.0(1) | 2.0(2) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) |
| Alanine    | 2.8(3) | 1.0(1) | 2.0(2) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) |
| Valine     | 0.6(1) |
| Methionine | 0.6(1) |
| Isoleucine | 0.9(1) | 1.0(1) | 0.6(1) |
| Leucine    | 1.0(1) | 1.1(1) | 0.9(1) | 0.9(1) | 2.0(2) |
| Tyrosine   | 2.0(2) |
| Phenylalanine | 1.0(1) | 2.0(2) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) |

YIELD (% Theoretical)

| TOTAL | 12 | 6 | 2 | 12 | 10 | 6 | 17 | 6 | 8 | 2 | 8 |

Table 1B

Amino acid composition of tryptic peptides from bovine A1 protein*  

| Amino Acid | T17 | T18 | T19 | T20 | T21 | T22 | T23 | T24 | T25 | T26 | T27 |
|------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Lysine     | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) |
| Histidine  | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) |
| Arginine   | 1.3(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) |
| Aspartic Acid | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) |
| Threonine  | 0.9(1) | 0.9(1) | 0.9(1) | 0.9(1) | 0.9(1) | 0.9(1) | 0.9(1) | 0.9(1) | 0.9(1) | 0.9(1) | 0.9(1) | 0.9(1) |
| Serine     | 2.8(2) | 0.9(1) | 0.9(1) | 0.9(1) | 0.9(1) | 0.9(1) | 0.9(1) | 0.9(1) | 0.9(1) | 0.9(1) | 0.9(1) | 0.9(1) |
| Glutamic Acid | 2.0(2) | 1.0(1) | 2.0(2) | 1.0(1) | 2.0(2) | 1.0(1) | 2.0(2) | 1.0(1) | 2.0(2) | 1.0(1) | 2.0(2) | 1.0(1) |
| Proline    | 0.9(1) |
| Glycine    | 2.0(2) | 1.0(1) | 2.0(2) | 1.0(1) | 2.0(2) | 1.0(1) | 2.0(2) | 1.0(1) | 2.0(2) | 1.0(1) | 2.0(2) | 1.0(1) |
| Alanine    | 2.0(2) | 1.0(1) | 2.0(2) | 1.0(1) | 2.0(2) | 1.0(1) | 2.0(2) | 1.0(1) | 2.0(2) | 1.0(1) | 2.0(2) | 1.0(1) |
| Valine     | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) |
| Methionine | 0.6(1) |
| Isoleucine | 0.9(1) | 0.9(1) | 0.9(1) | 0.9(1) | 0.9(1) | 0.9(1) | 0.9(1) | 0.9(1) | 0.9(1) | 0.9(1) | 0.9(1) | 0.9(1) |

YIELD (% Theoretical)

| TOTAL | 12 | 9 | 38 | 58 | 64 | 70 | 54 | 68 | 20 | 13 | 19 |

* The composition of Peptides T1, T2, T3, T9, T10, T11, and T12 has been reported previously (11, 20). Peptides T4, T6A, T7, T8, T13, T14, T15, T16, T17, T18, T20, T21, T22, T23, and T24 were eluted from the Dowex column (Fig. 1). Peptides T5, T6, T16A, T19, T25, T26, and T27 were isolated from a peptide map.

* In Peptide T16A 1.4 moles of arginine and 0.3 mole of dimethylarginine per 2.0 moles of leucine were found.

* For Peptides T18 and T19, the tryptophan value was quantitatively determined on the amino acid analyzer following hydrolysis with aminopeptidase M.
mapping (Fig. 3), were subsequently isolated. The other eight peptides were presumably retained on the column. Peptide T1, a neutral peptide because of the acetylated NH2 terminus (11), was probably not detected with the ninhydrin procedure; other peptides, however, such as Peptide T2, are relatively basic, having a charge +2, and may not have eluted. It appeared generally that arginine-containing peptides were bound more strongly than lysine-containing peptides. Peptide T19 is relatively large (18 residues) and hydrophobic and was probably strongly bound to the Dowex beads. It is fortunate, however, that under the conditions used the Lys-Pro linkage was partially cleaved, giving Peptide T18, which was obtained in a 9% yield. In the usual peptide mapping procedure, therefore, Peptide T18 is not observed; only Peptide T19.

For most of the tryptic peptides, a yield of 15 to 70% of theoretical was obtained, a value high considering the losses on paper electrophoresis or chromatography (Table 1). In some cases, however, the yield was lower, either due to adsorption on the Dowex 50 column or incomplete hydrolysis as in the case of Peptide T18 and Peptides T10 and T11 where the Lys-Asp linkage is partially resistant to hydrolysis by trypsin (29).

**SEQUENCE STUDIES ON TRYPIC PEPTIDES**

Peptide T1 (Residues 1 to 6): Acetyl-Ala-Ser-Ala-Gln-Lys—The sequence of this ninhydrin-negative peptide was established as described earlier (11) using the peptide fragment CB1 cleaved at methionine residue 20 with CNBr.

Peptide T2 (Residues 6 to 10): Arg-Pro-Gln-Arg—The sequence of this peptide was determined previously (11) as part of Peptide CB1.

Peptide T3 (Residues 11 and 12): Ser-Lys—The sequence is obvious from specificity of trypsin.

Peptide T4 (Residues 13 to 24): Tyr-Leu-Ala-Ser-Ala-Thr-Met-Asp-His-Ala-Arg—The sequence of the first 8 residues was determined previously (11) as part of Peptide CB1. The remainder of the sequence was determined in three ways. Direct Edman degradation of Peptide T4 confirmed the sequence from tyrosine to methionine as well as aspartic acid as the 9th residue. Direct Edman degradation of Peptide CB2 (from CNBr (reduced) gave Asp-(Ala)3, thus showing aspartic acid and alanine to occupy the first and third positions from the methionine residue. It had been established earlier (11) that Peptide CB2 was linked to Peptide CB1 through the methionyl residues. Finally, Peptide T4, when treated with carboxypeptidase for 30 min, gave arginine (1.08), alanine (0.55), and histidine (0.17). This information thus establishes the sequence of the COOH-terminal region as Met-Asp-His-Ala-Arg.

Peptide T5 (Residues 25 to 30): His-Gly-Phe-Leu-Pro-Arg—The sequence of this peptide was determined with the subtractive Edman method. Step 1: His, 93%; Step 2: Phe, 67%; Step 3: Gly, 54%; Step 4: Leu, 58%.

Peptide T6 (Residues 31 to 32): His-Arg—The sequence is established by the specificity of trypsin.

Peptide T6A (Residues 31 to 42): His-Arg-Asp-Thr-Phe-Asp-(Leu, Arg, Ser, Gly)—The sequence of this peptide was determined by direct Edman degradation over the first 5 residues as established from these data that the Arg-Asp linkage between Peptides T6 and T7 was not split. Such linkages are hydrolyzed more slowly by trypsin because of the carboxyl group (29).

Peptide T7 (Residues 33 to 42): Asp-Thr-Gly-Ile-Leu-Arg—Seven steps of the direct Edman degradation gave Asp-Thr-Gly-Ile-Leu-Asp-Ser. Use of the subtractive Edman technique gave the Asp-Thr-Gly-Ile- sequence for the NH2 terminal region and thus confirms isoleucine at the fourth position. Step 1: Asp, 42%; Step 2: Thr, 78%; Step 3: Gly, 37%; Step 4: Ile, 57%. Carboxypeptidase action revealed the sequence of the COOH-terminal region as -Leu-Gly-Arg. These data are sufficient to establish the sequence. The nonamidated form of the aspartic acid was inferred from the mobilities on electrophoresis (Fig. 3).

Peptide T8 (Residues 43 to 48): Phe-Phe-Gly-Ser-Asp-Arg—Four steps of the direct Edman procedure established the sequence of this peptide. The nonamidated form of the aspartic acid was inferred from the mobility on electrophoresis (Fig. 3).

Peptide T9 (Residues 49 to 52): Gly-Ala-Pro-Lys—The sequence was reported previously as part of peptic Peptide R (20).

Peptide T10 (Residues 53 to 57): Gly-Ser-Gly-Lys—The sequence of this peptide was also previously reported as part of peptic Peptide R (20).

Peptide T11 (Residues 58 to 64): Asp-Gly-His-Ala-Ala-Arg—The sequence of this peptide was also previously reported as part of peptic Peptide R (20).

Peptide T12 (Residues 65 to 71): Thr-Thr-His-Tyr-Gly-Ser-Leu-Pro-Gln-Lys—The sequence of this peptide was also previously reported as part of peptic Peptide R (20).

Peptide T13 (Residues 72 to 91): Ala-Glu-Gly-His-Pro-Gln-Glu-Asp-Val-Val-His-Ser-Phe-Lys—The determination of the sequence of this peptide was reported previously as part of peptic Peptide R (20) except for the COOH-terminal Phe-Lys segment. Carboxypeptidase treatment for 5 min gave relative values of Lys, 1.0; Phe, 1.9; His, 0.4; and Val, 0.3. Thus the COOH-terminal region appears to be Phe-Lys-Phe-Lys.

Peptide T14 (Residues 92 to 97): Asn-Ile-Val-Thr-Pro-Arg—The direct Edman procedure gave Asn-Ile-Val-Thr- for the first 4 residues, thus establishing the sequence of this peptide. The sequence was confirmed by the subtractive Edman procedure. Step 1: Asp, 78%; Step 2: Ile, 68%; Step 3: Thr, 72%; Step 4: Thr, 80%. The amidated aspartic residue is further suggested by the mobility of the peptide in the group with charge -1.

Peptide T15 (Residues 98 to 105): Thr-Pro-Pro-Pro-Ser-Gln-Gly-Lys—Using six steps of the direct Edman degradation, the sequence Thr-Pro-Pro-Pro-Ser-Gln- was established. Amino acid analysis of the material following this procedure gave: Lys, 1.0; Gly, 1.0; Glu, 0.2.

Peptide T16 (Residues 106 to 107): Gly-Arg—The sequence is evident from the specificity of trypsin. It was not determined whether the basic residue was arginine of the monomethylarginine derivative. No trace of the dimethylarginine was seen.

Peptide T16A (Residues 106 to 113): Gly-Arg(Methyl); or (Gly,2 Leu,2 Ser)—The indirect Edman procedure gave: Step 1: Gly, 40%; Step 2: Arg, 45%; Step 3: no change. These data define the partial sequence as shown. It has been established (23) that position 2 is occupied by methylated arginine residues (monomethyl- and dimethylarginine derivatives). It appears likely that the resistance of the methylated arginine site to trypsin digestion is due to the methylation, since, under the conditions used, even a highly resistant Lys-Pro linkage was partially cleaved producing Peptide T18.

Peptide T17 (Residues 108 to 113): Gly-Leu-Ser-Leu-Arg
Two steps of the direct Edman procedure gave Gly-Leu. The subtractive Edman procedure confirmed this: Step 1: Gly, 40%; Step 2: Leu, 33%. Ser, 80%; Step 3: Ser, 53%; Step 4: Leu, 63%. Carboxypeptidase (30 min) gave Arg, 1.0, and Ser, 0.2.

**Peptide T18 (Residues 11 to 19): Phc-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Lys**—The first 3 residues of Peptide T18 were established by the subtractive Edman technique, which gave Gly-Ala-Glu—glycine. The sequence of Peptide T18 was then established by carboxypeptidase treatment. Amino compound M treated for 10 hours gave the relative values: Lys, 1.0; Ser, 0.1; and Gly, 0.1. Aminopeptidase M was not helpful for the determination of the sequence as shown (Step 1: Ser, 95%; Step 2: Ala, 65%).

**Peptide T19 (Residues 11 to 19): Phc-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Lys-Pro-(2Gly, 1Phe)—Tyr-Glu-Gly-Arg**—This hepta-decapeptide was subjected to the Edman degradation; nine steps gave the sequence from NH2-terminal Phe to Lys. The presence of lysine at position was confirmed by the subtractive Edman procedure. The remainder of the sequence was determined by using peptide obtained after 2-hour digestion with chymotrypsin. The peptides were resolved by peptide mapping. Three peptides were obtained with the following sequences:

**T19-1** Phe-Ser-Trp

**T19-2** Gly-Ala-Glu-(3Gly, 1Glu, 1Lys, 1Pro)-Phe-Tyr

**T19-3** Gly-Gly-Arg

The sequence of Peptide T19-1 was shown by subtractive Edman degradation (NH2-terminal Phe) and carboxypeptidase (COOH-terminal Trp). The first 3 residues of Peptide T19-2 were shown by direct Edman degradation; the COOH-terminal Tyr was found by hydrazinolysis. The sequence of Peptide T19-3 was shown by carboxypeptidase which released relatively 1.0 mole of Arg and 0.2 mole of Gly in 30 min.

**Peptide T20 (Residues 131 to 139): Ala-Ser-Asp-Tyr-Lys**—The first 3 residues, Ala-Ser-Asp, were obtained by the direct Edman procedure. The subtractive Edman procedure confirmed this result: Step 1: Ala, 18%; Step 2: Ser, 86%; Step 3: Asp, 45%.

**Peptide T21 (Residues 196 to 204): Ser-Ala-His-Lys**—The direct and subtractive Edman procedures both gave Ser-Ala for the sequence of the NH2 terminus, thus establishing the sequence shown (Step 1: Ser, 95%; Step 2: Ala, 65%).

**Peptide T22 (Residues 140 to 142): Gly-Leu-Lys**—The direct and indirect procedures both gave glycine at the NH2 terminus of this tripeptide: Step 1: Gly, 90%.

**Peptide T23 (Residues 143 to 152): Gly-His-Asp-Ala-Glu-Gly-Thr-Leu-Ser-Lys**—This decapeptide was subjected to eight steps of the direct Edman procedure which established the sequence of the first 8 residues. Carboxypeptidase treatment (2 hours) gave the relative values: Lys, 1.0; Ser, 0.11; and Leu, 0.11.

**Peptide T24 (Residues 153 to 165): He-Phe-Lys**—The first 3 residues of Peptide T24 were established by the direct Edman procedure, which gave NH2-terminal isoleucine, and thereby the sequence as shown. Aminopeptidase M was not helpful because it released equal amounts of isoleucine and phenylalanine.

**Peptide T25 (Residues 166 to 170): Leu-Gly-Arg**—The direct Edman technique gave Leu-Gly at the NH2 terminus, which establishes the sequence shown. The indirect Edman confirmed the NH2-terminal Leu with a 60% reduction in leucine after one step.

**Peptide T26 (Residues 160 to 162): Asp-Ser-Arg**—The NH2 terminus of this tripeptide is aspartic acid as shown by the direct Edman procedure. The indirect Edman also established Asp at the NH2 terminal end: Step 1: Asp, 74%.

**Peptide T27 (Residues 163 to 169): Ser-Gly-Ser-Pro-Met-Ala-Arg**—Five steps of the direct Edman procedure established the Ser-Gly-Ser-Pro-Met sequence and thus the sequence of the peptide. Carboxypeptidase treatment for 30 min gave relative values of Arg, 1.0; Ala, 0.38. No other residues were liberated.

The subtractive Edman procedure confirmed the sequence of the first 3 residues: Step 1: Ser, 32%; Step 2: Gly, 23%; Step 3: Ser, 50%.

**Hydrolysis with Pepin and Purification of Pepin Peptides**—The bovine A1 protein (3.6 g) was treated with pepsin at 37°C for 18 hours (as described elsewhere (10)) and lyophilized. The digest was taken up in 0.001 N ammonium acetate, pH 7.0, and applied to a column of Cellex-P, as shown in Fig. 1 of Reference 20. The elution profile of the peptides was monitored by absorbance measurement at 223 nm. High voltage electrophoresis of selected sections from each peak was then performed. Based on these results, the elution profile was divided into 13 peptide fractions as follows: PA (tubes 60 to 80); PB (tubes 135 to 140); PC (tubes 141 to 145); PD (tubes 146 to 157); PE (tubes 158 to 164); PF (tubes 165 to 170); FG (tubes 180 to 190); FH (tubes 196 to 204); PI (tubes 205 to 220); PJ (tubes 223 to 253); PK (tubes 240 to 259); PL (tubes 353 to 364); PM (tubes 365 to 380); PN (tubes 360 to 430); PO (tubes 500 to 516). Tubes common to each fraction were pooled and lyophilized.

The peptic peptide fractions were desalted on a column (105 × 3.2 cm) of Sephadex G-10 (55 cm upper portion) and G-25 (90 cm lower portion). The elution was carried out at 15°C with 0.1 M acetic acid; 8 to 10 ml per tube were collected. The elution pattern was followed by measuring the absorbance at 223 and 280 nm. The purity and identity of the peptides were evaluated by high voltage electrophoresis at pH 4.7 and by paper chromatography. Appropriate tubes containing subfractions of peptides were combined and lyophilized. Desalted mixtures containing two or more peptides were further purified either by preparative paper chromatography or high voltage electrophoresis.

**Peptide P1** was derived from Fraction PM, one of the last peaks to elute from the Cellex-P column. On gel filtration, most of the material of this fraction eluted in a single peak. On preparative paper chromatography, one major peptide band was found with traces of three other peptides: 30 mg were eluted. This material gave one band on high voltage electrophoresis. Thus Fraction PM is composed primarily of one peptide component.

**Peptide P2** was also found in one of the last fractions to elute from the Cellex-P column, Fraction PN, and thus is one of the most basic peptides. On gel filtration, one major peak was observed, which on paper electrophoresis resolved into one main peptide, which migrated more rapidly than three minor bands. The major peptide was purified by preparative electrophoresis: 7 mg were obtained. This material appeared homogeneous.

**Peptide P3** was found in Fraction PC, one of the first peaks eluted from Cellex-P. One major peak was observed on gel filtration which resolved into six peptides on paper electrophoresis. This neutral peptide (0.3 mg) appeared homogeneous on paper chromatography.

**Peptide P3A** was present in the first Cellex-P fraction PA; on gel filtration, it was found in the second of two main peaks. On paper electrophoresis it is separated clearly from six other peptides, moving as an acidic peptide toward the anode. The peptide (27 mg) was eluted from the paper in 41% theoretical yield.

**Peptide P4** was derived from Cellex-P Fraction PG. After gel filtration the material eluting just prior to the single main peak was subjected to paper chromatography; six bands were...
Peptide P5 was located in Cellex-P Fraction PO, the last fraction to elute from the column. Peptide P5 (refored to elsewhere at Peptide R) was isolated in relatively high yield (45%) from this fraction as described elsewhere (20).

Peptide P6 was found in Cellex-P Fraction PH. On gel filtration, this peptide eluted in the first of two peaks. From paper electrophoresis, 39 mg of Peptide P6 were obtained; only a trace of another peptide was seen. This peptide, therefore, comprises 95% of the first peak found on gel filtration.

Peptide P7 was found in Cellex-P Fraction PJ. On gel filtration, this peptide (8 mg) was found in the shoulder prior to the single main peak. It migrated slowly as a homogeneous band on electrophoresis.

Peptide P8 was obtained by Cellex-P chromatography in phosphate-saline buffer as described earlier (10). It appeared homogeneous from the Cellex-P column.

Peptide P9 was derived from Cellex-P Fraction PE. On gel filtration, this peptide eluted in the major front peak in homogeneous form with yield of 37 mg, 30% theoretical. A minor second peak was also observed.

Peptide P10 was obtained primarily from Fraction PD of the Cellex-P eluate. Gel filtration of Fraction FD revealed one main peak which contained several peptides including traces of Peptides P8 and P9. Peptide P10 was obtained in homogeneous form by paper chromatography. The yield was 37 mg.

Peptide P10A was obtained from Cellex-P Fraction PK following gel filtration. A single major peak was obtained, which on paper electrophoresis separated into five peptides, Peptide P13 migrating slightly faster than the other two peptide components. A minor second peak was also observed.

Peptide P10 was obtained primarily from Fraction PD of the Cellex-P eluate. Gel filtration of Fraction FD revealed one main peak which contained several peptides including traces of Peptides P8 and P9. Peptide P10 was obtained in homogeneous form by paper chromatography. The yield was 37 mg.

Peptide P11A was found in Cellex-P Fraction PH along with Peptides P9 and P10. On gel filtration, Peptide P12 was the last peptide to elute from the column. All peptides containing tryptophan, Peptides P11, P11A, P12, P12A, appeared to be retarded on gel filtration, and thus eluted later than smaller peptides. Peptide P12 was purified by paper electrophoresis; 19 mg were obtained.

Peptide P12A was located primarily in Cellex-P Fraction PF. On gel filtration this peptide eluted as a small trailing band after the large single peak. On paper chromatography, five bands were observed but only the slowest, Peptide P12A, was recovered in adequate quantity (4.8 mg) for study.

Peptide P13 was found in Cellex-P Fraction FC, and eluted on gel filtration in the single main peak. It was resolved on paper electrophoresis into five peptides, Peptide P13 migrating slightly toward the cathode and Peptide P8 migrating slightly toward the anode. Approximately 8.2 mg were recovered.

Peptide P14 was found in Cellex-P Fraction PO along with the large Peptide P5. This peptide was easily isolated following paper chromatography, as shown in Fig. 2 of Reference 20, where it migrated more rapidly than the other two peptide components. Based on the weight of the peptide eluted from the paper, the yield was 18% of theoretical.

The amino acid compositions of the peptic peptides are shown in Table II. It should be noted that Peptides P10 and P10A are identical except for the replacement in the latter peptide of 1 arginine residue by the dimethylarginine derivative. It is evi-
dent that the methylation of the single arginine residue accounts for the differences in chromatographic properties of these peptides. Because of the broad specificity of pepsin activity, other peptides were observed but were not studied because of low yield or the presence of impurities.

**SEQUENCE STUDIES ON PEPTIDE PEPTIDES**

**Peptide P1** (Residues 1 to 13): N-Acetyl-Ala-Ser-Ala-Gln-Lys-Arg-Pro-Ser-Gln-Arg-Ser-Lys-Tyr—The NH₂-terminal residue was not detected by either the direct Edman or dansylation procedures. Carboxypeptidase treatment (2 hours; 37°) gave 0.9 mole of tyrosine, 0.8 mole of lysine, 0.4 mole of serine, and 0.2 mole of arginine. The blocked NH₂ terminus and the sequence of the COOH-terminal region identify this peptide as the NH₂ terminus of the A1 protein. It is also found as part of Peptide CB1, the sequence of which has been reported (11).

**Peptide P2** (Residues 14 to 57): Leu-Ala-Ser-Ala-Thr-Met-Asp-His-Ala-Ala-Arg-His-Gly-Phe-Leu-Pro-Arg-His-Arg-Asp (Asp, Thr)-Gly-Ile-Leu—As shown in Table II, Peptide P2 contains 1 methionine residue, 3 histidines, but no lysine and thus is unusual. When this peptide (5 mg) was treated with CNBr (11), two peptides were subsequently isolated by electrophoresis (Table III): Peptide P2-CN1, a neutral peptide and a highly basic peptide, Peptide P2-CN2.

The sequence of Peptide P2 was established as shown by the data in Table III. Peptide P2-CN2 was derived from the NH₂-terminal region since it contained homoserine, and was shown by Edman degradation to have the Leu-Ala-Ser-Ala-Thr-Met sequence previously demonstrated (11). Direct Edman degradation of Peptide P2-CN2 positions the tryptic peptides derived from this peptide. The Met-Asp sequence is evident. Following tryptic Peptide P2-CN2-T1 are Peptides T2 and T3, thus leaving Peptide T4, the only peptide with Ile, at the COOH-terminal position. It has the terminal sequence of Gly-Ile-Leu as shown by carboxypeptidase.

**Peptide P3** (Residues 58 to 85): Asp Ser Leu Gly-Arg-Phe—Four steps of the direct Edman degradation established the NH₂-terminal sequence as Asp-Ser-Leu-Gly. Hydrolysis with carboxypeptidase A for 1 hour released phenylalanine (1 mole) only. When carboxypeptidase B was then added, arginine (1.0 mole) and glycine (0.5 mole) were released in 10 min.

**Peptide P3A** (Residues 58 to 80): Asp-Ser-Leu—The NH₂-terminal aspartic acid was found by direct Edman procedure. The COOH-terminal leucine was found on hydrazinolysis.

**Peptide P4** (Residues 41 to 53): Gly-Arg-Phe—The direct Edman procedure gave glycine; the COOH-terminal phenylalanine was identified by hydrazinolysis.

**Peptide P5** (Residues 44 to 80): Phe-Gly-Ser-Asp-Ary-Gly-Ala-Pro-Asp-Gly-Ser-Gly-Arg-His-Asp-His-Ala-Ala-Ala-Thr-Thr-His-Tyr-Gly-Ser-Leu-Pro-Gln-Lys-Ala-Gln-Gly-His-Arg-Pro-Gln-Glu-Asn-Pro-Val-Val-His-Phe—The sequence of this peptide, referred to as Peptide R, has been reported previously (20).

**Hydrazinolysis** of Peptide R proceeded further, and amino acid analysis revealed the following composition: Phe, 1.0 mole; His, 0.35; Val, 0.4; and Leu, 0.7.

**Peptide P6** (Residues 69 to 89): Gly-Ser-Leu-Pro-Gln-Lys-Ala-Gln-Gly-His-Arg-Pro-Gln-Glu-Asn-(Pro,Val)-Val-His-Phe—The sequence of NH₂-terminal region was determined with 16 steps of the direct Edman procedure. Carboxypeptidase treatment for 2 hours gave molar ratios of Phe, 0.35; His, 0.52; and Val, 0.23.

**Peptide P7** (Residues 72 to 89): Pro-Gln-Lys-Glu-Ala-Gln-Gly-His-Arg-Pro-Gln-Glu-Asp-(Glu, Asp, Pro, Val)-Val-His-Phe—Using the direct Edman procedure, 11 residues of the NH₂-terminal region were determined. Carboxypeptidase treatment for 1 hour gave molar ratios of Phe, 0.35; His, 0.52; and Val, 0.23.

**Peptide P8** (Residues 85 to 89): Pro-Val-His-Phe—Four steps of the direct Edman procedure gave Pro Val Val His. Hydrazinolysis revealed COOH-terminal phenylalanine.

**Peptide P9** (Residues 90 to 95): Phe-Lys-Asn-Ile-Val-Thr—The NH₂-terminal sequence of Phe-Lys-Asn-Ile was determined with four steps of the direct Edman degradation. Treatment with carboxypeptidase for 1 hour released Thr, 1.0; Val, 0.7; His, 0.2 mole per mole of peptide.

**Peptide P10** (Residues 69 to 111): Pro-Arg-Thr-Pro-Pro-Ser-Gln-Gly-Gly-Ala-Gly-Gly-Ser-Leu—The sequence of this peptide was determined from the data shown in Table IV. Hydrolysis with carboxypeptidase released nearly 1 mole of leucine, and some serine. The Edman degradation gave the sequence of the first 11 residues. The additional data needed to determine the entire sequence were obtained from tryptic peptides (5 mg of peptide, 5 hours, 37°). The tryptic digest was resolved by paper electrophoresis into five peptides. The Pro-Arg peptide is derived from the NH₂-terminal region. Peptide P10-T9 is positioned from the Edman degradation results, which also positions the Gly-Arg dipeptide. The tetrapeptide, P10-T4, occupies the COOH terminus since it is the only peptide containing leucine. These data were sufficient to establish the complete sequence of Peptide P10.

**Peptide P10-T5** is of interest because it contains the uncleaved Arg-Gly linkage which, under normal conditions, would be hydrolyzed by trypsin to give Peptides P10-T3 and P10-T4. Based on the recoveries of these peptides, it was found that only 60% of the Arg-Gly linkage had been hydrolyzed, thus showing a relatively high resistance to trypsin. Since it is this site, arginine residue 107, at which methylation occurs (23) it is likely that this
residue exists as the dimethyl derivative in Peptide P10, a modification which could render it partially resistant to trypsin. Direct evidence for dimethylarginine in this peptide was not obtained since it cannot be distinguished from arginine by the short basic column of the Beckman AutoAnalyzer; no dimethyl-arginine was observed.

Peptide P10A has the sequence identical with Peptide P10, except that 1 arginine residue is present entirely as the dimethyl derivative. When treated with trypsin, as described for Peptide P10, the peptide analogous to Peptide P10-T5 was found, but Peptides P10-T3 and P10-T4 were not found. These data show that the arginine residue 107 is present in this peptide as the dimethyl derivative, and is highly resistant to hydrolysis by trypsin.

**Peptide P11** (Residues 112 to 125): Ser-Arg-Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Lys-Pro-Gly-Phe—This tetradecapeptide, previously referred to as Peptide E (8), was the first encephalitogenic peptide to be isolated from the A1 protein. Its sequence was originally reported to contain 2 additional residues (8), and since been corrected (13). The data shown in Table V establish the sequence. Ten steps of the direct Edman procedure give the sequence through glutamine. At the 11th step, however, glycine predominated over lysine and an unequivocal choice could not be made. The COOH-terminal residue was identified as Phe by hydrazinolysis.

The COOH-terminal portion of Peptide P11 was established by the isolation of Peptide P11-T1, a neutral peptide in 30% yield following treatment with trypsin (2 mg of peptide, 30 hours, 37°, 1:1 ratio of peptide to enzyme). It is apparent that, under these conditions, trypsin slowly hydrolyzes a Lys-Pro linkage, giving a Pro-Gly-Phe tripeptide. This finding parallels that of Carnegie (16), who found that in the analogous peptide from the human A1 protein an Arg-Pro linkage is slowly hydrolyzed.

**Peptide P11A** (Residues 112 to 133): Ser-Arg-Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Lys-(Pro, Gly, Phe)-Gly-Tyr-Gly-Gly-Arg-Ala-Ser-Asp—This peptide, previously referred to as Peptide E1 (8), was one of the first encephalitogenic peptides along with Peptide P11 to be derived from the A1 protein. It has an NH2-terminal region identical with Peptide P11 as shown by the 11 steps of the direct Edman procedure (Table V). This peptide differs from Peptide P11 only by an extension of the COOH-terminal region. As shown in Table V, the sequence of this region was determined by using chymotryptic peptides. Thus the COOH-terminal Phe of Peptide P11 is further extended in Peptide P11A as follows: -Phe-Gly-Tyr-Gly-Gly-Arg-Ala-Ser-Asp.

**Peptide P12** (Residues 115 to 128): Ser-Trp-Gly-(Ala, Glu, Gly, Lys, Pro)-Phe-Gly-Tyr—This 13-residue peptide is from the same tryptophan region as Peptides P11 and P11A and appears to have the identical sequence based on amino acid analysis and the partial sequence determination. This peptide begins with Ser-Trp-Gly as shown by direct Edman procedure. Carboxypeptidase (1 hour) treatment gave: Tyr (0.6), Gly (0.19), Phe (0.10). Thus Peptide P12 has the same COOH-terminal sequence as Peptide P11A-C3, a chymotryptic peptide derived from Peptide P11A.

**Peptide P12A** (Residues 115 to 133)—This peptide lacks the NH2-terminal tripeptide Ser-Arg-Phe present in Peptide P11A but otherwise is identical as shown by amino acid composition. Three steps of the Edman degradation gave Ser-Trp-Gly, thus showing an identity with Peptide P112 over 13 residues.

**Peptide P13** (Residues 125 to 134): Gly-Gly-Arg-Ala-Ser-Asp-Tyr—The sequence of Gly-Gly-Arg-Ala-Ser- was obtained with an identification of the Asp residue as the monomethyl derivative. When treated with trypsin, as described for Peptide P10, the peptide analogous to Peptide P10-T5 was found, but Peptides P10-T3 and P10-T4 were not found. These data show that the arginine residue 107 is present in this peptide as the dimethyl derivative, and is highly resistant to hydrolysis by trypsin.

**Table IV**

| Peptide P10 Sequence | Peptide P11 Sequence |
|----------------------|----------------------|
| Ser-Arg-Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Lys-Pro-Gly-Phe- | Ser-Arg-Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Lys-Pro-Gly-Phe- |
| Pro-Arg-Thr-Pro-Pro-Ser-Gln-Gly-Lys- | Pro-Arg-Thr-Pro-Pro-Ser-Gln-Gly-Lys- |
| P10-T1-Pro-Arg-Gly-Gln-Leu- | P10-T1-Pro-Arg-Gly-Gln-Leu- |
| P10-T3-Pro-Arg-Trp-Tyr-Phe-Pro-Arg-Gly-Gln-Lys- | P10-T3-Pro-Arg-Trp-Tyr-Phe-Pro-Arg-Gly-Gln-Lys- |
| Carboxypeptidase | Carboxypeptidase |
| Direct Edman degradation (11 steps) | Direct Edman degradation (11 steps) |
| Residue after Step 10 | Residue after Step 10 |
| Tryptic Peptides | Tryptic Peptides |
| P10-T1 | P10-T1 |
| P10-T2 | P10-T2 |
| Edman | Edman |
| P10-T3 | P10-T3 |
| P10-T4 | P10-T4 |
| Edman | Edman |
| P10-T5 | P10-T5 |

**Table V**

| Peptide P11 | Peptide P11A |
|-------------|--------------|
| Sequence | Sequence |
| Ser-Arg-Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Lys-Pro-Gly-Phe- | Ser-Arg-Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Lys-Pro-Gly-Phe- |
| Edman degradation (10 steps) | Edman degradation (10 steps) |
| Hydrazinolysis | Hydrazinolysis |
| Tryptic peptides | Tryptic peptides |
| P11-T1 (15% yield) | P11-T1 (15% yield) |
| Edman | Edman |
| Peptide P11A | Peptide P11A |
| Sequence | Sequence |
| Ser-Arg-Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Lys-(Pro, Gly, Phe)-Gly-Tyr-Gly-Gly-Arg-Ala-Ser-Asp | Ser-Arg-Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Lys-(Pro, Gly, Phe)-Gly-Tyr-Gly-Gly-Arg-Ala-Ser-Asp |
| Carboxypeptidase (3 hrs) | Carboxypeptidase (3 hrs) |
| Chymotryptic peptides | Chymotryptic peptides |
| P11A-C1 | P11A-C1 |
| Edman | Edman |
| P11A-C2 | P11A-C2 |
| P11A-C3 | P11A-C3 |
| Carboxypeptidase (2 hrs) | Carboxypeptidase (2 hrs) |
| P11A-C4 | P11A-C4 |
| Edman | Edman |
| Gly-Gly-Arg-Ala | Gly-Gly-Arg-Ala |

**Table V**

| Amino acid sequences of peptic Peptides 111 and 11A |
|---------------------------------------------------|
| **Peptide P11** | **Peptide P11A** |
| Sequence | Sequence |
| Ser-Arg-Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Lys-Pro-Gly-Phe- | Ser-Arg-Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Lys-Pro-Gly-Phe- |
| Edman degradation (10 steps) | Edman degradation (10 steps) |
| Hydrazinolysis | Hydrazinolysis |
| Tryptic peptides | Tryptic peptides |
| P11-T1 (15% yield) | P11-T1 (15% yield) |
| Edman | Edman |
| Peptide P11A | Peptide P11A |
| Sequence | Sequence |
| Ser-Arg-Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Lys-(Pro, Gly, Phe)-Gly-Tyr-Gly-Gly-Arg-Ala-Ser-Asp | Ser-Arg-Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Lys-(Pro, Gly, Phe)-Gly-Tyr-Gly-Gly-Arg-Ala-Ser-Asp |
| Carboxypeptidase (3 hrs) | Carboxypeptidase (3 hrs) |
| Chymotryptic peptides | Chymotryptic peptides |
| P11A-C1 | P11A-C1 |
| Edman | Edman |
| P11A-C2 | P11A-C2 |
| P11A-C3 | P11A-C3 |
| Carboxypeptidase (2 hrs) | Carboxypeptidase (2 hrs) |
| P11A-C4 | P11A-C4 |
| Edman | Edman |
| Gly-Gly-Arg-Ala | Gly-Gly-Arg-Ala |
highly specific reagent which cleaves solely at the COOH-tryptophanyl linkage giving two peptide fragments, Peptides L and T. Al protein. The same peptide was isolated (27) in higher yield following treatment of the Al protein with BNPS-skatole, a reagent not containing a tyrosine and several glycine residues, are derived from the COOH-terminal region. The first 12 residues of the NH2 terminus were determined by the Edman degradation. The Gly–His–Asp–Ala sequence of Peptide P14 T4 was also obtained from Edman degradation.

To obtain further data, Peptide P14 (5 mg) was treated with trypsin (120 µg) for 6 hours at 37°C. The tryptic peptides were resolved by high voltage electrophoresis. All of the expected peptides were found. Peptide P14 T3 moved identically with trypsin (120 µg) for 6 hours at 37°C. The tryptic peptides

**Peptide T (residues 117 to 170)**

Peptide T was used in this study to position the peptides located in the carboxyl-terminal region. When the A1 protein was treated with N-bromosuccinimide, the COOH-tryptophanyl linkage was cleaved, and a large peptide, referred to as Peptide T, was subsequently isolated (26). This peptide occupies the 54 residues of the COOH-terminal region of the A1 protein, from tryptophan to arginine, since it begins at the NH2 terminus with Gly–Ala–Glu–Gly–Gln- and terminates at the other end with Ala Arg Arg (26), the same COOH-terminal region found for the A1 protein. The same peptide was isolated (27) in higher yield following treatment of the A1 protein with BNPS-skatole, a highly specific reagent which cleaves solely at the COOH-tryptophanyl linkage giving two peptide fragments, Peptides L and T.

The NH2-terminal region of Peptide T, derived from BNPS-skatole degradation, was evaluated with the direct Edman procedure; seven steps were successful, giving Gly–Ala–Glu–Gly–Gln–Lys–Pro. This result confirms the sequence found in tryptic Peptide T18 and peptic Peptide P12, both of which are derived from the tryptophan region.

**Tryptic Peptides**—Peptide T was treated with trypsin under the same conditions as the A1 protein. Nine peptides and arginines were found on peptide mapping as shown in Fig. 4. Although 10 to 50% of the NH2-terminal residue of each peptide was destroyed by the ninhydrin treatment, the composition of each peptide was determined and, in some cases, subtractive Edman degradation was performed. The tryptic peptides were easily identified by comparisons of their composition with the analogous tryptic peptides obtained directly from the A1 protein, and are designated as such on the peptide map (Fig. 4). The only peptide not obtained from the tryptic digest of the A1 protein, Peptide TT1, must originate from the NH2 terminus of Peptide T; the composition is shown in Table VII, along with the chymotryptic peptides.

**Chymotryptic Peptides**—Peptide T was digested with chymotrypsin under the same conditions as the tryptic digestion. Seven peptides were produced and clearly resolved as shown by peptide mapping (Fig. 4). The composition of these peptides is given in Table VII.

**Sequence of Peptide T**—From the tryptic and chymotryptic peptides, the sequence of Peptide T can be directly determined, utilizing in addition information from certain peptic peptides. The sequence of Peptide T is shown in Fig. 5. It is evident by comparison with tryptic Peptide T18 and T19 and peptic Peptides P11, P11 A, P12, and P12A that Peptides TC1 and TT1, which contain a tyrosine and several glycine residues, are derived from the NH2-terminal region. Peptides TC1 and TT1 differ only by the Gly–Gly–Arg sequence present in the COOH-terminal region of Peptide TT1. Peptide TT1 is, in fact, identical with that part of Peptide T19 following the tryptophan residue. It is evident that Peptide TC2 has the composition which conforms precisely to the sequence Gly–Gly–Arg–Ala–Ser–Asp, a sequence established as part of tryptic Peptides T19 and T20 and

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**Table VI**

*Ammonia acid sequence of Peptide P14*

| Sequence | Tyr-Lys-Ser-Ala-His-Lys-Gly-Leu-Lys-Gly-His-Asp-Ala-(Glu,Gly,Thr,Leu,Ser,Lys,Ile,Phe,Lys, | |
|----------|---------------------------------|---|
| Carboxypeptidase | 2 hrs; 1.6 moles Arg and 0.7 mole Ala/mole peptide | |
| Direct Edman (2 steps) | Tyr-Lys-Ser-Ala-His-Lys-Gly-Leu-Lys-Gly-His-Asp | |
| Tryptic peptides | | |
| P14-T1 | Tyr (1.0); Lys (1.0) | |
| P14-T2 | Ser (1.1); Ala (1.3); His (0.0); Lys (1.0) | |
| P14-T3 | Gly (1.0); Leu (1.1); Lys (1.0) | |
| P14-T4 | Gly (2.0); His (0.8); Asp (1.0); Ala (1.0); Glu (0.9); Thr (1.1); Leu (1.0); Ser (1.0); Lys (0.9) | |
| Edman | Gly–His–Asp–Ala | |
| P14-T5 | Ile (1.0); Phe (0.9); Lys (0.8) | |
| P14-T6 | Leu (1.0); Gly (2.2); Arg (0.0) | |
| P14-T7 | Asp (0.9); Ser (0.8); Arg (1.0) | |
| P14-T8 | Ser (1.8); Gly (1.0); Pro (0.8); Met (0.6); Ala (1.0); Arg (1.1) | |
| Carboxypeptidase | Ser–Gly–Ser–Pro | |
| P14-T9 | Ser (1.1); Ala (1.3); His (0.9) | |
Peptide Peptides P11A and P12A and is, in fact, identical with peptic Peptide P13. This tyrosine residue constitutes the NH₂-terminal residue of peptic Peptide P14, a large peptide which occupies the remaining sequence. The direct Edman degradation established the sequence of Peptide P14 over 13 residues, and thus shows the position of tryptic peptides TT2 (T20), TT3 (T21), TT4 (T22), and part of TT5 (T23). Also included through Peptide TT5, which ends in -Ser-Lys (residue 36), are Peptides TC2, TC3, and TC4. Thus only Peptides TC5, TC6, and TC7 remain. Peptide TC7 is identical with the COOH-terminal residues of the A1 protein, Ala-Arg-Arg, a tripeptide which is released upon splitting the COOH-methionyl linkage with CNBr (11). Thus Peptide TC7 is identical with the sequence of residues 44 to 89 (Peptide P5 or Peptide R) which was elucidated previously (20). Peptide R is joined at both terminal positions by Phe-Phe linkages as shown by tryptic Peptide T8 at residue 36, and T13 at the COOH terminus.

With four steps of the Edman procedure, the NH₂-terminal region of Peptide TC6 was found to be Lys-Leu-Gly-Gly- and thus the union between Peptides TT6 (T24) and TT7 (T25). Peptide TT8 (T26) must follow since Peptide TT9 (T27) contains methionine. Peptides T27 and P14-T8 are in fact identical as shown by Edman degradation. These data are sufficient to establish the sequence of Peptide T as shown in Fig. 5.

### Table VII

| Amino acid | TC1 | TC2 | TC3 | TC4 | TC5 | TC6 | TC7 | TC8 |
|------------|-----|-----|-----|-----|-----|-----|-----|-----|
| Lys        | 0.8 (1) | 1.5 (2) | 1.0 (1) | 1.0 (1) | 0.0 (1) | 1.0 (1) |
| His        | 1.0 (1) | 1.0 (1) | 1.0 (1) | 1.8 (2) | 1.8 (2) | 1.0 (1) |
| Arg        | 1.0 (1) | 1.0 (1) | 1.0 (1) | 1.0 (1) | 1.0 (1) | 1.0 (1) |
| Asp        | 1.0 (1) | 1.0 (1) | 1.0 (1) | 1.0 (1) | 1.0 (1) | 1.0 (1) |
| Thr        | 1.0 (1) | 1.0 (1) | 1.0 (1) | 1.0 (1) | 1.0 (1) | 1.0 (1) |
| Ser        | 1.1 (1) | 0.8 (1) | 1.0 (1) | 1.0 (1) | 2.8 (3) | 1.8 (2) |
| Glu        | 1.9 (2) | 0.2 | 1.0 (1) | 1.1 (1) | 0.9 (1) | 0.9 (1) |
| Pro        | 1.1 (1) | 0.2 | 1.0 (1) | 1.1 (1) | 0.9 (1) | 0.9 (1) |
| Gly        | 3.7 (4) | 2.0 (2) | 1.0 (1) | 2.0 (2) | 2.7 (3) | 5.3 (6) |
| Ala        | 1.0 (1) | 1.3 (1) | 1.0 (1) | 1.0 (1) | 1.0 (1) | 1.2 (1) |
| Met        | 0.7 (1) | 0.7 (1) | 0.7 (1) | 0.7 (1) | 0.7 (1) | 0.7 (1) |
| Ile        | 1.1 (1) | 1.1 (1) | 1.1 (1) | 1.1 (1) | 1.1 (1) | 1.1 (1) |
| Leu        | 0.9 (1) | 0.9 (1) | 0.9 (1) | 0.9 (1) | 0.9 (1) | 0.9 (1) |
| Tyr        | 1.0 (1) | 1.0 (1) | 1.0 (1) | 1.0 (1) | 1.0 (1) | 1.0 (1) |
| Phe        | 0.9 (1) | 0.9 (1) | 0.9 (1) | 0.9 (1) | 0.9 (1) | 0.9 (1) |

| Total      | 11 | 7 | 7 | 9 | 4 | 13 | 3 | 14 |

The strategy used for positioning the various tryptic and peptic peptides to give the complete amino acid sequence of the 170 residues of the A1 protein can best be followed by reference to Fig. 5. The sequence from residues 38 to 43 was determined with peptic Peptides P3 and P4, and tryptic Peptide T7 and T8 because of the results from tryptic Peptide T6 and tryptic Peptide T6A where the Arg-Asp linkage was not fully hydrolyzed by trypsin. The sequence from residues 38 to 48 was determined with peptic Peptides P3 and P4, and tryptic Peptide T7 and T8. The sequence of residues 44 to 50 was elucidated previously (20). Peptide R is joined at both terminal positions by Phe-Phe linkages as shown by tryptic Peptide T8 at the NH₂-terminal end.

### Complete Amino Acid Sequence

The amino acid sequence of Peptide T is shown with the tryptic peptides denoted by TT and the chymotryptic peptides denoted by TC. The peptides and amino acids are numbered in order beginning at the NH₂-terminal end.

**Fig. 5.** The amino acid sequence of Peptide T is shown with the tryptic peptides denoted by TT and the chymotryptic peptides denoted by TC. The peptides and amino acids are numbered in order beginning at the NH₂-terminal end.
The strategy used in this study for determining the sequence of the bovine A1 protein was determined in part by the highly basic character of this protein. It was often necessary, as in the case of histone IV (28), to utilize preparative paper electrophoresis and chromatography to obtain homogeneous peptides. Pepsin was particularly helpful because it provided large peptides, useful not only for positioning the tryptic peptides, but also for localizing disease-inducing sites. Although only 19 of the 27 possible tryptic peptides were recovered from the Dowex 50 column, the remaining tryptic peptides were easily identified and isolated from peptide maps of the A1 protein or Peptide T. The high solubility and open conformation of the A1 molecule no doubt facilitated tryptic hydrolysis and lead to unusually well defined peptide maps. Although 80 to 90% of the available linkages are cleaved by trypsin in 30 min, it is of interest that the Lys-Asp linkage (Nos. 57 to 58) and Arg-Asp linkage (Nos. 33 to 34) are more slowly hydrolyzed by trypsin than are conventional linkages as shown by recovery of some of the uncleaved peptides. The COOH-terminal Arg-Arg sequence appeared to be cleaved by trypsin under the conditions used, unlike the situation in histone IV (28). The high solubility and open conformation of the A1 protein or Peptide T. The high solubility and open conformation of the A1 molecule no doubt facilitated tryptic hydrolysis and lead to unusually well defined peptide maps. Although 80 to 90% of the available linkages are cleaved by trypsin in 30 min, it is of interest that the Lys-Asp linkage (Nos. 57 to 58) and Arg-Asp linkage (Nos. 33 to 34) are more slowly hydrolyzed by trypsin than are conventional linkages as shown by recovery of some of the uncleaved peptides. The COOH-terminal Arg-Arg sequence appeared to be cleaved by trypsin under the conditions used, unlike the situation in histone IV (28). The hydrolysis of the Lys-Pro linkage (Nos. 122 to 123) and the Arg-Pro linkage (Nos. 79 to 80) by trypsin was particularly helpful in determining the sequences in those regions. Peptide T18, resulting from cleavage of the Lys-Pro linkage, was isolated from the Dowex 50 column whereas the larger unhydrolyzed fragment, Peptide T19, was apparently retained. The sequence of residues 80 to 84 was difficult to determine; hydrolysis of the Arg-Pro linkage in Peptide T18 gives two peptides which helped to confirm this sequence (20).

Depending on the degree of methylation of arginine residue 107, it is apparent that the sequence reported for the bovine A1 protein is unique (Fig. 6); no variants were found and no evidence of trace peptides other than those from incomplete or secondary cleavage was discovered. All of the expected tryptic peptides were found on peptide mapping, a factor which has proven useful in subsequent studies to determine the phylogenetic variation of the A1 protein sequence (15). The question arises, therefore, concerning the reported microheterogeneity of the A1 protein at pH 10.5 (20). This phenomenon cannot be attributed to the state of methylation of the single arginine residue 107 since the protein would still have a high positive charge at that pH, and it is unlikely that the difference in pK between arginine and monomethylarginine, the major derivative, would be significant. A more reasonable interpretation is that deamination occurs either during acid extraction or at alkaline pH during assay. Deamination of glutamine or aspartic residues may occur more readily if adjacent to a basic residue (31). In the A1 molecule, there are several such potential sites of deamidation: Glu-Lys or Glu-Arg (residues 4 to 5, 9 to 10, 73 to 74, 121 to 122) and Lys-Asn (91 to 92). Small quantities of the deamidated forms of Peptides T12, T14, T18 and T19 were observed in some preparations.

An alternate explanation of the microheterogeneity comes from possible degradation in situ because of the open conformation of the A1 protein and its high susceptibility to proteolysis (9). Traces of lysosomal enzymes might cause limited hydrolysis at the most vulnerable linkages; degradation of the A1 protein occurs in situ (2) in bovine spinal cord. As a consequence of the significant turnover of the A1 protein, (half-life 21 days (32)), the additional bands seen on gel electrophoresis could reflect the presence of large peptide fragments.

What properties of the A1 protein, associated with its role as a structural protein of the myelin membrane, can be inferred from the amino acid sequence? A rare feature of the sequence is the methylated arginine residue (No. 107), the only methylated residue detected in the molecule and the first localization of methylated arginine in a protein sequence (23). Both the A1 protein (33) and histones (34) serve as acceptor proteins for methylation by enzymes from brain and other tissues. The phylogenetic importance of the methylated arginine is illustrated by its presence at the analogous position in the A1 proteins of many species including turtle, chicken, rat, rabbit, guinea pig, cow, monkey, and human (23). In accord with the open conformation of this molecule, it is likely that the methylation of this highly specific arginine residue involves the recognition of a unique segment of the polypeptide chain by the appropriate methylase.

Near the middle of the polypeptide chain there is a Pro-Arg-Thr-Pro sequence. Although other conformations are possible, the restrictions imposed by the 4 proline residues could induce a sharp U-shaped bend in the molecule (29) and thereby induce the polypeptide chain to fold back on itself. Such an interpretation would explain the relatively low axial ratio of 10:1 found for the A1 protein by viscosity studies (5), a lower ratio than would be predicted for a completely open conformation lacking significant secondary or tertiary structure. The proposed open double chain structure (23) could be stabilized within the myelin membrane by interaction of the A1 protein with lipid or other proteins, such as proteolipid, or perhaps by cross-chain interaction within the A1 molecule itself. The close proximity of methylated arginine residue to the triproline region suggests a cooperative function; i.e. the methylated arginine is more non-polar than arginine and might participate in stabilization of the...
proposed double chain structure by either cross-chain interaction with the two proximal phenylalanine side chains (see Fig. 2 of Reference 23) or by interaction with lipids. It is of interest that the triproline sequence occurs infrequently in proteins, and has been reported (35) in rabbit IgG in which it constitutes the hinge region.

Adjacent to the triproline sequence is found threonine residue 98. This threonine residue is the sole focus for glycosylation in the Al protein by the polypeptide N-acetylgalactosaminyl transferase from the submaxillary glands (36); a GalNAc-O-Thr linkage is formed. Many other proteins were tested for acceptor activity (21); in addition to the Al protein, only the denuded polypeptide chain of the submaxillary glycoprotein, the natural acceptor for this enzyme, was functional. The question arises whether the Al protein, which contains no carbohydrate, may be functional as part of the myelin membrane.

The sequence of the Al protein allows relevant interpretation of the structural role of the Al protein within the myelin membrane. If it can be assumed that the unfolded form of the Al protein in solution prevails in situ, then the Al molecule appears ideally designed to promote maximum interaction with other components such as phospholipids. It is likely that this interaction is primarily electrostatic; the basic groups are distributed quite randomly without any obvious periodicity and could interact with the phosphate groups of lipids. The basic polypeptide chain may in fact serve as a type of template which directs phospholipids to the positive regions. It is of interest that, in the Al protein, regions of 8 to 10 residues exist in which basic residues are absent; nonpolar and even negative charges are found in these gaps and could provide a separate focus for attraction of lipids or possibly proteins such as proteolipids.

The Al protein is the agent in the central nervous system which is responsible for induction of experimental allergic encephalomyelitis. In defining the immunopathological role of the Al protein, the amino acid sequence again assumes major significance because of the unfolded conformation of the polypeptide chain. Unlike most proteins, therefore, peptide fragments derived from the Al protein are themselves immunogenic; i.e. they induce a delayed hypersensitive response, a process involving the specific sensitization of lymphocytes that ultimately leads to the disease state (2, 12–14). The g-residue segment (Peptide T12) surrounding the single tryptophan residue is the major disease-inducing site in guinea pigs (18). In rabbits, both Peptide P5 (referred to as Peptide R (20)) and Peptide T18 are highly encephalitogenic (19, 20). Although Peptide T5 contains 45 residues, it is of interest that a small region exists that has a close similarity to the triprophen region:

| 68 | 74 |
|---|---|
| Tyr-Gly-Ser-Leu-Pro-Gln-Lys (Peptide T18) | Try Glu Ala Glu Gly Gln Lys |
| 116 | 122 |

It has been shown (14) that the tryptophan, glutamine, and lysine residues are essential for encephalitogenic activity in guinea pigs. The sequence similarities in these regions suggest that similar requirements may exist for disease induction in rabbits with the exception that the requirements for the aromatic residue are less specific; i.e. tyrosine can substitute for tryptophan. In this regard, preliminary evidence for encephalitogenic activity of this region (Peptide T12) in rabbits has been reported (37). In the guinea pig, the tryptophan requirement appears absolute (13, 14); both Peptides P5 and T12 are inactive. Moreover, it is possible that other peptide sequences quite distinct from these regions may be capable of disease induction in other species such as monkey, rat, dog, and even human. This subject is currently being investigated in our laboratory.

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