The NH₂- and COOH-terminal Amino Acid Sequence of Nuclear Protein A24*

(Received for publication, April 1, 1976, and in revised form, May 17, 1976)

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The NH₂- and COOH-terminal sequence of nuclear protein A24 has been determined by automatic Edman degradation and carboxypeptidase A and B digestion. Protein A24 is of interest because it is composed in part of histone 2A (Goldknopf, I. L., and Busch, H., (1975) Biochem. Biophys. Res. Commun. 63, 951-960). The sequence of the first 37 NH₂-terminal residues is:

\[
\text{Met-Gln-Ile-Phe-Val-Lys-Leu-Thr-Gly-Lys-Thr-Ile-Thr-Leu-Glu-Val-Glu-Pro-Ser-Asp-Ile-Glu-Asn-Val-Lys-Ala-Lys-Ile-Gln-Asp-Lys-Glu-Ile-Pro-}
\]

This sequence is not homologous to any known histone sequence. It contains regions of internal homology (italics). The COOH-terminal amino acid sequence is the same as that of histone 2A, namely:

\[-\text{His-His-Lys-Ala-Lys-Gly-Lys-COOH.}\]

MATERIALS AND METHODS

Protein A24 is a non-histone chromatin protein with histone-like characteristics (1). The protein was initially observed by two-dimensional polyacrylamide gel electrophoresis in 0.4 M H₂SO₄ extracts of nucleoli (2). It was subsequently found to decrease in amount in nucleoli undergoing hypertrophy induced by thioacetamide or liver regeneration (3, 4).

After purification and characterization (1), protein A24 was found to contain the tryptic and chymotryptic peptides of histone 2A as well as additional peptides (5). Furthermore, protein A24 contains the blocked acetyl-Ser-Gly-Arg-NH₂-terminal sequence (6) identical to that of histone 2A (7). Since protein A24 also contains a single methionine NH₂ terminus (1), it was suggested that protein A24 contains an additional non-histone sequence linked to the histone 2A sequence (5). Because the NH₂ terminus of the histone 2A sequence was blocked by an acetyl group, the unblocked sequence was amenable to automatic Edman degradation without prior separation of the two polypeptides. This study was initiated to determine whether the NH₂-terminal sequence of the unblocked portion of the protein is homologous to histone 2A or other histones and to determine the COOH-terminal sequence(s). It was found that the NH₂-terminal sequence is unique and is not homologous to that of any known histone but that protein A24 contains a single COOH-terminal sequence which is identical to that of histone 2A.

*These studies were supported by the Cancer Research Center Grant CA-10893, P.3, awarded by the National Cancer Institute, Department of Health, Education, and Welfare.
COOH-terminal Amino Acid Sequence Analysis—Protein A24 and histone 2A were digested with carboxypeptidase B (0.02 unit of carboxypeptidase B/mmol of protein) at 37°C for 60 min in 0.2 M NaCl and 0.05 M ethylenediaminetetraacetic acid, pH 8.5. The digests were then boiled for 15 min to destroy the enzyme and aliquots were diluted with 0.2 M sodium citrate, pH 2.2, and analyzed on a Beckman 121 amino acid analyzer to determine released COOH-terminal amino acids (13). The remainder of the digest was then subjected to carboxypeptidase A digestion (0.004 unit of carboxypeptidase A/mmol of protein) for 60 min at 37°C. Released COOH-terminal amino acids were again analyzed.

RESULTS

Protein A24 was subjected to automatic Edman degradation in three different runs with samples of 67 to 130 nmol of protein. Repetitive yields were approximately 96% of the stable The amide functions of glutamine or asparagine were released COOH-terminal amino acids were again analyzed.

**TABLE I**

Automatic Edman degradation of protein A24

| Cycle | Prominent amino acid | Yield* by amino acid analysis | Amino acid present in 2nd largest amount | Yield* by amino acid analysis | Amino acid present in most prominent cycle | Yield* by amino acid analysis |
|-------|----------------------|-------------------------------|----------------------------------------|-------------------------------|------------------------------------------|-------------------------------|
|       |                      | nmol                          | nmol                                   | nmol                          | nmol                                     | nmol                          |
| 1     | Met*                 | 1.8                           | Glu                                     | 1.4                           |                                         |                               |
| 2     | Gln                   | 77.5                          | Thr                                     | 18.0                          |                                         |                               |
| 3     | Ile*                 | 7.2                           | Phe                                     | 5.9                           |                                         |                               |
| 4     | Phe                  | 8.2                           | Val                                     | 4.2                           |                                         |                               |
| 5     | Val                  | 4.2                           | Lys                                     | 4.2                           |                                         |                               |
| 6     | Lys                  | 2.6                           | Thr                                     | 2.6                           |                                         |                               |
| 7     | Thr*                 | 4.0                           | Leu                                     | 4.0                           |                                         |                               |
| 8     | Leu                  | 4.7                           | Thr                                     | 5.2                           |                                         |                               |
| 9     | Thr                  | 11.4                          | Leu                                     | 5.4                           |                                         |                               |
| 10    | Gly                  | 7.4                           | Asp                                     | 8.7                           |                                         |                               |
| 11    | Lys                  | 6.4                           | Thr                                     | 6.0                           |                                         |                               |
| 12    | Thr                  | 17.7                          | Lys                                     | 8.7                           |                                         |                               |
| 13    | Ile                  | 14.4                          | Thr                                     | 8.2                           |                                         |                               |
| 14    | Thr                  | 17.6                          | Leu                                     | 5.0                           |                                         |                               |
| 15    | Leu                  | 7.9                           | Asp                                     | 4.9                           |                                         |                               |
| 16    | Glu*                 | 8.6                           | Val                                     | 1.6                           |                                         |                               |
| 17    | Val                  | 8.9                           | Glu                                     | 8.3                           |                                         |                               |
| 18    | Glu                  | 6.8                           | Pro                                     | 3.8                           |                                         |                               |
| 19    | Gly                  | 36.0                          |                                         |                               |                                         |                               |
| 20    | Ser*                 | 12.4                          | Ser                                     | 10.4                          |                                         |                               |

*Values for amino acids recovered after hydrolysis of PTH-derivatives in 57% HI at 130°C were corrected for destruction by factors calculated from analyses of standard amounts of hydrolyzed PTH-derivatives.

*Values for threonine by amino acid analysis were calculated as the sum of isoleucine and alloisoleucine.

*Values for threonine by amino acid analysis were calculated as the result of reductive hydrolysis.

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*Glycine background was not calculable because of glycyl residues on carboxyls of glutamic acid.

Identification of residues at each cycle required amino acid analysis after HI hydrolysis and gas chromatography of PTH-derivatives. Table I indicates that yields were variable for both systems even after correction for losses during hydrolysis, especially for the less stable amino acids serine, threonine, and lysine. Overlap between steps was generally less than 5% but began to increase gradually at certain steps after cycle 9 (e.g. 12, 16, 19, 20, 21, 22, etc.). However, the overlap did not prevent positive identification of any of the 37 residues.

The amide functions of glutamine or asparagine were assigned to residues 2, 25, and 31 by the presence of the isolated N-ethylmorpholine acetate, pH 8.5. The digests were then boiled for 15 min to destroy the enzyme and aliquots were diluted with 0.2 M sodium citrate, pH 2.2, and analyzed on a Beckman 121 amino acid analyzer to determine released COOH-terminal amino acids (13). The remainder of the digest was then subjected to carboxypeptidase A digestion (0.004 unit of carboxypeptidase A/mmol of protein) for 60 min at 37°C. Released COOH-terminal amino acids were again analyzed.

**TABLE I**

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|-------|----------------------|-------------------------------|----------------------------------------|-------------------------------|------------------------------------------|-------------------------------|
|       |                      | nmol                          | nmol                                   | nmol                          | nmol                                     | nmol                          |
| 1     | Asp                  | 47.9                          | Ala                                     | 23.4                          |                                         |                               |
| 2     | Gly                  | 30.0                          |                                         |                               |                                         |                               |
| 3     | Thr                  | 43.2                          | Asp                                     | 17.0                          |                                         |                               |
| 4     | Ile                  | 26.9                          | Leu                                     | 9.7                           |                                         |                               |
| 5     | Glu                  | 23.5                          | Lys                                     | 7.3                           |                                         |                               |
| 6     | Gly                  | 25.5                          |                                         |                               |                                         |                               |
| 7     | Asp                  | 47.9                          | Glu                                     | 13.0                          |                                         |                               |
| 8     | Gly                  | 11.14                         |                                         |                               |                                         |                               |
| 9     | Val                  | 30.5                          | Leu                                     | 9.9                           |                                         |                               |
| 10    | Lys                  | 25.6                          | Glu                                     | 10.2                          |                                         |                               |
| 11    | Ala                  | 43.5                          | Leu                                     | 7.1                           |                                         |                               |
| 12    | Lys                  | 38.4                          | Ala                                     | 8.6                           |                                         |                               |
| 13    | Ile                  | 33.1                          | Lys                                     | 13.2                          |                                         |                               |
| 14    | Glu                  | 24.5                          | Leu                                     | 8.1                           |                                         |                               |
| 15    | Gly                  | 2.4                           |                                         |                               |                                         |                               |
| 16    | Asp                  | 17.2                          | Glu                                     | 12.1                          |                                         |                               |
| 17    | Lys                  | 20.6                          |                                         | Asp                            |                                         |                               |
| 18    | Gly                  | 18.0                          |                                         | Asp                            |                                         |                               |
| 19    | Pro                  | 21.0                          |                                         |                               |                                         |                               |
| 20    | Ser                  | 17.5                          |                                         |                               |                                         |                               |

*Values for amino acids recovered after hydrolysis of PTH-derivatives in 57% HI at 130°C were corrected for destruction by factors calculated from analyses of standard amounts of hydrolyzed PTH-derivatives.

*Values for threonine by amino acid analysis were calculated as the sum of isoleucine and alloisoleucine.

*The free carboxyl groups of glutamic and aspartic acid were modified by attachment of glycine ethyl ester. The presence of greatly increased amounts of glycine in addition to glutamic or aspartic acid after HI hydrolysis indicates that the residue contains the free carboxyl rather than the amide function.

*Serine is converted to alanine by HI hydrolysis. Positive identification of serine was done by gas chromatography.

*Glycine background was not calculable because of glycyl residues on carboxyls of glutamic acid.
in glycine by amino acid analysis (greater than 0.6 mol/mol of parent amino acid) indicated the presence of glutamic or aspartic acid at residues 16, 18, 21, 25, 32, and 34. Some residues of glutamic or aspartic acid were confirmed by gas chromatography after silylation; however, problems of reproducibility and background limited the usefulness of this procedure.

Several PTH-derivatives are destroyed or converted to other amino acids by HI hydrolysis. Methionine is completely destroyed, but was positively identified at Step 1 by gas chromatography. Threonine yields γ-aminobutyric acid, which was readily identified with the amino acid analyzer. Serine is destroyed, but was identified on the gas chromatogram at residue 20. Isoleucine residues were identified as a mixture of isoleucine and alloisoleucine.

Table II shows the patterns of free amino acids obtained upon digestion of protein A24 and histone 2A with carboxypeptidase B followed by carboxypeptidase A. The known (14) COOH-terminal amino acid sequence and rate-limiting steps for carboxypeptidase B and A digestions of histone 2A are shown in Fig. 1. The values in Table II obtained by setting nanomoles of lysine at 1.0 after carboxypeptidase B and at 3.0 after carboxypeptidase A digestions show essentially identical patterns for both proteins and are consistent with protein A24 having the same COOH-terminal sequence as histone 2A indicated in Fig. 1. Quantitative hydrazinolysis indicated that lysine is the sole COOH-terminal amino acid of protein A24 and its molar yield is identical to that of histone 2A (6).

**DISCUSSION**

This study indicates that protein A24 has a single COOH-terminal amino acid sequence which is identical to histone 2A and a unique NH2-terminal sequence which is unlike that of histone 2A (7) or any known histone sequence (15). Protein A24 therefore appears to be a unique protein for which the linkage of the non-histone and histone polypeptides is presently unknown.

Interestingly, the A24 NH2-terminal sequence exhibits internal homology. Homologous tetrapeptides are present from residues 6 through 15 in which lysine and threonine residues are spaced by pentapeptide regularity:

\[
\text{Lys-Thr-Lys-Thr Lys-Thr-Ile-Lys-COOH}
\]

This sequence may have been generated by gene duplication as previously observed in protamines (16) and in haptoglobins (17).

This sequence contains an equal number of free carboxyl groups and amino groups (six each), effectively resulting in a net charge of zero for this part of the molecule. It is of interest that no arginine was found in this NH2-terminal sequence. The complete sequence of this structure and its linkage to the histone 2A portion of protein A24 requires further studies.

The results of studies on the COOH-terminal sequence by carboxypeptidase digestion (Table II) and quantitative hydrazinolysis as noted above provide evidence that lysine is the sole COOH-terminal amino acid, and thus that protein A24 has a Y-shape (6). However, the possibility is not excluded that some unknown linkage could exist that is resistant to these procedures. Further studies on branch structures and the complete amino acid sequence of this molecule will be necessary to eliminate the possibility that it may have an H-shape.

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J. Biol. Chem. 1976, 251:5901-5903.

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