Structures of Amidohydrolases

AMINO ACID SEQUENCE OF A GLUTAMINASE-ASPARAGINASE FROM ACINETOBACTER
GLUTAMINASIFICANS AND PRELIMINARY CRYSTALLOGRAPHIC DATA FOR AN ASPARAGINASE FROM ERWINIA CHRYSANTHEMI*

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Shuji Tanaka‡, Elizabeth A. Robinson‡, Ettore Appella‡, Maria Miller$, Herman L. Ammon‡, Joseph Roberts, Irene T. Weber‡, and Alexander Wlodawer§

From the ‡Laboratory of Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205, the §Department of Chemistry and Biochemistry and Center for Advanced Research in Biotechnology, University of Maryland, College Park, Maryland 20742, and the $Crystallography Laboratory, BRI-Basic Research Program, National Cancer Institute-Frederick Cancer Research Facility, Frederick, Maryland 21701

The complete amino acid sequence of a glutaminase-asparaginase from Acinetobacter glutaminasificans, for which a preliminary tertiary structure is available from crystallographic analysis, has been determined by automated Edman degradation of fragments produced by chemical and proteolytic cleavages. The protein consists of 331 amino acid residues and has a molecular weight of 35,500. The pattern of hydrophilic and hydrophobic regions is typical of a globular protein.

A new crystal form of an Erwina chrysanthemi 1125 asparaginase is reported. The space group is monoclinic C2, with unit cell parameters of: a = 107.8, b = 91.7, c = 129.2 Å and β = 91.7°. A V_n of 2.25 Å³ dalton was calculated for one tetramer of 35,100-dalton subunits per asymmetric unit. X-ray intensity data have been obtained to 2.2 Å resolution. The point group symmetry of the Er. chrysanthemi tetramer is 222 from self-rotation function calculations. The relative orientations of an A. glutaminasificans glutaminase-asparaginase model and the Er. chrysanthemi asparaginase tetramer have been determined with the cross-rotation function, and translation function calculations have revealed a plausible location for the asparaginase tetramer in the crystal.

We are investigating the structures of several bacterial amidohydrolases with the ultimate aim of correlating the detailed atomic models with their enzymatic activity and efficacy as anti-tumor agents. A preliminary structure of an Acinetobacter glutaminase-asparaginase from glutaminasificans has been published recently (Ammon et al., 1988). The completion of this structure was prevented, in part, by the lack of primary structure information for the enzyme. The determination of the amino acid sequence of A. glutaminasificans glutaminase-asparaginase, the next stage in the structural investigation, is reported here. In addition, we report the crystallization of an Erwina chrysanthemi asparaginase, for which a primary structure is available (Minton et al., 1986) in a form suitable for determination of a high resolution structure.

Interest in the structures of amidohydrolases stems from their utilization in cancer therapy. Several amidohydrolases have been under investigation as potential anti-tumor agents over the last 20 years. The administration of L-asparaginase (L-asparagine amidohydrolase, EC 3.5.1.1) leads to the regression of certain lymphomas and leukemias in experimental animals and in humans (Oettgen et al., 1967; Kidd, 1970; Broome, 1981; Prager et al., 1982). The Escherichia coli and Er. chrysanthemi (previously known as Erwinia carotovora) enzymes are used clinically in the treatment of acute lymphoblastic leukemia (Oettgen et al., 1967; Hruschkesy et al., 1976), and other amidohydrolases have been in clinical trials. Asparaginase-sensitive tumor cells generally show a diminished capacity to synthesize L-asparagine, because of relatively low levels of L-asparaginase synthetase, and therefore require an exogenous supply of the amino acid for protein biosynthesis. It is presently unclear, however, exactly how asparaginase kills sensitive tumor cells, and the biochemical basis for asparaginase sensitivity has not been firmly established (Kef er et al., 1985).

L-Glutamine, like L-asparagine, is not an essential component in the human diet. The activity of a glutaminase-asparaginase against asparaginase-resistant cells (Schmid and Roberts, 1974) and in asparaginase-resistant patients (Spier and Wade, 1976) has been demonstrated. Recently, a combination of a glutaminase-asparaginase from Pseudomonas 7A, 6-diazo-5-oxonorleucine (DON), and acivicin has been shown to be active against human mammary and colon tumors in vitro, and in tumors growing in nude mice (Roberts, 1983). The glutaminase activity (approximately 3–5%) of E. coli asparaginase has a role in the inhibition of cell growth and in cell toxicity (Wu et al., 1978; Hakimi and Boaseman, 1979). The simultaneous elimination of both amino acids might be therapeutically advantageous.

Preliminary crystal data and molecular replacement studies based on the tentative model of A. glutaminasificans glutaminase-asparaginase have been published for the enzymes from Pseudomonas 7A (Ammon et al., 1983), Vibrio sacciogenes (Ammon et al., 1985), and E. coli (Ammon et al., 1988). Complete primary structures now are available for asparaginases from E. coli A-1-3 (Maita et al., 1974, 1979; Maita and Matsuda, 1980) and Er. chrysanthemi 1066 (Minton et al., 1986). The Er. chrysanthemi 1125 sequence is identical to

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The abbreviations used are: DON, 6-diazo-5-oxonorleucine; AgGA, A. glutaminasificans glutaminase-asparaginase; E4A, E. chrysanthemi asparaginase; E4A, E. coli asparaginase; PGA, Pseudomonas 7A asparaginase; PTH, phenylthiohydantoin; SDS, sodium dodecyl sulfate.

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RESULTS AND DISCUSSION

Amino Acid Sequence of A. glutaminasificans Glutaminase-Asparaginase—The sequencing results for A. glutaminasificans glutaminase-asparaginase are summarized in Fig. 1. The amino acid sequence of A. glutaminasificans glutaminase-asparaginase consists of 331 residues; the calculated molecular weight is 35,500. The sequence was obtained from analyses of the intact enzyme, and from characterization of peptides generated by cleavages at methionyl, lysyl, or arginyl residues (designated CB, K, and CT, respectively). In addition, two CB fragments were subdigested at aspartyl residues (designated D).

Cleavage of A. glutaminasificans glutaminase-asparaginase with cyanogen bromide was the major mode of fragmentation. One fragment, corresponding to residues 151-153, was not recovered. The other fragments were sequenced in part or in entirety. Sequence analysis of peptides K-2 through K-7 and peptides CT-1 through CT-4 served to align the CB fragments and complete their sequences. The sequence of CB-1-D2 joined K-2 and CT-1 and identified residues 66-72. The sequence of CB-6-D2 overlapped CT-3 and CT-4. The fractionation of the peptides, the amino acid compositions, and the sequence data are presented in Figs. 1M-5M and Tables 1M-4M, in the Miniprint.

The results presented here differ in two positions from the amino-terminal sequence (residues 1-60) previously reported (Holcenberg et al., 1978). Residues 34 and 50 were identified as asparagine and aspartic acid, respectively, versus aspartic acid and threonine in the present work. We identified residue 34 as aspartic acid in the degradation of CB-1; no asparagine was detected. In addition, cleavage of CB-1 with the aspartylendopeptidase Pseudomonas fragi-protease yielded CB-1-D2 commencing with Asp-34. Residue 50 was identified as threonine by us in both the degradation of CB-1-D2 and of K-2. There is no apparent explanation for these discrepancies.

Er. chrysanthemi Asparaginase Crystals—The crystallization of an Erwinia asparaginase in the orthorhombic space group P212121 was reported nearly 20 years ago (North et al., 1969). Our new crystal form of Er. chrysanthemi asparaginase is monoclinic, space group C2, with unit cell parameters of a = 107.8 Å, b = 91.7 Å, c = 129.2 Å, β = 81.7°. The space group and cell parameters were determined from x-ray data collected with a Nicolet Proportional Counter and were confirmed by precession photography. Four 35,000-dalton subunits (Minton et al., 1986) per crystal asymmetric unit yield a Vm (Matthews, 1968) of 2.25 Å³/dalton. The value is similar to those observed for other amidohydrolases, which range from Vm = 2.26 for E. coli asparaginase (Ammon et al., 1988) to 2.75 for A. glutaminasificans glutaminase-asparaginase (Ammon et al., 1985).

Rotation and Translation Function Calculations—The rotation function (R) is a measure of the overlap of two Patterson functions (P1, P2), following rotation of one (P2) of them (Rossman and Blow, 1962). We have utilized the self-rotation function to investigate the noncrystallographic (molecular) symmetry of the Er. chrysanthemi asparaginase tetramer, and the cross-rotation function to deduce the relative orientations of the Er. chrysanthemi asparaginase and A. glutaminasificans glutaminase-asparaginase tetramers. Additionally, translation function calculations were used to determine a probable location for the Er. chrysanthemi asparaginase tetramer in the unit cell.

A three-dimensional, self-rotation function for Er. chrysanthemi asparaginase was calculated with a Crowther fast rotation program (Crowther, 1972) with all Eix² - 1 coefficients (5,706 terms) in the 10-4.7-Å range and a radius of integration of 30 Å. Since the average value of Exi² is 1, this corresponds to the use of an origin-removed Patterson function. The Exi² - 1 R map was superior to other maps calculated on different sets of Eix² coefficients. The R map was clear and contained only three maxima, roughly one-third the height of the origin, self-superposition peak (Table 1). Conversion of the fast rotation function αβγ peak positions to the ϕ,ψ,φ polar coordinate system showed that the peaks corresponded to rotation dyads; the angles between the dyads were 90 ± 0.3°. These data are consistent with three mutually perpendicular 2-fold rotation axes and indicate that the Er. chrysanthemi asparaginase tetramer has 222 point group symmetry in the crystal.

The Crowther self-rotation function results were confirmed with the Rossmann program (Rossmann and Blow, 1962). This program performs calculations directly in polar coordinates (κ, θ, φ), and a search for 2-fold symmetry in the self-rotation function is accomplished by fixing κ at 180°, and scanning the θ and φ coordinates. The calculations used the 1000 largest Eix² coefficients in the 10-4.7-Å resolution range with a radius of integration of 30 Å. Each point in the map was weighted by sin(φ), which is proportional to the area of the R map represented by the (ψ,φ) point. The asymmetric unit of the R map contained the three peaks shown in Table 2 which, following appropriate ϕ,ψ symmetry and coordinate system transformations can be seen to correspond closely to the Crowther self-rotation function maxima in Table 1.

Cross-rotation function calculations were carried out with the Crowther program, with Er. chrysanthemi asparaginase as P1 and A. glutaminasificans glutaminase-asparaginase (Ammon et al., 1988) as P2. The number of A. glutaminasificans glutaminase-asparaginase Exi² - 1 coefficients was 3,641. The R map contained two peaks (height above the map average, α, β, γ) at (8.6 α, 85.9, 5.3, 175.7°) and (4.4 α, 154.9, 79.1, 148.4°). A. glutaminasificans glutaminase-asparaginase crystallizes in the space group I222 with one subunit per asymmetric unit, and thus the molecular (tetramer) dyads are coincident with the unit cell axes. The similarity of the self- and cross-rotation function results was tested by transforming...
The angles between the rotated re-oriented constructed from the cross-function angles, and comparing dyads with the each of the original A. glutaminasificans glutaminase-asparaginase dyads (at 1.0, 0.1, 0, 0 1) by a rotation matrix constructed from the cross-function angles, and comparing the re-oriented A. glutaminasificans glutaminase-asparaginase dyads with the Er. chrysanthemi asparaginase dyads obtained from the self-rotation function $\varphi_1$ values listed in Table 1. The angles between the rotated A. glutaminasificans glutaminase-asparaginase and the self-rotation dyads (following appropriate symmetry transformations) were $(1.0)/(peak 1) = 1.1 (0.1)/(peak 2) = 0.6$, and $(0.0 1)/(peak 3) = 1.3$.

An approximate location for the Er. chrysanthemi asparaginase tetramer in the unit cell was determined with the use of an appropriately rotated A. glutaminasificans glutaminase-asparaginase model and the TFTRANS translation function.

![Diagram of A. glutaminasificans glutaminase-asparaginase structure](image-url)
TABLE 1
Crowther program self-rotation function maxima and angles between the molecular axes

| Peak Ht | \(\alpha\) | \(\beta\) | \(\gamma\) | \(\alpha'\) | \(\beta'\) | \(\gamma'\) |
|---------|---------|---------|---------|--------|--------|--------|
| 1       | 5.2    | 76.9°   | 64.2°   | 140.0° | 180.0° | 121.1° |
| 2       | 4.6    | 172.9°  | 171.6°  | 7.1°   | 180.0° | 97.1°  |
| 3       | 4.4    | 85.1°   | 116.5°  | 94.9°  | 180.0° | 147.9° |

*Molecular axes.*

|        | Angle |
|--------|-------|
| 1/2    | 90.1° |
| 1/3    | 89.9° |
| 2/3    | 90.3° |

*Peak height (Ht) in \(\sigma\) above the average map value. The origin peak was 13.9 \(\sigma\).*

*Fast rotation function angles. The alignment of the Er. chrysanthemi asparaginase reciprocal axes with the rotation function axes was \(b^*\) along \(Z\), and \(\alpha^*\) along \(Y\). The application of the \(\alpha,\beta,\gamma\) Eulerian angles is termed the “YZV” convention to indicate the order in which the new axes are selected for successive rotation.*

*Spherical polar angles. The application of the \(\alpha,\beta,\gamma\) Eulerian angles is termed the “YZV” convention to indicate the order in which the new axes are selected for successive rotation.*

The orientation and location of the model in the unit cell were refined with the program TRAREF (Huber and Schneeberger, 1985). The calculations used structure factors for the Er. chrysanthemi asparaginase tetramer in an orthogonal tetrameric P1 cell with 131 \(\AA\) cell edges, and \(x\)-ray data in the 10–6–\(\AA\) resolution range. The final R value was 0.441 (\(\alpha\), \(\beta\), \(\gamma\)) = (31.76, 85.33, 81.73°) and (x, z) = (0.2790, 0.2364).

\[\begin{bmatrix} \alpha_{\beta}\gamma & \gamma \end{bmatrix} = \begin{bmatrix} -\sin(\beta) \cos(\beta) & 0 \\ \cos(\beta) & 1 \end{bmatrix} \begin{bmatrix} \alpha_{\beta}\gamma & \gamma \end{bmatrix} \]

The \(\alpha_{\beta}\gamma\) matrix formalism used here is the transpose of the matrix given in Table 1a of Rossmann and Blow (1962) with \(\theta = \alpha\), \(\beta\), \(\gamma\).

**FIG. 2.** Comparison of amino acid sequences of three amidohydrolases. The three sequences are: 1) A. glutaminasificans glutaminase-glutaminase (top line); 2) E. coli asparaginase (middle line); 3) Er. chrysanthemi (bottom line). The one-letter code for amino acid type is used. The A. glutaminasificans glutaminase-asparaginase sequence is numbered, and residues in boxes are identical in all three sequences. *Indicates residues labeled by irreversible active site inhibitors.

**Comparison of Asparaginase Sequences—**The amino acid sequences of A. glutaminasificans glutaminase-asparaginase, Er. chrysanthemi asparaginase (Minton et al., 1986), and E. coli asparaginase (Maita et al., 1974, 1979, 1980) have been aligned as illustrated in Fig. 2. Although the E. coli asparaginase sequence (321 residues) is 10 residues shorter than A. glutaminasificans glutaminase-asparaginase (331 residues), their alignment shows no insertions or deletions larger than 2 residues. The Er. chrysanthemi sequence (327 residues) has deletions in three places, the largest being 5 residues long. There are 103 residues that are identical in all three sequences, and 34 residues are similar. A. glutaminasificans glutaminase-asparaginase and E. coli asparaginase are the most similar pair with 149 identical and 42 homologous amino acids. E. coli asparaginase and Er. chrysanthemi asparaginase have 143 identical and 43 similar residues; while A. glutaminasificans glutaminase-asparaginase and Er. chrysanthemi asparaginase are less alike, with 137 identical and 32 similar residues. This is surprising since Er. chrysanthemi asparaginase and E. coli asparaginase are classed as asparaginas with relatively low glutaminase activity, while A. glutaminasificans glutaminase-asparaginase is active as both an asparaginase and a glutaminase. There are several places where the three sequences have a series of identical residues. Near the amino terminus, for example, there are eight consecutive identical amino acids (residues 8–15 in A. glutaminasificans glutaminase-asparaginase) which are also found in the amino-terminal residues of Pseudomonas TA glutaminase-asparaginase (Holcenberg et al., 1978), and Proteus vulgaris asparaginase.\(^5\) Residues 87–94 are also identical in all

\(+ \frac{\pi}{2}, \theta = \beta, \text{and} \delta = \gamma - \frac{\pi}{2}\). The central matrix is required to convert from the Crowther program coordinate system used for monoclinic unit cells (i.e. \(\alpha Y^* b^* Z\)) to the system \((b^* Y, c^* Z)\) used in TRAREF.

\(^5\) G. Matsuda (1986), personal communication.
Proteins are more similar in sequence than the carboxyl-terminal halves. Many identical residues are found in the residue 302 to 331. Overall, the amino-terminal halves of the proteins are more similar in sequence than the carboxyl-terminal halves.

Prediction of Secondary Structure—The method of Chou and Fasman (1978) was used to predict the secondary structures for the three sequences (Fig. 3). The insertions and deletions shown for the sequence alignment mostly occur between or near the ends of elements of predicted secondary structure. The two exceptions are the single-residue deletion in E. coli asparaginase corresponding to A. glutaminasificans glutaminase-asparaginase 112 which is in the middle of a β-strand; the single-residue deletion in Er. chrysanthemi asparaginase at A. glutaminasificans glutaminase-asparaginase 265, and the single-residue deletion in E. coli asparaginase corresponding to A. glutaminasificans glutaminase-asparaginase 313, which are predicted to be in α-helices. Five α-helices are predicted in very similar positions in all sequences. Eleven β-strands of at least 3 residues in length are predicted to be in similar positions. We place greater significance on predicted elements of secondary structure that are common to all sequences, since the enzymes fold into similar secondary structures as shown by the correlation of the diffraction patterns of these enzymes.

Correlation with the Preliminary Crystal Structure of A. glutaminasificans Glutaminase-Asparaginase—A preliminary structure for the Acinetobacter enzyme has been determined from 2.9-Å resolution x-ray data (Ammon et al., 1988). The present model for the A. glutaminasificans glutaminase-asparaginase structure has 331 residues in two chains, numbered as residues 1–273 and 300–357. This numbering is arbitrary at present, because the connectivity is not yet fully established. In particular, several of the surface connections between elements of secondary structure are still ambiguous. The A. glutaminasificans glutaminase-asparaginase subunit folds into two domains; residues 1–130 and 255–273 form the amino-terminal domain, while residues 131–254 and 300–357 form the carboxyl-terminal domain. The amino-terminal domain consists of a five-stranded β-sheet surrounded by five α-helices, a common feature in other protein structures (Richardson, 1981). The carboxyl-terminal domain was difficult to interpret, although it clearly contains three α-helices. The α-helical content is 27%, and at least 12% of the residues form β-strands in the preliminary model. The predicted secondary structure (Fig. 2) shows 41% α-helix and 35% β-strand. The sizes and shapes of the amino acid side chains in the amino-terminal sequence of A. glutaminasificans glutaminase-asparaginase are consistent with those initially assigned on the basis of the x-ray structure. We expected that the common helices which were predicted for all three sequences would aid in establishing the connectivity for the A. glutaminasificans glutaminase-asparaginase structure. When the amino terminus of the A. glutaminasificans glutaminase-asparaginase sequence was aligned with the 1st residue in the A. glutaminasificans glutaminase-asparaginase structure, the positions of five of the predicted α-helices for A. glutaminasificans glutaminase-asparaginase were close to helices (α2, α4, α6, α7, and α8) in the preliminary tracing of the A. glutaminasificans glutaminase-asparaginase structure. Only the predicted common helices starting at A. glutaminasificans glutaminase-asparaginase 259 and at A. glutaminasificans glutaminase-asparaginase 304 appear in similar positions in the preliminary structure (Ammon et al., 1988), so that the secondary structure predictions were not very helpful. Even if there are α-helices in the five common positions predicted, a total of eight are seen in the preliminary structure. The connectivity has not yet been fully established and will require refitting the correct sequence into the preliminary A. glutaminasificans glutaminase-asparaginase structure.

The Active Site of Amidohydrolases—Possible active site residues in amidohydrolases have been identified from the reaction of E. coli asparaginase with an asparagine analog, DONV (Peterson et al., 1977; Handschumacher, 1977), and the reaction of A. glutaminasificans glutaminase-asparaginase and Pseudomonas 7A glutaminase-asparaginase with the glutamine analog DON (Holcenberg et al., 1978). The DON-reactive Thr-12 of A. glutaminasificans glutaminase-asparaginase has been tentatively assigned to the α1-helix at the start of the amino-terminal domain; there is evidence that the DON binding site is part of the catalytic site for glutamine and asparagine (Holcenberg et al., 1978; Steckel et al., 1983). This residue is within the conserved 8-residue fragment found at the amino termini of A. glutaminasificans glutaminase-asparaginase, Pseudomonas 7A glutaminase-asparaginase, Er. chrysanthemi asparaginase, E. coli asparaginase, and the P. vulgaris asparaginase sequences, but the corresponding residue in E. coli asparaginase is not labeled by either the DONV or DON reagents (Handschumacher, 1977). A portion of the
E. coli asparaginase active site has been tentatively identified as involving a Ser-Thr-Ser fragment at residues 117-119, which our secondary structure predictions indicate should be in a region of coil. The E. coli asparaginase-DONV labeling experiment suggests that the most probable site for DONV binding is Thr-118 or Ser-119. Ser-119 is aligned with Ala-126 in E. coli asparaginase, and Thr-118 aligns with threonine in both Er. chrysanthemi asparaginase and A. glutaminasificans glutaminase-asparaginase and Er. chrysanthemi asparaginase which are in progress in our laboratories.

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Fig. 14. Reverse phase HPLC of CB fractions of Agla. Conditions, solvent and flow rate are as given under **Fractionation of peptides**. Gradient: 0 to 10% B in 50 min.

**Table** 1A. **Amoeboid composition of glycogen glycogen fraction of Agla**

| AA     | N/C1 | N/C2 | N/C3 | N/C4 | N/C5 |
|--------|------|------|------|------|------|
| A             | 1.2  | 0.7  | 1.6  | 7.0  | 2.7  |
| G         | 0.11 | 2.9  | 3.4  | 4.0  | 3.1  |
| Ser       | 0.4  | 0.3  | 0.2  | 0.1  | 0.1  |
| Glu       | 4.5  | 4.6  | 4.2  | 2.7  | 2.2  |
| His       | 0.6  | 0.8  | 0.1  | 0.1  | 0.1  |
| Arg       | 1.7  | 1.7  | 1.7  | 1.7  | 1.7  |
| Thr       | 0.1  | 0.1  | 0.1  | 0.1  | 0.1  |
| Tyr       | 0.2  | 0.2  | 0.2  | 0.2  | 0.2  |
| Lys       | 1.9  | 1.9  | 1.9  | 1.9  | 1.9  |
| Pro       | 0.1  | 0.1  | 0.1  | 0.1  | 0.1  |
| Thr       | 0.1  | 0.1  | 0.1  | 0.1  | 0.1  |
| Ser       | 0.1  | 0.1  | 0.1  | 0.1  | 0.1  |
| Glu       | 4.5  | 4.6  | 4.2  | 2.7  | 2.2  |
| His       | 0.6  | 0.8  | 0.1  | 0.1  | 0.1  |
| Arg       | 1.7  | 1.7  | 1.7  | 1.7  | 1.7  |
| Thr       | 0.1  | 0.1  | 0.1  | 0.1  | 0.1  |
| Tyr       | 0.2  | 0.2  | 0.2  | 0.2  | 0.2  |
| Lys       | 1.9  | 1.9  | 1.9  | 1.9  | 1.9  |
| Pro       | 0.1  | 0.1  | 0.1  | 0.1  | 0.1  |
| Thr       | 0.1  | 0.1  | 0.1  | 0.1  | 0.1  |
| Ser       | 0.1  | 0.1  | 0.1  | 0.1  | 0.1  |
| Glu       | 4.5  | 4.6  | 4.2  | 2.7  | 2.2  |
| His       | 0.6  | 0.8  | 0.1  | 0.1  | 0.1  |
| Arg       | 1.7  | 1.7  | 1.7  | 1.7  | 1.7  |
| Thr       | 0.1  | 0.1  | 0.1  | 0.1  | 0.1  |
| Tyr       | 0.2  | 0.2  | 0.2  | 0.2  | 0.2  |
| Lys       | 1.9  | 1.9  | 1.9  | 1.9  | 1.9  |
| Pro       | 0.1  | 0.1  | 0.1  | 0.1  | 0.1  |

**Fig. 15** Reverse phase HPLC of CB fractions of Agla. Conditions are given under **Fractionation of peptides**. Gradient: 0 to 10% B in 50 min.

**Table** 1B. **Amoeboid composition of glycogen glycogen fraction of Agla**

| AA     | N/C1 | N/C2 | N/C3 | N/C4 | N/C5 |
|--------|------|------|------|------|------|
| A             | 1.2  | 0.7  | 1.6  | 7.0  | 2.7  |
| G         | 0.11 | 2.9  | 3.4  | 4.0  | 3.1  |
| Ser       | 0.4  | 0.3  | 0.2  | 0.1  | 0.1  |
| Glu       | 4.5  | 4.6  | 4.2  | 2.7  | 2.2  |
| His       | 0.6  | 0.8  | 0.1  | 0.1  | 0.1  |
| Arg       | 1.7  | 1.7  | 1.7  | 1.7  | 1.7  |
| Thr       | 0.1  | 0.1  | 0.1  | 0.1  | 0.1  |
| Tyr       | 0.2  | 0.2  | 0.2  | 0.2  | 0.2  |
| Lys       | 1.9  | 1.9  | 1.9  | 1.9  | 1.9  |
| Pro       | 0.1  | 0.1  | 0.1  | 0.1  | 0.1  |
| Thr       | 0.1  | 0.1  | 0.1  | 0.1  | 0.1  |
| Ser       | 0.1  | 0.1  | 0.1  | 0.1  | 0.1  |
| Glu       | 4.5  | 4.6  | 4.2  | 2.7  | 2.2  |
| His       | 0.6  | 0.8  | 0.1  | 0.1  | 0.1  |
| Arg       | 1.7  | 1.7  | 1.7  | 1.7  | 1.7  |
| Thr       | 0.1  | 0.1  | 0.1  | 0.1  | 0.1  |
| Tyr       | 0.2  | 0.2  | 0.2  | 0.2  | 0.2  |
| Lys       | 1.9  | 1.9  | 1.9  | 1.9  | 1.9  |
| Pro       | 0.1  | 0.1  | 0.1  | 0.1  | 0.1  |

**Fig. 16** Reverse phase HPLC of CB fractions of Agla. Conditions are given under **Fractionation of peptides**. Gradient: 0 to 10% B in 50 min.
### TABLE 4M. Sequence data for peptides from AgA.

| Position | Residue | Cycle Yield (picomol) | K-H |
|----------|---------|-----------------------|-----|
| 1        | Lys     | 111                   | Pro 16 | 155 |
| 2        | Arg     | 112                   | Ile 15 | 186 |
| 3        | Val     | 113                   | Thr  6 | 147 |
| 4        | Val     | 114                   | Leu  10| 160 |
| 5        | Val     | 115                   | Val  14| 148 |
| 6        | Ile     | 116                   | Asp  71|     |
| 7        | Val     | 117                   | Ser  103|    |
| 8        | Ala     | 118                   | Met  125|    |
| 9        | Thr     | 119                   | Arg  116| 52  |
| 10       | Gly     | 120                   | Pro  207| 38  |
| 11       | Gly     | 121                   | Ser  84 | 44  |
| 12       | Thr     | 122                   | Thr  108| 41  |
| 13       | Ile     | 123                   | Ala  138| 71  |
| 14       | Ala     | 124                   | Ser  49 | 42  |
| 15       | Gly     | 125                   | Ser  132| 54  |
| 16       | Ala     | 126                   | Ala  109| 52  |
| 17       | Gly     | 127                   | Asp  57 | 29  |
| 18       | Ser     | 128                   | Gly  58 | 24  |
| 19       | Pro     | 129                   | Pro  48 | 22  |
| 20       | Ser     | 130                   | Leu  50 | 19  |
| 21       | Thr     | 131                   | Asp  46 | 14  |
| 22       | Ser     | 132                   | Ser  35 | 19  |
| 23       | Ala     | 133                   | Ser  13 | 13  |
| 24       | Thr     | 134                   | Ala  50 | 12  |
| 25       | Tyr     | 135                   | Ala  19 | 9   |
| 26       | Ser     | 136                   | Leu  137| 27  |
| 27       | Ala     | 137                   | Ser  21 | 8   |
| 28       | Ala     | 138                   | Ala  23 | 9   |
| 29       | Lys     | 139                   | Ser  8  |     |
| 30       | Val     | 140                   | Ser  9  |     |
| 31       | Pro     | 141                   | Asp  8  |     |
| 32       | Ser     | 142                   | Glu  7  |     |
| 33       | Asp     | 143                   | Ala  9  |     |
| 34       | Ala     | 144                   | Lys  6  |     |
| 35       | Leu     | 145                   | Ser  6  |     |
| 36       | Ile     | 146                   | Lys  5  |     |
| 37       | Lys     | 147                   | Gly  5  |     |
| 38       | Ala     | 148                   | Pro  207|     |
| 39       | Ala     | 149                   | Val  170|     |
| 40       | Val     | 150                   | Met  216|     |
| 41       | Pro     | 151                   | Val  147|     |
| 42       | Gin     | 152                   | Leu  164|     |
| 43       | Val     | 153                   | Met  184|     |
| 44       | Asn     | 154                   | Asn  158|     |
| 45       | Asp     | 155                   | Asp  142|     |
| 46       | Leu     | 156                   | Ser  115|     |
| 47       | Ala     | 157                   | Ile  85 |     |
| 48       | Asn     | 158                   | Phe  98 |     |
| 49       | Gin     | 159                   | Ala  89 |     |
| 50       | Thr     | 160                   | Ala  116|     |
| 51       | Gly     | 161                   | Arg  87 |     |
| 52       | Ile     | 162                   | Ser  86 |     |
| 53       | Gin     | 163                   | Val  53 |     |
| 54       | Ala     | 164                   | Thr  28 |     |
| 55       | Leu     | 165                   | Val  27 |     |
| 56       | Gin     | 166                   | Gly  7 |     |

**Structures of Amidohydrolases**
### Structures of Amidohydrolases

| 57 | Val | 132 | 27 | 167 | Ile | 230 |
|----|-----|-----|----|-----|-----|-----|
| 58 | Ala | 132 | 25 | 168 | Aan | 221 |
| 59 | Ser | 95  | 29 | 169 | Ile | 212 |
| 60 | Glu | 57  | 18 | 170 | His | 142 |
| 61 | Ser | 77  | 17 | 171 | Thr | 93  |
| 62 | Ile | 55  | 14 | 172 | His | 123 |
| 63 | Thr | 19  | 7  | 173 | Ala | 171 |
| 64 | Asp | 24  | 9  | 174 | Phe | 146 |
| 65 | Lys | 26  | 9  | 175 | Val | 133 |
| 66 | Glu | 2   | 176 | Ser | 42  |
| 67 | Leu | 9   | 177 | Gin | 103 |
| 68 | Leu | 11  | 178 | Trp | 8  |
| 69 | Ser | 7   | 179 | Gly | 86  |
| 70 | Leu | 8   | 180 | Ala | 94  |
| 71 | Ala | 5   | 181 | Leu | 77  |
| 72 | Arg | 7   | 182 | Giv | 75  |
| 73 | Gin | 6   | 183 | Thr | 24  |
| 74 | Val | 4   | 184 | Leu | 59  |
| 75 | Aaa | 4   | 185 | Val | 53  |
| 76 | Asp | 31  | 186 | Glu | 64  |
| 77 | Leu | 24  | 187 | Giv | 47  |
| 78 | Val | 24  | 188 | Lys | 40  |

### Amino Acid Fragments

- **K**: Whole protein
- **G**: Cyanogen bromide fragments
- **K**: Lysylendopeptidase peptides
- **C**: Citraconilic acid tryptic peptides
- **D**: Aspartylendopeptidase peptides

| 79 | Lys | K+2 | 12 | 189 | Pro | 31 |
|----|-----|-----|----|-----|-----|-----|
| 80 | Pro | 225 | 13 | 190 | Tyr | 24 |
| 81 | Ser | 184 | 19 | 191 | Trp | 23 |
| 82 | Val | 73  | 9  | 192 | Phe | 30 |
| 83 | Met | 163 | 16 | 193 | Arg | 17 |
| 84 | Lys | 139 | 12 | 194 | Ser | 13 |
| 85 | Gly | 121 | 21 | 195 | Ser | 19 |
| 86 | Val | 125 | 12 | 196 | Val | 6  |
| 87 | Ile | 135 | 11 | 197 | Lys | 7  |
| 88 | Ile | 139 | 13 | 198 | Lys | 11 |
| 89 | Thr | 76  | 7  | 199 | His | 30 |
| 90 | His | 29  | 4  | 200 | Thr | 47 |
| 91 | Aaa | 89  | 14 | 201 | Aan | 51 |
| 92 | Aaa | 72  | 6  | 202 | Aan | 52 |
| 93 | Asp | 85  | 7  | 203 | Ser | 19 |
| 94 | Thr | 50  | 5  | 204 | Glu | 37 |
| 95 | Met | 29  | 86 | 205 | Phe | 35 |
| 96 | Glu | 485 | 65 | 206 | Aan | 33 |
| 97 | Glu | 437 | 62 | 207 | Ile | 25 |
| 98 | Thr | 52  | 34 | 208 | Glu | 26 |
| 99 | Ala | 195 | 63 | 209 | Lys | 67 |
| 100 | Phe | 172 | 45 | 210 | Ile | 228 |
| 101 | Phe | 112 | 21 | 211 | Giv | 191 |
| 102 | Leu | 96  | 9  | 212 | Gly | 184 |
| 103 | Ser | 84  | 23 | 213 | Asp | 90 |
| 104 | Leu | 82  | 24 | 214 | Ala | 219 |
| 105 | Val | 129 | 25 | 215 | Leu | 208 |
| 106 | Val | 80  | 26 | 216 | Pro | 182 |
| 107 | His | 33  | 27 | 217 | Gly | 166 |
| 108 | Thr | 25  | 28 | 218 | Val | 169 |
| 109 | Asp | 45  | 29 | 219 | Gin | 171 |
| 110 | Lys | 93  | 220 | Ile | 163 |