PREDICTED SECONDARY STRUCTURE OF CYTOPLASMIC ASPARTATE AMINOTRANSFERASE FROM PIG HEART

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1. Introduction

Aspartate aminotransferase (L-aspartate-2-oxoglutarate aminotransferase, EC 2.6.1.1.) is widely distributed in animal and plant tissues as well as bacteria [1–3] and represents an important link between carbohydrate and amino acid metabolism.

Most of the studies on aspartate aminotransferase (AAT) have used the cytoplasmic pig heart enzyme [4–6]. It is a dimer, formed by two identical subunits of mol. wt 46 200. Each subunit is made of one polypeptide chain of 412 amino acid residues. The primary structure has been established [5–7], however the secondary and tertiary structures are still unknown, even though preliminary data on the crystallization of the pig and chicken heart AAT, as well as the low resolution structure of chicken heart AAT, have been published [8–10].

A number of amino acid residues have been identified from the active site of this enzyme, namely, Lys-258 [11,12], Tyr-40 [13] and Cys-390 [14]. The secondary structure could provide useful information on the enzyme conformation, and on the topology of the active site.

The use of statistical information derived from the primary structure to predict the secondary structure of a protein is now widely accepted [15]. That the nature and sequence of the amino acids are the main factors that determine the folding of the polypeptide chain has been supported by experimental evidence. The more successful [16], predictive method introduced by Chou and Fasman [17,18], is used here.

2. Methods and results

The prediction of secondary structure by inspection of the primary structure consists of a series of rules, based on the assignment of conformational parameters (normalized frequencies) to each of the 20 amino acids [17,18]. Even though originally, only the normalized frequencies with which each of the 20 amino acids appeared in α-helical regions and β-sheets seemed to be important, it appeared that the participation of the amino acids in the so-called β-turns gives the protein a globular rather than linear character [19].

A computer program that lists both the individual helical, β-sheet and β-turn conformational parameters, as well as the probability products for groups of 6, 5 and 4 amino acid residues, respectively, was obtained from K. K. Kannan, Wallenberg Lab., Univ. Uppsala. This program allows the prediction of the secondary structure using the criteria in [17,18], and also permits the modification proposed in [20] which uses the product of the conformational parameters of the group of residues involved in the structure, instead of the average value proposed in [17,18] (fig.1). It can be seen that both methods gave similar results, but with discrepancies, the product method was preferred.

The predicted secondary structure of aspartate aminotransferase from pig heart is represented in fig.2, in relation to its primary structure. Fig.3 is the usual 'arrow and cylinder' representation of one possible model of the secondary structure of AAT, drawn more or less to scale but constrained to 2-dimensions for the sake of clarity. Care has been taken to leave β_2, β_{14} and the turn after α_{13} together, since 3 residues of the active site, namely, Tyr-40, Cys-390 and Lys-258 lie respectively on β_2, β_{14} and on the turn. These 3 structural patterns have been marked on fig.3.

The amount of α-structure predicted by this

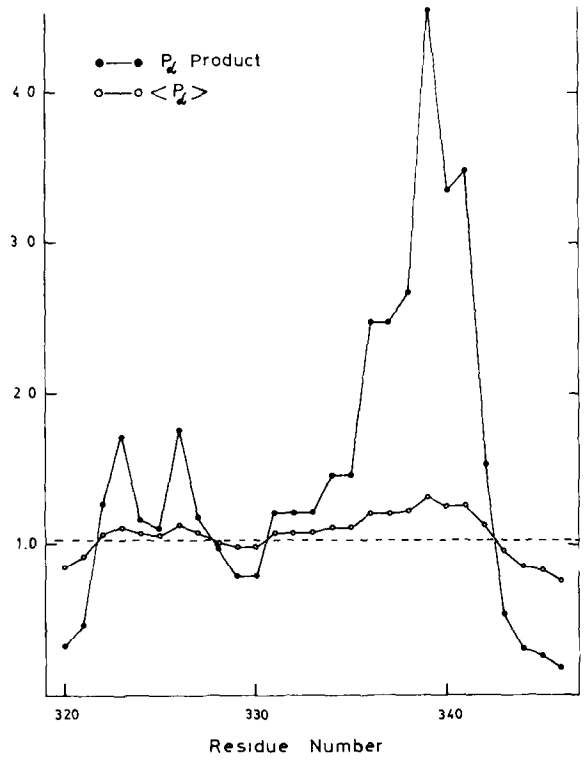

Fig.1. Prediction of α-helix for cytoplasmic AAT from pig heart, over amino acid residues 320–346. P_α product is the product of the conformational parameters P_α of 6 adjacent amino acids. $< P_\alpha >$ is the arithmetic mean of 6 adjacent amino acids. The P_α product clearly differentiate the secondary structure forming and non-forming regions.

method is 36.7%; 29.4% of β-structure and 16.5% of the amino acids are involved in turns.

3. Discussion

The predicted values for α- and β-structure, agree well with data obtained from CD and ORD which gave 37% α-helix for the cytoplasmic enzyme and 40% α-helix for the mitochondrial enzyme, together with a high content of α+β structure [1]. High values for both helical and β-structures as found in this enzyme have been found also in alcohol dehydrogenase from horse liver with 30% helical structure and 34% β-structure and in aspartate carbamoyltransferase

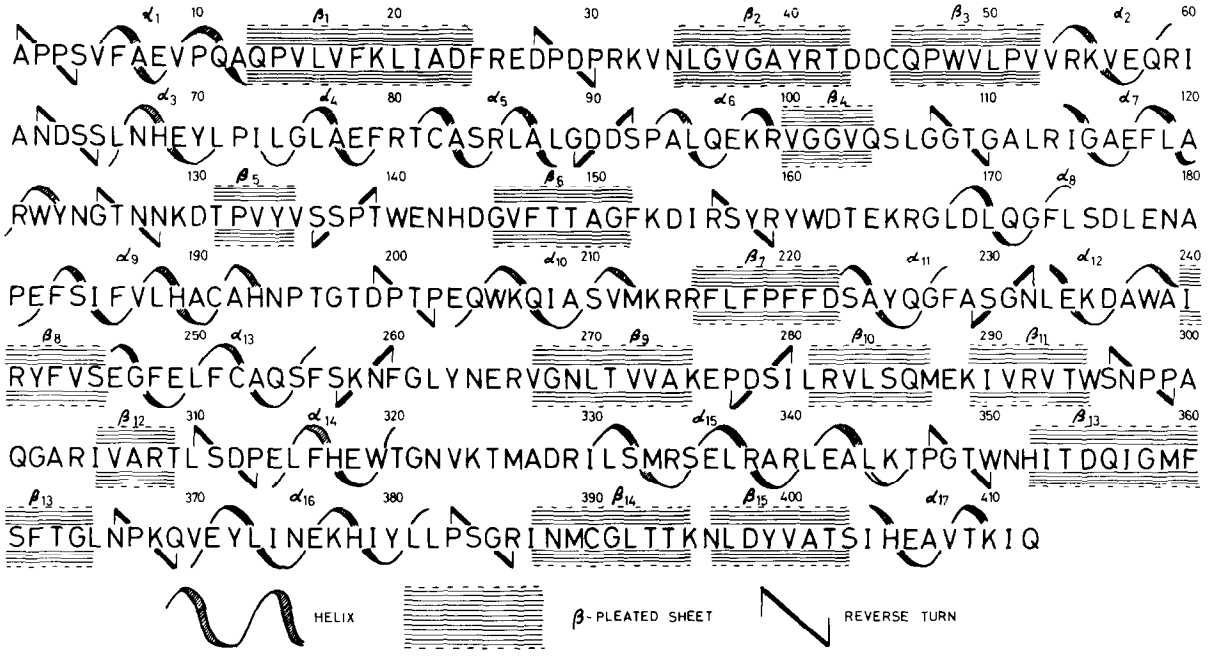

Fig.2. Schematic diagram of the regions of secondary structure of cytoplasmic AAT from pig heart. The one-letter symbol has been used in the primary structure representation according to the code: A, Ala; V, Val; L, Leu; I, Ile; P, Pro; F, Phe; W, Trp; M, Met; G, Gly; S, Ser; T, Thr; C, Cys; Y, Tyr; N, Asn; Q, Gln; D, App; E, Glu; K, Lys; R, Arg; H, His; B, Asx; Z, Glx.

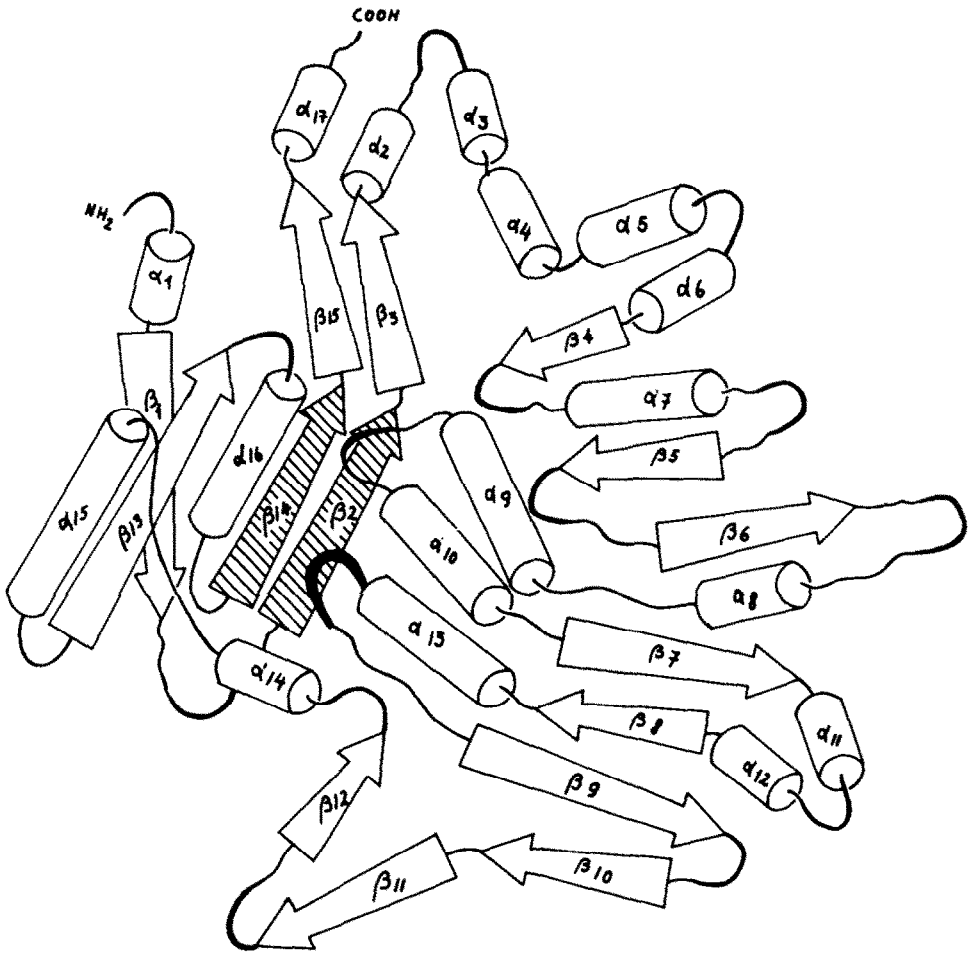

Fig.3. A possible model of the predicted secondary structure of cytoplasmatic AAT from pig heart in the usual 'cylinder and arrow' representation. The arrow lengths have been scaled to 3.5 A/AA, the cylinder lengths are 1.5 A/AA, the random coiled and β-turn regions have been scaled to 3.0 A/AA. The model has been forced to 2 dimensions for the sake of clarity. β_2, β_{14} and the β-turn after α_{13} should be a part of the active site.

from *Escherichia coli* [20]. These two enzymes present a secondary structure with >1 catalytic domain.

The model based on the predicted secondary structure of AAT shows a α/β topology/packing diagram according to the classification in [21]. The characteristic feature of this group of proteins is that most of the β-strands are separated by α-helix. This type of packing would be illustrated by the groups $\beta_4, \alpha_7, \beta_5, \beta_6, \alpha_8, \beta_7$ and $\beta_{13}, \alpha_{16}, \beta_{14}$ in our model (fig.3). In this group of proteins, with >300 amino acid residues, the structure can be separated in >1 domain [21]. This might be also the case of AAT with 412 amino acid residues. The model, schematized in fig.3, illustrates this possibility, since it shows two main β-structure domains:

(i) That which would contain the active site, is a β-pleated sheet formed by the strands $\beta_1, \beta_{13}, \beta_{14}, \beta_{12}$ and β_{11};

(ii) That where a pleated sheet would be formed by the strands $\beta_4, \beta_5, \beta_6, \beta_7, \beta_8, \beta_9$ and β_{10}.

The possibility of >1 domain deserves further investigation, because, notwithstanding the specificity of pig heart cytoplasmic AAT for its natural substrates, a variety of amino- and oxo-compounds, whether metabolically relevant or not, have been found capable

of serving as substrates [24]. Cytoplasmic AAT from pig heart can also utilize aromatic amino acids as substrates [23,25]. Phenylalanine transaminase activity of the pig heart AAT was confirmed [25], where competitive inhibition of the transamination of aspartate by phenylalanine and other aromatic amino acids (as well as by methionine) was observed. Similar observations have been made in [27].

The low resolution tertiary structure of cytoplasmic AAT from chicken heart appeared in [10]. It is not possible to compare directly our predicted secondary structure with the results of the chicken heart AAT, for several reasons:

(1) The chicken and pig enzymes have important differences in: the amino acid composition; the kinetic constants; the immunological response. All these facts indicate that there are great structural dissimilarities between the AAT enzymes from different animal species [28].
(2) The chain tracing in the low resolution tertiary structure of the chicken heart enzyme is not complete yet, and as pointed out, 'the course of the chain is reliable only on the position of the helical segments' [10].
(3) The complete primary structure of the chicken heart enzyme is not yet known, so that a prediction of the secondary structure of this molecule is not possible.

The only obvious similarity in the chicken and pig AAT secondary structure at this point, seems to be the high α-helix content.

Although X-ray diffraction results on tertiary structure will be definitive, the predictions of secondary structure presented here might stimulate the initiation of new studies that can explain the mechanism of action of this molecule.

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References

[1] Braunstein, A. E. (1973) The enzymes, 3rd edn, 9 379–480.
[2] Campos-Cavieres, M. and Munn, E. A. (1973) Biochem. J. 135, 683–693.
[3] Orlacchio, A., Campos-Cavieres, M., Pashev, I. and Munn, E. (1979) Biochem. J. 177, 583–593.
[4] Martinez-Carrión, M., Turano, C., Chiancone, E., Bossa, F. Giartosio, A., Riva, F. and Fasella, P. (1967) J. Biol. Chem. 242, 2397–2409.
[5] Ovchinnikov, Yu. A., Egorov, C A., Aldanova, N. A., Feigina, M. Yu., Lipkin, V. M., Abdulaev, N. G., Grishin, E. V., Kiselev, A. P., Modyanov, N. N., Braunstein, A. E., Polyanovsky, O. L. and Nosikov, V. V. (1973) FEBS Lett. 29, 31–34.
[6] Doonan, S., Doonan, H. J., Vernon, C. A., Walker, J. M., Airoldi, L. P., Bossa, F., Barra, D., Carloni, M., Fasella, P. and Riva, F. (1975) Biochem. J. 149, 497–506.
[7] Doonan, S., Doonan, H. J., Hanford, R., Vernan, C. A., Walker, J. M., Bossa, F., Barra, D., Carloni, M., Fasella, P., Riva, F. and Walton, P. L. (1974) FEBS Lett. 38, 229–233.
[8] Arnone, Rogers, P. H., Schmidt, J., Han, C. N., Harris, C. M., Metzler, D. E. (1977) J. Mol. Biol. 112, 509–513.
[9] Gehring, H., Christen, P., Eichele, G., Glor, M., Jansonius, J. N., Reinier, A. S., Smit, J. D. G. and Thaller, C. (1977) J. Mol. Biol. 115, 97–101.
[10] Borisov, V. V., Borisov, S. N., Kachalova, G. S., Sosfenov, N. I., Vainshtein, V. K., Torchinsky, Y. M. and Braunstein, A. E. (1978) J. Mol. Biol. 125, 275–292.
[11] Morino, Y. and Watanabe, T. (1969) Biochemistry 8, 3412–3417.
[12] Campos-Cavieres, M. and Milstein, C. P. (1975) Biochem. J. 147, 275–281.
[13] Demidkina, T. V., Bocharov, A. L., Polyanovsky, O. L. and Karpeisky, M. Ya. (1973) Molekul. Biol. (Engl. transl.) 7, 372–380.
[14] Torchinsky, Yu. M., Zufarova, R. A., Agalarova, M. B. and Severin, E. S. (1972) FEBS Lett. 28, 302–304.
[15] Robson, B. and Suzuki, E. (1976) J. Mol. Biol. 107, 327–356.
[16] Garnier, J., Osguthorpe, D. J. and Robson, B. (1978) J. Mol. Biol. 120, 97–120.
[17] Chou, P. Y. and Fasman, G. D. (1974) Biochemistry 13, 211–222.
[18] Chou, P. Y. and Fasman, G. D. (1974) Biochemistry 13, 222–245.
[19] Chou, P. Y. and Fasman, G. D. (1977) J. Mol. Biol. 115, 135–175.
[20] Dufton, P. Y. and Hilder, R. C. (1977) J. Mol. Biol. 115, 177–193.
[21] Monaco, H. L., Crawford, J. L. and Lipscomb, W. N. (1978) Proc. Natl. Acad. Sci. USA 75, 5276–5280.

[22] Levitt, M. and Chothia, C. (1976) Nature 261, 552–558.
[23] Novogrodsky, A. and Meister, A. (1964) Biochim. Biophys. Acta 81, 605–608.
[24] Evangelopoulos, A. E. and Sizer, I. W. (1965) Chim.-Chron. 30, 65–67.
[25] Miller, J. E. and Litwack, G. (1971) J. Biol. Chem. 246, 3234–3240.
[26] Schrawder, E. and Martinez-Carrión, M. (1972) J. Biol. Chem. 247, 2486–2492.
[27] Gelehrter, T. D. and Spencer, C. J. (1975) in: Isoenzymes (Markert, C. L. ed.) vol. 1, pp. 433–450, Academic Press, London, New York.
[28] Schrawder, E. J. and Martinez-Carrión, M. (1973) J. Biol. Chem. 248, 2140–2146.