MULTIPLE FORMS OF ASPARTATE AMINOTRANSFERASE. THE FORMATION OF ψ-AAT

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Received 1 December 1971

1. Introduction

Purified preparations of pig heart cytoplasmatic AAT** show the presence of multiple forms indicated as α, β, γ and δ in the order of increasing anionic character [1], although they have an identical molecular weight, amino acid composition and peptide map [2]. Previous work from our laboratory showed that the AAT subforms are characterized by different isoelectric points [3]. In non-aged enzyme preparations, herein indicated as i-AAT (i = initial), the subforms α and β, in which the coenzyme is mainly bound in an "active" way [2], are the most abundant and account for 70–80% of the total protein content. In an attempt to test the electrophoretic behaviour of AAT subforms after incubation under different conditions of pH and ionic strength we have found a procedure which allows the transformation of i-AAT into an enzyme preparation containing mainly γ and δ subform-moving species, herein indicated as ψ-AAT (ψ = pseudo). A recent paper by Arrio-Dupont et al. [4] has prompted us to report this procedure.

2. Experimental

Aspartate aminotransferase was prepared essentially according to the method B of Martinez-Carrion et al. [2]. The enzyme preparation had a specific activity of 930 units as assayed according to Banks et al. [5], and showed 5 enzymatically active bands upon starch gel electrophoresis [2, 3]†. The α-subform was obtained by isoelectric focusing as already described [3]. The protein concentration was evaluated spectrophotometrically at 278 nm by using $E^{1\%}_{1\,cm}$ = 14.80 [6].

Standard conditions to obtain ψ-AAT were as follows: 6 ml of aspartate aminotransferase (6.4 mg/ml) were dialysed by stirring at 37° for 24 hr in a stoppered flask against 2 mM 2-oxoglutarate, 1.6 M ammonium sulphate (ultrapure) which had been brought to pH 9.6 by addition of concentrated ammonia solution. After the incubation, the enzyme was dialysed in the cold against several changes of 0.05 M acetate buffer pH 5.4. A slight precipitate was removed by centrifuging for 20 min at 40.000 *g*. CM-Sephadex chromatography (1.25 × 30 cm column) was then performed under the conditions described by Martinez-Carrion et al. [2]. Besides some protein

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** AAT: aspartate aminotransferase (L-aspartate 2-oxo glutarate aminotransferase, EC 2.6.1.1).

† Identical electrophoretic patterns have been consistently observed even when the enzyme has been purified in the presence of 10^{-4} M dithiothreitol [4].

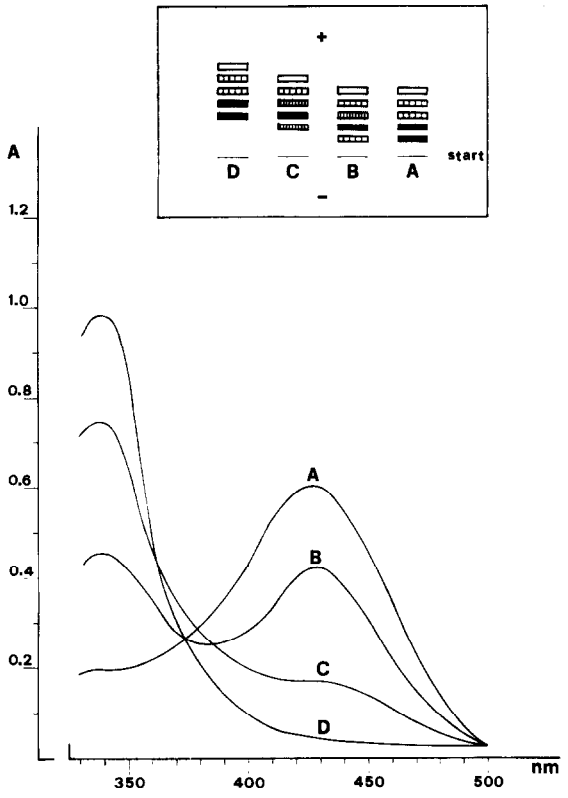

Fig. 1. Visible absorption spectra and protein electrophoretic patterns (inset) of samples of AAT incubated for different times in the presence of ammonium sulphate pH 9.2 (see the experimental section). The incubation times were: A, 0 hr; B, 1 hr; C, 5 hr; D, 48 hr. Spectra were recorded at a protein concentration of 6.4 mg/ml, after dialysis against 0.05 M acetate buffer pH 5.4.

material eluted with the front, the main protein fraction was eluted in correspondence with fraction I of Martinez-Carrion et al. (see fig. 1 in [2]). The protein was precipitated by the addition of an equal volume of cold acetone, dissolved in acetate buffer, dialysed against water, and then deionized as previously described [6]. The pyridoxal-phosphate content was determined spectrophotometrically at 388 nm, by using an $\epsilon = 6{,}000$, on the addition of 4 N KOH to the deionized enzyme solution to give a final alkali concentration of 0.1 N.

3. Results and discussion

Incubation of i-AAT in the presence of 1.6 M $(NH_4)_2SO_4$ at pH 9.2 (NH_4OH) causes a change in the visible absorption spectrum (0.05 M acetate buffer, pH 5.4), a concomitant fall in enzyme activity and a drastic variation in the electrophoretic pattern (fig. 1). After 24 hr the enzyme loses 98% of its original activity, shows a sharp maximum in the visible region centered at 340 nm (the A_{280}/A_{250} ratio remains unchanged) and gives an electrophoretic pattern in which none of the α- and β-subforms are evident, but in which γ- and δ-subforms are predominant (faster moving species showing exceedingly low enzymatic activity, are present as well).

Further incubation does not decrease the relative amounts of the γ and δ species in respect to the faster moving ones, but denaturation occurs and an insoluble precipitate is formed. Attempts to effect the transformation of i-AAT into ψ-AAT by using different buffers in the presence of different neutral salts to achieve $\mu \sim 4.8$, or different bases to achieve pH 9.2 in the presence of 1.6 M $(NH_4)_2SO_4$, were unsuccessful. For example, incubation of i-AAT in borate buffer pH 10.4, 4.8 M KCl, does not cause any significant variation of the electrophoretic pattern, even after 48 hr.

The conditions reported are so far the best found for the transformation of i-AAT into ψ-AAT. Following the procedure described in the experimental section 29.3 mg of ψ-AAT are obtained; the enzyme preparation has a specific activity of 18.2 units and shows an electrophoretic pattern identical to that of sample D in fig. 1. As far as the coenzyme binding is concerned: i) the absorption maximum at 340 nm does not depend upon the pH; ii) the coenzyme cannot be removed by the procedure described by Scardi et al. [7]; iii) the activity is not improved by the addition of an excess of pyridoxal-phosphate; iv) on treatment with alkali, the coenzyme is released in an amount of 0.94 moles per 47,000 g. Therefore, in ψ-AAT the coenzyme seems to be totally bound to the apoprotein moiety through a substituted aldimine bond, as suggested for the γ- and δ-subforms [2].

Pure α-subform treated under the conditions described above is transformed into an enzyme which has electrophoretic and spectral properties identical

to the ψ-AAT obtained from i-AAT. All of the faster moving subforms can be generated from the one α-subform.

Furthermore, in contrast with some findings reported by Arrio-Dupont et al. [4], the addition to the incubation mixture of thiol protecting agents, e.g. 0.1 M mercaptoethanol or 1 mM dithiothreitol, does not inhibit the transformation at all.

In conclusion, the procedure described in this report affords an enzyme preparation containing mainly those subforms which are scarcely present in the native AAT, thus allowing a detailed investigation of their chemical and physico-chemical properties.

Acknowledgements

The authors are grateful to Prof. A. Ballio for his interest, advice and valuable comments throughout this work. This investigation was supported by a grant of Italian Research Council (C.N.R.) to LCSN.

References

[1] M. Martinez-Carrion, F. Riva, C. Turano and P. Fasella, Biochem. Biophys. Res. Commun. 20 (1965) 206.
[2] M. Martinez-Carrion, C. Turano, E. Chiancone, F. Bossa, A. Giartosio, F. Riva and P. Fasella, J. Biol. Chem. 242 (1967) 2397.
[3] G. Marino, M. De Rosa, V. Buonocore and V. Scardi, FEBS Letters 5 (1969) 347.
[4] M. Arrio-Dupont, I. Cournil and P. Duie, FEBS Letters 11 (1970) 144.
[5] B.E. C. Banks, S. Doonan, A.J. Lawrence and C.A. Vernon, European J. Biochem. 5 (1968) 528.
[6] M. Bergami, G. Marino and V. Scardi, Biochem. J. 110 (1968) 528.
[7] V. Scardi, P. Scotto, M. Iaccarino and E. Scarano, Biochem. J. 88 (1963) 172.