CARBOHYDRATE COMPONENTS OF ASPARTATE TRANSAMINASE: MULTIPLE FORMS OF THE ENZYME FROM PIG HEART CYTOSOL

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1. Introduction

Cytoplasmic aspartate transaminase (L-aspartate:2-oxoglutarate aminotransferase, EC 2.6.1.1) occurs in cells in several subforms separable by ion-exchange chromatography or gel electrophoresis [1–5]. Catalytic activity of the subforms, which usually are designated by Greek letters, diminishes in the following sequence: $\alpha > \beta > \gamma > \delta$. By means of peptide mapping and amino acid analysis Martinez-Carrion et al. have shown that the primary structures of the subforms are identical [4]. It has been suggested that the subforms may represent distinct conformational variants (conformers) arising in the course of aging of the initial form (α), which has the highest activity [2].

The following study has been carried out to test a suggestion that the enzyme subforms may differ in content of some low molecular protein-bound component. It has actually been found that each subform contains carbohydrate (no less than 1 mono- or oligosaccharide unit per peptide chain). The low-activity γ-subform, exhibiting the greatest anionic mobility, is shown to contain sialic acids.

2. Materials and methods

Separation of subforms of aspartate transaminase from pig heart cytosol was carried out in the course of the purification procedure developed previously [6]. Protein solution obtained after treatment with ammonium sulphate (0.6 saturation) was dialysed against 0.005 M acetate buffer, pH 5.4, and applied to a CM-cellulose column equilibrated with the same buffer. The column was washed with initial buffer, then the molarity of eluting buffer was increased stepwise (at constant pH value, 5.4). Four discrete fractions containing aspartate transaminase were obtained which were eluted at the following concentrations of acetate buffer: 1) 0.005 M; 2) 0.05 M; 3) 0.06 M; 4) 0.2 M. Each fraction was subjected to subsequent purification on DEAE-Sephadex columns, followed by a second run of chromatography on CM-cellulose equilibrated with 0.005 M acetate buffer. Elution of α, β and γ-subforms was achieved by increasing the molarity of acetate buffer stepwise (0.005–0.05 M). Under these conditions, one subform [11], designated hereafter as 'ω', was tightly bound on CM-cellulose, and could be eluted by 0.2–0.4 M acetate buffer pH 5.4. The γ-form was characterized by its highest affinity for DEAE-Sephadex. When 0.005 M acetate buffer pH 5.4 was applied, this form remained at the top of the column and could be eluted only at concentrations of acetate buffer exceeding 0.05 M. To verify the purity of the subforms, polyacrylamide gel electrophoresis was used in glycine–pyridine buffer, pH 7.5.

Composition of neutral sugars was determined by paper chromatography. An aliquot of the protein (3–4 mg) was subjected to acid hydrolysis (3 N HCl, 3 hr 100°C). Hydrochloric acid was removed; thereafter the hydrolysate was batch treated with Dowex 50 × 8 (H^+-form), to remove amino acids and peptides (amino sugars were likewise bound by the resin). Chromatography was carried out in the system pyridine:isoamyl alcohol:H_2O (1:1:0.8 v/v) on FN4 paper (G.D.R.). The chromatogram was treated with alkaline $AgNO_3$ solution. The content of neutral sugars in acid hydrolysates was determined by the orcinol method [8]. Quantitative analysis was based on comparative analysis of the spectra of products formed in the

reaction between orcinol and the hydrolysate, orcinol and pure sugars. The 'least square' procedure was applied.

To estimate the content of sialic acids, mild hydrolysis of the enzyme subforms was performed (0.1 N H_2SO_4, 80°C, 60 min); the sulphuric acid was neutralized with an equivalent amount of $Ba(OH)_2$. Sialic acids were identified by their behaviour in the system mentioned above. The content of sialic acid was estimated by a quantitative resorcinol method elaborated by Svennerholm [9]. *N*-Acetylneuraminic acid was used as a marker substance for chromatography and quantitative analysis. The content of amino sugars in the aspartate transaminase subforms was determined by the Elson–Morgan procedure as modified by Zessi [10]. An aliquot of the protein was hydrolysed in 4 N HCl (100°C, 4 hr) and neutralized with NaOH solution in the cold.

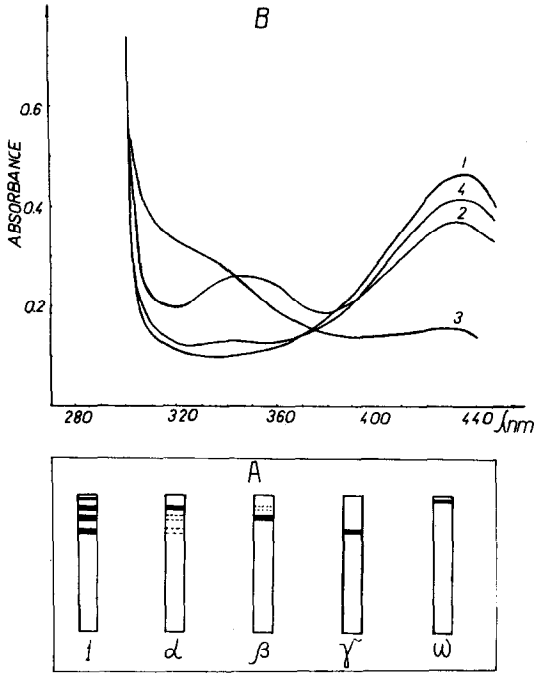

Fig. 1. Characterisation of aspartate-aminotransferase subforms. A) Electrophoretic patterns of transaminase on polyacrylamide gel: 1-enzyme before separation into subforms; α, β, γ, ω-electrophoretic patterns of the corresponding subforms; B) Absorption spectra of subforms. Concentration of protein: 3.8 mg/ml; pH 5.3; curves 1, 2, 3, 4: α, β, γ and ω-subforms, respectively.

Table 1
Characterization of aspartate transaminase subforms.

Subform	ω	α	β	γ
Specific activity[a]	33 000	45 000	34 000	11 000
Glucose[b]	3	1	1	3
Galactose[b]	–	–	–	7
Mannose[b]	3	1	1	5
Xylose[b] + Rhamnose	1	–	1	8
N-Acetyl-hexose-amine[b]	– [d]	0.27	1.2	6.7
Sialic acids[b]	0.1	0.17	0.25	2.6
ϵ_{580}/mg in carbazole reaction[c]	8.5	(1.0)	1.8	1.2

[a] Units per mg, cf. ref. [6].
[b] Number of residues per one transaminase protein subunit (mol wt. 46 500).
[c] Relative values of $A_{580\ nm}$ (α-form = 1.0) in the carbazole reaction.
[d] Was not quantitatively estimated.

The total carbohydrate content was estimated using the reaction with carbazole [11].

Spectra were read in either SF-4A (USSR) or Specord (GDR) spectrophotometers.

3. Results and discussion

By rechromatography on CM-cellulose and DEAE-Sephadex four discrete aspartate transaminase subforms were obtained. Their anionic mobilities on CM-columns may be written in the following sequence: $\omega \ll \alpha < \beta \ll \gamma$. The same sequence is shown by the mobilities of the subforms towards the anode during acrylamide gel electrophoresis (fig. 1A). The electrophoretic data show that ω and γ forms are homogeneous whereas the β form conatins a small amount of the α form, and that minor admixtures of β and γ forms are present in the α-form samples. Spectra of the four subforms are shown in fig. 1B. Specific activities are indicated in table 1.

Comparisons of acidity values, spectral properties

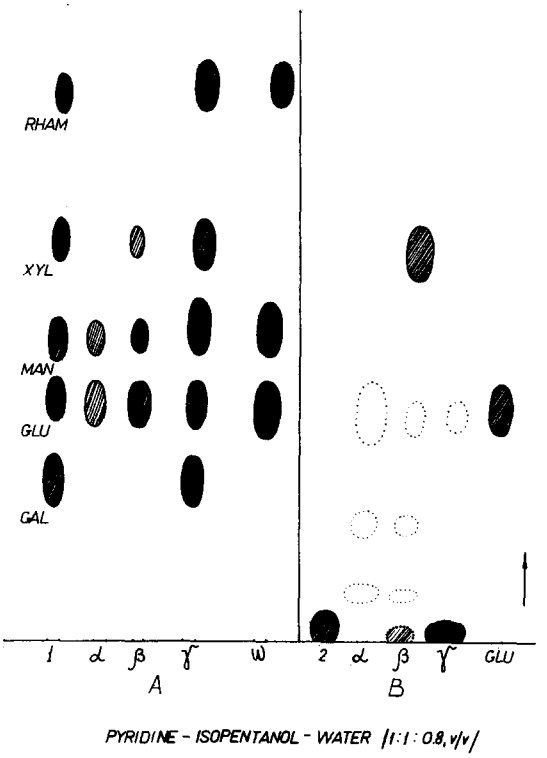

Fig. 2. Chromatographic distribution of carbohydrates bound to various subforms. Intensities and dimensions of spots correlate with sugar contents of transaminase subforms. A) Acid hydrolysis: 3 N HCl, 3 hr, 100°C. Amounts of protein (μmol): α-form, 0.08; β-form, 0.08; γ-form, 0.02; ω-form, 0.018; B) Acid hydrolysis: 0.1 N H_2SO_4, 60 min, 80°C. Amounts of protein, 0.1 μmol. Blanks: 1–sugar mixture (0.05 μmol of each); Glu, glucose; 2-*N*-acetylneuraminic acid (0.05 μmol).

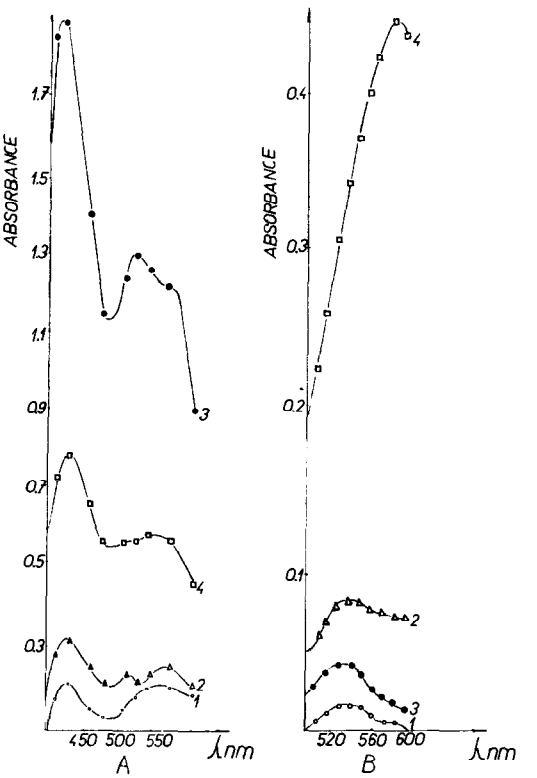

Fig. 3. Reactions of subforms for carbohydrates: A) Orcinol reaction; B) Reaction with carbazole; 1) (○—○) α-form; 2) (△—△) β-form; 3) (●—●) γ-form; 4) (□—□) ω-form.

and specific enzymatic activities indicate that the subforms described in this paper correspond to subforms of cytosol aminotransferase obtained by other authors [3–5].

By means of chromatography various neutral sugars as well as sialic acid were detected in the enzyme subforms. It can be seen from the chromatogram (fig. 2) that the diversity and quantitative content of sugars increase in the following order: α, β, ω, γ. Semiquantitative analysis was performed for sialic acid using *N*-acetylneuraminic acid as the standard and for amino sugars with *N*-acetylglucosamine as standard.

Spectra of the products formed in the reaction of subform hydrolysates with orcinol are given in fig. 3A; they characterise the contents of neutral sugars detected in the chromatograms. The spectra were used for quantitative evaluation of glucose, mannose, xylose, galactose and rhamnose. Results of the quantitative analysis of carbohydrate contents are presented in table 1. The data given here agree with the results of paper chromatography. It is noteworthy, that a positive correlation exists between the anionic properties of ω, α, β and γ-forms and sialic acid content. Under conditions of mild hydrolysis (0.1 N H_2SO_4, see under 'Methods') sialic acid is practically the only component cleaved from the carbohydrate of the γ-form (fig. 2); sugars which might interfere with sialic acid estimation in the resorcinol reaction are not released (see fig. 2B). The preferential release of sialic acid residues (about 3 per 1 enzyme subunit) from the γ-form may indicate that these residues are located at the distal end of the carbohydrate chain. Hydrolysis of the β-form under similar conditions yielded mainly xylose.

The content of sugars is high in the ω-form, which is tightly bound on CM-cellulose and generally discarded in the course of enzyme isolation. It is possible that this subform contains not only the sugars indicated in fig. 2 and table 1, but other carbohydrate components which have not been identified. An indication that this may be the case is an intense green colour ($\lambda_{max\ 580\ nm}$) of the carbazole product which is not usual for this reaction. Other subforms of the enzyme are characterised by a less intense reaction typical of neutral sugars ($\lambda_{max\ 530\ nm}$).

Carbohydraye components of ω, α, β and γ-subforms are gradually (but not completely) cleaved from the protein moiety at pH values $\geqslant 8$; this feature is similar to the behaviour of the alkali-labile O-glycosidic bond. This property may result in the appearance of artifactuai intermediate forms of the enzyme under experimental conditions.

The data obtained in this work indicate a possible reason for the differences in ionic charge of distinct aminotransferase subforms. However, these results do not explain spectral and catalytic differences existing between the enzyme forms.

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