FURTHER MICROHETEROGENEITY OF CYTOPLASMIC ASPARTATE AMINOTRANSFERASE FROM PIG HEART

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1. Introduction

A preparation of pure cytoplasmic aspartate aminotransferase (AAT, E.C. 2.6.1.1) from pig heart can be fractionated by CM-Sephadex chromatography into various subforms indistinguishable with respect to coenzyme content, molecular weight, peptide maps, amino acid composition [1]. We decided to study the carbohydrate content of cytoplasmic AAT, to see whether this might provide evidence for structural differences between the various subforms.

2. Materials and methods

AAT was prepared according to a modification by Martinez-Carrion et al. (method B) [1] of the Jenkins' procedure [2]. Apo-AAT was prepared according to Scardi et al. [3]. Subforms were separated according to Martinez-Carrion et al. [1]. Starch gel electrophoresis of various fractions was performed at pH 7.4, according to Banks et al. [4]. Carbohydrate content was determined using the phenol-sulfuric acid method [5], with glucose as standard.

3. Results

Method B [1] gives, after elution from DEAE-cellulose column, a mixture of several protein fractions all provided with AAT activity; the carbohydrate content of the mixture varies for different preparations between 0.7 and 1.7%. As will be shown later, at this stage the preparation contains in varying proportions two distinct classes of subforms, one of which contains an appreciable amount of carbohydrate; this nonhomogeneity of the preparation may be responsible for the variations in the carbohydrate content. However with a given preparation the distribution of carbohydrate essentially paralleled that of the protein eluted from the column (fig. 1). Neither concentration of the enzyme by acetone precipitation and dialysis nor the treatment for preparing the apoenzyme affected the carbohydrate content.

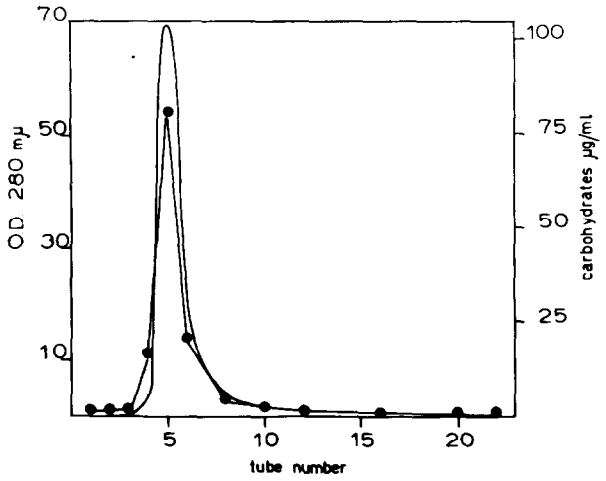

Fig. 1. Chromatography on DEAE-cellulose of AAT prepared according to method B [1].
——— protein elution monitored at 280 mμ
●——● carbohydrate content expressed as μg/ml.

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The mixture of AAT subforms was fractionated by chromatography on CM-Sephadex as described previously [1] and the carbohydrate content of the various fractions (fractions II and III were collected together) was found to be 0.20% for fraction I, 0.12% for fraction II + III and 0.18% for fraction IV. The highest of these values corresponds to about one-half mole of hexose per mole of enzyme monomer (assumed molecular weight 47,000 [1]). Since after the elution of these fractions a yellow band was observed on the top of the column, elution was continued with 0.5 M sodium acetate buffer at pH 5.55. With this eluant, a previously neglected component (fraction V) emerges from the column. The protein was precipitated in the cold with 100% acetone (V/V) and dialyzed against water. Its spectral properties were similar to those of fraction II + III of AAT; the specific activity was 78% of that of fraction IV (i.e., the most active of the previously known fractions); the carbohydrate content was 11.8%. Starch gel electrophoresis at pH 7.4 (fig. 2) did not show substantial differences between the pattern of fraction V and that of the mixture of subforms obtained by method B. All the bands possessed enzymic activity, detected by the method of Dekker and Rau [6].

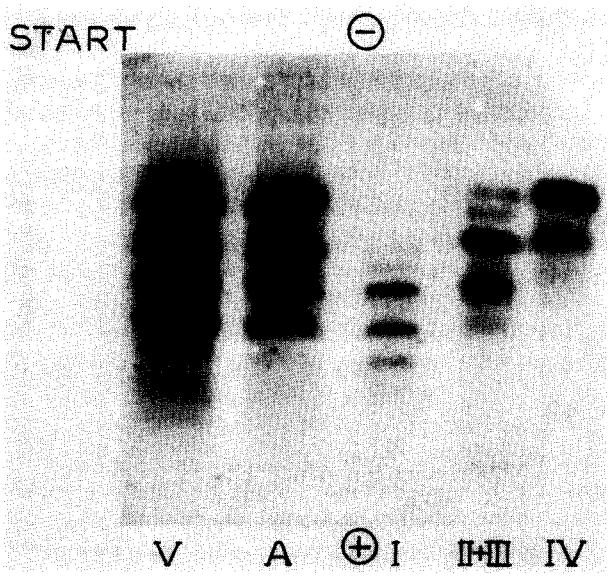

Fig. 2. Starch gel electrophoresis of the proteins present in the enzyme preparation (A) obtained by method B [1], and in its chromatographic fractions I, II + III, IV and V.

Since the purification includes chromatography on ion-exchange celluloses, it seemed possible that during these steps cellulose derivatives could have bound themselves to the AAT molecules, thus producing the carbohydrate containing species (fraction V). In order to investigate this possibility, fraction IV, which contains only traces of carbohydrates, was rechromatographed on a CM-cellulose column (25 × 1.3 cm) under the usual conditions (method B) [1]. The enzyme was recovered quantitatively from the column and showed no increase of the carbohydrate content. The same was observed for fraction I and fraction II + III after the same treatment.

Moreover AAT was prepared according to another modification of the Jenkins' procedure (method A) [1], which does not involve the use of cellulose or dextran derivatives. The enzyme so obtained showed a carbohydrate content of 5.5%. This enzyme was rechromatographed on CM-cellulose under the usual conditions (method B) [1]. With 0.06 M buffer most of the enzyme was eluted and showed a carbohydrate content of 1%, which remained constant during the following treatment: acetone precipitation (100% V/V); dialysis; DEAE–cellulose chromatography and further acetone precipitation. A yellow band remained on top of the CM–cellulose column and could be eluted with 0.5 M sodium acetate buffer, pH 5.38. This material, after acetone precipitation and dialysis, showed a carbohydrate content of 10.2%, but was contaminated by traces of material absorbing at 410 mμ. It was then further purified by chromatography through DEAE-cellulose which removed the contamination. The enzyme so obtained showed a carbohydrate content of 9.5% and a specific activity of 79% of the enzymatic fraction containing 1% carbohydrate.

4. Discussion

Present results suggest two conclusions. In the first place, the amount of carbohydrate found in the subforms prepared by the standard method [1] is so low (less than one mole, expressed as glucose, per mole of bound coenzyme) that it can probably be attributed to small contaminants; consequently, the differences among these subforms are presumably not due to bound carbohydrates of the type detected by the phenol-sulfuric acid method, which is sensitive to

hexoses, pentoses, disaccharides, oligo- and polysaccharides [5]. In the second place, these results constitute evidence for the existence of a new set of cytoplasmic subforms of AAT, which are characterized by a relatively high carbohydrate content. Such protein contains approximately 10% of the total activity present in the preparation obtained by method B. The carbohydrate containing subforms have a specific activity which is only a little less (about 20%) than that of the carbohydrate-free subforms. The amino-acid composition is probably the same, since previous analysis had revealed no difference in the composition of the individual carbohydrate-free subforms relative to the mixture of all subforms obtained by method B [1,4]. The problem of the origin of the carbohydrate containing subforms of AAT is still open. It cannot be excluded that carbohydrates bind to the protein at some time during the preparation, although this does not seem very likely, considering the relative constancy of the carbohydrate content found in various preparations, its high affinity for the protein (the carbohydrate content is not affected by dialysis and by repeated precipitation in ammonium sulfate or acetone), and the finding of a high carbohydrate content also in AAT preparation isolated by methods, which do not involve contact with carbohydrate containing material, such as dextran and cellulose columns. Moreover, repeated chromatography on such media does not alter the carbohydrate content. It seems probable that a sizeable fraction of the cytoplasmic AAT molecules are naturally bound to carbohydrate. The nature and possible biological role of these glycoproteic forms of AAT are presently under investigation. Other examples of isoenzymes differing only in their carbohydrate content are known, the most notable being perhaps bovine pancreas ribonuclease A and B [7]; a glycohemoglobin, with a hexose residue attached to the N-terminal has also been described [8]. Finally, Plapp and Cole [9], using the phenol-sulfuric acid reaction, have recently shown that the only structural difference between the multiple forms of bovine liver β-glucuronidase consists in the different amount of carbohydrate bound to the protein.

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