Journal of Chromatography, 143 (1977) 519—521
Biomedical Applications

CHROMBIO. 052

Note

Purification of alanine aminotransferase from human serum on a cycloserine-derivatized agarose

T.K. KORPELA

Department of Biochemistry, University of Turku, 20500 Turku 50 (Finland)

(Received January 20th, 1977)

In a previous paper [1] we described an approach designed to find a simple affinity chromatographic method for purifying alanine aminotransferase (E.C. 2.6.1.2) with gels replaced with inhibitors of the enzyme. Some of the derivatized agaroses separated bovine albumin from commercial pig-heart alanine aminotransferase (AlaAT) reasonably well.

The purpose of the present work was to investigate the applicability of the technique to the purification of crude AlaAT. A cycloserine derivative of agarose was used because it is easy to prepare and because its properties have been previously studied most thoroughly, although the gel was not the best of those tested with respect to separation of AlaAT from albumin [1]. The results demonstrate that AlaAT can be highly purified from crude sources in a single run. It is suggested that the large and serrated activity peak obtained is due to partly resolved sub-forms of the enzyme.

MATERIALS

Sepharose 4B 200, D-cycloserine and pig-heart AlaAT were purchased from Sigma (St. Louis, Mo., U.S.A.). Cyanogen bromide was obtained from Eastman-Kodak (Rochester, N.Y., U.S.A.). The other reagents were of commercial and analytical grade. Two samples of human serum were obtained from the Central Hospital of the University of Turku and were stored at —20°.

METHODS

D-Cycloserine was coupled to the cyanogen bromide-activated Sepharose (0.1 g of cyanogen bromide per millilitre of the gel) at pH 9.5 [2]. After washing, the derivatized gel was poured into a glass column of 12 mm I.D. The gel (settled volume about 75 ml) was equilibrated at 8° with 100 ml of 0.1 *M* sodium chloride solution containing 0.025 *M* sodium phosphate buffer, pH 6.0. The serum sample (0.5 ml) was applied to the gel and the elution was carried out at 8° with the above buffer solution at an elution rate of about 35 ml/h. The fractions (1.85 ml) were collected with an ISCO Model 820 fraction collector. Between runs, the gel was washed with 50 ml of 1 *M* sodium chloride solution before the equilibration.

The AlaAT activity was measured as the change in absorbance of NADH [1] and aspartate aminotransferase (AspAT) activity with the 2,4-diphenylhydrazine method [3]. The absorbance at 280 nm indicates the protein concentration.

RESULTS

Fig. 1 shows the elution curves of two human sera (I and II) chromatographed on the cycloserine-derivatized agarose. As can be estimated (see AlaAT I or II and the protein), several hundred-fold purification was achieved.

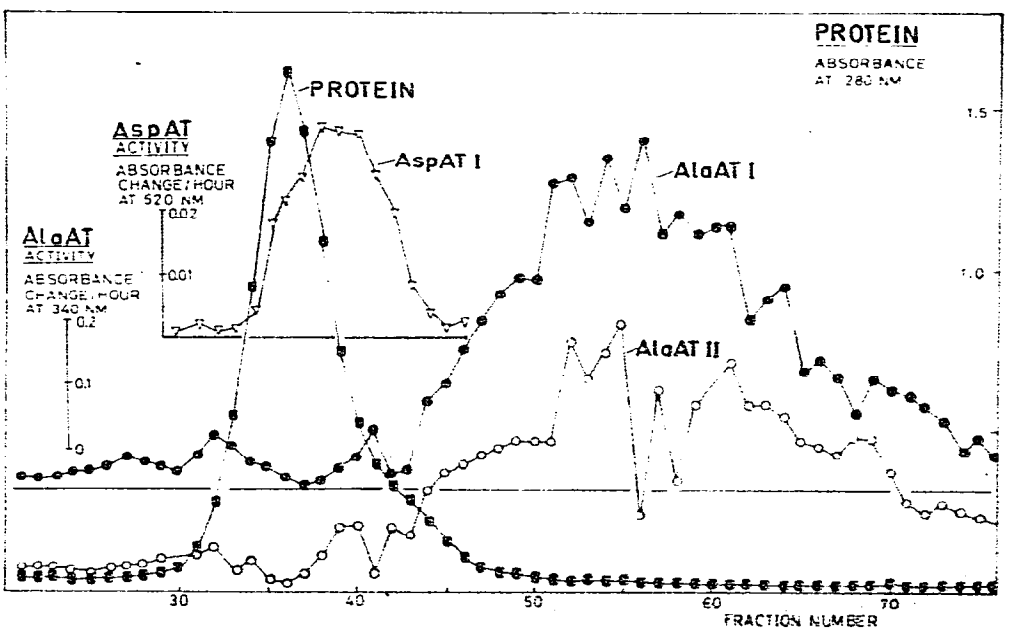

Fig. 1. Chromatography of two samples (I and II) of human serum on a cycloserine-derivatized Sepharose 4B gel. Samples I and II had aspartate aminotransferase activities 10 and 5 times the normal value, respectively. The fraction volume was 1.85 ml.

The recovery of AlaAT and serum protein was 95±5%. Commercial pig-heart AlaAT containing 5 mg of bovine serum albumin gave a sharper activity curve (maximal activity in the 54th fraction) than serum AlaAT. The behaviour of *Escherichia coli* crude protein on chromatography was similar to that of serum protein. As shown in Fig. 1, AspAT (I) formed one sharp peak that separated from the bulk of protein only slightly.

The activity of AlaAT in Fig. 1 increased smoothly between fractions 40 and 50, then saw-toothed activity patterns appeared in both curves. The serrated shape is due to the chromatographic process; the maximal difference between points of duplicate determinations was only 0.03 absorbance unit. The presence of reducing or oxidizing agents in the sera, which could disturb the measurement of AlaAT activity, was not excluded, but their retardation into the fractions of AlaAT seems improbable.

DISCUSSION

The results suggest that cycloserine-derivatized agarose is suitable for the purification of AlaAT from sources other than serum also. If a better resolution from the bulk of the protein is desired, aminooxyalanine bound to aminoethylagarose can presumably be used [1]. The advantages of the affinity chromatographic method, other than speed and simplicity, lie in the fact that the fractions contain all naturally existing forms of AlaAT.

The properties of cytosolic and mitochondrial AlaAT are different in several mammals [4, 5]. The fact that samples I and II had high AspAT activities (see Fig. 1) indicates that they contained some AlaAT originating from damaged cells and thus both cytosolic and mitochondrial enzymes. The effect of mercaptoethanol on the chromatography of rat-liver AlaAT on Sephadex G-200 suggests a subunit structure [6]. An insect cytoplasmic AlaAT exists as both a monomer and a tetramer, whereas the mitochondrial enzyme exists mainly as a dimer [7]. Consideration of these results in the present work suggests that the sera contained multiple forms of the enzyme. Because partially purified pig-heart AlaAT gave a sharper peak than crude AlaAT from serum, it is possible that the large serrated shape of the activity curve in Fig. 1 is due to partly resolved multiple forms of the enzyme. This fact might have clinical use in indicating of cell damage.

REFERENCES

1 T.K. Korpela, A.E. Hinkkanen and R.P. Raunio, J. Solid-Phase Biochem., 1 (1977) 215.
2 P. Cuatrecasas, J. Biol. Chem., 245 (1970) 3059.
3 J. King, Practical Clinical Enzymology, Van Nostrand, London, 1965, pp. 130—132.
4 G. DeRosa and R.W. Swick, J. Biol. Chem., 250 (1975) 7691.
5 J. Orlicky and M. Ruscak, Physiol. Bohemoslov., 25 (1976) 223.
6 H.L. Segal, P.W. Gatehouse, and S. Hopper, in E.E. Snell, A.E. Braunstein, E.S. Severin and Yu. M. Torchinsky (Editors), Pyridoxal Catalysis: Enzymes and Model Systems, Interscience, London, 1968, pp. 215—253.
7 S.D. Mane and K.N. Mehrotra, Experientia, 32 (1975) 154.