MULTIPLE FORMS OF SUPERNATANT GLUTAMATE-ASPARTATE TRANSAMINASE FROM PIG HEART

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Holo glutamate-aspartate transaminase from pig heart has been purified by Lis (1958) and by Jenkins et al.(1959), These preparations contain essentially the supernatant enzyme (Wada and Morino, 1964). Preparations homogeneous in the ultracentrifuge and in free boundary electrophoresis had an $O.D._{280m\mu}/O.D._{362m\mu}$ ratio of 10 at pH 9; this property was often used as a routine criterium for purity. Hetereogeneity of preparations which met these specifications has recently been reported (Jenkins,1965). It is the purpose of the present communication to show that such preparations contain multiple forms of the supernatant enzyme differring in specific activity, spectrophotometric properties and motility on starch gel electrophoresis.

Experimental. GOT prepared according to Jenkins et al.(1959) using succinate buffer instead of maleate (Turano et al.,1964), when analyzed by starch gel electrophoresis (Barret et al.,1962) was found to contain three major components, hereafter indicated as α, β and γ in order of increasing motility (Figure 1). The three components were separated by two successive chromatographies. 500 mg of protein in 30 ml of 0.02 M sodium acetate buffer, pH 5.4 were placed on a CM-sephadex column (4 x 40 cm)

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Note: Abbreviations used, Glutamate-aspartate transaminase - GOT
Pyridoxal phosphate - PLP

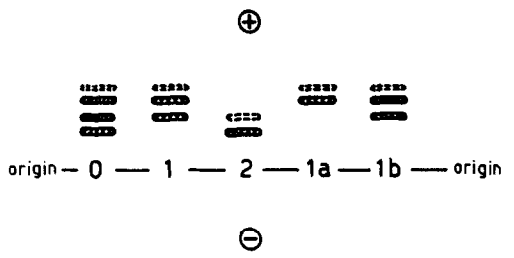

Fig. 1. Starch gel electrophoresis patterns. 0) original enzyme preparation containing the α, β and γ bands and traces of a faster moving component. 1) chromatographic fraction 1, containing the β and γ bands and traces of the fast component. 2) chromatographic fraction 2, containing predominantly the α band. 1a) chromatographic fraction 1a, containing predominantly the γ band. 1b) chromatographic fraction 1b, containing predominantly the β band and traces of the γ band and of the faster moving component. The motility of the bands contained in fraction 1b is the same before and after incubation with PLP.

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equilibrated with the buffer. The column was washed with the same buffer at increasing concentrations according to the following pattern: 100 ml of 0.04 M, 200 ml of 0.06 M, and a linear gradient with 1 liter of 0.06 M in the mixing chamber and 1 liter of 0.11 M in the reservoir. One protein peak (fraction 1) emerged with 300 - 600 ml; a second peak (fraction 2) emerged with 800 - 1200 ml. On starch gel electrophoresis fraction 1 gave the β and γ bands, while fraction 2 gave essentially the α band (Fig. 1). Fraction 1 was resolved according to Scardi et al. (1963) and re-chromatographed on a CM-sephadex column (2 x 12 cm) equilibrated with 0.02 M sodium acetate buffer, pH 5.4. The column was washed extensively with the same buffer at a concentration of 0.07 M. A protein peak (fraction 1a), containing mostly the γ band, emerged with the washing. The molarity of the eluting buffer was then raised to 0.2 M and a second protein peak (fraction 1b) containing mostly the β band was obtained. The electrophoretic patterns, enzymic activities and spectral

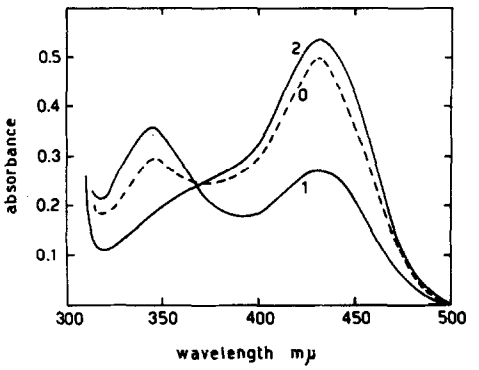

Fig. 2. Variation of the enzyme absorption spectrum with fractionation on CM-sephadex. 0) enzyme (5.0 mg/ml), prior to the first chromatography. 1) fraction 1 (3.9 mg/ml). 2) fraction 2 (4.6 mg/ml). All spectra were recorded in 0.05 M sodium acetate buffer, pH 5.4.

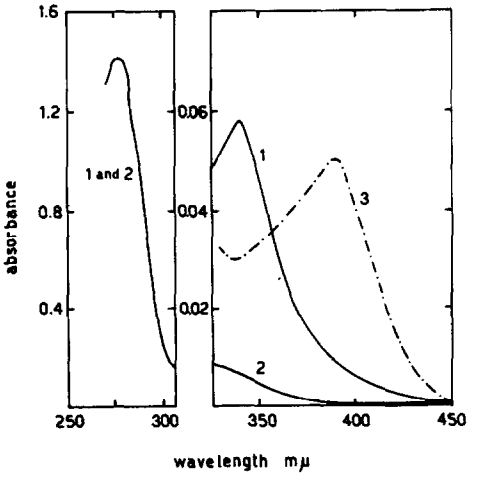

Fig. 3. Absorption spectra of the fractions (0.1 % solutions) obtained from the first chromatography on CM-sephadex and submitted to the resolving treatment of Scardi et al. (1963). 1) fraction 1, pH=5.4. 2) fraction 2, pH=5.4. 3) fraction 1, in 0.1 M NaOH.

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properties of the various fractions are reported in figures 1 to 3 and in Table I.

TABLE I

PROPERTIES ON THE PROTEIN FRACTIONS PRESENT IN GOT PREPARATIONS

Enzyme preparation	Electrophoretic bands (Fig.1)	GOT specific activity (o)	Spectral properties pH=5.4 max mμ	$E^{1\%}_{cm}$	pH=8.5 max mμ	$E^{1\%}_{cm}$
Supernatant GOT	α , β , γ	70	340 430	0.58 1.00	340 362	(oo) 1.45
Fraction 1	β , γ	40	340 430	0.90 0.70	340 362	(oo) 0.87
Fraction 1a (ooo)	γ	1	340	1.40	340	1.40
Fraction 1b (ooo)	β , traces of γ	80	430	1.14	362	1.68
Fraction 2	α , traces of β	100	430	1.15	362	1.70

(o) Micromoles of oxalacetate formed per minute per mg of protein at 25°C under the assay conditions described by Lis (1958).

(oo) Only a shoulder is visible at 340 mμ.

(ooo) Fractions 1a and 1b recovered from the second chromatography were incubated for 30 min at 25°C with 1 x 10^{-3}M PLP in 0.2 M sodium acetate buffer, pH 5.4; excess PLP was removed by chromatography on Sephadex G-25; the protein fractions obtained by this treatment were then used for determining the properties reported in the Table. Some preparations of fraction 1b presented a small peak or shoulder at 340 mμ, depending upon the amount of the contaminating traces of the γ band present in the preparation.

Results and discussion. The above results show that currently used preparations of GOT contain at least three distinct proteins, two of which (fractions 2 and 1b) are enzymatically active and, on the basis of their spectra (Figs. 2 and 3; Table I), can be thought to bind PLP as an apoenzyme-coenzyme Schiff base (Guirard and Snell, 1964). The third component (fraction 1a), though provided with negligible GOT activity, can be reasonably assumed to be structurally rela-

ted to the other two for the following reasons: a) the amino acid compositions of each of the three components are identical to that reported by Turano et al. (1963) for a GOT preparation containing a mixture of them; b) the three fractions do not present immunological hetereogeneity when tested by the Ouchterlony (1949) technique; c) the three fractions have an identical PLP content. It also seems reasonable to think that PLP is bound to the inactive component in a peculiar way since: a) at pH 5.4 its coenzyme absorption maximum is at 340 mμ and not at 430 mμ as in the α and β components (Table I; Fig. 3); b) the maximum at 340 mμ is not affected by resolution according to Scardi et al. (1963) (Fig.3) nor by the addition of glutamate and ketoglutarate nor by treatment with $NaBH_4$, nor by shifts of pH between 5 and 8.5 (for the effects of these treatments on the 430 mμ peak of GOT, see Guirard and Snell (1964)); c) the 340 mμ peak is shifted to 390 mμ by treatment with 0.1 M NaOH (Fig.3) (indicating release of free PLP, which can be recovered by chromatography on Sephadex G 25).

A detailed study of the structural and functional properties of the three components is under way. At present it is not clear whether these different forms are biologically determined or just reflect the isolation procedures. It is worth noting, however, that α GOT predominated in all the preparations so far studied, being approximately 2/3 of the total enzyme on a protein basis. When resolved (Fig.3), this form appears to be the first reported apoGOT devoid of spectral bands indicative of bound coenzyme. We finally wish to point out that the multiple forms of GOT described in the present paper are all to be ascribed to the supernatant enzyme, since we have found (unpublished results) that also the enzyme isolated from mitochondria contains multiple subforms, all of which can be easily distinguished from the

supernatant enzymes, because, under the conditions used, they migrate on starch gel electrophoresis towards the cathode.

Hence, it can be concluded that there exist multiple forms of both supernatant and mitochondrial enzyme. This fact would explain some of the reported discrepancies (Henson and Cleland, 1964; Nisselbaum and Bodansky, 1964; Boyd, 1961) on the kinetic behaviour of the isozymes of GOT. Finally, the present findings could help in the interpretation of part of the spectrophotometric results obtained by Evangelopoulos and Sizer (1963) on an enzyme preparation containing a large proportion of material absorbing at 340 mμ.

REFERENCES

Barret, R.J., Friesen, H., and Astwood,E.B., J. Biol. Chem., 237, 432 (1962)

Boyd, J.W., Biochem. J., 81, 434 (1961)

Evangelopoulos, A.E., and Sizer, I.W., Proc. Natl. Acad. Sci., 49, 638 (1963)

Guirard, B.M., and Snell, E.E., in M. Florkin and E.H. Stotz (Editors), Comprehensive Biochemistry, Vol. 15, page 138, Elsevier Publishing Company, Amsterdam (1964)

Henson, C.P., and Cleland, W.W., Biochemistry, 3, 338 (1964)

Jenkins, W.T., Yphantis, D.A., and Sizer, I.W., J. Biol. Chem., 234, 51 (1959)

Jenkins, W.T., in preparation (1965)

Lis, H., Biochim. et Biophys. Acta, 28, 191 (1958)

Nisselbaum, J.S., and Bodansky, O., J. Biol. Chem., 239, 4232 (1964)

Ouchterlony, O., Acta Pathol. Microbiol. Scand., 26, 507 (1949)

Scardi, V., Scotto, P., Iaccarino, M., and Scarano, E., Biochem. J., 88, 172 (1963)

Turano, C., Giartosio, A., Riva, F., and Vecchini, P., I.U.B. Symposium on Pyridoxal Catalysis,page 149, Pergamon Press, Oxford (1963)

Turano, C., Giartosio, A., Riva, F., and Fasella, P., Biochem. and Biophys. Res. Comms., 16, 221 (1964)

Wada, H., and Morino, Y., Vitamins and Hormones, 22, 411 (1964)