REVERSIBLE DISSOCIATION OF SUCCINYLATED ASPARTATE TRANSAMINASE INTO SUBUNITS

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It has been shown in previous work that the dimeric molecule of pig heart aspartate transaminase (L-aspartate: 2-oxoglutarate aminotransferase, EC 2.6.1.1) dissociates into subunits on treatment with sodium dodecylsulfate (Torchinsky and Shpikiter, 1963) and at extreme pH values (at pH's below 3 or above 11; Polyanovsky and Shpikiter, 1965). In either case dissociation was irreversible and the enzyme was inactivated as a result of release of pyridoxal phosphate and denaturation. Further evidence was obtained with the technique of polarization of fluorescence (Polyanovsky and Ivanov, 1964) indicating that the enzyme undergoes dissociation into subunits at pH 5-9 in dilute solutions (20-30 μg protein per ml), while retaining full catalytic activity.

Since very much lower concentrations of enzyme protein are usually employed in the assay of transaminase activity, it appears that the subunit (monomer) of aspartate transaminase is probably catalytically active.

In order to provide additional evidence to confirm the formation of active transaminase subunits, it was desirable to achieve experimental conditions that would make possible the reversible dissociation of the enzyme at higher protein concentrations, and so allow direct observation of the process in the ultracentrifuge.

To this aim aspartate transaminase was subjected to acylation with succinic anhydride (Habeeb et al., 1958), a treatment known to alter the macromolecular structure of proteins and to promote dissociation into subunits of proteins that possess quaternary structure (Klotz et al., 1963).

Enzyme preparations. High-purity native aspartate transaminase was obtained from pig hearts in the absence of maleate, as described previously (Polyanovsky and Telegdi, 1965). A 1% aqueous solution of the enzyme was succinylated by the portionwise addition of dry succinic anhydride to a final concentration of 3%, adding dry Tris to keep the pH within constant limits (from 7 to 8). The reaction mixture was kept for two hours at room temperature and left overnight in the refrigerator. On the next day low-molecular components were removed by gel-filtration through Sephadex G-25. The clear yellowish fraction of filtrate that contained the succinylated aspartate transaminase (SAT) was used for the experiments reported below.

Dissociation into subunits. In neutral solution (in water or 0.1 M Tris-acetate buffer, pH 6.5-7.0), at protein concentration of 0.5 per cent, SAT sedimented in the ultracentrifuge (Spinco, model E) as a homogenous protein with $S_{20,w} = 4.65$ (Fig.1a). At pH 8.0-8.5 a second component appeared ($S_{20,w} = 2.4$) (Fig.1b). The content of this slow component increased at higher pH values; near pH 10 the slower component predominates (Fig.1d,e).

Appearance of the slow component may be due to dissociation of the protein into subunits and/or increased dissymmetry of the transaminase molecules in alkaline solution. To ascertain the nature of the slow component, the frictional ratios (f/fo) of the succinylated enzyme were calculated from viscosity data at various pH values, measured with an Ostwald type viscosi-

meter. At pH 7.0 and 11.0 the f/fo ratios were 1.74 and 1.90, respectively; frictional ratios of the non-modified enzyme at the same pH values are equal to 1.35 and 1.90 (Polyanovsky and Shpikiter, 1965).

Using the equation $S/S_1 = M^{2/3}(f/fo)\ M_1^{2/3}(f/fo)_1$ (Lundgren and Williams, 1939) we calculated that the molecular weight of the slow component of SAT is one half of that of the original fast component in neutral solution. As calculated

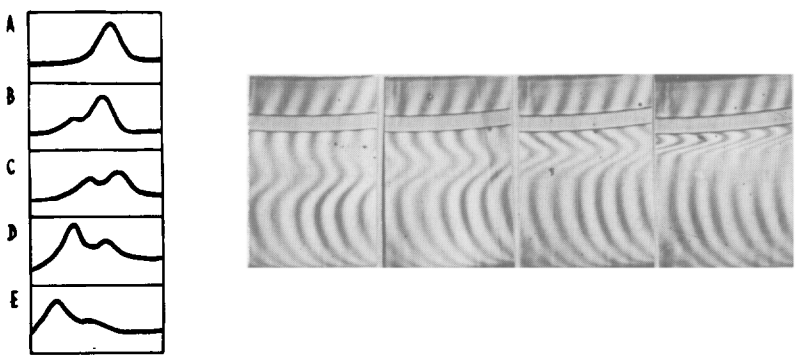

Fig. 1. Effect of pH on sedimentation pattern of SAT. A - E, 0,5% solution of SAT; Spinco, Model E, ultracentrifuge (schlieren technique); F, 0,07% solution of SAT; UCPA ultracentrifuge supplied with polarization interferometer; temperature in the all cases 20°.

	pH[x]	Buffer	Speed RPM	Times of exposure (min)[xx]
A	6,5	0,05M tris-acetate	56100	72
B	9,0	0,05M NaOH-glycine	56100	72
C	9,4	- " -	50740	90
D	10,2	- " -	50740	90
E	10,7	- " -	39460	120
F	9,0	- " -	50500	27,57,87,117

x) pH was measured before centrifugation experiments.
xx) Times after reaching constant speed.

according to the equation of Svedberg and Pedersen (1940) the molecular weight of SAT (fast component) is 117,000, in good agreement with the molecular weight 116,000 of native

aspartate transaminase (Jenkins et. al.,1959). These experimental data and calculations constitute proof that the slow component of SAT arising in alkaline media is the monomeric subunit of the enzyme.

It was further observed that the degree of dissociation of SAT increases upon dilution of the enzyme solution (see below, and Fig.1f).

Reassociation of the subunits. Neutralization of alkaline solutions of SAT resulted in reassociation of the subunits. A 0.5% solution of the enzyme was adjusted to pH 9.5. One portion of this solution was subjected to ultracentrifugal analysis immediately (Fig. 2a), and another portion after 2 hrs incubation followed by neutralization to pH 6.5. It is seen from Fig. 2c that the slow component (subunit) arising in alkaline medium disappears completely upon readjustment of the solution to pH 6.5, while the area of the fast peak (dimeric form) increases.

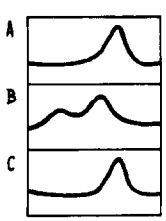

Fig.2. Reassociation of SAT after reneutralization A, pH 6.5; B, pH 9.5; C, solution B adjusted from pH 9.5 to pH 6.5. Exposures made at A, 80 min.; B, 85 min.; C, 80 min. after reaching constant speed (56100 RPM); temperature 20°. Spinco, Model E ultracentrifuge.

The catalytic activity of succinyl-transaminase. Transaminase activity was determined spectrophotometrically (Cammarata and Cohen, 1951) by measurement of the increase in O.D. at 280 mμ due to oxaloacetate formed by transamination between α-ketoglutarate and L-aspartate at 37°, in a 3 ml reaction sample containing equimolar amounts (20 μmoles) of both substrates and 0.2-0.3 μg/ml of enzyme protein.

In the region of optimal pH (8.5-9.3) SAT retains 65 per cent of the activity of the corresponding amount of native transaminase. It should be noted that the pH-activity curve in the alkaline range and the pH-curve of dissociation of the 0,5% solution of SAT do not coincide (Fig. 3).

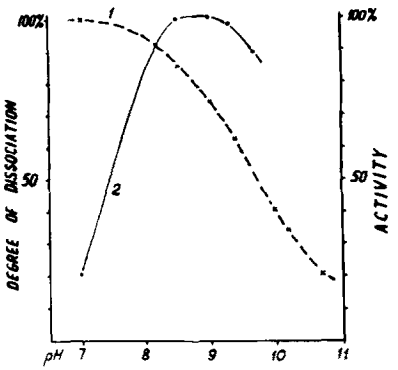

Fig.3. Effect of pH on catalytic activity and degree of dissociation of SAT into subunits.
1. Dissociation of enzyme derivative; 2. Initial velocity of transamination.

These data imply that the monomer of SAT is catalytically active: at the optimal pH the succinylated enzyme is already significantly dissociated, and at pH 9.7 the enzyme still has 90 per cent of the activity in the pH-optimum.

As mentioned earlier the dissociation of SAT is promoted not only by increase of pH but also by mere dilution of the protein solution. It seemed likely that in the reaction mixture for activity determination, containing the enzyme at very low concentration (0,2-0,3 μg/ml), SAT was completely dissociated. This conjecture was confirmed by the following experiment.

A 0.5 per cent solution of SAT was adjusted to pH 10.2 with NaOH-glycine buffer. Part of the solution was analyzed directly in the Spinco, model E ultracentrifuge (Fig.1d). Another portion of this solution was diluted seven-fold with 0,05 M NaOH-glycine buffer, pH 9.0 (this pH value is close to that of the enzyme assay mixture, vide infra), and was analyzed in the analytical

cell of an UCPA centrifuge (made in USSR; recording with the aid of a polarization interferometer as described by Tsvetkov, 1951). The sedimentation graph (Fig.1f) of the diluted enzyme solution revealed, from the very beginning of the run, the presence of only the monomeric component, sedimenting at constant velocity ($S_{20,w} = 2.4$).

It thus appears that under the specified experimental conditions even a moderate dilution of SAT solution resulted in its complete and rapid dissociation, inspite of a certain shift in pH of the solution to a less alkaline value (pH 9). In parallel with the ultracentrifugal run, activity measurements (in standard reaction mixture at pH 8.7) were performed on samples taken from the first portion of 0.5% SAT solution, kept at pH 10.2. During the first 20-30 minutes the activity was close to 100% of the starting value; a drop of 10-15 per cent was noted after 1.5-2 hrs owing to gradual denaturation of the enzyme in alkaline solution.

Summary. The molecule of succinylated aspartate-transaminase (mol.weight 117,000) dissociates into two subunits in alkaline solutions. Upon reneutralization to pH 6.5 the enzyme subunits reassociate. In the region of pH-optimum (pH 8.5-9.3) succinylated transaminase retains 65 per cent of the catalytic activity of native transaminase. The complete and rapid dissociation of succinyl-transaminase upon moderate dilution of its solutions in the range of optimal pH indicates that the succinylated enzyme is completely in the monomeric form under the conditions of activity determination.

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