Sedimentation properties of native and proteolysed preparations of ox glutamate dehydrogenase

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The concentration-dependent aggregation behaviour of purified ox liver and brain glutamate dehydrogenase preparations was compared with that of commercially-obtained preparations of the liver enzyme, which have recently been shown to have suffered proteolytic cleavage. Although there were no significant differences in these effects, the presence of 3 mM-GTP and 3 mM-NADH had markedly different effects on the two types of preparation. In this situation, at higher protein concentrations the commercially obtained preparations existed in a higher degree of aggregation than those which had not suffered proteolysis. Studies of the effects of GTP and NADH concentrations on the sedimentation coefficients at a fixed enzyme concentration suggested these effects to be largely due to differences in the affinities of the two preparations for nucleotides.

The structural and kinetic properties of ox liver glutamate dehydrogenase [L-glutamate: NAD(P)\textsuperscript+ oxidoreductase (deaminating) EC 1.4.1.3] have been studied extensively (see Sund et al., 1975; Eisenberg et al., 1976, for reviews). Polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate (Weber & Osborn, 1969), sedimentation-equilibrium studies in the presence of 6 M-guanidinium chloride (Cassman & Schachman, 1971) and amino acid sequence analysis (Moo & Smith, 1973) have shown it to have a subunit \( M_r \) of 56000. At low protein concentrations (less than 1 mg/ml) the \( M_r \) has been determined by several techniques (Reisler et al., 1970; Markau et al., 1971; Cohen et al., 1976) to be about 320000, indicating it to exist as a hexamer. A hexamer structure has also been shown by cross-linking studies (Hucho et al., 1975).

At higher protein concentrations, sedimentation-equilibrium (Cassman & Schachman, 1971), sedimentation-velocity (Markau et al., 1971) and light-scattering techniques (Markau et al., 1971; Cohen et al., 1976) have shown reversible aggregation of the hexamer to form higher aggregates. The enzymes prepared from pig and human liver have been found to polymerize in solution to similar extents to that from ox liver (Dessen & Pantaloni, 1969; Kubo et al., 1959) but that from rat liver either does not polymerize above the hexamer at all (King & Frieden, 1970) or only to a limited extent (Ifleander & Sund, 1972).

We have recently shown that commercially available preparations of ox liver glutamate dehydrogenase have suffered limited proteolysis (McCarty et al., 1980), and the studies reported in this paper were designed to see whether this had affected the aggregation properties of the enzyme. The similarity between the reported amino acid sequences of the enzyme preparations from ox and rat liver (see Smith et al., 1975) suggests that quite small differences in sequence could cause large differences in the aggregation behaviour. Thus it was decided to examine the variation of sedimentation coefficient with protein concentration using both the native and commercially available preparations of the ox liver enzyme. The effects of the nucleotides GTP and NADH, which have been reported to affect the polymerization of the enzyme (Markau et al., 1971), were also studied.

Materials and methods

Materials

Glutamate dehydrogenase was purified from fresh ox brain and liver as described by McCarthy et al. (1980). Ox liver glutamate dehydrogenase preparations were also obtained from Boehringer-
Mannheim, as a solution in 50% (v/v) glycerol, and from Sigma, as a crystalline suspension in (NH₄)₂SO₄. GTP and NADH were obtained from Boehringer, and all other chemicals were obtained from BDH.

Sedimentation analyses

These experiments were carried out using a Spinco Model E analytical ultracentrifuge and a two-cell titanium rotor (An-H). The ultracentrifuge was operated at the ambient temperature (17–21°C) and at either 42112 or 44770 rev./min. Average temperatures were known to ±0.03°C. The odometer readings were used to calculate the speed of rotation accurately. One of the two cells contained a wedge window and, in most of the work, the centre-pieces, of 12 mm path length, were double-sectored and of aluminium-filled Epon. The second sector in each cell contained the appropriate solvent (plus additives, if used) and provided an accurate baseline for each of the schlieren patterns, obtained with the phase plate set at 45°C. Photographs were taken at 8 min intervals on Ilford FP4 film strips, exposed and developed under standard conditions. In evaluating the second moments of the schlieren patterns, measurements were performed on a two-dimensional projection microscope made by Precision Gridding Ltd. and equipped with Shardlow micrometers.

All experiments were performed in 30 mM-sodium phosphate/150 mM-NaCl/0.1 mM-EDTA, pH 7.0, I 0.2. Before use, enzyme solutions were extensively dialysed against this buffer to remove the (NH₄)₂SO₄ or glycerol which had been added to the enzyme solutions for storage. Protein concentration was measured by determining the A₂₈₀ using quartz cells of 2 mm pathlength, and taking the absorption of a 1 mg/ml solution of the enzyme to be 0.97 cm⁻¹ (Olson & Anfinsen, 1952). The effect of adding 3 mM-GTP and 3 mM-NADH on the viscosity of this buffer was measured by using a capillary viscometer held in a water bath maintained at 20°C. Addition of these concentrations of the nucleotides increased the buffer viscosity by 1%; this small increase was ignored in calculating the sedimentation coefficients.

Data analysis

As shown by Goldberg (1953), the migration of the position corresponding to the square root of the second moment of the protein boundary pattern may be identified with the average sedimentation of molecules in the bulk of the solution. These values were calculated from the schlieren traces as described by Coates (1970), taking approx. 20 points for each schlieren pattern. Values for the sedimentation coefficients were obtained from linear regression analysis of the square root of the second moment versus time, and were corrected to the viscosity and density of water at 20°C. The partial specific volume of all protein samples was taken to be 0.75 ml/g as previously determined for the ox liver enzyme (Olson & Anfinsen, 1952; Eisenberg & Tomkins, 1968).

Results and discussion

Fig. 1 shows variation of sedimentation coefficient with protein concentration for the glutamate dehydrogenase preparations, in the absence of added nucleotides. All the protein samples showed an increase in sedimentation coefficient with protein concentration consistent with aggregation occurring. There was no significant difference between the association behaviour of the ox liver enzyme obtained from Boehringer-Mannheim and those from ox liver and brain purified by the method of McCarthy et al. (1980).

Fig. 2 shows the concentration-dependence of the sedimentation coefficients in the presence of 3 mM-NADH and 3 mM-GTP. In this case the commercially obtained preparations clearly polymerized to a greater extent than those purified in the laboratory, particularly at higher concentrations. Linear regression analysis showed there to be no significant difference between the sedimentation

![Graph](image-url)

Fig. 1. Dependence of the sedimentation coefficients of ox glutamate dehydrogenase preparations on the protein concentration

Details of the experimental procedures used are given in the text. The preparations used were: □, ox brain and ▽, ox liver glutamate dehydrogenase preparations obtained by the procedure of McCarthy et al. (1980); ○ and ●, ox liver glutamate dehydrogenase preparations obtained from Boehringer-Mannheim and Sigma respectively.
Fig. 2. Dependence of the sedimentation coefficients of ox glutamate dehydrogenase on the protein concentration in the presence of 3 mM-GTP and 3 mM-NADH

Details of the experimental procedures used are given in the text. The preparations used were: □, ox brain and ▼, ox liver glutamate dehydrogenase preparations obtained by the procedure of McCarthy et al. (1980); ○ and □, ox liver glutamate dehydrogenase preparations obtained from Boehringer-Mannheim and Sigma respectively.

<table>
<thead>
<tr>
<th>[GTP] (mM)</th>
<th>[NADH] (mM)</th>
<th>10^{13} \times s_{20,w} (s)</th>
</tr>
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<tbody>
<tr>
<td>0.7</td>
<td>0.7</td>
<td>14.7</td>
</tr>
<tr>
<td>3.0</td>
<td>3.0</td>
<td>14.4</td>
</tr>
<tr>
<td>7.0</td>
<td>7.0</td>
<td>13.9</td>
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Table 1. Effects of varying GTP plus NADH concentrations on the sedimentation coefficients of ox liver glutamate dehydrogenase preparations

The initial protein concentration in all determinations was 2.0 mg/ml. Sample A was prepared by the method of McCarthy et al. (1980) and sample B was obtained from Boehringer-Mannheim.

The coefficients at infinite dilution ($S_{20,w}^0$). Values of $(13.5 \pm 0.3) \times 10^{-13}$ s and $(13.6 \pm 0.3) \times 10^{-13}$ s were obtained for the laboratory purified ox liver and brain enzymes respectively; the ox liver enzyme preparation obtained from Boehringer-Mannheim gave a value of $(13.4 \pm 0.3) \times 10^{-13}$ s.

Huco et al. (1975) showed proteolysis of the N-terminal region to affect the action of allosteric nucleotides on glutamate dehydrogenase. The observation that the commercially available enzyme preparation from Boehringer-Mannheim has undergone proteolysis in this region would be consistent with the N-terminal region being involved in this process. The altered extent of depolymerization observed could be due either to the enzyme preparations differing in their affinities for the nucleotides or to the enzyme–nucleotide complexes differing in their self-association constants. This was examined by determining the sedimentation coefficients of the enzyme preparations, at a fixed protein concentration of 2 mg/ml, in the presence of varying concentrations of GTP and NADH. The results of this experiment are shown in Table 1 and show that, as the GTP and NADH concentrations increased, the $s_{20,w}$ value for the commercially obtained enzyme approached closer to that of the enzyme purified as described by McCarthy et al. (1980). This suggested that the difference in sedimentation coefficients between the two types of enzyme preparation might be due to a difference in affinity for the nucleotides used, although this experiment did not eliminate the possibility that the enzyme–nucleotide complexes formed from the two preparations differed slightly in their association constants as well.

In the presence of 3 mM-GTP and 3 mM-NADH, the beef liver enzyme obtained from Sigma was depolymerized to a smaller extent than the enzyme from Boehringer-Mannheim (see Fig. 2). Fractionation of this preparation by using GTP–Sepharose as described by Godinot et al. (1974) (see also McCarthy et al., 1980) gave rise to two peaks of eluted enzyme activity, whereas only a single peak was obtained when the enzyme from Boehringer-Mannheim was used.

This series of experiments showed that the proteolysis that had occurred in the commercial preparations of ox liver glutamate dehydrogenase had little or no effect on the concentration-dependent association in the absence of GTP and NADH, but appeared to affect the interaction of GTP and NADH with the enzyme. This suggests that further effects of this proteolysis may be found if the kinetic and allosteric properties of the preparations are re-examined.

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References


