PN 10083

Metal-ion requirement for pyridine nucleotide-induced disaggregation of glutamate dehydrogenase

There has been a great deal of interest in the relationship of the activity of glutamate dehydrogenase (L-glutamate: NAD(P) oxidoreductase (deaminating), EC 1.4.1.3.) to its state of aggregation (*e.g.* see refs. 1–6). It was reported previously that disaggregation of crystalline beef-liver glutamate dehydrogenase occurred in the presence of concentrations of DPNH above $4 \cdot 10^{-4}$ M (ref. 1). Recently, however, we have found that DPNH or TPNH alone were not sufficient to induce the disaggregation of the enzyme, but would do so if Zn^{2+} or certain other divalent cations were also present. The details of these experiments are reported below.

Nucleotides and beef-liver glutamate dehydrogenase (as a suspension of crystals in $(NH_4)_2SO_4$) were purchased from the Sigma Chemical Company. Measurements of weight average molecular weight of the enzyme were made by light scattering using the Aminco photomultiplier microphotometer (calibrated with Ludox, E. I. du Pont de Nemour and Co., Inc.)[7] on solutions of the enzyme which had been clarified by centrifugation at 5000 × *g* for 60 min. The other reagents were freed of particulate matter either by centrifugation, or by filtration through millipore filters. Sedimentation velocity experiments were performed in the Spinco Model E analytical ultracentrifuge as described previously[2].

In Fig. 1 the recriprocal of the weight average molecular weight determined by

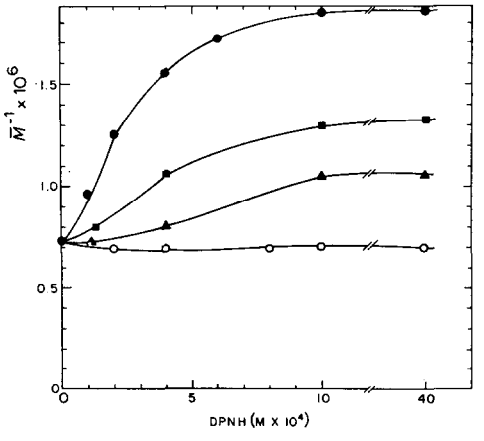

Fig. 1. Effects of DPNH and Zn^{2+} on weight average molecular weight ($\bar{M}$) of glutamate dehydrogenase. Light-scattering measurements were made at room temperature on reaction mixtures containing crystalline glutamate dehydrogenase 0.84 mg/ml, 0.1 M NaCl, 0.02 M Tris–HCl (pH 7.75), and DPNH and $ZnCl_2$ as follows: ○—○, no $ZnCl_2$; ▲—▲, $4 \cdot 10^{-6}$ M $ZnCl_2$; ■—■, $8 \cdot 10^{-6}$ M $ZnCl_2$; ●—●, $1.6 \cdot 10^{-5}$ M $ZnCl_2$.

light scattering, is plotted as a function of DPNH with and without varying concentrations of Zn^{2+}. As shown, even $4 \cdot 10^{-3}$ M DPNH had no significant effect on the molecular weight of the enzyme when Zn^{2+} was not present. However, the inclusion of molar concentrations Zn^{2+} as little as a 5-fold of that of the enzyme (assuming a molecular weight of 10^6) in the same reaction mixtures permitted DPNH to cause extensive lowering of the weight-average molecular weight. As expected,

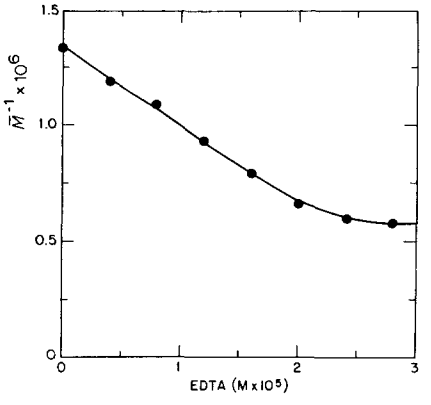

Fig. 2. Reaction mixture contained 0.76 mg/ml glutamate dehydrogenase, $4 \cdot 10^{-4}$ M DPNH, $1.6 \cdot 10^{-5}$ M $ZnCl_2$, and EDTA as shown in 0.025 M Tris–HCl (pH 7.75) and 0.1 M NaCl.

the effect of Zn^{2+} and DPNH could be completely reversed by the addition of EDTA (Fig. 2). Identical results were obtained when TPNH was substituted for DPNH. The weight average molecular weight of the enzyme was not affected by Zn^{2+} in the absence of the nucleotides except at very high concentrations (*i.e.* $> 10^{-3}$ M). CO^{2+}, Mn^{2+} and Cu^{2+} (in the presence of DPNH and TPNH) also promoted the disaggregation of the enzyme, although considerably higher concentrations of thesemetals were required than of the Zn^{2+}.

Sedimentation-velocity experiments confirmed the requirement for the metal

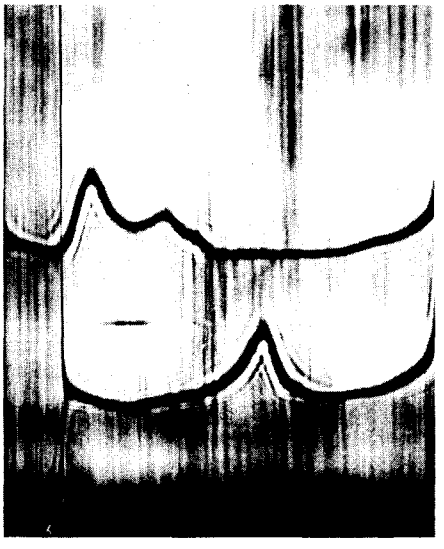

Fig. 3. Effects of DPNH and Zn^{2+} on the sedimentation velocity of glutamate dehydrogenase. Experimental mixtures contained 4 mg/ml crystalline glutamate dehydrogenase in 0.022 M Tris–HCl (pH 7.75), and $1 \cdot 10^{-3}$ M DPNH. The lower curve contained $2 \cdot 10^{-4}$ M EDTA while the upper curve contained $1 \cdot 10^{-4}$ M $ZnCl_2$. Sedimentation was from left to right at 59 780 rev./min and 25°.

ion in the DPNH induced disaggregation of the enzymes (Fig. 3). In this experiment the $s_{20,w}$ of the enzyme in the presence of $5 \cdot 10^{-4}$ M DPNH alone was 25.8 but was 12.5 when $1 \cdot 10^{-4}$ M Zn^{2+} was added.

It appears, therefore, that DPNH and TPNH can promote the reversible dissociation of glutamate dehydrogenase into subunits in the presence of Zn^{2+} or certain other metal ions. Other investigations have shown that DPNH is also involved in steroid or GTP-induced structural modifications of glutamate dehydrogenase[6,8].

A small amount of Zn^{2+} has been reported to be tightly bound to the crystalline enzyme[9]. The relationship of this intrinsic Zn^{2+} to the Zn^{2+}-dependent DPNH disaggregation is not clear at present. However, further investigations on the mechanism may establish some relationship between the two.

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