MODIFICATION BY SUCROSE OF THE CATALYTIC ACTIVITY AND PHYSICAL PROPERTIES OF GLUTAMIC DEHYDROGENASE

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Summary: Sucrose (and a variety of glycols) caused inhibition of the glutamate specific catalytic activity of glutamate dehydrogenase and stimulation of the monocarboxylic acid activity. In addition, sucrose prevented the concentration dependent aggregation of the enzyme and produced a sharp increase in stability toward heat denaturation. These studies suggest the potential usefulness of sucrose in studying protein structure transitions, but demand caution in the use of such media for separation and characterization of macromolecules unless possible effects on structure are taken into account.

Introduction

The extensive importance of solvent structure in determining protein conformations in solution has been emphasized repeatedly. Although there is not complete agreement on the exact nature of the solvent-solute relationships, the effects of solvent modifications on protein structure are of considerable interest.

Sucrose in aqueous solution is used widely in fractionating cells, in gradient density centrifugation of proteins and nucleic acids, and as a chromophore perturbant for protein spectroscopy. The present report shows that sucrose in concentrations commonly employed for such purposes produces considerable changes in the conformation and catalytic activity of the enzyme glutamate dehydrogenase (L-glutamate: NAD(P) oxidoreductase (deaminating), EC

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1.4.1.3). This enzyme, whose catalytic efficiency, relative substrate specificity, and conformation in solution are influenced by a variety of reagents, has been used often as a model for enzyme regulation, (for example see ref. 1-6). Reagent effects may be rationalized in terms of an equilibrium between different conformations, of which one exhibits optimal activity for glutamate $\rightleftharpoons$ alpha ketoglutarate interconversion and another for the analogous reductive amination of certain monocarboxylic L-amino acids (6). Thus, ADP favors the glutamate active form and inhibits reaction of monocarboxylic substrates while the converse is true for GTP, Zn++, and diethylstilbestrol (5) (6).

In previous studies the equilibrium has been followed conveniently by molecular weight measurements since the glutamate active form of the enzyme usually aggregates in solution as the protein concentration is raised. The present finding that sucrose and certain other glycols affect the structure and activity of this enzyme may assist in defining the nature of the changes in tertiary interactions involved in its regulation and in its aggregation.

Experimental and Results

Glutamate dehydrogenase from beef liver was purchased from Sigma Chemical Company as a crystalline suspension in $(NH_4)_2SO_4$, and enzyme grade sucrose was obtained from Mann Chemical Company. Light scattering experiments and enzyme assays were performed as described previously (7).

Effects on Catalytic Activity - Sucrose in the enzyme reaction mixture in concentrations as low as 0.5 M produced substantial inhibition of the reductive amination of alpha ketoglutarate and a reciprocal stimulation of the reaction with alpha ketobutyrate as substrate (Figure 1). Examination of the effect as a function of NADH and alpha keto acid concentrations showed that the change in kinetic

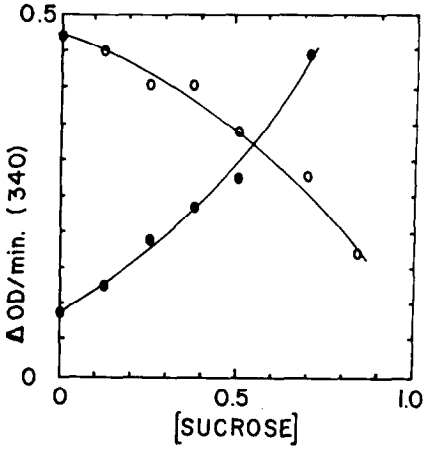

Figure 1. Effects of sucrose on the catalytic activity of glutamate dehydrogenase. All reaction mixtures contained: 0.02 M tris buffer pH 7.4, 0.1 M NH_4Cl, 1x 10^{-4} M NADH, and sucrose as shown. The open circles contained 0.002 mg/ml enzyme and 2 x 10^{-3} M alpha ketoglutarate, and the closed circles contained 0.1 mg/ml enzyme and 2 x 10^{-3} M alpha ketobutyrate. Rates for the alpha ketobutyrate reaction were multiplied x 5.

activity resulted from changes in V_{max} rather than K_m for these substrates. Thus, sucrose at large concentrations in the medium appeared to mimic in a general way the kinetic effects of GTP (8), diethylstilbestrol (9) and Zn (10), all of which cause decreased alpha KG and increased monocarboxylic acid activity. Dextran, glycerol, ethylene glycol, propylene glycol, and glucose produced effects on enzyme kinetics similar to those observed with sucrose.

Effect of Sucrose on Enzyme Structure - It is well established that the catalytic activity of glutamate dehydrogenase is sensitive to changes in enzyme conformation (1-6,11). It was of interest therefore, to examine the effects of sucrose on the physical properties of the enzyme. Figure 2 is a light scattering experiment showing that sucrose prevented the concentration dependent aggregation of the enzyme. Since ADP favors the aggregation process and antagonizes the effects of GTP, DES and Zn^{++}, it was important to determine whether ADP could promote enzyme aggregation in the presence of sucrose. In these ex-

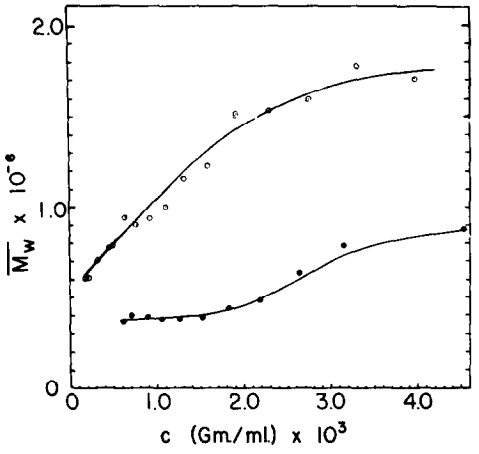

Figure 2. Effect of sucrose on concentration dependent aggregation of glutamate dehydrogenase. Experiment performed as described previously (7) in 0.1 M PO_4 buffer pH 7.4, and (closed circles) with 1.4 M sucrose.

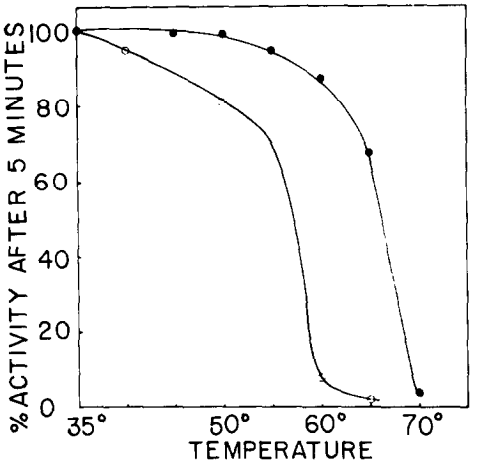

Figure 3. Effect of sucrose on heat stability of glutamate dehydrogenase. Enzyme at a concentration of 0.4 mg/ml was incubated for 5 minutes at temperature shown in 0.1 M PO_4 buffer pH 7.4 (open circles), or in 40% sucrose in the same buffer (closed circles). A 50 fold dilution was then made for enzyme assay into a reaction mixture containing 2 x 10^{-3} M alpha ketoglutarate, 0.1 M NH_4Cl, and 1 x 10^{-4} M NADH in 0.1 M PO_4 buffer pH 7.4.

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periments, 5 x 10^{-4} M caused an increase in catalytic activity but had no effect on enzyme aggregation in the presence of 1.5 M sucrose.

Previous studies have indicated that changes in enzyme con-

formation resulting in decreased alpha ketoglutarate activity and increased monocarboxylic acid activity and decreased tendency to aggregate in solution were accompanined by a decreased enzyme stability (6). Sucrose, in contrast, was found to cause a drastic increase in stability as shown in figure 3. This is also in keeping with the known ability of various glycols to stabilize proteins (12,13). This also contrasts with the effects of high salt concentration which stabilizes the enzyme but favors the glutamate active conformation.

Discussion

These experiments have shown that both the kinetic and physical properties of glutamate dehydrogenase are modified by addition of sucrose. The sucrose apparently favors a conformation of the enzyme which shows a decrease in glutamate dehydrogenase activity, a reciprocal increase in monocarboxylic amino acid dehydrogenase activity, increased thermal stability, and a loss in aggregability normally seen at elevated enzyme concentrations. The inability of the enzyme to aggregate in response to ADP, even though the nucleotide produced changes in catalytic activity showed that changes in the aggregation equilibrium while serving as useful indices of reagent induced changes in enzyme conformation, are not necessarily linked to such reagent effect. The sucrose effect may therefore assist in distinguishing between these 2 types of structure transitions. The mechanism by which sucrose modifies enzyme properties is not clear. A reasonable explanation is that sucrose changes the nature of the solvent, and modifies solvent-protein interactions. Recent studies with this enzyme have reported somewhat similar effects of D_2O (14) and DMSO.(15)

These observations with sucrose may prove useful in studying enzyme solution properties and structure transitions and in providing stable conditions for specific modification of enzyme structure. It is

also obvious from these studies that the use of sucrose gradients in protein separation and characterization, particularly of particle size, must be interpreted with caution. Even sephadex gel filtration may yield anomalous estimates of particle size since dextran was shown in these experiments to act in a manner similar to sucrose. Similarly, sucrose 'perturbation' of the spectral properties of proteins must take into account possible effects of sucrose on protein structure.

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