DIFFERENT STRUCTURAL FORMS OF REVERSIBLY DISSOCIATED GLUTAMIC DEHYDROGENASE: RELATION BETWEEN ENZYMATIC ACTIVITY AND MOLECULAR WEIGHT[1]

Carl Frieden

Department of Biological Chemistry, Washington University School of Medicine
St. Louis 10, Missouri

Received January 30, 1963

Beef liver glutamic dehydrogenase is known to undergo a freely reversible, concentration dependent, association-dissociation reaction (Olson and Anfinsen, 1952, Frieden, 1958, Kubo et al., 1959)[2]. In a variety of reports, it has been clearly demonstrated that the extent of association or dissociation may be influenced by compounds which also influence the initial velocity of the enzymatic reaction. Such compounds include the coenzymes for the enzymatic reaction (Frieden, 1959), various purine nucleotides including ADP, ATP (Frieden, 1959), GDP, GTP (Frieden, 1962b) in the presence of coenzyme, several sterols (Yielding and Tomkins, 1960) and other aromatic ring structures such as ortho phenanthroline (Frieden, 1958) and phenanthridine (Yielding and Tomkins, 1962). All of the data reported show that those compounds which cause complete dissociation of the enzyme, as measured by a decrease of about two fold in the sedimentation coefficient at relatively high protein concentration, also inhibit the enzyme activity, as measured at much lower enzyme concentrations, under initial velocity conditions. When dissociation is prevented, most notably by ADP, the

---

1. Supported by RG 8117, National Institutes of Health, U. S. Public Health Service.

2. It should be noted that the enzyme may be irreversibly dissociated into a large number of peptide chains of low molecular weight. This dissociation leads to irreversible loss of enzymatic activity and is definitely a different process (Frieden, 1962a). This paper is not concerned with irreversible dissociation nor with the individual peptide chains which arise from such dissociation.

enzymatic activity is not lost. As a consequence of these results, it was originally proposed that the enzymatically active enzyme was associated and that enzyme which was dissociated was inactive (Frieden, 1959).

Since changes in the molecular weight of the enzyme due to the reversible association-dissociation reaction have been observed to occur mostly in the range of 0.1-3 mg/ml, the question of whether or not the enzyme could be associated under usual conditions of enzymatic assay (< 0.01 mg/ml) may be raised. Recently, Fisher et al. (1962) have shown both the associated and dissociated forms of the enzyme to be equally active by measuring the enzymatic activity and the molecular weight of the enzyme at high enzyme concentrations in the presence of a large amount of inhibitor.

The purpose of the present paper is two fold: First, to show that the enzyme remains dissociated under conditions of the enzymatic assay and therefore that the enzyme subunit is catalytically active and, secondly, to show that compounds which cause dissociation at high enzyme concentrations do so as a result of an alteration in the configuration of the enzyme subunit. This structurally altered subunit is essentially enzymatically inactive and does not undergo association at any enzyme concentration.

In Fig. 1 are shown plots of the reciprocal weight average molecular weight, $\overline{M}_w$, determined by light scattering, as a function of enzyme concentration. Comparison of the molecular weight dependence in the presence and absence of TPNH (lower two curves) clearly shows that TPNH does not cause complete association of the enzyme at all enzyme concentrations. Thus, the enzyme does not associate at very low enzyme concentrations, but TPNH does alter the equilibrium constant of the association-dissociation reaction such that the subunits may associate more readily as the enzyme concentration is raised. Similar results are obtained for TPN, DPN and low DPNH levels. Since α-ketoglutarate, glutamate or NH_4^+ ions do not measurably affect $\overline{M}_w$, the conclusion must be that catalytically active enzyme is dissociated at low enzyme concentrations.

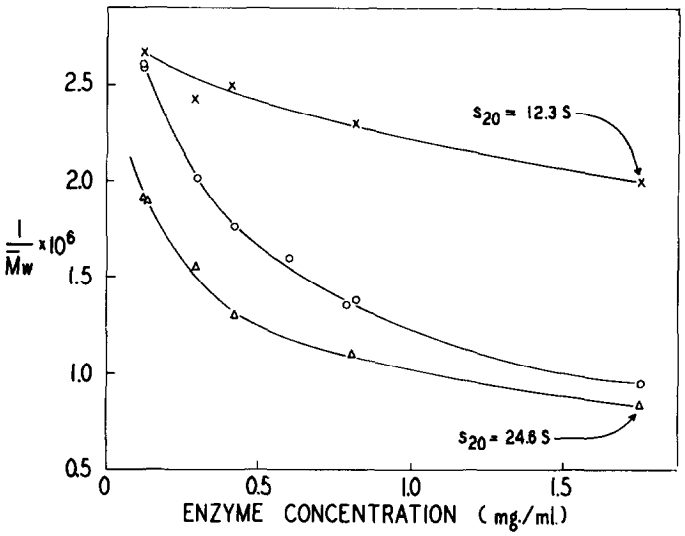

Fig. 1. Reciprocal weight average molecular weight plotted as a function of enzyme concentration. The experiments were performed in 0.1 M Tris-acetate buffer, pH 8, 10^{-4} ethylene diaminetetraacetate, 18°. In all experiments, solutions were made 0.001 M in phosphate to prevent any inactivation. Circles represent the experiments performed in the absence of coenzyme and nucleotide. Lowest curve (triangles), $\bar{M}_w$ at 30-40 x 10^{-6} M TPNH. The uppermost curve (crosses) represents experiments in the presence of both TPNH and guanosine triphosphate (GTP) both approximately 30-40 x 10^{-6} M. The sedimentation coefficients shown are those determined at the highest enzyme concentration (1.75 mg/ml) using the same enzyme solution as used in the light scattering experiment.

It has been previously noted that guanosine triphosphate (GTP), a potent inhibitor of the enzymatic reaction ($K_i < 10^{-6}$ M), will, in the presence of TPNH, cause dissociation of the enzyme as measured in the ultracentrifuge (Frieden, 1962b). In Fig 1 it is shown that association of the enzyme is prevented by the relatively low levels of GTP and TPNH used in this experiment (30-40 x 10^{-6} M). At the highest enzyme concentration, 1.75 mg/ml, sedimentation coefficients were also measured. The value of 12.3 S obtained in the presence of both GTP and TPNH is close to the minimum value of the sedimentation coefficient which may be obtained for reversibly dissociated enzyme (Frieden, 1958, 1959). The value of 24 S obtained in the presence of TPNH with no GTP is somewhat less than the maximum value of 26-27 S observed for fully associated enzyme (Olson and Anfinsen, 1952). GTP itself at this concentration has very little effect on the extent of association.

In another effort to determine changes in molecular weight as a function of enzyme concentration, experiments at several enzyme levels were performed in the partition cell of the ultracentrifuge (Svedberg and Pederson, 1940). The enzyme activity was measured before and after the experiment and is directly proportional to enzyme concentration. Control experiments, run under the same conditions, but not in the ultracentrifuge, indicated that there was almost no loss of activity during the run. Results of typical experiments are shown in Table I.

Table I. Sedimentation Coefficients Determined in Partition Cell

All experiments in 0.1 M phosphate, pH 7.2 - 7.3

Enzyme Conc. mg/ml	0.05		0.1		0.44		1.1	
Coenzyme	None	20 μM TPNH	None	160 μM TPNH	None	650 μM DPN	None	160 μM TPNH
s_{20} (Svedberg units)	12.5	13.8	13.2	14.7	18.1	19.1	17.5*	18.5*

*Sedimentation coefficients calculated measuring concentration by activity or by movement of peaks agreed within 10-15%.

In agreement with the data of Fig. 1, coenzymes increase the ability of the subunits to associate as measured by the increase in the value of the sedimentation coefficient, but do not cause complete association ($s_{20} = 26$) at any enzyme level up to 1.1 mg/ml.

Discussion: The previous observations that coenzymes cause association of subunits at high enzyme concentrations (> 2 mg/ml) simply reflect the ability of the coenzyme to cause the subunits to associate more readily. It may be estimated from Fig. 1 that the equilibrium constant for the reaction may be changed by perhaps 2 - 4 fold in the presence of TPNH. Whether such

a change in the equilibrium constant reflects a configurational change or some change in the forces between subunits due to bound coenzyme is not clear. Although factors which affect the association-dissociation reaction in the absence of coenzyme have been studied, it is still not certain what forces are primarily involved in the association of subunits (Frieden, 1962a).

Association of the enzyme is prevented in the presence of both TPNH and GTP. Simple charge repulsion due to purine nucleotide binding may be ruled out since association is not prevented in the presence of TPNH and ATP (Frieden, 1959). Thus, a configurational change in the subunit due to GTP binding appears to be responsible for the inability of the subunit to undergo association. Under the same conditions, the enzyme loses almost all of its enzymatic activity. These two processes, alteration of enzyme configuration and loss of enzymatic activity, are directly related to one another. Absolute correlation of the two processes will be described elsewhere. It should be pointed out, however, that experiments utilizing quenching of protein fluoresence by TPNH show that TPNH is even more tightly bound to the enzyme in the presence of GTP than in its absence. Where GTP is displaced by ADP, enzymatic activity is restored and association of the subunits may again occur, showing that the configurational change is reversible.

Although association per se is not required for enzymatic activity, the relationship between the ability to dissociate the enzyme as measured at high enzyme levels, and the inhibition of enzymatic activity is still valid. As pointed out above, this correlation holds for many other compounds which have been tested. Breakdown of the correlation would be expected when measuring the enzymatic activity at very high enzyme levels (>0.1 mg/ml) or if the compounds used did not completely prevent association of the subunits but only changed the equilibrium constant of the association-dissociation reaction to a small extent.

In the cell, the amount of glutamic dehydrogenase which is present in the enzymatically active form or in the structurally altered, enzymatically inactive, form may very well depend upon the levels of the various purine nucleotides present. It seems likely that the ability to alter the enzymatic activity in this way may be important in certain metabolic control systems *in vivo*.

Further work on the question of why the altered subunit, which does not undergo association, is essentially enzymatically inactive is in progress.

REFERENCES

Fisher, H. F., Cross, D. G., and McGregor, L. L. Nature 196, 895 (1962).
Frieden, C., Biochim. et Biophys. Acta, 27 431 (1958).
Frieden, C., J. Biol. Chem., 234 809, 815 (1959).
Frieden, C., J. Biol. Chem., 237 2396 (1962a).
Frieden, C., Biochim. et Biophys. Acta, 59 484 (1962b).
Kubo, H., Iwatsubo, M., Watasi, H. and Soyama, T., J. Biochem., Tokyo, 46 1171 (1959).
Olson, J. A. and Anfinsen, C. B., J. Biol. Chem., 197 67 (1952).
Svedberg, T. and Pederson, K. O., (1940) The Ultracentrifuge, Oxford Univ. Press, London and New York.
Yielding, K. L. and Tomkins, G., Proc. Natl. Acad. Sci. U. S. 46, 1483 (1960)
Yielding, K. L. and Tomkins, G., Biochim. et Biophys. Acta, 62 327 (1962).