Reversible Alteration of the Structure of Enzymes in Acidic Solution[+]

by

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Received November 21, 1962

It has been recently demonstrated (Deal and Van Holde, 1962; Stellwagen and Schachman, 1962a, 1962b; and Deal, et al., 1962) that rabbit muscle aldolase dissociates in acidic solution into three unfolded polypeptide subunits, which upon neutralization reassociate and refold to form a product with the gross structure and catalytic and immunological activity of the native protein.

Since these results suggested that the native configuration of even so complex a molecule could be specified by its amino acid sequence, it was of interest to examine the generality of this phenomenon. Accordingly, a preliminary investigation was undertaken with a number of enzymes of moderate to high molecular weight. Several of these had previously been reported to be inactivated in acid solution.

Experimental procedure. Crystalline pig heart fumarase was used. Crystalline yeast enolase was kindly supplied by Dr. F. Wold, and crystalline

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+ Supported by a grant from the National Institutes of Health; taken in part from the thesis submitted by W. C. Deal to the University of Illinois in 1962, in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Chemistry.

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suspensions of the rabbit muscle enzymes, lactic dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase and α-glycerophosphate dehydrogenase were Sigma Chemical Co. products. Crystalline aldolase was prepared from rabbit muscle by the method of Taylor et al. (1948).

The enzymes were dialyzed overnight against a pH 6.9 buffer containing 0.01 M citrate (Na), 0.05 M NaCl, 0.001 M EDTA, and 0.001 M mercaptoethanol. The protein concentrations were determined by absorption at 280 mμ. The enzyme solutions were diluted with buffer to a protein concentration in the neighborhood of 0.17%. This was followed by acidification, with 0.5 M or 1.0 M citric acid, to a final pH of 2.6 - 2.9 and a final enzyme concentration of about 0.15%. Sedimentation velocity experiments using a Kel-F centerpiece were made at 3.0° C with a speed setting of 52,640 RPM. Sedimentation equilibrium experiments utilized a double sector cell with a filled-epon centerpiece. Standard assays were employed (Colowick and Kaplan, 1955). In all cases, the acidified enzyme solutions were allowed to stand for at least one hour before neutralization, which was carried out by direct addition of base or by dilution into neutral pH assay medium.

Results and Discussion. The enzyme solutions were all clear upon acidification and remained so upon standing in the cold for several weeks. Results of sedimentation experiments and assays of catalytic activity are shown in Table 1 together with previous results on aldolase for comparison. A pronounced change in sedimentation coefficient suggesting structural modification was observed for every enzyme except glyceraldehyde-3-phosphate dehydrogenase. The stability of this enzyme seems especially remarkable in view of the fact that it possesses at least two polypeptide chains and may have no disulfide bonds (Czok and Bücher, 1960). In at least two cases (aldolase and lactic dehydrogenase) the molecular weight data indicate dissociation into

Table 1

Enzyme	Native		In Acidic Solution[1]		Recovery of Activity on Neutralization
	$S_{20} \times 10^{13}$	$M \times 10^{-3}$	$S_{20} \times 10^{-3}$	$M \times 10^{-3}$	
					%
Aldolase	7.7[2]	149[3]	1.93[2]	M_w=51[2] M_z=52[2]	70-90[4]
Enolase	5.8[5]	67[5]	2.30	M_w=120[6] M_z=121[6] M_z=127[7]	80
α-glycerophosphate dehydrogenase	4.9[8]	78 - 87[8]	3.00 and 4.30	M_z=93	50
Fumarase	9.1[9]	220[9]	2.60	----[10]	20
Glyceraldehyde-3-phosphate dehydrogenase	7.7[11]	140[11]	7.70[12]	----	0
Lactic dehydrogenase	6.9[8]	135[13]	1.78	M_w=70	0

1 pH = 2.6 - 2.9

2 Deal, et al. (1962)

3 Taylor and Lowry (1956)

4 The maximum value of 90% has been reported by Butler et al., (1962)

5 Malmstrom (1961)

6 At 3 mg protein/ml

7 At 1.5 mg protein/ml

8 Quoted by Czok and Bücher (1960)

9 Quoted by Alberty (1961)

10 Not homogeneous

11 Fox and Dandliker (1956)

12 Peak not sharp. Same average S after several days

13 The molecular weight of beef heart LDH (S_{20}=7.05) has been reported as 135,000. (Quoted by Czok and Bucher 1960). Also the molecular weight of rat liver LDH has been given as 126,000 (Davisson et al., 1953)

unfolded subunits. In this context it is of interest that Appella and Markert (1961) have reported that beef heart lactic dehydrogenase dissociates into sub-

units of molecular weight 35,000 in guanidine hydrochloride, and that Millar (1962) has stated that this enzyme undergoes a concentration dependent dissociation involving a monomer of molecular weight 72,000.

The appearance of two peaks in the sedimentation pattern of α-glycerophosphate dehydrogenase probably results from a structural modification rather than dissociation since no decrease in average molecular weight is observed. The molecular weight and sedimentation coefficient of enolase in acidic solution suggests a very highly unfolded dimer.

Although the structure of the enzymes was extensively altered under acidic conditions, the catalytic activity of several enzymes was partially recovered upon neutralization. As shown in Table 1, aldolase, enolase, glycerophosphate dehydrogenase and fumarase recovered significant activity. In all instances, the activity was regained in a characteristic time dependent fashion (similar to that reported for aldolase, Deal et al., 1962) with a half time of a few minutes. That the activity observed was not the result of the persistance of some unmodified enzyme through the acidification and neutralization was demonstrated in the following manner: The addition of urea to a concentration of 2.3 M in the assay medium had little effect upon the activity of the native enzyme, but prevented completely the recovery of activity of acidified samples. If unchanged molecules had remained, activity should have been elicited immediately.

The two hydrogenases which showed no recovery of activity are deserving of further study. In the case of glyceraldehyde-3-phosphate dehydrogenase, irreversible loss of activity occurred even though no change in the average sedimentation coefficient was observed. Possible explanations for such irreversible changes are apparent: First, a cofactor may dissociate, and not recombine effectively with the neutralized enzyme; for example, recovery of

activity from a metallo protein might be minimal under the conditions employed. Glutathione has been reported to be involved in the active site of glyceraldehyde-3-phosphate dehydrogenase (Racker and Krimsky, 1952). The addition of glutathione in the present experiments, however, did not effect the loss of activity of the enzymes in acid solutions. It is possible that some of the effects observed with glycerophosphate dehydrogenase may be a result of dissociation of thiamic acid (Van Eys et al., 1959). Second, there may be a direct effect of hydrogen ion on the structure of the molecule, either by hydrolysis of particularly sensitive bonds, or by catalysis of the formation of a new structure.

In conclusion, the results suggest that for a number of these enzymes the native configuration is so thermodynamically favored that it can be spontaneously regained by a sizeable proportion of the molecules which have been unfolded and/or dissociated. While the formation of catalytically ineffective forms is competitive under the conditions employed here, it seems likely that this may be further decreased.

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