Reversible Dissociation of Yeast Glyceraldehyde 3-Phosphate Dehydrogenase by Adenosine Triphosphate*

George M. Stancel† and W. C. Deal, Jr.

ABSTRACT: Tetrameric yeast glyceraldehyde 3-phosphate dehydrogenase undergoes a time-dependent inactivation in the presence of adenosine 5′-triphosphate as a result of dissociation into monomeric subunits. Optimal conditions, which yield complete inactivation in 5 hr are: (1) 1–2 mM adenosine 5′-triphosphate, (2) 0°, (3) protein concentrations of 0.03–0.1 mg/ml, (4) pH 9.0, and (5) 0.1 M β-mercaptoethanol. Transition points (half-maximal loss of activity in 5 hr) are: (1) 0.5 mM adenosine 5′-triphosphate, (2) 12°, (3) 0.5 mg/ml, and (4) pH 8.6. Adenosine 5′-monophosphate and adenosine 3′,5′-monophosphate do not dissociate the enzyme. They, and all the substrates of the reaction, partially protect the enzyme from dissociation. Dissociation and inactivation are completely reversed by warming to 17°. Reassembly is greatly stimulated by adenosine 5′-triphosphate and by 10% sucrose. Optimal reassembly conditions are: (1) 0.04 mg/ml of protein, (2) pH 7.0, (3) 1–2 mM adenosine 5′-triphosphate, (4) 17°, (5) 10% sucrose, and (6) 0.1 M β-mercaptoethanol. Inactivation and dissociation apparently result from electrostatic repulsion. The results are discussed in terms of a possible role for this enzyme in the regulation of glycolysis. Since this dissociation produces fairly compact subunits, association of folded monomers to tetramers may be studied independently of the polypeptide folding.

Numerous examples of the effects of adenine nucleotides on the structure and activity of various enzymes of carbohydrate metabolism have previously been reported (Atkinson, 1965, 1966; Wood, 1966; Stadtman, 1966; Scrutton and Utter, 1968). Recent investigations in this laboratory have demonstrated that ATP and other adenine-containing compounds have pronounced effects upon the structure and catalytic activity of yeast glyceraldehyde 3-phosphate dehydrogenase (GADP).[1] Glyceraldehyde 3-phosphate dehydrogenase is *inactivated* by ATP as a result of dissociation of the native tetrameric enzyme into monomeric subunits (Stancel and Deal, 1968; Stancel, 1969). ATP and other adenine nucleotides also produce an instantaneous *inhibition* of the catalytic activity of yeast glyceraldehyde 3-phosphate dehydrogenase (Yang and Deal, 1969a), which is measured before any appreciable dissociation occurs.

This paper reports the reversal of the dissociation of yeast glyceraldehyde 3-phosphate dehydrogenase by ATP (Stancel and Deal, 1968) and a detailed characterization of the dissociation and reassembly processes (Stancel, 1969). Other papers (Constantinides and Deal, 1968, 1969) from this laboratory show that rabbit muscle glyceraldehyde 3-phosphate dehydrogenase is also reversibly dissociated into subunits by ATP and that the characteristics of that system are greatly different from the yeast system.

There were several strong incentives for characterizing the dissociation of yeast glyceraldehyde 3-phosphate dehydrogenase by ATP. First, the process might be involved in metabolic control; other work in this laboratory has shown that incubation of glyceraldehyde 3-phosphate dehydrogenase with ATP greatly increases the susceptibility of the enzyme to digestion and inactivation by chymotrypsin (Yang and Deal, 1969b). Also, Williamson (1965, 1967) has shown that in rat liver and heart, the glyceraldehyde 3-phosphate dehydrogenase reaction is far from equilibrium and pointed out that it is a potential control point for glycolysis. If, as expected, the reaction is also not in equilibrium in yeast and rabbit muscle, then a remarkable situation exists, because glyceraldehyde 3-phosphate dehydrogenase constitutes 20% of the total soluble protein in yeast (Krebs *et al.*, 1953), and about 10% in rabbit muscle (Cori *et al.*, 1948).

In contrast to most dissociating agents, which extensively unfold the polypeptide chains of yeast glyceraldehyde 3-phosphate dehydrogenase, ATP produces a fairly compact subunit. This system seems ideal to investigate several important biochemical questions: (1) Can individual subunits exhibit catalytic activity? (2) Do subunits associate after synthesis or during synthesis? (3) Do metabolites affect the association of subunits?

Results

Except for the particular variable under study, all experiments in this section used the following procedures and conditions. The enzyme (0.2 mg/ml) was inactivated and dissociated at 0° for 5 hr in solutions containing 0.1 M Tris-HCl (pH 8.5), 0.1 M β-mercaptoethanol, and 4 mM ATP. Control enzyme samples were treated identically, except ATP was omitted.

* From the Department of Biochemistry, Michigan State University, East Lansing, Michigan 48823. *Received June 9, 1969.* This is paper X in a series entitled Metabolic Control and Structure of Glycolytic Enzymes. Paper IX is the work of Constantinides and Deal (1969). A report of this work has been presented earlier (Stancel, 1969). Supported in part by grants from the National Institute of General Medical Sciences, U. S. Public Health Service (GM-11170), and the Michigan Agricultural Experiment Station (Hatch 932; Publication No. 4731).

† National Institutes of Health predoctoral trainee (Training Grant 2 T-1 GM 1091-06).

[1] The abbreviations used are: GAP, D-glyceraldehyde 3-phosphate; GAPD, D-glyceraldehyde 3-phosphate dehydrogenase:NAD oxidoreductase (phosphorylating) (EC 1.2.1.12).

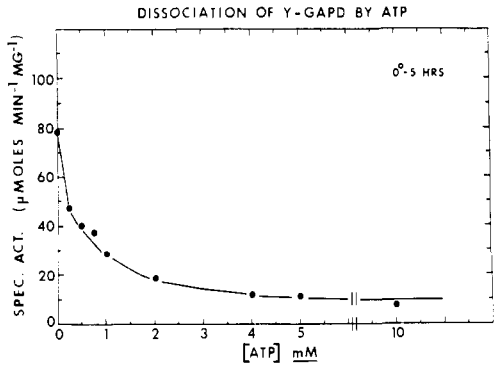

FIGURE 1: Activity of glyceraldehyde 3-phosphate dehydrogenase (0.2 mg/ml) after incubation for 5 hr at 0° with ATP in 0.1 M Tris (pH 8.5) and 0.1 M β-mercaptoethanol. An ammonium sulfate suspension of the enzyme was centrifuged, the pellet was taken up in 0.1 M Tris (pH 8.5) and 0.1 M β-mercaptoethanol, and the enzyme was diluted into a series of samples with varying amounts of ATP.

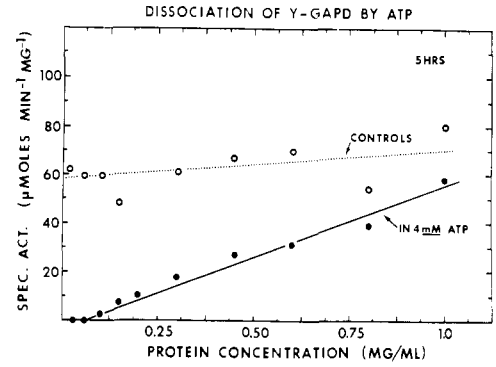

FIGURE 3: Effect of protein concentration on inactivation of glyceraldehyde 3-phosphate dehydrogenase by 4 mM ATP. See text and legend of Figure 1.

Effect of ATP Concentration on Inactivation and Dissociation of Yeast Glyceraldehyde 3-Phosphate Dehydrogenase. Yeast glyceraldehyde 3-phosphate dehydrogenase showed a time-dependent loss of activity upon incubation with ATP. As seen in Figure 1, at ATP concentrations of 2 mM or higher, about 75% of the activity was lost after 5 hr. At higher concentrations the curve leveled off. Half-maximal loss of activity occurred at an ATP concentration of 0.4–0.5 mM, a value which may be taken as the approximate dissociation constant for the enzyme–ATP complex. The residual ATP added into the assay with the enzyme was too low to inhibit the enzyme (Yang and Deal, 1969a); therefore, the activity loss was due to structural changes which were not immediately reversible.

The inactivation by ATP was accompanied by dissociation of the enzyme into a 2.8S species, while the enzyme without ATP sedimented as the tetrameric 7.6S species. This is shown by the sucrose density gradient pattern given in Figure 2. It should be noted that since the 2.8S species was enzymatically inactive, a reassembly process (see later section) was used to detect the otherwise inactive enzyme in the gradient; the fractions collected after centrifugation were warmed to 23°.

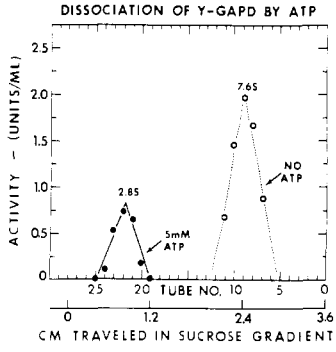

FIGURE 2: Sucrose density gradient centrifugation of glyceraldehyde 3-phosphate dehydrogenase (0.25 mg/ml) after 9-hr incubation at 0° with or without 5 mM ATP in the standard dissociation medium (Figure 1). The samples were then centrifuged for 22 hr at 0° in the sucrose gradients with the same composition. The fractions were collected at 0°, warmed to 23° for 2 hr, and assayed. Beef heart lactic dehydrogenase was used as a marker. See text for further details.

Effect of Protein Concentration. Dissociating–associating systems are expected to exhibit a strong dependence upon protein concentration. The results shown in Figure 3 indicate that the inactivation of yeast glyceraldehyde 3-phosphate dehydrogenase by ATP increased as the protein concentration was lowered. Inactivation was complete at protein concentrations lower than 0.1 mg/ml, and inversely related to protein concentration in the concentration range 0.1–1.0 mg/ml. In contrast, the control enzyme with no ATP was inactivated only slightly at lower protein concentrations. These data provided further evidence that the inactivation involved dissociation into subunits.

Effect of Temperature. The dissociation showed a marked dependence upon temperature as illustrated in Figure 4; the specific activity of the control samples remained essentially constant over the temperature range studied. Although no activity was lost at 23°, a conformational change occurred as indicated by a difference spectrum.[2]

Effect of pH. Enzyme samples were incubated for 5 hr at various pH values in 0.1 M Tris buffers. As shown in Figure 5, inactivation by ATP was favored at high pH; the control samples exhibited constant activity over the pH range examined.

The pH-dependent transition is centered at about pH 8.6 (Figure 5). The pH dependence was not a result of titration of the Tris buffer, since samples in Tris buffers of fixed pH (8.5), but varying concentrations (0.02–0.2 M), gave similar results, as did other buffers.

Specificity of the Dissociation Process for ATP. Several competitive inhibitors (Yang and Deal, 1969a), including ATP, ADP, AMP, and 3′,5′-AMP, were examined for dissociating ability. Samples of glyceraldehyde 3-phosphate dehydrogenase (0.2 mg/ml) were incubated at 0° for 5 hr in 0.1 M Tris buffer (pH 8.1) and 0.1 M β-mercaptoethanol with 1 mM concentrations of either ATP, ADP, AMP, or 3′,5′-AMP. The control was treated similarly but no nucleotide was added. As shown in Figure 6, AMP and 3′,5′-AMP had no appreciable effect. The ADP and ATP samples lost 20 and 60%, respectively, of the control activity. These results indicated that the additional

[2] G. M. Stancel, and W. C. Deal, Jr., unpublished results.

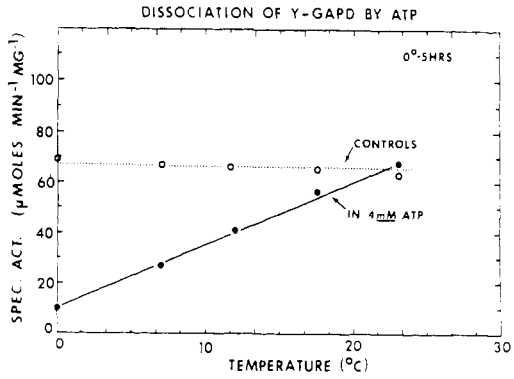

FIGURE 4: Effect of temperature of incubation on inactivation of glyceraldehyde 3-phosphate dehydrogenase by 4 mM ATP. See text and legend of Figure 1.

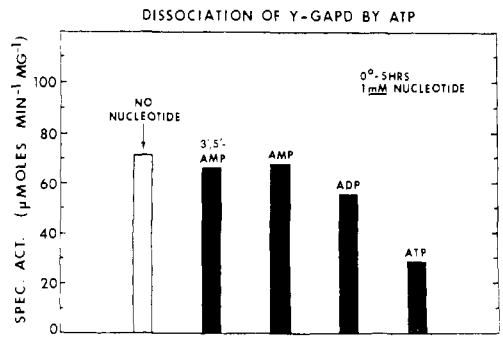

FIGURE 6: Activity of glyceraldehyde 3-phosphate dehydrogenase (0.2 mg/ml) after incubation at 0° for 5 hr with 1 mM adenine nucleotides in 0.1 M Tris (pH 8.1) and 0.1 M β-mercaptoethanol.

phosphoryl groups on the ATP molecule were responsible for the dissociation.

Protective Effects of 3',5'-AMP, AMP, ADP, and Substrates. In light of the findings in the preceding section and the discovery that ATP and other adenine nucleotides are competitive inhibitors with respect to NAD (Yang and Deal, 1969a), it was of interest to determine whether the substrates and above compounds protected glyceraldehyde 3-phosphate dehydrogenase from dissociation by ATP. As in the previous experiment, samples of glyceraldehyde 3-phosphate dehydrogenase (0.2 mg/ml) were incubated with or without added nucleotides. One control contained *no* ATP; a second control contained *only* 1 mM ATP. The test samples all contained 1 mM ATP *plus* 1 mM additional nucleotide (or substrate), except for the 10 mM phosphate sample. All these compounds protected the enzyme from inactivation (Figure 7). The order of effectiveness was 3',5'-AMP > NAD > AMP > P_i > ADP > glyceraldehyde-3-phosphate.

Reversal of the Inactivation and Dissociation of Yeast Glyceraldehyde 3-Phosphate Dehydrogenase. The temperature dependence of the dissociation process (Figure 4) suggested a temperature-dependent equilibrium existed and that activity might be regained by warming the dissociated enzyme. The initial reassembly studies yielded only about 30% of the control value upon warming the samples to 23°. With 10% sucrose, there was essentially complete recovery. The sucrose effect is nonspecific, since maltose, fructose, or glucose also increased recovery of activity. Studies with different concentrations of sucrose indicated the maximal effect was achieved with about 7.5–10% sucrose. A sucrose concentration of 10% was used to provide a safe margin of error. Other experiments indicated that the reassembled enzyme had the same K_m, sedimentation coefficient, and electrophoretic mobility on polyacrylamide disc electrophoresis as the native enzyme.

This surprising observation that the dissociation was completely reversed, even in the presence of the dissociating agent, raised the question of what role, if any, ATP played in the reassembly process.

To answer this question a sample of glyceraldehyde 3-phosphate dehydrogenase (0.25 mg/ml) was completely dissociated by a 9-hr incubation at 0° in 5 mM ATP, and three aliquots were withdrawn. One aliquot was layered on a sucrose gradient without ATP. If ATP were *not* required for reassembly, the collected fractions from this aliquot should regain enzymatic activity if warmed to 23° before being assayed. This was *not* observed; only a trace of activity was recoverable in the monomer position, as indicated by the lower solid curve in Figure 8.

A second aliquot of the original dissociated enzyme was layered on a gradient that contained 5 mM ATP. When the fractions collected from this sample were warmed and assayed, substantial activity was recovered (Figure 8, upper solid curve).

The third aliquot was layered on a gradient without ATP,

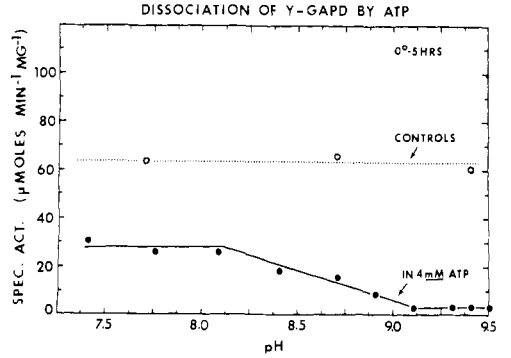

FIGURE 5: Effect of pH of Tris buffer on inactivation of glyceraldehyde 3-phosphate dehydrogenase by 4 mM ATP. See text and legend of Figure 1.

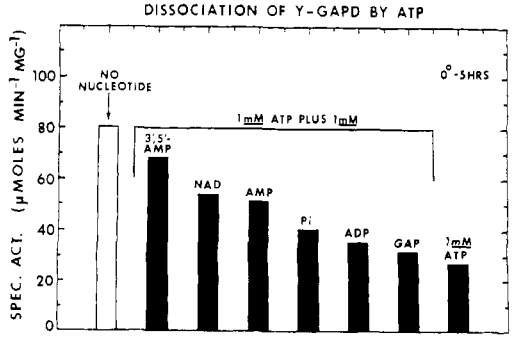

FIGURE 7: Protective effects of adenine nucleotides and substrates against inactivation of glyceraldehyde 3-phosphate dehydrogenase by ATP. See text and legend of Figure 5.

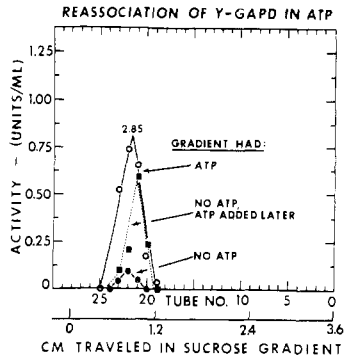

FIGURE 8: Effect of ATP on reactivation of inactive 2.8S monomers. The samples were dissociated at 0° for 9 hr in the standard dissociation medium (Figure 1) but with 5 mM ATP. They were centrifuged for 22 hr at 0° in sucrose density gradients with the same composition, except ATP was omitted where indicated. The fractions were collected at 0° and then incubated for 2 hr at 23°. See text for further details.

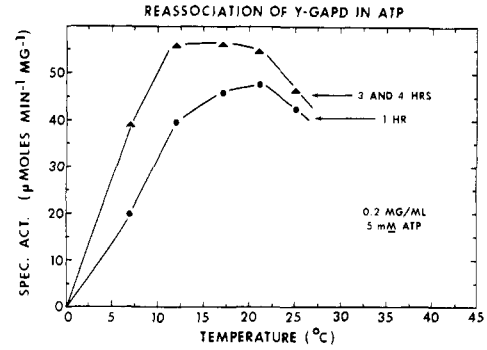

FIGURE 10: Effect of temperature on reassociation of glyceraldehyde 3-phosphate dehydrogenase subunits. Only one curve is drawn for the 3- and 4-hr incubations, since they were identical. See legend of Figure 9.

but concentrated ATP was added to the collected fractions (to a final concentration of 5 mM) before they were warmed to 23°. The dashed line (Figure 8) shows that ATP greatly increased activity recovery over that in its absence.

Thus ATP did not inhibit the reassembly and both ATP and 10% sucrose had to be present in the reassembly solution to achieve maximal recovery of activity.

For all the following reassembly studies, the standard dissociation procedure was changed to a 12–14-hr incubation at 0° in 10 mM ATP, 0.02 M Tris (pH 8.5), and 0.1 M β-mercaptoethanol with 0.4 mg/ml of protein. The dissociated enzyme samples were then diluted into a reassembly mixture at 0°. The higher concentrations of ATP and protein in the dissociation mixture compensated for the dilution, so that the final concentrations under which reassembly occurred were the same as those for the previously described dissociation experiments. The standard final reassembly conditions were: 0.2 mg/ml of glyceraldehyde 3-phosphate dehydrogenase, 0.1 M β-mercaptoethanol, 5 mM ATP, 10% sucrose, and 0.2 M Tris (pH 7.0).

The activity recovery in the following experiments was only 80–85% of that previously observed (Figure 4), presumably owing to the longer incubation times (12–14 hr), since the subunits appeared to undergo a slow, irreversible side reaction (half-time approximately 24 hr) at 0°. The longer incubation times were required to obtain complete dissociation at the higher protein concentrations used.

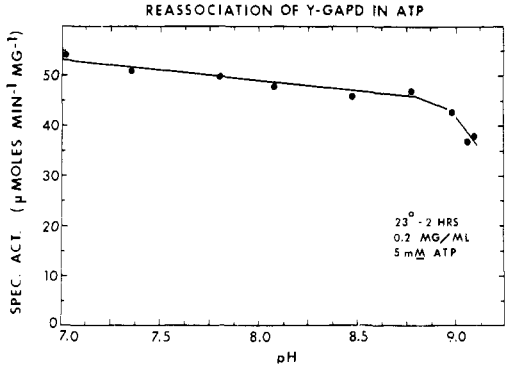

FIGURE 9: Effect of pH (0.2 M Tris buffers) on reassociation of glyceraldehyde 3-phosphate dehydrogenase subunits (0.2 mg/ml) by warming to 23° for 2 hr. The samples also contained 5 mM ATP, 0.1 M β-mercaptoethanol, and 10% sucrose, as described in Methods.

Effect of pH on Reassembly. After incubation at 0°, aliquots of the inactive monomers were diluted 1:1 into a series of reversal mixtures at 0° containing 0.4 M Tris buffers of varying pH. The samples were warmed at 23° for 2 hr and assayed. As shown in Figure 9, there was a slight dependence of the reassembly upon pH in the range of 7.0–8.5 and at higher pH values the activity recovery decreased even more. Other experiments showed that activity recovery was less in imidazole than in Tris buffers at given values of pH.

Effect of Temperature on Reassembly. Inactive monomers were diluted into the reassembly mixture at 0° and warmed to various temperatures for 1 hr. There was a sharp dependence of activity recovery upon temperature (Figure 10). These samples were also assayed after 3 and 4 hr at the indicated temperatures and equilibrium appeared to be established within 2–3 hr in the temperature range studied. Presumably tetramers, dimers, and monomers are all present in this equilibrium mixture in this temperature range. Since incubation at 17° appeared to yield maximum reassembly under these conditions (Figure 10) it was used as the optimal reassembly temperature. This is very near the optimal temperature for reassembly of the urea-dissociated subunits (Deal, 1969).

Effect of Protein Concentration on Reassembly. Aliquots were removed from a stock solution of inactive monomers (0.4 mg/ml) and diluted with various amounts of dissociation medium at 0° before the 1:1 dilution into the reversal mixture. This kept all parameters except protein concentration constant. After the dilution into the reassembly mixture, the samples were placed at 17° and assayed after 1 hr and 2.5 hr. The results indicated a requirement for a minimum protein concentration, 0.04 mg/ml, for maximal reassembly (Figure 11). The same value was obtained for the reassembly of yeast glyceraldehyde 3-phosphate dehydrogenase dissociated by 8 M urea (Deal, 1969). This probably reflects the concentration dependence of a dissociation–reassociation equilibrium.

Effect of ATP Concentration on the Reassembly. For this experiment, the ATP concentration in the dissociation medium was lowered, since we wished to study reassembly at much

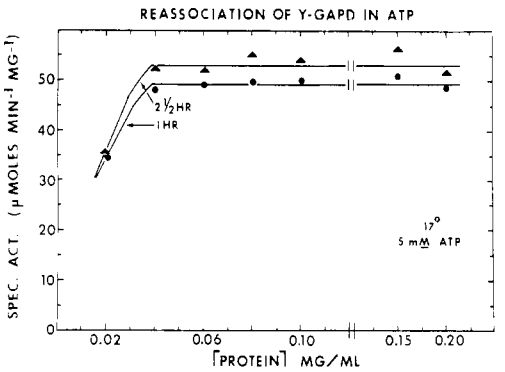

FIGURE 11: Effect of protein concentration on reassociation of glyceraldehyde 3-phosphate dehydrogenase subunits. See legend of Figure 9 and Methods.

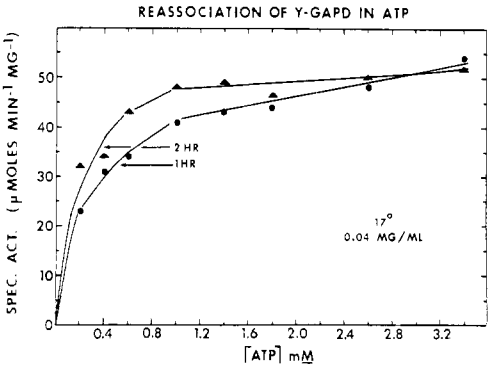

FIGURE 12: Effect of ATP concentration on reassociation of glyceraldehyde 3-phosphate dehydrogenase subunits. See legend of Figure 9 and Methods.

lower ATP concentrations in order to obtain larger differences in reassembly. Enzyme samples were incubated at 0° with 2 mM ATP for 14 hr. Aliquots were withdrawn and first diluted fivefold at 0° into a series of solutions which were similar to the dissociation medium, but contained varying amounts of ATP. A second dilution (1:1) of these solutions at 0° with the reassembly mixture then yielded a series of similar solutions containing 0.04 mg/ml of glyceraldehyde 3-phosphate dehydrogenase and various amounts of ATP. The samples were then incubated at 17°. Maximal activity was recovered with 1–2 mM ATP; half-maximal activity was recovered with 0.3–0.4 mM ATP (Figure 12). These values are in excellent agreement with those obtained for the dependence of the dissociation process upon ATP concentration (Figure 1).

Discussion

Mode of Action of ATP. Native glyceraldehyde 3-phosphate dehydrogenase does not undergo any appreciable dissociation when incubated at 0° under the conditions employed in the work reported here. Likewise, at 23° ATP does not significantly dissociate yeast glyceraldehyde 3-phosphate dehydrogenase. However, it does cause a conformational change since an ultraviolet difference spectrum is observed[2] and the rate of degradation of the enzyme by chymotrypsin is greatly increased (Yang and Deal, 1969b). Thus, neither ATP alone nor low temperature alone can shift the equilibrium of yeast glyceraldehyde 3-phosphate dehydrogenase toward dissociation, but together they do, under proper conditions.

Two major questions are: (1) How does the binding of ATP by the enzyme alter the folding of the polypeptide chains? (2) What types of forces involved in subunit bonding would be sufficiently decreased by lowering the temperature to lead to dissociation?

Electrostatic repulsion between the negatively charged phosphate portion of ATP and negatively charged groups at the active center of the glyceraldehyde 3-phosphate dehydrogenase molecule appear to be responsible for conformational changes produced by ATP as indicated by the following: (1) Reassembly experiments with urea-dissociated yeast glyceraldehyde 3-phosphate dehydrogenase indicate that it contains a region of electrostatic charge near its active center which prevents proper refolding unless neutralized by NAD or high salt (Deal, 1969). (2) The order of the strength of binding of the adenine nucleotides to glyceraldehyde 3-phosphate dehydrogenase (Yang and Deal, 1969a) is 3′,5′-AMP > AMP > ADP ~ ATP, but their net charge and dissociation ability is exactly opposite. (3) Mg^{2+}, which chelates with the negatively charged phosphate portion of ATP, prevents inactivation of yeast glyceraldehyde 3-phosphate dehydrogenase by ATP in yeast glyceraldehyde 3-phosphate dehydrogenase solutions containing both ATP and chymotrypsin (Yang and Deal, 1969b). (4) Dissociation is favored at higher pH, with a transition point at pH 8.6.

This transition point suggests the involvement of a cysteine residue in the dissociation process. A sulfhydryl group has been reported to be involved in the binding of NAD (Racker, 1965), and ATP is a competitive inhibitor with respect to this substrate (Yang and Deal, 1969a). Also, a different sulfhydryl group forms a covalent acyl thioester with the substrate in the oxidation reaction (Racker, 1965).

It appears that the loss of critical hydrophobic bonds at 0° is the factor which, coupled with the ATP-induced electrostatic repulsion, causes dissociation of the enzyme into subunits. Kauzmann (1959) has discussed the reasons for expecting hydrophobic bonds to be more stable at room temperature than at 0°. A less likely explanation for the temperature dependence is that one of the ionizable groups in the enzyme undergoes a temperature-dependent change in pK value.[3]

Thus, dissociation appears to proceed as follows: (1) conformational changes occur in the individual subunit polypeptide chains as a result of electrostatic repulsions when ATP is bound. (2) This change in the folding of the individual polypeptide chains alters the contact sites between subunits in such a manner that hydrophobic interactions assume a more critical responsibility for maintaining the native tetrameric structure. (3) Lowering the temperature to 0° is then sufficient to weaken hydrophobic interactions at the contact site between subunits to such an extent that dissociation occurs.

It is also interesting that the temperature dependence of reassociation (Figure 10) does not exactly parallel that of dissociation (Figure 3). It thus appears that the presence of 10%

[3] Lauffer (1962) has shown that electrostatic interactions influence the polymerization of tobacco mosaic virus protein (TMV protein). He has also shown that the pH of unbuffered solutions of TMV protein show a temperature-dependent change of pH, but it is not clear whether this electrostatic effect is the cause of, or the result of, the accompanying temperature-dependent polymerization.

sucrose in the reassociation mixture alters the temperature dependence of the equilibrium. This may result from an effect of the sucrose on the water structure, since alterations in water structure would be expected to affect hydrophobic interactions.

Throughout this discussion we have implicitly assumed the interaction of ATP and glyceraldehyde 3-phosphate dehydrogenase to involve binding, followed by dissociation. Tanford (1964) has pointed out, however, that in some cases the process may be reversed; *i.e.*, dissociation may occur first, followed by binding of the denaturing agent. In such a case, the denaturing agent binds preferentially to the dissociated product, thereby shifting the equilibrium of the dissociation process by removing the product.

It seems unlikely that this occurs in this system, since ATP seems to bind to the native enzyme as tightly as it does to the subunits. This is based on the midpoints of graphs as a function of ATP concentration for (1) chymotryptic inactivation at pH 7.0 and 25° (Yang and Deal, 1969b), (2) dissociation at pH 8.5 and 25°, and (3) reassembly at pH 7.0 and 17°.

It is difficult at present to calculate an equilibrium constant for this process, since dimers are almost certainly involved in the conversion of the inactive monomers into active tetramers and it is not known whether the dimers are catalytically active or not. However, if the dimer is assumed to be inactive, the equilibrium constants at 5.5° (and the conditions of Figure 10) for a dimer–tetramer and a monomer–tetramer equilibrium are 0.1 and 0.001 mg per ml, respectively.

It is interesting that ATP serves as a catalyst in the reassembly reaction at 17° and perhaps in the dissociation reaction at 0°; this is in addition to whatever effect it may have on the equilibrium of the dissociation. Further work is in progress on the mechanism by which ATP stimulates the reactivation and reassociation processes.

As discussed in a previous paper (Stancel and Deal, 1968), the 2.8–3.0S dissociation product appears to be a fairly compact monomer, since that sedimentation coefficient is appropriate for the molecular weight of 38,000 obtained from subunit analysis using urea (Deal, 1963; Deal and Holleman, 1964; W. C. Deal, Jr., in preparation). The sedimentation coefficient seems too low for a dimer, since it would have to be extremely unfolded to exhibit such a small sedimentation coefficient. It is interesting that a dimer has not been detected in the dissociation. This suggests that the monomer–monomer interaction is somewhat dependent upon the dimer–dimer interaction for stability and is immediately broken when the dimer–dimer interaction is broken.

The effect of ATP on yeast glyceraldehyde 3-phosphate dehydrogenase appears to be similar to the effect of oxygen on hemoglobin. The subunits of tetrameric hemoglobin undergo a marked reorientation with respect to each other when oxygen is bound (Muirhead and Perutz, 1963), apparently due to conformational changes in individual subunits upon oxygenation (Edsall, 1968). The binding of oxygen also increases the susceptibility of hemoglobin to proteolytic degradation (Zito *et al.*, 1964).

Biological Significance of the Dissociation Process for Metabolic Control. Since the *in vivo* ATP concentration of yeast is 1–2 mM (Betz and Moore, 1957), it seems likely that the interaction of ATP and glyceraldehyde 3-phosphate dehydrogenase may be physiologically significant. However, the dissociation of yeast glyceradehyde 3-phosphate dehydrogenase into subunits by ATP occurs at low temperatures and occurs most readily at higher pH values. Also, rapid but subtle conformational changes occur when ATP is bound, but dissociation occurs much more slowly. The rapid subtle conformational changes increase the rate of inactivation of yeast glyceraldehyde 3-phosphate dehydrogenase by chymotrypsin at 23° and neutral pH (Yang and Deal, 1969b). This effect seems to have the greatest potential as a control mechanism, and offers an additional mechanism other than induction–repression or activation–inhibition mechanisms to control metabolic processes. It is also very significant that 3′,5′-AMP and 5′-AMP alone do not dissociate yeast glyceraldehyde 3-phosphate dehydrogenase and, in fact, protect it from dissociation by ATP. The possible physiological significance of these and other effects is discussed in more detail elsewhere (Yang and Deal, 1969b).

If we assume that the 2.8S component is a folded monomer, then the results from the reassociation experiments suggest that folded monomers *can* associate to a tetrameric enzyme. This would be consistent with, but would not prove, that *in vivo* synthesis of this polymeric enzyme may involve two processes: the assembly and folding of the individual polypeptide chains followed by a separate step, the association of folded monomers to a catalytically active polymeric enzyme. A more thorough discussion of this topic has recently been presented elsewhere (Deal, 1969).

There are very pronounced differences between the effect of ATP on yeast glyceraldehyde 3-phosphate dehydrogenase and its effect on the rabbit muscle enzyme. The rabbit muscle enzyme is dissociated to a much greater extent at high protein concentrations (10 mg/ml) and is much more specific for ATP (Constantinides and Deal, 1968, 1969).

Materials and Methods

Enzyme Preparation and Assay. The materials and methods for preparation and assay of yeast glyceraldehyde 3-phosphate dehydrogenase were those described previously (Deal, 1969), with the following minor modifications. A stock assay solution was prepared by mixing the following (final assay concentrations in parentheses): (1) 12 ml of 0.2 M Tris, pH 8.0 (100 mM); (2) 1.2 ml of 0.2 M sodium arsenate (10 mM); (3) 2.4 ml of 0.01 M NAD (1 mM); (4) 3.6 ml of 0.06 M cysteine hydrochloride (9 mM); (5) 1.2 ml of 3.0 M KCl (150 mM); and (6) 1.2 ml of H_2O. The final pH was 7.8. For assay, 0.36 was added to a cuvet, then 0.01 ml of enzyme solution (0.2 mg/ml) and the contents were mixed. The enzyme reaction was then initiated by addition of 0.03 ml of a stock glyceraldehyde 3-phosphate solution (0.02 M), to give a final volume of 0.4 ml.

Reagents. Reagent grade β-mercaptoethanol was obtained from Eastman. ATP (sodium salt) was obtained from Sigma and stored dessicated under a vacuum at −20°. Stock solutions were prepared at 0.04–0.05 M concentrations, adjusted to pH 7.5–8.5, and stored frozen until used. Normally this storage period was not longer than 30–60 days. ADP, AMP, and 3′,5′-AMP were all obtained from Sigma (sodium salts) and were prepared as was the ATP. All other reagents used were the highest quality reagent grades commercially available.

Sucrose Density Centrifugation. Sucrose density gradient centrifugation was performed using the method of Martin and Ames (1961) with slight modifications which are described elsewhere (Constantinides and Deal, 1969). Samples were

centrifuged at 40,000 rpm in a Beckman Model L preparative ultracentrifuge with either a SW39 or a SW50 rotor for 22 hr at 0–2°.

Dissociation and Reassembly. For the dissociation experiments, the enzyme (0.2 mg/ml) was incubated for 5 hr at 0° in 4 mM ATP, 0.1 M Tris buffer (pH 8.5), and 0.1 M β-mercaptoethanol.

For the reassembly experiments, glyceraldehyde 3-phosphate dehydrogenase (0.4 mg/ml) was normally dissociated by a 12–14-hr incubation at 0° in 10 mM ATP, 0.02 M Tris (pH 8.5), and 0.1 M β-mercaptoethanol. The samples were completely inactivated by this treatment.

Reassembly was accomplished by diluting 0.5 ml of the inactive monomers with 0.5 ml of reversal mixture at 0° and warming to 23 or 17° for 2 hr. The reversal mixture contained 0.4 M Tris buffer (pH 6.2), 20% sucrose, and 0.1 M β-mercaptoethanol. After dilution, the resulting mixture contained 0.2 mg/ml of glyceraldehyde 3-phosphate dehydrogenase, 0.1 M β-mercaptoethanol, 5 mM ATP, 0.21 M Tris, 10% sucrose, and had a pH of 7.0.

References

Atkinson, D. E. (1965), *Science 150*, 851.

Atkinson, D. E. (1966), *Ann. Rev. Biochem. 35*, 85.

Betz, A., and Moore, C. (1957), *Arch. Biochem. Biophys. 120*, 268.

Constantinides, S. M., and Deal, W. C., Jr. (1968), 156th National Meeting of the American Chemical Society, Atlantic City, N. J.

Constantinides, S. M., and Deal, W. C., Jr. (1969), *J. Biol. Chem.* (in press).

Cori, G. T., Selin, M. W., and Cori, C. F. (1948), *J. Biol. Chem. 173*, 705.

Deal, W. C., Jr. (1963), *Federation Proc. 22*, 290.

Deal, W. C., Jr. (1969), *Biochemistry 8*, 2795.

Deal, W. C., Jr., and Holleman, W. H. (1964), *Federation Proc. 23*, 264.

Edsall, J. T. (1968), *in* Structural Chemistry and Molecular Biology, Rich, A., and Davidson, N., Ed., San Francisco, Calif., Freeman, p 88.

Kauzmann, W. (1959), *Advan. Protein Chem. 14*, 1.

Krebs, E. G., Rafter, G. W., and Junge, J. M. (1953), *J. Biol. Chem. 200*, 479.

Lauffer, M. A. (1962), The Molecular Basis of Neoplasia, Houston, Texas, University of Texas, p 180.

Martin, B. N., and Ames, R. G. (1961), *J. Biol. Chem. 236*, 1372.

Muirhead, H., and Perutz, M. F. (1963), *Nature 199*, 633.

Racker, E. (1965), Mechanisms in Bioenergetics, New York, N. Y., Academic, p 21.

Scrutton, M. C., and Utter, M. F. (1968), *Ann. Rev. Biochem. 37*, 249.

Stadtman, E. R. (1966), *Advan. Enzymol. 28*, 41.

Stancel, G. M. (1969), *Federation Proc. 28*, 490 (1969).

Stancel, G. M., and Deal, W. C., Jr. (1968), *Biochem. Biophys. Res. Commun. 31*, 398.

Tanford, C. (1964), *Brookhaven Symp. Biol. 17*, 154.

Williamson, J. R. (1965), *J. Biol. Chem. 240*, 2308.

Williamson, J. R. (1967), *7th Intern. Congr. Biochem., Tokyo, 7*, 863.

Wood, W. A. (1966), *Ann. Rev. Biochem. 35*, 521.

Yang, S. T., and Deal, W. C., Jr. (1969a), *Biochemistry 8*, 2806.

Yang, S. T., and Deal, W. C., Jr. (1969b), *Biochemistry 8*, 2814.

Zito, R., Antonini, E., and Wyman, J. (1964), *J. Biol. Chem. 239*, 1804.