METABOLIC CONTROL AND STRUCTURE OF GLYCOLYTIC ENZYMES. V[1]

DISSOCIATION OF YEAST GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE INTO SUBUNITS BY ATP[2]

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Numerous examples of the effects of adenine nucleotides on the regulation of carbohydrate metabolism have been reported (for recent reviews, see Atkinson, 1965, 1966; Stadtman, 1966, and Wood, 1966). Great emphasis has been placed on the influence of ATP, ADP, AMP and 3',5'-AMP on the activity of phosphorylase, phosphofructokinase and fructose diphosphatase and their potential ability to produce major fluctuations in the rate of glycolysis (Krebs and Fischer, 1962; Cori, 1942, Mansour and Mansour, 1962, Passonneau and Lowry, 1962, Williamson, 1965; Taketa and Pogell, 1963, Bonsignore et al., 1963). The possibility of similar (although perhaps lesser in magnitude) effects of these adenine nucleotides on other glycolytic enzymes has largely been ignored. We have, therefore, undertaken a study of the effects of metabolites on the control of several important glycolytic enzymes, including the glyceraldehyde-3-phosphate dehydrogenases (GAPD) from yeast, rabbit muscle, and liver. Our initial studies have shown that ATP produces two separate, major effects with yeast GAPD, both of which affect its activity. One effect, which is due to slow structural change and which leads to loss of activity, is described in this communication. The other effect, which is due to ATP competition with NAD for binding sites, and which leads to instantaneous inhibition of activity is to be described elsewhere (Shih-Tzy Yang and W.C. Deal, Jr., submitted for publication).

[1]Paper IV in this series covers the NAD-dependent reversal of the urea dissociation of yeast GAPD into subunits (W.C. Deal, Jr.; submitted for publication).

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The main purpose of this paper is to report the phenomenon of destabilization of yeast GAPD, as evidenced by *in vitro* inactivation and dissociation of the enzyme into 1.6S subunits. One strong incentive for characterizing the process was the possibility that the destabilization and presumed increased susceptibility to proteolytic degradation might be a mechanism for control *in vivo*.

MATERIALS AND METHODS

A separate paper[1] describes the preparation and assay of the bakers yeast (Red Star) enzyme, which involves slight modifications of Krebs methods (Krebs, 1955). The adenine nucleotides were obtained from Sigma (St. Louis, Mo.) as the sodium salts; NAD was obtained from Sigma and the P-L labs, (Minneapolis, Minn.). Sedimentation velocity experiments were performed at 56,100 r.p.m. in a Spinco model E analytical ultracentrifuge using wedge and plane single-sector capillary synthetic-boundary cells.

RESULTS AND DISCUSSION

LOSS OF ACTIVITY UPON INCUBATION WITH ATP AND PRODUCTION OF A SLOWER-MOVING COMPONENT IN POLYACRYLAMIDE DISC ELECTROPHORESIS. The initial tests for inhibition of yeast GAPD by ATP revealed that after incubation with ATP, the enzyme showed a marked loss of activity (assayed in the absence of ATP). The fact that the loss of activity of GAPD increased significantly with time of incubation with ATP suggested that marked structural changes were occurring with time. To define the structural change, initially a study of the effect of ATP concentration was conducted with an arbitrarily selected incubation time of 4½ hours. Protein samples (0.2 mg/ml) were incubated at 0° in 0.2 M tris (HCl) buffer, pH 8.0, with varying amounts of ATP. After 4½ hours aliquots were removed and assayed in the standard assay. Since all but a negligible residual amount of ATP was diluted out, any activity loss should have been due to structural changes which were not immediately reversible. As seen in Figure 1, at ATP concentrations of 2 mM or higher, about 50% loss of activity occurred after 4½ hours incubation at 0°. The maximum ATP effect appears to be exerted

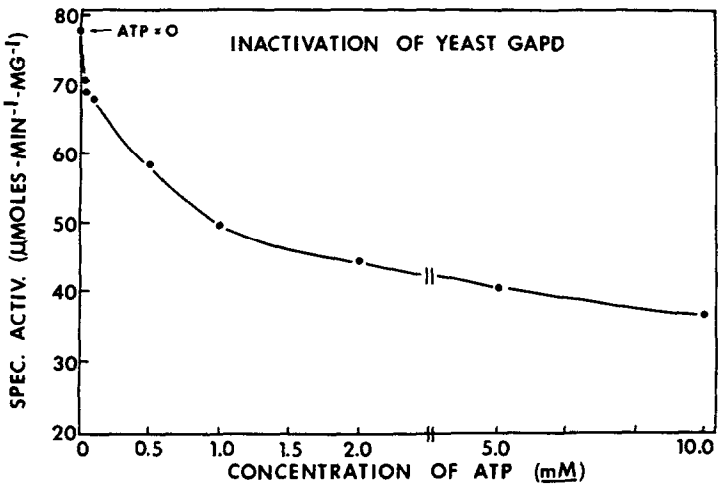

Figure 1 Activity of yeast glyceraldehyde-3-phosphate dehydrogenase after 4½ hour incubation with ATP. See text.

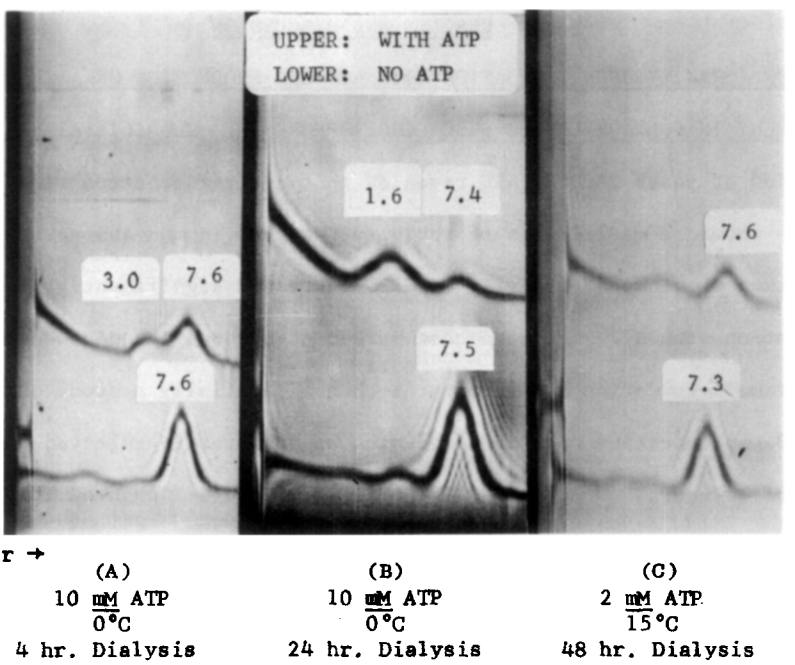

Figure 2 Dissociation of yeast glyceraldehyde-3-phosphate dehydrogenase after incubation with ATP. Run at temperatures near those of incubation. See text.

by concentrations of 1-2 mM; above this the curve levels off.

After activity measurements were performed as above, aliquots were removed from the tubes and subjected to polyacrylamide ($6\frac{1}{2}\%$) disc electrophoresis at pH 8.3. The sample without ATP showed only a single native band. All other samples showed an additional, slower-moving band whose concentration apparently was greater at higher concentrations of ATP. Again the maximum change appeared to be exerted by all concentrations greater than 2 mM ATP. In contrast, incubation of GAPD with an NAD concentration equal to that of the ATP prevented, to a large extent, the loss of activity and the appearance of the more slowly moving band upon electrophoresis.

This set of experiments provided the first direct evidence that ATP produced a marked structural change in the enzyme. A tempting explanation of the data was that ATP might cause dissociation of the enzyme into subunits, since this enzyme has been shown to be dissociated into subunits by urea (Deal, 1963; Deal and Holleman, 1964; W.C. Deal, Jr., in preparation). However, the alternative possibility that the slower-moving species was an aggregated product of the native enzyme could not be ruled out.

PRODUCTION OF A 1.6S SUBUNIT. To decide between these possibilities, sedimentation velocity experiments were performed. It was desirable for the sedimentation experiments to increase the protein concentration to 2.5 mg/ml. The concentration of the dissociating agent, ATP, was made higher to help compensate for the unfavorable effect of increased protein concentration on dissociation. Samples of GAPD (2.5 mg/ml) were dialyzed at 0° for $4\frac{1}{2}$ or 24 hours against a solvent containing 10 mM ATP, 0.15 M KCl and 0.02 M imidazole, pH 7.5. Native enzyme, treated in a similar manner, but without ATP, served as a control. As seen in Figure 2A and 2B, the ATP-treated samples showed, in addition to the usual 7.5S native peak, a slower-moving peak, suggesting a dissociation into subunits. It should be noted that the sedimentation coefficients of the slower moving peaks were $s_{20,w} = 3.0S$ and 1.6S, respectively, for the samples incubated $4\frac{1}{2}$ hours and 24 hours. One explanation for this change is that the enzyme

(1) first undergoes only a limited unfolding, (2) then dissociates, and (3) then unfolds rather extensively. It is interesting that the sedimentation coefficient of 3.0S is consistent with that expected for a folded monomeric subunit of molecular weight about 35,000 and the value of 1.6S is identical to that found for unfolded subunits in urea (Deal, 1963; Deal and Holleman, 1964; W.C. Deal, in preparation).

To determine whether this might be a physiologically significant process, two conditions used in the previous experiments were changed to more closely approximate in vivo conditions. The ATP concentration was lowered to 2 mM and the experiment was run at 15-16°. As seen in Figure 2C, incubation of the enzyme with ATP under these conditions for about 48 hours produced about 20-25% dissociation. Although, the dissociation at 15° with 2 mM ATP was not as rapid or extensive as that at 0° with 10 mM ATP, there clearly was a major destabilization and dissociation.

MECHANISM OF DISSOCIATION AND THE OVERALL CONTROL OF GAPD. Because of structural similarities it seems reasonable to suspect that ATP is binding at an NAD site, and that the additional phosphate and additional negative change on the terminal phosphate of ATP may be responsible for the destabilization.

The conditions of ATP concentration (Betz and Moore, 1967), salt, temperature, pH and protein concentration expected in vivo in yeast are reasonably close to those described in this report. Furthermore, since only a small structural change may be necessary to increase GAPD susceptibility to yeast proteolytic enzymes, the destabilization by ATP could be a physiologically significant process. Studies concerning the role that this process might play in the metabolism of yeast are in progress.

For comparison, it is interesting to note that rabbit muscle GAPD shows dissociation by ATP (S.M. Constantinides, and W.C. Deal, Jr., in preparation), by urea (Deal and Holleman, 1964; W.C. Deal, Jr., in preparation), and by low temperatures at low protein concentration (Constantinides and Deal, 1967; S.M. Constantinides, and W.C. Deal, Jr., in preparation) and all of these

dissociation processes are inhibited by NAD.

The stabilization by NAD of yeast GAPD against ATP-induced destabilization and dissociation provides a mechanism involving NAD in control of degradation of this enzyme. These effects, together with the recent results (Deal, 1967; Deal and Constantinides, 1967; W.C. Deal, Jr., submitted for publication) suggesting a role for NAD in accelerating the rate of synthesis of the enzyme, provide mechanisms for a logical, systematic, and effective control of both synthesis and degradation of GAPD by NAD, with the degradative control being shared antagonistically with ATP.

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