REVERSIBLE INACTIVATION AND INHIBITION OF LIVER FRUCTOSE-1, 6-DIPHOSPHATASE BY ADENOSINE NUCLEOTIDES

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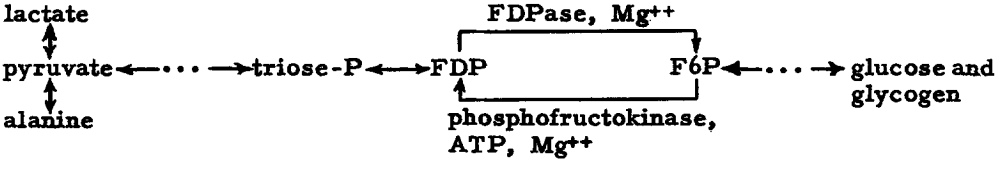
Studies on fructose-1, 6-diphosphatase (FDPase) in crude liver preparations at physiological pH by a sensitive spectrophotometric procedure have revealed the following: (a) a K_m for FDP of 5.5×10^{-6} M and an even lower value of 1.2×10^{-6} M in aldolase-free preparations, (b) marked inhibition by FDP concentrations greater than 0.1 mM, and (c) activities of 5-7 units (μmoles of product/min.)/g of tissue in rabbit liver and 10-13 units/g in rat liver at 30° and pH 7.5. Much lower values (0.2-0.35 units/g) have been found for liver phosphofructokinase under similar conditions (1, 2).

Scheme I



During glyconeogenesis in liver, these relative activity levels would favor carbohydrate formation. However, a fully active FDPase with such a low K_m would be extremely wasteful during glycolysis or glycogenolysis in liver and result in a decreased net conversion of carbohydrate to lactate. These consid-

erations and the recent report of the role of adenosine nucleotides as activators of phosphofructokinase (1) stimulated us to look for related effects on FDPase. Two possible mechanisms for the control of FDPase activity by adenosine nucleotides are reported in this communication.

Reversible inactivation by ATP - FDPase was assayed spectrophotometrically at 30° and pH 7.5 by following TPNH formation at 340 mμ in the presence of excess phosphohexoisomerase and G6P-dehydrogenase. Accurate measurements with low substrate concentrations were obtained with a Gilford multiple sample absorbance recording system set at maximum sensitivity so that full-scale deflection of the 10 inch recording chart corresponded to an absorbancy of 0.1. Routinely, 0.1 mM FDP, 50 mM Tris·HCl (pH 7.5), 0.17 mM TPN^+, 20 mM mercaptoethanol, 10 mM $MgSO_4$, excess phosphohexoisomerase and G6P-dehydrogenase, and FDPase were present in the assay system in a final volume of 3 ml. Maximum activities were found at FDP concentrations of 0.03 to 0.1 mM, 60% inhibition occurring at 1.0 mM and 75% at 10 mM FDP. Weber (3) previously had reported inhibition of FDPase by high substrate concentrations at pH 7.4.

Incubation of undialyzed rat or rabbit liver homogenates or high-speed supernatants (1:1,500 dilution of liver) in such a system in the presence of 1 mM ATP for 10 minutes before addition of substrate resulted in a 40-57% decrease of enzyme activity. With 5 mM ATP, the inactivation was 81-86%. There was little or no decrease in reaction rate if the ATP was added after the substrate. ATP had no effect on the complete reaction system with F6P as substrate. Furthermore, a similar inactivation occurred when dialyzed supernatant was first treated with ATP in the absence of added phosphohexoisomerase and G6P-dehydrogenase and then assayed either spectrophotometrically or by measurement of the release of inorganic phosphate. The extent of inactivation was found to be dependent on temperature, time of incubation, and nucleotide concentration (Fig. 1). ADP was found to have a similar effect (see Nucleotide specificity).

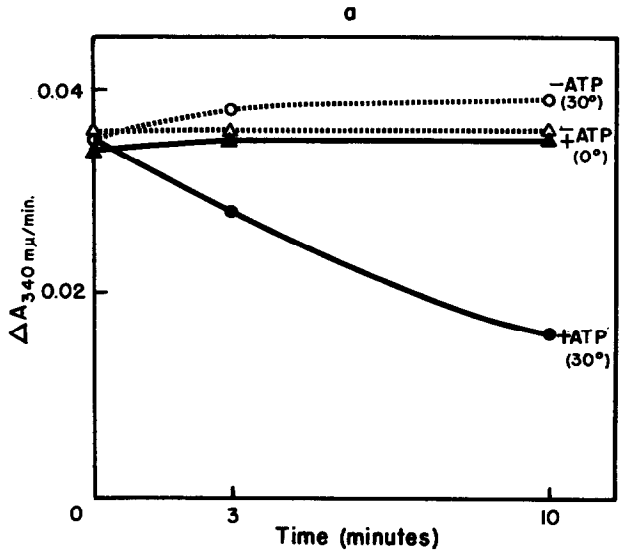
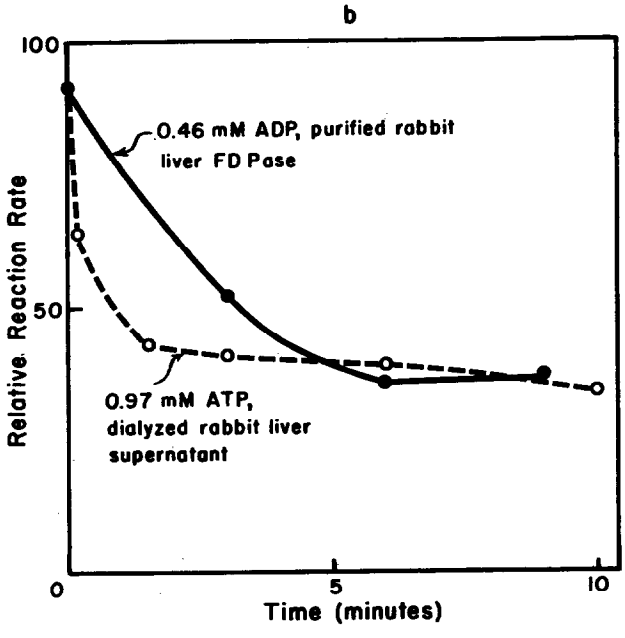
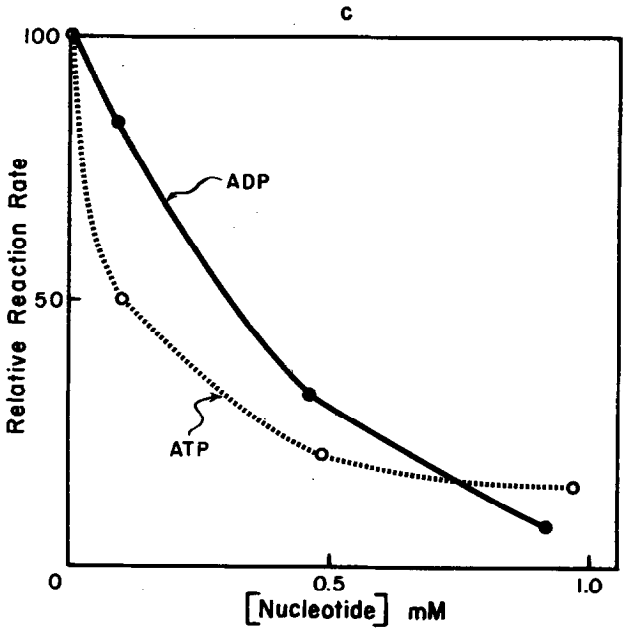


Figure 1. a. Effect of temperature on ATP inactivation of FDPase. 0.1 ml dialyzed rabbit liver supernatant, 15 mM $MgSO_4$, 20 mM mercaptoethanol, 50 mM Tris, ± 15 mM ATP were incubated in a final volume of 1 ml at 0° or 30°. At timed intervals, 0.2 ml aliquots were added to the complete reaction mixture at 30°. In all cases, the values shown are the maximal velocities attained before 30% substrate utilization. In samples incubated without ATP, an equal amount of ATP was added to the final reaction system immediately after enzyme.



b. Effect of time on ATP and ADP inactivation of FDPase. Rabbit liver dialyzed supernatant or purified FDPase were incubated at 30° for timed intervals ± nucleotide in the complete system minus FDP. The reaction was started by substrate addition.



c. Effect of [ATP] and [ADP] on inactivation of purified rabbit liver FDPase. ATP or ADP at the concentrations indicated were incubated for 10 minutes at 30° in the complete system minus FDP. The reaction was started by substrate addition.

In order to test for the possible enzymatic nature of the inactivation process, rabbit liver FDPase was purified 340-fold by high-speed centrifugation, dialysis, adsorption on CM-cellulose, and elution with dilute solutions of FDP (4). This preparation (specific activity of 805 μmoles/hr./mg. protein) was inactivated 86% by 1 mM ATP in 10 minutes, whereas only 40% inactivation was found with the crude extract. Purification of rat liver FDPase by dialysis and heating at pH 4.5 also yielded preparations which were inactivated to a greater extent by ATP under otherwise constant conditions. The increased inactivation upon purification is probably caused by removal of interfering proteins, since the addition of either inactive protein not retained by CM-cellulose or crystalline bovine serum albumin reduced the inactivation.

The presence of phosphate ions in concentration as low as 2.5 mM completely prevented inactivation by 1 mM ATP. After treatment with ATP at 30°, the inactivated rat liver enzyme could be dialyzed overnight at 4° against 20 mM mercaptoethanol with no change in activity. A control sample,

treated identically except for the omission of ATP, retained its activity. Complete restoration of FDPase activity in the ATP-inactivated, dialyzed sample was attained by incubation at 30° for 10 minutes in the complete reaction system with 10 mM phosphate buffer in place of Tris. There was a slower recovery in Tris, and in both cases reactivation was immediately stopped upon addition of FDP. EDTA appears to function similarly to phosphate both in preventing ATP inactivation and in accelerating reactivation. So far, there has been no evidence for the involvement of any enzyme either in the inactivation or reactivation process.

Reversible inhibition by adenosine-5'-monophosphate (AMP) - Low concentrations of AMP produce a marked inhibition of FDPase at neutral pH, and this may represent a second mechanism for controlling the rate of this reaction. 0.1 mM AMP inhibited the enzyme activity 35% and more than 95% inhibition occurred at a concentration of 0.3 mM. AMP had no effect on the complete reaction system with F6P as substrate. The AMP inhibition could be clearly distinguished from ATP inactivation by the following criteria: (a) Addition of 1 mM AMP to the system after starting the reaction with FDP resulted in complete inhibition of FDPase activity, whereas ATP at this concentration had no effect on the reaction rate. (b) Treatment of FDPase with AMP followed by dilution resulted in complete restoration of activity, whereas the inactivation by ATP was not reversed either by dilution or overnight dialysis at 4°. (c) Incubation of the enzyme with AMP in phosphate buffer before FDP addition produced the same degree of inhibition as that found in Tris buffer, whereas phosphate completely prevented ATP inactivation. (d) There was no measurable inorganic phosphate released from ATP by dialyzed rat liver supernatant when assayed with or without the addition of Mg ions.

Nucleotide specificity - Incubation of dialyzed rat liver supernatant for 10 minutes at 30° with nucleotides or nucleosides present at 1 mM concentration gave the following decreases in reaction rate: ATP : 79%; UTP : 31-48%; CTP, GTP, CDP, GDP, or GMP : 10-28%; UDP, UMP, CMP, uridine,

cytidine, adenosine, or guanosine : no decrease. ADP decreased the reaction rate 96%. This was presumably caused by the combined action of ADP and AMP formed by myokinase, since the rate continued to decrease in the presence of substrate. With the purified rabbit liver enzyme, ADP was still as effective an inactivator as ATP (Fig. 1c). This was not due to AMP formation or contamination of the ADP with AMP, since the decreased rate remained after dilution to a level where an AMP concentration equivalent to that of the ADP would not have been effective and phosphate prevented the inactivation. DPN^+, cyclic-3', 5'-AMP, and pyrophosphate had no effect either as inactivators or inhibitors of FDPase.

When tested by addition to the reaction system immediately after FDP, only AMP was found to inhibit the enzyme. At 1 mM concentration, 2'-AMP, 3'-AMP, UMP, CMP, GMP, IMP, ATP, GTP, UTP, CTP, UDP, GDP, or CDP were without effect. ADP did not inhibit the purified enzyme when tested in phosphate buffer.

Discussion - It is not known whether the inactivation of FDPase involves the covalent addition of part of the ATP or ADP to the enzyme molecule, such as is found with glycogen phosphorylase (5) and UDPG-glycogen transglucosylase (6). In these two cases, phosphatases and ATP-requiring kinases exist which catalyze the dephosphorylation and phosphorylation of the enzymes. It may rather be that only conformational changes of the enzyme occur, possibly with the formation of enzyme subunits. Many of the properties of the FDPase-inactivation system, including an increased degree of inactivation with increased dilution of the enzyme,[1] are similar to those of known examples of reversible enzyme dissociation into subunits. These include G6P-dehydrogenase (7), aldolase (8), and glutamic dehydrogenase (9, 10). In the latter case, adenine nucleotides reverse the splitting of the enzyme by various agents including DPNH and steroid hormones. Both the revers-

[1] Taketa and Pogell, unpublished results.

ible inactivation and inhibition of FDPase appear to be in accord with current generalized concepts of cellular control mechanisms (11).

It is of particular interest that AMP is both a potent activator of phosphofructokinase (1) and a potent inhibitor of FDPase. Thus, at concentrations of 0.3 mM, there would be a maximal activation of the former enzyme and inhibition of the latter, and maximal glycolytic rates could occur. Calculations from published data give values for the AMP concentration of liver of this order of magnitude (12, 13). Lowering the AMP concentration would reverse the situation and be favorable for glyconeogenesis. This would be an attractive site for glucocorticoid action.

Dr. Joseph Mendicino, Ohio State U. (private communication), has found similar inhibitions of a specific FDPase from kidney by both substrate and AMP. This enzyme also appears to be inactivated by ATP. Dr. M. F. Utter, Western Reserve U. (private communication), has found that AMP specifically inhibits both yeast and liver FDPase.

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