*INHIBITION OF GLUCOSE 6-PHOSPHATE DEHYDROGENASE BY ADENOSINE 5'-TRIPHOSPHATE**

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D-glucose 6-phosphate dehydrogenase (G6PDH) catalyzes the oxidation of glucose 6-phosphate (G6P) to 6-phosphogluconate-δ-lactone and thereby constitutes the step which introduces hexoses into the "pentose cycle" pathway. This well-known enzyme, which is widely distributed in nature, was purified to varying degrees from different sources (for literature see refs. 1 and 2). The yeast enzyme,[3, 4] which is now commercially available in a high degree of purity, is extensively used for analytical purposes in biochemical research, especially for the assay of ATP, NADP, hexoses, sugar phosphates, and several kinases. While employing yeast G6PDH in a coupled reaction for the analysis of a bacterial hexokinase-ATP system, we have noticed some inhibition of the expected initial rate of NADPH formation when compared to that observed in control systems with G6P. A search for the cause of this phenomenon has indicated that ATP exerted an inhibitory effect on the reaction catalyzed by G6PDH. This observation is described in the present communication.

Materials and Methods.—Crystalline yeast G6PDH and hexokinase were obtained from Boehringer und Soehne, GmbH. G6P, NADP, and ATP were preparations supplied by Boehringer and by Sigma Chemical Co. Nucleotides, sugar phosphates, and other biochemical reagents were purchased from Calbiochem, Boehringer, and Pabst Laboratories Biochemicals, Inc.

NADPH formation in the G6PDH reaction was followed at 340 mμ using quartz cells with a 10-mm light path in the Gilford model 2000 multiple-sample automatic recording spectrophotometer, temperature controlled at 25° or 30°. Reaction was usually initiated by the addition of NADP as the last component. In G6PDH-hexokinase-coupled systems, the reaction was started by the addition of hexokinase.

A unit of enzyme activity was defined as that amount of enzyme which produced one μmole of NADPH/min at V_{max} conditions at the pH in which the particular set of experiments was performed.

Results.—Inhibition by ATP: Inclusion of ATP in a standard G6PDH reaction system resulted in a significant inhibitory effect on the rate of oxidation which was also pH-dependent (Fig. 1). It is clear that the inhibition is much more pronounced at neutral pH values, whereas it is not so apparent between pH 8.0 and 8.5, the range usually employed for the assay of this enzyme system. Inhibition by ATP could be relieved competitively with G6P (Fig. 2). Mg^{++} strongly diminished the inhibition by ATP (Fig. 3). It was found that maximum change in the magnitude of inhibition by ATP occurred with about an equimolar concentration of Mg^{++} (Fig. 3). Similar to what has been observed by Glaser and Brown,[3] Mg^{++} did not change appreciably the V_{max} of the reaction at pH 7.3, but caused a slight change in the apparent K_m for G6P from 4.0×10^{-5} M to 7.0

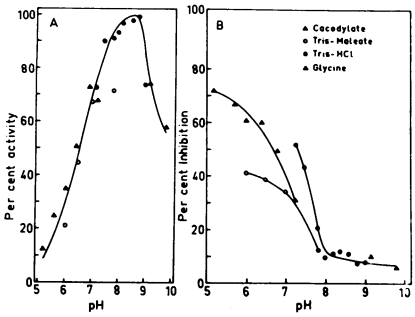

FIG. 1.—Effect of pH on G6PDH activity and its inhibition by ATP. Reaction mixture (1.0 ml) contained (in μmoles): buffer, 125; G6P, 0.2; NADP, 0.5; G6PDH, 0.6–1.2 μg protein (0.043–0.086 units as assayed at pH 8.0) with or without 2 mM ATP. Rates of change in absorption at 340 mμ were recorded at 25°. (*A*) Relative initial rates of reaction; (*B*) per cent of inhibition of the initial rates by ATP at each pH value.

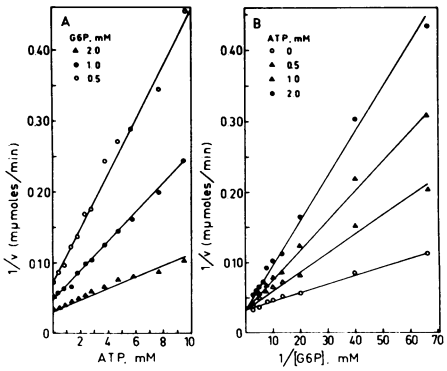

FIG. 2.—Competitive inhibition of G6PDH reaction by ATP in absence of Mg^{++}. Reaction system (1.0 ml) at 30° contained (in μ-moles): Tris-HCl pH 7.35, 100; NADP, 0.55; variable amounts of ATP and G6P as indicated, and 0.033 unit enzyme. (*A*) Plot of reciprocal values of initial rates at varying concentrations of inhibitor; (*B*) double reciprocal plot of the G6PDH reaction in presence of ATP. K_m (G6P), 4.0×10^{-5} *M*; K_i (ATP), 5.5×10^{-4} *M*.

10^{-5} *M* (compared to K_m values of 6.9×10^{-5} *M* and 5.8×10^{-5} *M* in absence or presence of Mg^{++} at pH 8.0, ref. 3). The K_i value for ATP was found to be 5.5×10^{-4} *M*, whereas that of Mg-ATP was 2.7×10^{-3} *M*. Thus, as a competitor with G6P under the assay conditions described, ATP in the absence of Mg^{++} is three times more efficient than Mg-ATP.

Variation in NADP concentrations between 0.1 and 1.0 mM did not appreciably alter the inhibitory effect of ATP on the rates of G6P oxidation by G6PDH. It was reported that K_m values of NADP and of G6P at pH 8.0 were not significantly influenced by the concentration of the second substrate.[3, 5] In some experiments, however, it was noticed that in yeast G6PDH system at pH 7.4, the apparent K_m value determined for one substrate shows some dependence on the concentration of the second one.[6] It is thus expected that the apparent K_i values for ATP as a competitor to G6P will vary slightly when measured at different NADP concentrations.[6] An exact kinetic analysis of the G6PDH system in terms of a two-substrates reaction system [7–9] has yet to be performed.

Effect of other metabolites: Other nucleoside 5′-triphosphates were found to inhibit the G6PDH reaction. In a reaction mixture containing Tris-HCl at pH 7.3, 100 mmoles/liter; G6P, 0.05 mmole/liter; NADP, 0.41 mmole/liter; and G6PDH, 0.025 unit/ml, the presence of 2 mM ATP inhibited the initial rate of oxidation by 70 per cent, whereas 2 mM CTP, GTP, or ITP inhibited it by 40–50 per cent and 2 mM UTP or TTP inhibited it by only 20 per cent. The detailed kinetics of the inhibition for these materials has not been reported.

Many other nucleotides, sugars, and other metabolites were tested for their ability to affect the G6PDH reaction or to relieve the inhibition exerted by ATP. In a reaction system composed as described above, 2 mM ATP inhibited the rate of oxidation 66 per cent. Addition of 4 mM glucose 1-phosphate, mannose

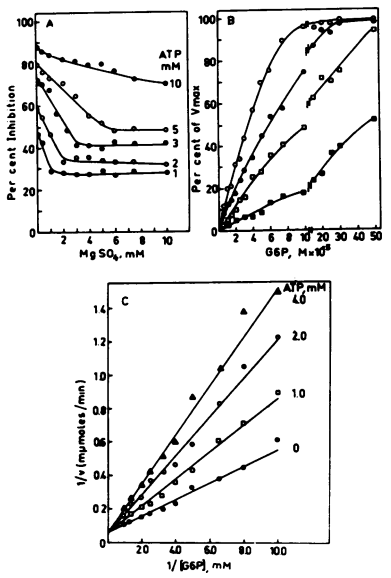

FIG. 3.—Effect of Mg^{++} on the inhibition of G6PDH by ATP. (*A*) Influence of varying concentrations of Mg^{++} in relation to different ATP concentrations on the initial rates of the reaction. Reaction system (1.0 ml) at 30° contained (in μmoles): Tris pH 7.3, 100; G6P, 0.05; NADP, 0.37; $MgSO_4$ and ATP, as indicated. G6DPH was added to give a V value of 22 mμmoles NADPH/min in the absence of both Mg^{++} and ATP. (*B*) Influence of Mg^{++} and ATP on the initial rate of NADPH formation by G6PDH in relation to G6P concentration. Reaction system (1.0 ml) at 30° contained (in μmoles): Tris pH 7.3, 100; NADP, 0.36; with or without 5 mM $MgSO_4$ and 4 mM ATP. G6PDH was added to give a V_{max} of 10 mμmoles NADPH/min. ○, Reaction without ATP and Mg^{++}; ●, with Mg^{++} without ATP; ■, with ATP, without Mg^{++}; □, with both Mg^{++} and ATP. (*C*) Double reciprocal plot representation of the initial rates obtained for reaction systems composed as in (*B*) with 5 mM $MgSO_4$ and variable concentrations of G6P and ATP as indicated. K_m (Mg-G6P) 7.0×10^{-5} *M*; K_i (Mg-ATP) 2.7×10^{-3} *M*.

6-phosphate, fructose 1-phosphate, 6-phosphogluconate, fructose 6-phosphate, fructose diphosphate, acetylphosphate, phosphoenolpyruvate, fumarate, lactate, pyruvate, citrate, succinate, DL-isocitrate, L-aspartate, ethanol, D-gluconate, pyrophosphate, or 0.4 mM sedoheptulose diphosphate did not alter the magnitude of ATP inhibition. Addition of 4 mM 5′-AMP or ADP, 2 mM IDP, TDP, or UDP and 1 mM 3′,5′-cyclic AMP also did not influence the inhibition exerted by 2 mM ATP in a similar G6PDH reaction system.

Orthophosphate, which at high concentrations is known to inhibit the G6PDH reaction by competition with NADP (refs. 3, 10, 11), did not influence the magnitude of inhibition by ATP. For example, in a standard reaction system (composed as described above), in which 50 mM orthophosphate buffer at pH 7.2 was present, the rate of G6P oxidation decreased 67 per cent compared to systems assayed in Tris or ethanolamine buffers. Nevertheless, inclusion of 2 mM ATP caused in all cases an inhibition of 60–65 per cent compared to the initial rates observed for each buffer without ATP. Conceivably, inhibition by ATP in this case was superimposed, but was probably independent of the inhibition by orthophosphate.

Inhibition in a coupled reaction: G6PDH is extensively used in assay systems coupled with hexokinase for the purpose of determination of glucose, ATP, or the estimation of hexokinase activity. In most of these assay systems, an excess of enzyme reagents is added and the Mg^{++} concentration and pH levels of 8.0–8.5 will considerably minimize the significance of ATP inhibition on the rates of the G6PDH reaction. However, quite often in our own experience, assay conditions may prevail when the inhibition by ATP is apparent (Fig. 4). In this experiment it is evident that a lag in the NADPH formation occurs and that its extension is directly proportional to the initial ATP concentration. The reaction rate tends later to approach the maximal expected rate when the relative concentration of G6P in the system increases with time of incubation.

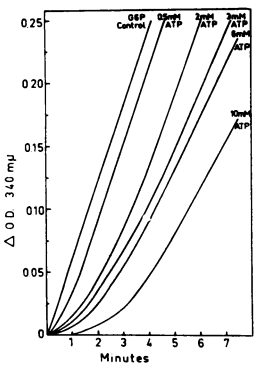

FIG. 4.—Rates of G6P oxidation in a hexokinase-G6PDH coupled system. Reaction system (1.0 ml) at 30° contained (in μ-moles): Tris, pH 7.3, 100; glucose, 10; $MgSO_4$, 8; NADP, 0.54; G6PDH, 0.01 unit; hexokinase, 0.02 unit. ATP as indicated. Reaction was started by addition of hexokinase and followed in the automatic recorder. Control system was run with 1 mM G6P in absence of ATP.

Survey on the ATP effect on G6PDH from other sources: Preliminary studies have shown that the G6PDH activity present in several other biological specimens was inhibited by ATP in a competitive manner with G6P, similar to the observation made for the yeast enzyme. The systems in which this effect was observed were crude soluble preparations obtained from human erythrocytes, rat liver, *Gluconobacter cerinus*, and *Torula utilis*.

Discussion.—It has previously been observed[1] that G6PDH obtained from yeast as well as from other sources is inhibited or sometimes activated by relatively high concentrations of various ions. Such effects were observed for orthophosphate,[3, 10] sulfate,[5] and several cations.[5, 12, 13] These effects are probably of limited physiological importance. On the other hand, the inhibition of mammalian G6PDH by various steroids[14–16] and of the yeast and mammalian enzymesby palmityl-CoA[17, 18] could occur also in the living cell and play a part in controlling the level of G6PDH activity.

For obvious kinetic considerations, it has been suggested that the amount of G6P in the cell is a major factor which determines the rate of its own oxidation by G6PDH and thereby the influx of carbohydrate into the pentose phosphate pathway (for latest discussions, see refs. 19 and 20). A similar consideration also has been applied to the relative NADP/NADPH concentration in the cell.[1, 19, 21] In addition, at least in the case of the rabbit retina,[22] it has been suggested that unidentified control mechanisms linked to the respiratory system affect the rate of entry of glucose into the pentose phosphate cycle. The present study indicates that in addition to the ultimate concentration of G6P itself, its concentration relative to that of ATP and most probably to other nucleoside triphosphates in the cell is a determinant factor which governs the rate of the oxidation catalyzed by G6PDH. The K_i values at pH 7.3 obtained for ATP are within the fluctuating level of the intracellular concentration of ATP found in yeasts.[19, 23]

The regulating effect of ATP on G6PDH activity is similar in character to many recent observations which showed that the level of ATP and other adenine nucleotides controls several key enzyme reactions that channel carbohydrates through the glycolytic and oxidative pathways.[19, 23–32] Regulation by nucleoside triphosphates of the G6PDH reaction, which supplies NADPH and pentose phosphates for biosynthesis of fatty acids, purine bases, and nucleic acids, is thus complementary to the other control mechanisms known to regulate flow of intermediates through the major metabolic pathways.

In concluding, a word of caution should be added in view of the present findings. When performing spectrophotometrical kinetic studies involving a G6PDH-coupled enzyme reaction which includes ATP and very small amounts of enzymes (i.e., such as is very often used when coupled to the hexokinase system), care should

be taken to ensure that the observed initial rates of the reaction measured by NADPH formation were not influenced by the ATP present.

Summary.—Adenosine 5′-triphosphate, as well as other nucleoside triphosphates, inhibits glucose 6-phosphate dehydrogenase by competition with the substrate, glucose 6-phosphate. Apparent K_i values for ATP were 5.5×10^{-4} M in absence of Mg^{++} and 2.7×10^{-3} M in the presence of Mg^{++}. This case is an additional example of an enzyme activity which is controlled by adenine nucleotides, thus providing a sensitive, efficient mechanism for the regulation of metabolic pathways in the cell.

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