ALLOSTERIC REGULATION OF PHOSPHORIBULOKINASE ACTIVITY

Robert D. MacElroy

Exobiology Division
Ames Research Center, NASA
Moffett Field, California 94035

and

E. J. Johnson and M. K. Johnson

Dept. Microbiology
Tulane University Medical School
New Orleans, La.

Received February 16, 1968

Inhibition of ATP-dependent CO_2 fixation by AMP has been reported in several autotrophic organisms (Johnson and Peck, 1965; Mayeux and Johnson, 1966; Johnson, 1966; Gale and Beck, 1966). Johnson (1966) suggested that the site of AMP inhibition is phosphoribulokinase and that inhibition may occur through allosteric modification of the enzyme. In contrast, Gale and Beck (1966) presented data indicating that AMP inhibition of ATP-dependent CO_2 fixation in extracts of Thiobacillus ferrooxidans is competitive with ATP. The studies reported below suggest that in Thiobacillus thioparus phosphoribulokinase is cooperatively affected by ATP and that inhibition of the enzyme by AMP may not be solely competitive.

Materials and Methods

T. thioparus was grown with continuous neutralization at 30°C in the medium described by Santer, Boyer, and Santer (1959). Five g (wet weight) of cells were suspended in 45 ml of buffer (0.05 M Tris-HCl, pH 7.5, 0.10 M 2-mercaptoethanol) and passed through a French pressure cell at 20,000 psi. The homogenate was centrifuged at 20,000 × g for 30 minutes and the pellet discarded. The supernatant fraction was first brought to 50% saturation with

solid $(NH_4)_2SO_4$, the precipitate discarded, and then to 75% saturation. The resulting precipitate was collected and dissolved in 1 ml of buffer. This solution was passed through a G-100 Sephadex column (2.5 × 38 cm) and the first 10 ml of protein emerging after the void volume was collected.

Phosphoribulokinase activity was determined at 37°C with a Gilford Spectrophotometer according to the method of Hurwitz (1962). Lactic dehydrogenase and pyruvate kinase were obtained from Boehringer Bros. (New York); NADH was obtained from Sigma Chemical Co. (St. Louis). Ribulose-5-phosphate was prepared according to the method of Pontremoli and Mangiarotti (1962).

Results and Discussion

In the presence of ATP, phosphoribulokinase catalyzes the formation of ribulose-1,5-diphosphate (RuDP) from ribulose-5-phosphate (Ru5P) with the production of ADP:

$$Ru5P + ATP \longrightarrow RuDP + ADP$$

With saturating amounts of ATP the K_m for Ru5P was determined as 4×10^{-5} M by extrapolation from reciprocal plots according to the method of Lineweaver and Burk (1934) (Fig. 1). In the presence of AMP, the apparent

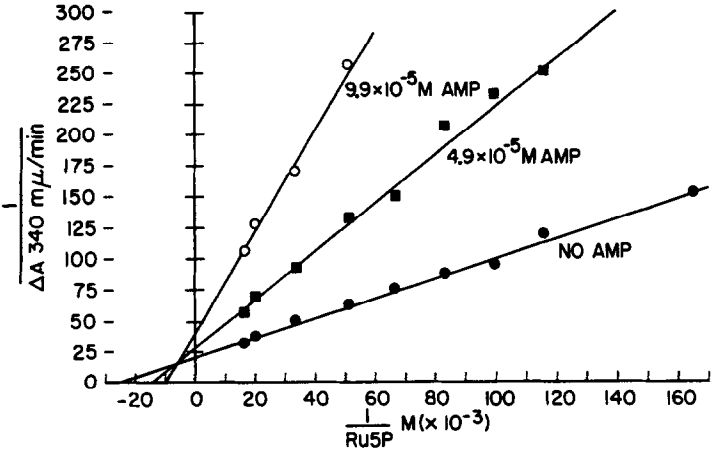

Fig. 1. Reciprocal plots of increasing concentrations of ribulose-5-phosphate in the presence and absence of AMP. Each reaction cuvette contained (in μmoles): PO_4^{3+} (as potassium phosphate buffer, pH 7.5) 300; $MgCl_2$, 50; NADH, 0.5; sodium phosphoenol pyruvate, 1.8; ATP, 15; 25 units each of lactic dehydrogenase and pyruvate kinase, and 5 μg of extract protein in a total volume of 3.0 ml. The reaction was followed at 340 mμ at 37°C.

K_m for Ru5P is increased and the V_{max} is decreased. The inhibition produced by AMP appears to be of a mixed type.

Velocity vs substrate plots were made at saturating concentrations of Ru5P and with varying concentrations of ATP. Under these conditions, sigmoidal curves were obtained (Fig. 2). The work of Monod, Wyman, and Changeux (1965)

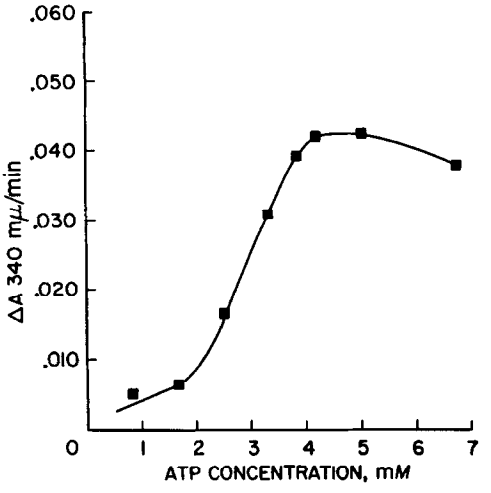

Fig. 2. The response of phosphoribulokinase, at saturating concentrations of ribulose-5-phosphate, to increasing concentrations of ATP. The reaction cuvettes contained the components listed in Figure 1, except that ATP was varied as indicated, 0.73 μmoles of ribulose-5-phosphate (Ru5P) was added, and 2.5 μg of protein was present in a total volume of 3.0 ml.

---

and of Kirtley and Koshland (1967) provides compelling evidence that sigmoidal saturation curves are characteristic of regulatory enzymes. The inhibition of enzymatic activity caused by AMP at various concentrations of ATP is complex: in general, increased inhibition is observed with increasing concentrations of AMP (Fig. 3). However, it has been consistently observed that inhibition is more pronounced the higher the concentration of the substrate (ATP) (Fig. 3).

The saturation curve for ATP indicates that ADP and, hence, ribulose diphosphate are produced at very low levels until the concentration of ATP is increased above a certain point. Since the rate of CO_2 fixation is dependent

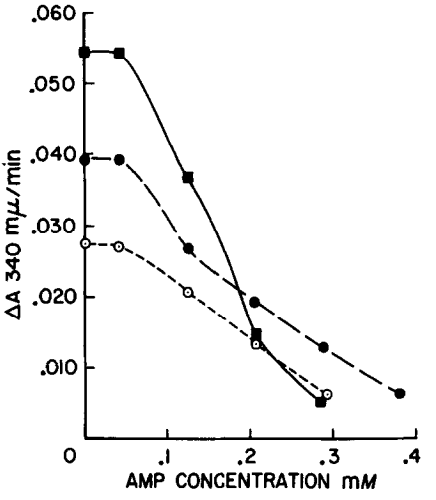

Fig. 3. Inhibition of phosphoribulokinase by AMP at three concentrations of ATP. The reaction mixture contained the components listed in Figure 1; the indicated amount of ATP and 0.73 μmoles of ribulose-5-phosphate in a total volume of 3.0 ml. ■ 5.1 mM ATP, ● 4.3 mM ATP, ⊙ 3.4 mM ATP.

upon the level of ribulose diphosphate, this cooperative phenomenon of phosphoribulokinase would function as a regulator of CO_2 fixation. A finer control of this function is provided by the inhibition caused by AMP. The kinetics of this inhibition have not been examined in sufficient detail to determine whether AMP is competitive with ATP at the same site, but the complexity of the kinetics indicates that while this is possible, an additional inhibition may be caused by the attachment of AMP at a specific site on the enzyme.

The effects reported here have also been observed in extracts of T. neapolitanus, and results obtained from other assay systems indicate that ADP is also effective as an inhibitor of phosphoribulokinase. Further work on phosphoribulokinase from several sources will be reported more extensively elsewhere.

REFERENCES

Gale, N. L. and Beck, J. V., Biochem. Biophys. Res. Commun. 24, 792 (1966).
Hurwitz, J., in Methods in Enzymology, S. P. Colowick and N. O. Kaplin (eds.), vol. 5, p. 258 (1962).
Johnson, E. J.. Arch. Biochem. Biophys. 114, 179 (1966).
Johnson, E. J. and Peck, H. D., J. Bacteriol. 80, 1041 (1965).
Kirtley, M. E. and Koshland, D. E., J. Biol. Chem. 242, 4192 (1967).
Lineweaver, H. and Burk, D., J. Am. Chem. Soc. 56, 658 (1934).
Mayeux, J. V. and Johnson, E. J., Bacteriol. Proc. No. P142, p. 94 (1966).
Monod, J., Wyman, J., and Changeux, J. P., J. Mol. Biol. 12, 88 (1965).
Pontremoli, S. and Mangiarotti, G., J. Biol. Chem. 237, 643 (1962).
Santer, M., Boyer, J., and Santer, V., J. Bacteriol. 78, 192 (1959).