Regulatory Roles of Cyclic 3',5'-AMP in Bacteria: Control of Malic Enzyme of Escherichia coli*

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Received April 7, 1969

A considerable amount of information is available regarding the regulatory functions of cyclic 3',5'-AMP in animals. The consensus has evolved that hormonal control in higher organisms is mediated largely through the agency of this nucleotide (Robison, Butcher and Sutherland, 1968). Despite the absence of hormonal regulation in bacteria, however, cyclic 3',5'-AMP has been shown to occur in Escherichia coli (Makman and Sutherland, 1965) and Brevibacterium liquefaciens (Okabayashi, Yoshimoto and Ide, 1963). Makman and Sutherland (1965) demonstrated that in E. coli during glucose starvation the concentration of cyclic 3',5'-AMP rose to levels as high as 10^{-4} M. Addition of an energy source to the starved bacteria led to an immediate excretion of the nucleotide and the return of in vivo levels to about 10^{-7} M. This fact combined with the observation that concentrations of cyclic 3',5'-AMP are higher in acetate grown compared to glucose grown cells (Makman and Sutherland, 1965) suggested to us that this nucleotide may control the activity of enzymes whose functioning is not necessary under conditions which lead to augmentation of cyclic 3',5'-AMP levels in vivo. One such enzyme in E. coli is the TPN-specific malic enzyme (malate: TPN oxidoreductase (decarboxylating); EC 1.1.1.40). We report below that cyclic 3',5'-AMP is an allosteric inhibitor of this enzyme. This, we believe, to be the

*Supported by a National Research Council of Canada grant.

first case reported in the literature where cyclic 3',5'-AMP is specifically shown to effect the activity of an enzyme from a microbial source.

Experimental - Malic enzyme from E. coli B cells was prepared and assayed as outlined earlier (Sanwal and Smando, 1969a, b). In the present work the enzyme from the DEAE-cellulose step (Sanwal and Smando, 1969a) was further purified by chromatography on a Sephadex G-200 column (2.5 x 40 cm) equilibrated with 0.05 M phosphate buffer, pH 7.0. Fractions showing a specific activity of higher than 42 were pooled and the enzyme was precipitated by the addition of 65% solid ammonium sulfate. The precipitate was dissolved in a small volume of 0.05 M phosphate, pH 7.0 and dialyzed overnight against the same buffer. This enzyme preparation was about 200-250 fold purified and had a specific activity of 58-65. It was used for all of the experiments reported below.

Results and Discussion - The inhibition of malic enzyme by cyclic 3',5'-AMP is shown in Fig. 1. It will be noted that the amount of the nucleotide required to cause half-maximal inhibition varies depending upon the concentration of malate used in the assay medium. Also, the inhibition curves obtained (Fig. 1) at all concentrations of malate are sigmoidal suggesting a multisite binding of cyclic 3',5'-AMP. The inhibition caused by cyclic 3',5'-AMP is specific. At a fixed malate concentration of 2.5 mM, the following compounds do not affect the enzyme activity: AMP (2 mM); ATP (2.5 mM); cyclic 2',3'-AMP (2 mM); ADP (1.5 mM); Coenzyme A (1.0 mM); Acetyl phosphate (4.0 mM).

As demonstrated earlier (Sanwal, Wright and Smando, 1968; Sanwal and Smando, 1969a, b) with less purified malic enzyme

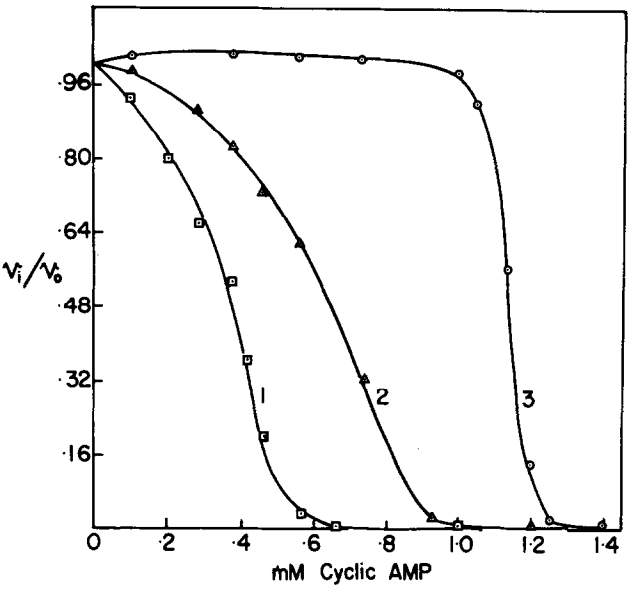

Fig. 1. The effect of cyclic 3',5'-AMP on the velocity of malic enzyme. The reaction mixture contained 0.05 M phosphate, pH 7.2, 0.077 mM TPN, 1 mM $MnCl_2$ and L-malate as indicated. 1 = 2 mM malate, 2 = 5 mM malate, 3 = 11.0 mM malate. v_o and v_i are initial velocities without and with the inhibitor respectively.

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preparations, the activity of the enzyme used in the present work is inhibited in an allosteric manner by acetyl-CoA, oxal-acetate and DPNH. In conformity with the earlier results also, the enzyme is 'desensitized' to these inhibitors when assays are performed in 0.6 M glycine, pH 7.0. In this regard the behaviour of cyclic 3',5'-AMP is completely different. In the presence of 0.6 M glycine in the assay medium neither the nature of the inhibition curve nor the extent of inhibition by cyclic 3',5'-AMP is altered to any significant extent.

We had shown earlier (Sanwal and Smando, 1969b) that the initial velocity plots for both of the substrates of malic enzyme

(TPN and malate) are hyperbolic in the absence of allosteric inhibitors but the plots for malate become sigmoidal in their presence (acetyl CoA, oxalacetate and DPNH). Such is, however, not the case with cyclic 3',5'-AMP. As can be seen from Fig. 2, cyclic 3',5'-AMP causes competitive inhibition when malate is the

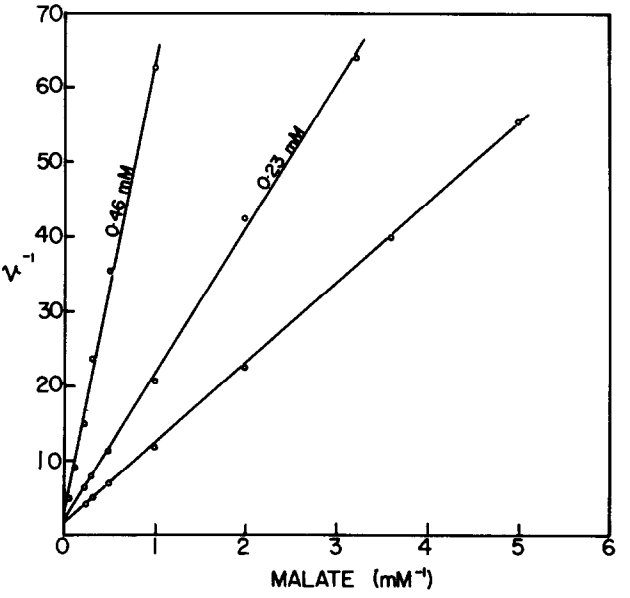

Fig. 2. Double reciprocal plots of initial velocity data with malate as the variable substrate and cyclic 3',5'-AMP as the inhibitor (concentration indicated above the lines). Concentration of other reactants is the same as in Fig. 1.

varied substrate, but the double reciprocal plots, unlike with other inhibitors mentioned earlier, remain linear. With TPN as the varied substrate, cyclic 3',5'-AMP (in common with other inhibitors) produces noncompetitive inhibition (Fig. 3).

While elucidation of the physical mechanism underlying the inhibitory effect of cyclic 3',5'-AMP will have to await the availability of malic enzyme in a homogeneous form, we feel that

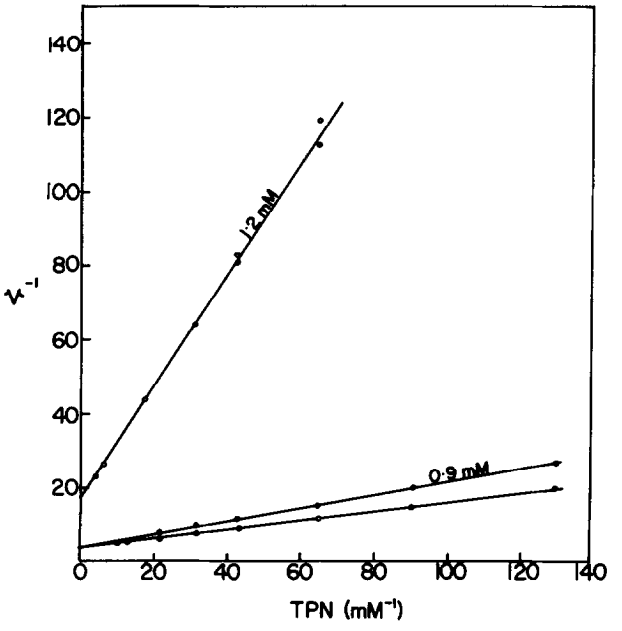

Fig. 3. Double reciprocal plots of initial velocity data with TPN as the variable substrate and cyclic 3',5'-AMP as the inhibitor. Malate concentration was 10 mM throughout.

the data presented here are sufficient to ascribe a physiological role to the inhibition of the enzyme by cyclic 3',5'-AMP. What this role might be is a matter of conjecture at the present time. Since malic enzyme is significantly inhibited at concentration (ranges ($\sim 10^{-4}$ M) of cyclic 3',5'-AMP which are only found in cells starved of an energy source (Makman and Sutherland, 1965) it is reasonable to suppose that this inhibition is directed towards prevention of wasteful (and unnecessary) reactions during starvation. Malic enzyme presumably qualifies as one of such dispensable enzymes. This presumption is supported by the observation (Raunio, 1966) that under normal conditions of growth, i.e., in the absence of the limitation of an energy source, E. coli secretes large quantities of pyruvate part of which possibly

arises by the withdrawal of malate from the Kreb's cycle by malic enzyme. Under normal conditions this diversion of malate to pyruvate may serve a useful purpose; for instance, this conversion may help generate TPNH for fatty acid synthesis. Under starvation conditions, however, when degradative processes take precedence over biosynthetic ones, the demand for reducing power may not be as great and it would help the cell economy if intermediates of Kreb's cycle are not drained away unnecessarily. The interpretation given above, if nothing else, has heuristic value and it is possible that one of the roles of cyclic 3',5'-AMP in microorganisms may be connected with mechanisms which help the organism to tide over unfavourable circumstances. This suggestion does not detract from the importance of other regulatory roles that cyclic 3',5'-AMP may have in E. coli such as its demonstrated role as a 'derepressor' of catabolic repression (Ullmann and Monod, 1968; Perlman and Pastan, 1968; Pastan and Perlman, 1968).

REFERENCES

Makman, R.S., and Sutherland, E.W., J. Biol. Chem., 240, 1309 (1965).
Okabayashi, T., Yoshimoto, A., and Ide, M., J. Bacteriol, 86, 930 (1963).
Pastan, I., and Perlman, R.L., Proc. Natl. Acad. Sci., 61, 1336 (1968).
Perlman, R.L., and Pastan, I., Biochem. Biophys. Res. Commun., 30, 656 (1968).
Raunio, R., Acta Chem. Scand., 20, 11 (1966).
Robison, G.A., Butcher, R.W., and Sutherland, E.W., Ann. Rev. Biochem., 37, 149 (1968).
Sanwal, B.D., Wright, J.A., and Smando, R., Biochem. Biophys. Res. Commun., 31, 623 (1968).
Sanwal, B.D., and Smando, R., J. Biol. Chem., 244, 1817 (1969a).
Sanwal, B.D., and Smando, R., J. Biol. Chem., 244, 1824 (1969b).
Ullmann, A., and Monod, J., Fed. Eur. Biochem. Soc. Letters, 2, 57 (1968).