ON THE EFFECT OF ADENOSINE 3',5' CYCLOPHOSPHATE ON THE KINASE OF UDPG:α-1,4-GLUCAN α-4-GLUCOSYL TRANSFERASE*

Fr. Huijing** and J. Larner

Department of Biochemistry, College of Medical Sciences
University of Minnesota, Minneapolis, Minnesota

Received March 28, 1966

Adenosine 3',5' cyclophosphate is known to stimulate the kinase which converts the glucose 6-phosphate independent (I) form of UDPG:α-1,4-glucan α-4-glucosyl transferase (EC 2.4.1.11) into the glucose 6-phosphate dependent form (D) (Friedman and Larner, 1963; Rosell-Perez and Larner, 1964; Appleman et al., 1964). This kinase can be distinguished from phosphorylase b kinase (Friedman and Larner, 1965). However, neither the sensitivity of the kinase for the cyclophosphate nor the mechanism of action of the cyclophosphate are known. The present paper assesses the sensitivity range and offers some information on the mechanism.

The transferase I kinase was tested in principle as described by Friedman and Larner (1965). To 0.1 ml of a solution containing ATP, $MgCl_2$ and adenosine 3',5' cyclophosphate (concentrations as indicated in Figures 1 and 2) was added 0.25 or 0.4 ml of a solution containing enzyme, 8 mM glycerophosphate pH 7.8, 2.5 or 5 mM caffeine, and 50 mM mercaptoethanol. After 7 min at 30° C, the kinase reaction was stopped by adding 0.1 ml EDTA, 100 mM, and 0.05 ml aliquots

- -

*This work was supported in part by a grant (No. AM 09071) from the National Institute of Arthritis and Metabolic Diseases of the U. S. Public Health Service.

**On leave of absence from the Department of Biochemistry of the University of Amsterdam, The Netherlands, recipient of a fellowship of the Netherlands Organization for the Advancement of Pure Research (Z.W.O.)

were then transferred to tubes containing 0.1 ml of the test mixture (without glucose 6-phosphate) for assay of transferase I activity as described by Villar-Palasi et al., (1966). The tubes were incubated for 15 min at 30° C and the incorporation of C^{14} glucose from UDPG into glycogen was determined. The activity of the transferase I kinase is expressed as a decrease in activity of the transferase I during the 7 minute kinase incubation, i.e., as a decrease in cpm incorporated into glycogen compared to controls where no ATP was added, or where EDTA was added before the reaction was started. Both control procedures gave comparable rates. In some tubes, the total (I+D) transferase activity was determined in the presence of glucose 6-phosphate. The total transferase activity did not change appreciably during the transferase I kinase test.

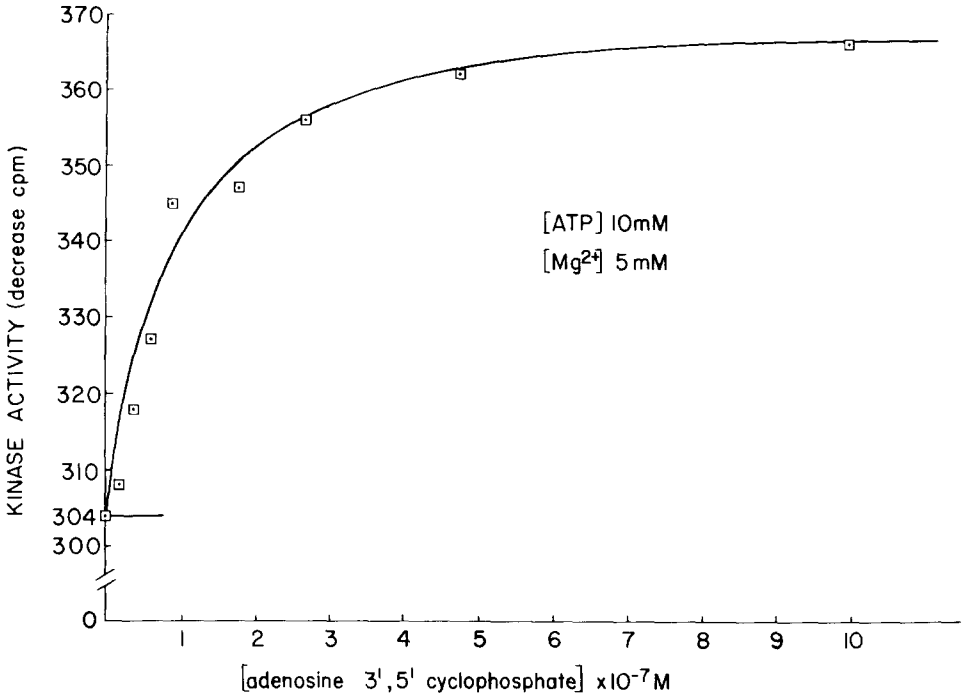

Fig. 1 Transferase I kinase activity at varying concentrations of adenosine 3',5' cyclophosphate.

0.4 ml of 51,000 x g supernatant containing 2.5 mM caffeine was added to 0.1 ml of a solution containing ATP and $MgCl_2$, final concentrations 10 mM and 5 mM and cyclophosphate as indicated.[2] The transferase I kinase reaction was allowed to proceed for 7 min, the transferase I activity was assayed for 15 min.

As a source of both transferase and its kinase rabbit muscle was homogenized in 3 volumes 50 mM Tris-HCl, pH 7.8 containing 5 mM EDTA; 100 mM KF and then centrifuged for 20 min at 12,000 x g. The resulting supernatant was centrifuged for 3 hours at 78,000 x g and the precipitate that was obtained from 10 g fresh muscle was homogenized in 5 ml of 8 mM glycerophosphate, pH 7.8 containing 50 mM mercaptoethanol. The enzyme solution was incubated for 45 min at 30° C and centrifuged for 45 min at 51,000 x g. To the clear supernatant, caffeine was added to a concentration of 2.5 or 5 mM.

Fig. 1 shows the increase in transferase I kinase activity caused by adenosine 3',5' cyclophosphate. Half maximal activation is obtained at approximately 6×10^{-8} M cyclophosphate. Under these assay conditions the maximal stimulation produced by the cyclic nucleotide represents a 20% increase in terms of the activity in the absence of added cyclic nucleotide.

Using a 12,000 x g supernatant of 10% homogenate of rat heart, half maximal activation is achieved with an identical concentration of the cyclic nucleotide and a total stimulation of the same magnitude is found. This sensitivity of the rat heart transferase I kinase is surprising in view of the fact that in the intact organ epinephrine, which increases the concentration of adenosine 3',5' cyclophosphate (Hammermeister et al., 1965; Robison et al., 1965), has no direct effect on the fraction of the transferase which is in the I form, (Williams and Mayer, 1965; Robison et al., 1965, Huijing, 1966). However, epinephrine causes a decrease in the glycogen concentration of the heart and this decrease results in a conversion of transferase D into I (Huijing, 1966).

The sensitivity of the transferase I kinase of skeletal muscle and heart for adenosine 3',5' cyclophosphate under these conditions is the same as that of the phosphorylase kinase system of skeletal muscle and liver (Posner et al., 1964; Butcher et al., 1965).

Figure 2 shows the activity of the transferase I kinase as a function of varying Mg^{2+} concentrations. Both curves with and without adenosine 3',5'

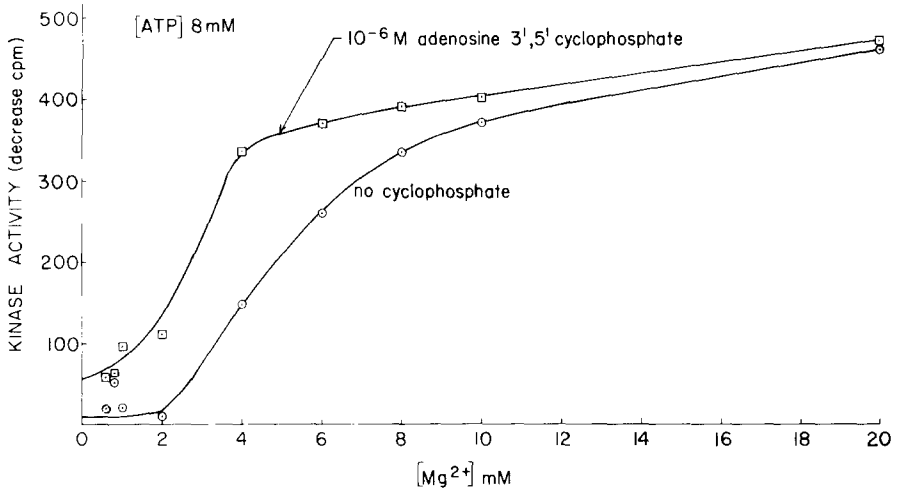

Fig. 2 Transferase I kinase activity at varying concentrations of $MgCl_2$.

0.25 ml of 51,000 x g supernatant containing 5 mM caffeine was added to 0.1 ml ATP (final concentration 8 mM) and adenosine 3',5' cyclophosphate where indicated. The transferase I kinase reaction was allowed to proceed for 7 min, the transferase I activity was assayed for 15 min.

cyclophosphate are sigmoidal. At low Mg^{2+} concentrations adenosine 3',5' cyclophosphate changes the rate from zero to a finite value. Under these conditions the cyclophosphate may exert a regulatory role. At 4 mM $MgCl_2$ it causes a 100% stimulation. At high Mg^{2+} concentrations there is no effect of the cyclophosphate under conditions where Mg^{2+} itself has maximally stimulated the kinase.

On the basis of kinetic analysis to be dealt with in a future publication it appears that these phenomena can be explained by the possibility that Mg^{2+} serves the dual role of activating the kinase, and being a substrate in the form of the Mg ATP complex. The activation, however, requires high concentrations of Mg^{2+}. Adenosine 3',5' cyclophosphate may act to increase the affinity of the Mg^{2+} for the activating site on the enzyme.

References

Appleman, H.M., Belocopitow, E. and Torres, H.H., Biochem. Biophys. Res. Comm., 14, 550 (1964).

Butcher, R.W., Ho, R.J., Meng, H.C. and Sutherland, E.W., J. Biol. Chem., 240, 4515 (1965).
Friedman, D.L. and Larner, J., Biochemistry, 2, 669 (1963).
Friedman, D.L. and Larner, J., Biochemistry, 4, 2261 (1965).
Hammermeister, K.E., Yunis, A.A. and Krebs, E.G., J. Biol. Chem., 240, 986 (1965).
Huijing, F., Fed. Proc., 25, 449 (1966).
Posner, J.B., Hammermeister, K., Bratvold, G.E. and Krebs, E.G., Biochemistry, 3, 1040 (1964).
Robison, G.A., Butcher, R.W., Øye, I., Morgan, H.E. and Sutherland, E.W., Mol. Pharmacol., 1, 168 (1965).
Rosell-Perez, M. and Larner, J., Biochemistry, 3, 75 (1964).
Villar-Palasi, C., Rosell-Perez, M., Hizukuri, S., Huijing, F. and Larner, J. in Methods in Enzymology (Ed. S.P. Colowick and N.O. Kaplan) Vol. VIII (64) (1966).
Williams, B.J. and Mayer, S.E., Fed. Proc., 24, 151 (1965).