DISSOCIATION OF RABBIT RED BLOOD CELL CYCLIC AMP-DEPENDENT PROTEIN KINASE I BY PROTAMINE

Mariano Tao

Department of Biological Chemistry
University of Illinois at the Medical Center
Chicago, Illinois 60612

Received October 14, 1971

Summary When protamine is used as the protein substrate for the rabbit red blood cell cyclic AMP-dependent protein kinase I, no dependency on cyclic AMP is observed. It appears that protamine is capable of interacting with the regulatory subunit of kinase I, leading to the dissociation of the regulatory subunit from the catalytic subunit. This releases the catalytic moiety for enzymic activity. The results suggest an alternative mechanism by which protein kinase I may be activated by protein-protein interaction without the participation of the cyclic nucleotide.

Since the isolation by Walsh et al. (1) of an adenosine 3':5'-cyclic monophosphate-dependent (cyclic AMP-dependent) protein kinase from rabbit skeletal muscle, work in this area has progressed rapidly. The mechanism of activation of these kinases by cyclic AMP appears to involve the dissociation of the enzyme into two dissimilar functional subunits. We have shown (2,3), as have others (4-8), that cyclic AMP activates the enzyme by binding to one of the subunits, the regulatory subunit, causing it to dissociate from the catalytic subunit. It is in this dissociated form that the catalytic subunit carries out its enzymic function.

Cyclic AMP-dependent protein kinases from different sources have been shown to phosphorylate a wide variety of proteins (1,2,9-11). However, the degree of stimulation of protein kinase activity by cyclic AMP seems to depend on the phosphoryl acceptor used (2,9-11). During the course of our studies on the properties of rabbit red blood cell protein kinases (2), we have found that when protamine, casein, or phosvitin is used as substrate, little if any

cyclic AMP effect is observed. In order to explain this phenomenon, we have investigated in greater detail the interaction between protamine and protein kinase I from rabbit red blood cells. In this communication, evidence is presented indicating that protamine may cause the dissociation of kinase I in the absence of cyclic AMP. Recently, Miyamoto et al. (12) also reported that bovine brain protein kinase may be dissociated by some protein substrates.

Materials and Methods

Materials.- ATP-γ-^{32}P and cyclic AMP-^{3}H were obtained from New England Nuclear. Cyclic AMP was purchased from Schwarz BioResearch. Calf thymus histone, lysine-rich histone, and salmon protamine were supplied by Sigma Chemical Co. All substrates were titrated with either KOH or HCl to pH 8.0-8.5 before being used.

Assay for protein kinase I.- Enzyme activity was determined as described previously (3) with slight modifications. The incubation mixture contained 0.2 M Tris-HCl, pH 8.5; 4 mM $MgCl_2$; 0.2 mM ATP-γ-^{32}P; 1.8 mg/ml of histone; $\pm 5 \times 10^{-6}$ M cyclic AMP; and enzyme protein in a final volume of 0.2 ml. Incubation was carried out at 37° for 5 min; and the reaction terminated by the addition of 0.6 mg of bovine serum albumin followed by 2 ml of 10% trichloroacetic acid (TCA). The protein precipitate was collected on Whatman GF/C glass fiber paper, washed with 20 ml of 10% TCA, and counted in 5 ml of Bray's solution (13). Protein concentration was determined by the method of Lowry et al. (14) using bovine serum albumin as standard.

Assay for cyclic AMP binding activity.- The binding of cyclic AMP-^{3}H to protein kinase I was assayed by the Millipore filtration technique as described previously (3).

Preparation of protein kinase I.- Erythrocytes were obtained from Pel-Freez Biological, Inc. Protein kinase I was eluted from a DEAE column at a concentration of about 0.1 M KCl as described previously (2). Further purification of kinase I was carried out by applying the dialyzed DEAE fractions (5 ml) to a Sephadex G-150 column (2.5 x 80 cm). The column had been pre-

viously equilibrated with a buffer containing 0.02 M Tris-HCl, pH 7.5, and 1 mM dithiothreitol (buffer A). Elution was carried out using buffer A at a flow rate of 4 ml/hr. The kinase activity peak fractions were pooled, precipitated with 50% $(NH_4)_2SO_4$, dissolved, and dialyzed in 0.05 M potassium phosphate buffer, pH 6.8, and applied to a hydroxylapatite column (2 x 5 cm). Kinase I was eluted using a linear gradient of 0.05 M to 0.25 M potassium phosphate buffer. The kinase peak fractions were pooled, precipitated with 50% $(NH_4)_2SO_4$, dissolved in buffer A, and dialyzed overnight against this buffer. The enzyme solution was stored in liquid nitrogen.

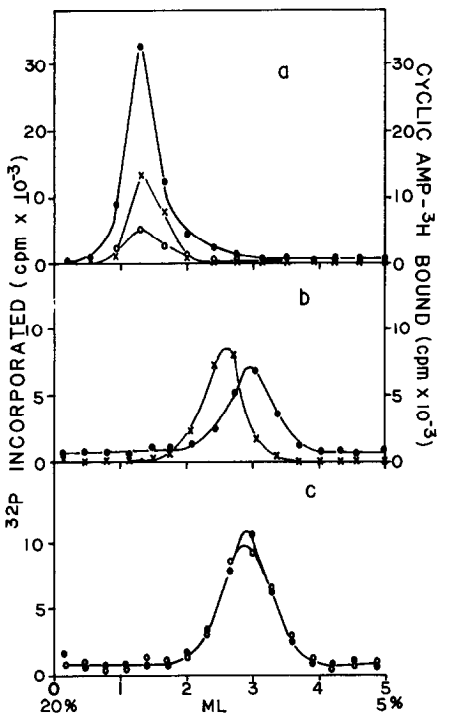

Figure 1. Sucrose density gradient centrifugation of kinase I under different conditions. Sucrose density gradient centrifugation was carried out in an SW50L rotor at 48,000 rpm for 15 hours at 1°C, according to the procedure of Martin and Ames (15). Three-drop fractions were collected from the bottom of the tube and 25 µl of alternate fractions was used for the determination of either kinase or cyclic AMP binding activity. The following solution was layered over a 5-20% sucrose gradient: (a). 0.1 ml of a solution containing 1 mg/ml of kinase I; (b) 0.1 ml of a solution containing 1 mg/ml of kinase I and 1 x 10^{-6} M cyclic AMP-^{3}H. The solution had been preincubated at 37°C for 3 min before applying to the gradient. The sucrose gradient also contained 1 x 10^{-6} M cyclic AMP-^{3}H; (c) 0.1 ml of a solution containing 1 mg/ml of kinase I which had been preincubated with 0.6 mg/ml of protamine. Kinase activity was determined either in the presence (●——●) or absence (○——○) of cyclic AMP. Cyclic AMP-^{3}H bound (×——×). Specific activity of ATP-γ-P^{32} = 26 cpm/pmole; cyclic AMP-^{3}H = 8.6 x 10^3 cpm/pmole. All the sucrose solutions were prepared in buffer A.

Results and Discussion

Figure 1 shows the sedimentation profile of kinase 1 in a sucrose density gradient under different conditions. The control experiment showed that the catalytic and binding activity of kinase I (Fig. 1a) sedimented as a single peak. When the enzyme was preincubated with cyclic AMP-^{3}H and centrifuged in a gradient containing cyclic AMP-^{3}H, the catalytic and the binding activity appear to dissociate from each other as shown in Figure 1b. This was previously suggested as the mechanism of activation of kinase I by cyclic AMP (2,3). A similar mechanism of activation of cyclic AMP-dependent protein kinases from other sources has also been proposed by several laboratories (4-8). Interestingly, when kinase I was preincubated with protamine and sedimented in a sucrose gradient (Figure 1c), the kinase activity was found to sediment at a position similar to that obtained in Figure 1b. The activity of this kinase peak was found not to be dependent on cyclic AMP. It was not possible to detect any cyclic AMP binding activity in the same sucrose gradient. It is possible that when the regulatory subunit is dissociated from the catalytic subunit, it becomes more labile. However, it was previously shown that the regulatory subunit appeared to be stabilized toward heat denaturation in the presence of cyclic AMP. Therefore, an experiment similar to Figure 1c was carried out except that cyclic AMP-^{3}H was included both in the preincubation mixture and in the sucrose gradient. Here, as shown in Figure 2, in addition to kinase activity, a cyclic AMP binding activity was also detected. However, the binding activity appeared to be sedimented at the bottom of the tube indicating the regulatory subunit had formed a complex with protamine. Protamine did not seem to affect the binding of cyclic AMP to the regulatory subunit. In contrast to the recent observation by Miyamoto et al. (12) on the behavior of bovine brain protein kinase, both histones "mixture" and lysine-rich histones had no effect on the sedimentation of kinase 1 (same as Figure 1a).

The results reported above suggest an explanation for the slight depen-

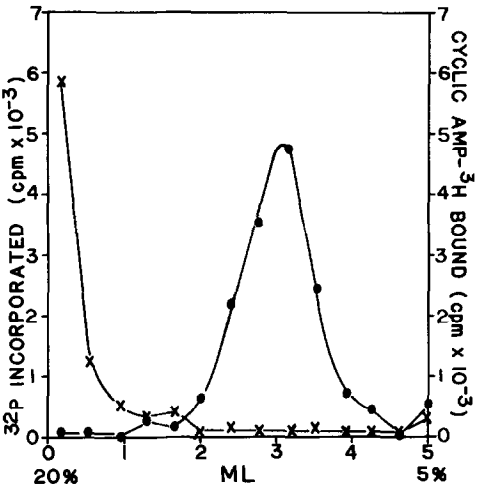

Figure 2. Sedimentation of kinase 1 preincubated with protamine and cyclic AMP-^{3}H. Centrifugation was carried out as described in Figure 1c except 10^{-6} M cyclic AMP-^{3}H was present in both the preincubation mixture and in the gradient. Kinase activity was determined in the presence of cyclic AMP (●——●); cyclic AMP-^{3}H bound (×——×). Specific activity of ATP-γ-^{32}P = 25 cpm/pmole; cyclic AMP-^{3}H = 8.6 x 10^{3} cpm/pmole.

dency on cyclic AMP for the phosphorylation of protamine (or perhaps other proteins) by protein kinase 1. The experiment clearly demonstrated that protamine could bind to the regulatory subunit of kinase 1 causing it to dissociate from the catalytic subunit, thereby releasing the latter for enzymic activity. This suggests an interesting alternative mechanism for the activation of kinase 1 without the participation of cyclic AMP.

Acknowledgments

I wish to acknowledge the valuable assistance of Mrs. Jane E. Baer and Patricia Hackett in carrying out the reported experiments and in the preparation of this manuscript. I also wish to thank Dr. Charalampos Arsenis for the use of his ultracentrifuge. This work was supported, in part, by Grant GB-27435 from the National Science Foundation.

References

1. Walsh, D. A., Ashby, C. D., Gonzalez, C., Calkins, D., Fischer, E. H., and Krebs, E. G. (1971), J. Biol. Chem. 246, 1977.

2. Tao, M., Salas, M. L., and Lipmann, F. (1970), Proc. Nat. Acad. Sci. U. S. A. 67, 408.

3. Tao, M. (1971), Arch. Biochem. Biophys. 143, 151.

4. Gill, G. N., and Garren, L. D. (1970), Biochem. Biophys. Res. Commun. 39, 335.

5. Gill, G. N., and Garren, L. D. (1971), Proc. Nat. Acad. Sci. U. S. A. 68, 786.

6. Kumon, A., Yamamura, H., and Nishizuka, Y. (1970), Biochem. Biophys. Res. Commun. 41, 1290.

7. Reimann, E. M., Brostrom, C. O., Corbin, J. D., King, C. A., and Krebs, E. G. (1971a), Biochem. Biophys. Res. Commun. 42, 187.

8. Erlichman, J., Hirsch, A. H., and Rosen, O. M. (1971), Proc. Nat. Acad. Sci. U. S. A. 68, 731.

9. Langan, T. A. (1968), Science, 162, 579.

10. Miyamoto, E., Kuo, J. F., and Greengard, P. (1969), Science 165, 63.

11. Kuo, J. F., Krueger, B. K., Sanes, J. R., and Greengard, P. (1970), Biochim. Biophys Acta. 212, 79.

12. Miyamoto, E., Petzold, G. L., Harris, J. S., and Greengard, P. (1971), Biochem. Biophys. Res. Commun. 44, 305.

13. Bray, G. A. (1960), Anal. Biochem. 1, 279.

14. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951), J. Biol. Chem. 193, 265.

15. Martin, R. G., and Ames, B. N. (1961), J. Biol. Chem. 236, 1372.