RABBIT RED CELL CYCLIC AMP-DEPENDENT PROTEIN KINASE I: REVERSIBLE SUBUNIT INTERACTION

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Summary: Cyclic AMP-dependent protein kinase I consists of two dissimilar functional subunits: a catalytic subunit and a cyclic AMP binding subunit. The interaction of the two subunits appears to be reversible.

Adenosine 3',5'-monophosphate-dependent (cyclic AMP-dependent) protein kinases have been isolated from a wide variety of sources (1-5). During the course of our investigation regarding the role of cyclic AMP in rabbit red blood cells, we have demonstrated the presence of two cyclic nucleotide-dependent protein kinases (6). The occurrence of multiple forms of protein kinases have subsequently been demonstrated in other systems (7-9). However, it is not known whether these enzymes phosphorylate similar or different intracellular substrates.

Our studies on the protein kinases in the red cell system led us to propose a mechanism for the action of cyclic AMP on protein kinase I (6,10). The enzyme appears to consist of two dissimilar functional subunits: a regulatory subunit and a catalytic subunit. The enzyme is inactive in its complex form. The activation process involves the binding of cyclic AMP to the regulatory subunit resulting in the dissociation of the complex. It is in this dissociated form that the catalytic subunit carries out its enzymic function. Cyclic AMP-dependent protein kinases isolated from other tissues were also found to be activated through a

similar mechanism (11-14). This communication further shows that the interaction of the regulatory and catalytic subunits of the red cell kinase I is fully reversible.

Cyclic AMP-dependent protein kinase I was isolated from rabbit erythrocytes as described (15). The catalytic subunit was prepared using the protamine binding procedure (15). As was shown previously, incubation of kinase I with protamine led to the dissociation of the catalytic subunit from the regulatory subunit. Protamine formed a heavy complex with the regulatory subunit causing it to separate completely from the catalytic moiety upon sucrose gradient centrifugation. The activity of the catalytic subunit thus isolated was found to be independent of cyclic AMP.

The method for the preparation of the regulatory component of kinase I was based on the observation that the two subunits may be differentially heat inactivated in the presence of a cyclic nucleotide (16).

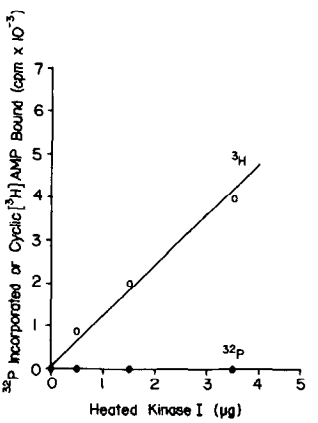

Fig. 1: The catalytic and binding activity of kinase I after heating in the presence of cyclic GMP. One ml of kinase I was heated in the presence of 2×10^{-4} M cyclic GMP at 53° C for 5 min. The heated enzyme was dialyzed against 2 liters of a buffer consisting of 0.02 M Tris-HCl, pH 7.5, and 1 mM dithiothreitol, for 5 hr. The final enzyme concentration was 0.05 mg/ml. Kinase activity (●-●) was determined in the presence of cyclic AMP using lysine-rich histones as the phosphoryl acceptor (15). The binding activity (o-o) was determined by Millipore filtrations as described previously (15). γ-^{32}P-ATP = 25 cpm/pmole. Cyclic ^{3}H-AMP = 4.3×10^{3} cpm/pmole.

Kinase I was heated with 2 x 10^{-4} M cyclic GMP at 53° C for 5 min. Under these conditions the catalytic activity was lost while cyclic GMP seems to have protected the binding activity. Cyclic GMP was used in place of cyclic AMP because the enzyme appears to have a weaker affinity for this cyclic nucleotide, thus facilitating the removal of the cyclic nucleotide by dialysis after heating. Figure 1 shows that the heated enzyme fraction after dialysis retained most of its regulatory subunit activity as measured by its ability to bind cyclic ^{3}H-AMP. The catalytic activity as measured by the enzyme's ability to phosphorylate lysine-rich histones using γ-^{32}P-ATP as the phosphoryl donor, appeared to be completely abolished.

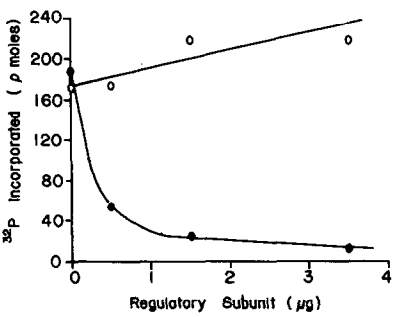

Fig. 2: Inhibition of the activity of the catalytic subunit of kinase I by the regulatory subunit. The catalytic subunit obtained from the dissociation of kinase I by protamine was isolated using sucrose gradient centrifugation (15). The activity of the catalytic subunit was determined using lysine-rich histones as the phosphoryl acceptor as a function of varying amounts of the regulatory subunit in the presence (o-o) and absence (•-•) of cyclic AMP. The regulatory subunit was isolated as described under Fig. 1. The concentration of the catalytic fraction in the assay mixture was 15 µg/ml. γ-^{32}P-ATP = 25 cpm/pmole.

When an increasing amount of this heated enzyme fraction, which contained only the regulatory subunit activity, was added to the catalytic subunit isolated from the protamine sucrose gradient, a progressive inhibition of the kinase activity was observed (Fig. 2). An inhibition of up to 85% of the kinase activity was obtained. No inhibition occurred when cyclic AMP was also present in the incubation mixture.

We conclude from these observations that the interaction of the regulatory and the catalytic subunits of kinase I is fully reversible. It is suggested that the catalytic activity of the protein kinase may be modulated in this manner *in vivo* in response to intracellular concentrations of cyclic AMP. Cyclic AMP-dependent protein kinases isolated from other sources also appear to undergo reversible subunit interactions (11-14).

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