DISSOCIATION AND CONCOMITANT ACTIVATION OF ADENOSINE 3',5'-MONOPHOSPHATE-DEPENDENT PROTEIN KINASE BY HISTONE*

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Summary:

Adenosine 3',5'-monophosphate-dependent (cAMP-dependent) protein kinase from bovine brain has been examined after sedimentation in a sucrose density gradient. The molecular weight of the catalytic and of the cAMP binding protein decreased in the presence of low concentrations of either histone or cAMP, indicating that the enzyme had dissociated into subunits. The dissociation in the presence of histone was accompanied by conversion of the enzyme activity from a cAMP-dependent to a cAMP-independent form.

The hypothesis that the numerous and diverse biological effects of cAMP are mediated through activation of a family of protein kinases, first proposed by our laboratory about two years ago (1,2), has received increasing experimental support. In view of the physiological significance of these cAMP-dependent protein kinases, a knowledge of factors regulating the activity of these enzymes is of considerable importance. Several laboratories (3-8) have recently presented evidence suggesting that cAMP-dependent protein kinase from various tissues consists of a regulatory (inhibitory) subunit, which binds cAMP, and a catalytic subunit, and that the mechanism by which cAMP activates protein kinases involves removal of the inhibitory subunit, yielding the active (cAMP-independent) form of the enzyme. In the course of studying purified cAMP-dependent protein kinase from bovine brain, we have observed, as with kinases from other tissues, that cAMP is capable

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of causing the dissociation of the enzyme into subunits. In addition, we have found that histone, a good substrate for the enzyme, is capable, at low concentrations, of causing the dissociation and concomitant activation of the enzyme.

The cAMP-dependent protein kinase used in this investigation was prepared from bovine brain by a modification (9) of the purification procedure of Miyamoto et al. (10). The two active peaks obtained after column chromatography on hydroxylapatite, designated Protein Kinases I and II, respectively, were used as enzyme preparations. Sucrose density gradient centrifugation was carried out according to the procedure described by Martin and Ames (11), using catalase (M.W. 232,000), glyceraldehyde-3-phosphate dehydrogenase (M.W. 140,000), and horse liver alcohol dehydrogenase (M.W. 80,000) as markers in each experiment. Protein kinase activity was assayed in a final volume of 0.2 ml as described previously (12). The reaction was started by the addition of γ-^{32}P-ATP. Mixed histone (calf thymus) was used as the histone preparation, except where noted. All histones were commercial preparations. cAMP binding activity of protein was determined by the method of Gilman (13) in the absence of protein kinase inhibitor.

When Protein Kinase I was preincubated in phosphate buffer, and then centrifuged in a sucrose density gradient, cAMP-dependent catalytic activity appeared as two peaks which ran in positions corresponding to molecular weights of 140,000 and 80,000 on our standard calibration curve (Fig. 1A). Under the same conditions, cAMP-dependent catalytic activity of Protein Kinase II appeared as a single peak with an apparent molecular weight of 140,000 (Fig. 1B). When either enzyme preparation was preincubated with either 200 μg per ml of histone or 5 x 10^{-6}M cAMP, and then centrifuged in a sucrose gradient containing that amount of histone or cAMP, the catalytic activity shifted to a position corresponding to a protein of approximately M.W. 80,000. When either enzyme preparation was preincubated with both

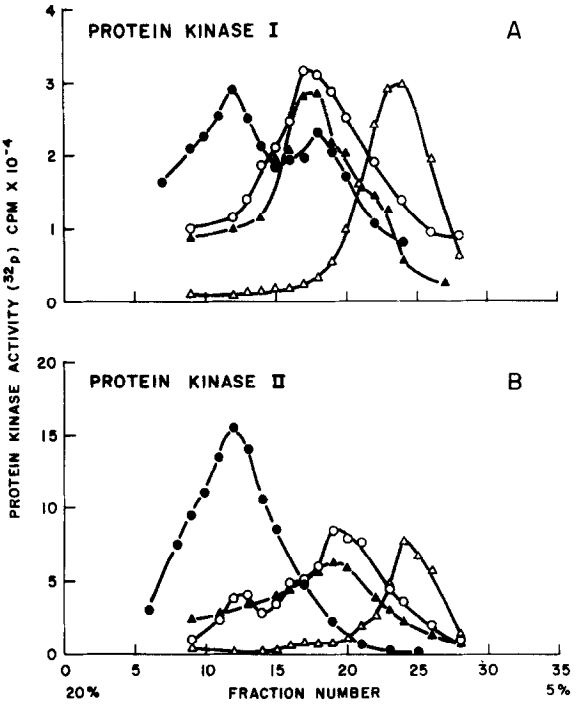

Fig. 1. Sucrose density gradient centrifugation of Protein Kinase I and Protein Kinase II in phosphate buffer. 0.22 ml of Protein Kinase I (1.6 mg/ml) or Protein Kinase II (1.5 mg/ml) was preincubated at 30° for 5 min in a solution of the same composition, except for the omission of sucrose, as was to be present in the respective centrifuge tube, and then was layered onto 4.8 ml of a 5-20% sucrose gradient. In addition to the 5-20% sucrose gradient, and 5 mM potassium phosphate buffer, pH 7.0, the centrifuge tubes contained the following components: •—•, none; ▲—▲, histone, 200 μg per ml; o—o, cAMP, 5 x 10^{-6}M; ▲—▲, histone, 200 μg per ml, plus cAMP, 5 x 10^{-6}M. After centrifugation was carried out at 37,500 x g for 16 hours in an SW 39L rotor at 3°, the bottom of the tube was punctured and 7-drop fractions (about 0.14 ml) were collected. Kinase activity was assayed in the presence of 5 x 10^{-6} M cAMP.

histone and cAMP and then sedimented in a sucrose gradient containing these components, enzyme activity appeared as a peak corresponding to protein of M.W. 40,000. Thus, preincubation of either Protein Kinase I or Protein Kinase II with histone caused a decrease in the apparent molecular weight, both in the presence as well as in the absence of cAMP.

Experiments were also carried out in which Protein Kinase I or Protein Kinase II was preincubated, and then centrifuged, in the presence of a mixture containing several of the components used in the assay of protein

kinase, instead of in 5 mM phosphate buffer. This mixture ("acetate-mixture") contained 50 mM acetate buffer, pH 6.0, 5 x 10^{-6}M ATP, 10 mM Mg acetate, 10 mM NaF, 2 mM theophylline, and 0.3 mM ethylene glycol bis (β-aminoethyl ether)-N,N'-tetraacetic acid (EGTA). The results obtained using the acetate-mixture, alone or with histone, were similar, respectively, to the results (Fig. 1) obtained with phosphate buffer alone or with histone. However, the ability of cAMP to dissociate either Protein Kinase I or II was enhanced in the presence of the acetate-mixture, as indicated by the fact that when either enzyme was preincubated with cAMP in the acetate-mixture, the protein kinase activity shifted entirely to a position of about M.W. 40,000.

When Protein Kinase I or II was preincubated with histone, in either phosphate buffer or the acetate-mixture, and then assayed in the absence of cAMP, a peak of protein kinase activity was observed in a position corresponding to about M.W. 40,000. (Such a peak was not present if histone was omitted during preincubation and centrifugation.) When the same fractions were assayed in the presence of cAMP, a shoulder (e.g. Fig. 1A), or even a distinct peak, of catalytic activity appeared on the low molecular weight slope of the M.W. 80,000 peak in about 90% of the experiments. This shoulder or peak could be quantitatively accounted for by the cAMP-independent protein kinase activity.

In all experiments in which we determined the effect of histone or of cAMP on the position of protein kinase activity in a sucrose gradient, we also determined cAMP-binding activity on the same fractions. Under all conditions in which a peak of protein kinase activity was found at a position corresponding to M.W. 140,000 or M.W. 80,000, a peak of cAMP-binding activity was also found in that position. These results indicate that both histone and cAMP caused the conversion of the cAMP-binding protein, as well as of the catalytic protein, to a substance of lower molecular weight. Under conditions in which the catalytic moiety of the protein kinase was converted

to a protein of about M.W. 40,000, cAMP-binding activity was largely lost, presumably due to instability of the free cAMP-binding subunit.

A few proteins other than histone were tested in the acetate-mixture for their ability to dissociate Protein Kinase II. In the presence of bovine serum albumin (500 μg per ml), fructose-6-phosphokinase (200 μg per ml), or poly-L-serine (200 μg per ml), the peak of catalytic activity remained at M.W. 140,000; in the presence of casein (3 mg per ml), the peak of catalytic activity shifted to M.W. 80,000; in the presence of protamine (500 μg per ml) or poly-L-lysine (200 μg per ml), most of the catalytic activity was lost; the small amount of surviving catalytic activity showed two peaks, one at M.W. 80,000 and one at M.W. 40,000. In these and all other studies in which preincubation and centrifugation were carried out in the absence of cAMP, so that cAMP-independent catalytic activity could be measured, all catalytic peaks at M.W. 140,000 and at M.W. 80,000 were cAMP-dependent, and all those at M.W. 40,000 were cAMP-independent.

Any interpretation of the present data in terms of subunit structure of the protein kinases from bovine brain must be made with reservation, in view of the limitations inherent in measuring molecular weights of proteins by sucrose gradient centrifugation. Nevertheless, the results do suggest plausible models for the quaternary structure of these protein kinases. For example, the data are compatible with a structure for brain protein kinase consisting of a tetramer, containing two catalytic and two regulatory (inhibitory) subunits, which splits in the presence of histone or cAMP to a dimer containing one catalytic and one regulatory subunit, and further, to separate catalytic and regulatory monomeric subunits.

The partial conversion of protein kinase, in the presence of histone, to a cAMP-independent form, observed in the sucrose gradient experiments, could also be demonstrated simply by preincubation of the enzyme with histone, followed by assay of protein kinase activity in the absence and presence of cAMP. Thus, preincubation of Protein Kinase I in the presence

of histone (1 to 40 μg per tube) caused a substantial increase in the activity of the enzyme in the absence of cAMP, with a corresponding decrease in the stimulatory effect of cAMP. In contrast, bovine serum albumin (100 μg per tube), while largely protecting the enzyme from denaturation during preincubation, neither increased the activity of the enzyme in the absence of cAMP nor affected the percent stimulation by cAMP. Preincubation of the enzyme with 40 μg per tube of any of fifteen commercial enzymes used as test proteins, in the presence or absence of bovine serum albumin, had no effect, apart from providing some protection against denaturation, on the ability of Protein Kinase I to phosphorylate histone in the absence or presence of cAMP. Some typical results are shown in Table 1. Under the same conditions as used in the experiment of Table 1, 40 μg per tube of protamine or poly-L-lysine caused an increase in cAMP-independent protein kinase activity, but poly-L-serine, casein, L-lysine and L-arginine did not.

TABLE 1

Specificity of activation of protein kinase by protein

Protein Kinase I (2.2 μg) was preincubated for 10 min at 30° in 10 μmoles acetate buffer, pH 6.0, 2 μmoles Mg acetate, 2 μmoles NaF, 0.4 μmole theophylline, 100 μg bovine serum albumin, and 0.06 μmole EGTA, in the presence of the test protein indicated, in a volume of 0.16 ml. Between preincubation and assay, all tubes were placed at 0° and were adjusted to contain 40 μg test protein plus 40 μg histone, in a final volume of 0.2 ml, except for tubes in the rows labeled histone, which were adjusted to contain 40 μg histone only. Cyclic AMP was then added, as indicated, and the reaction started by addition of γ-^{32}P-ATP.

Test Protein	Preincubation -Test Protein		Preincubation +Test Protein		No Preincubation	
	Assay		Assay		Assay	
(μg per tube)	-cAMP	+cAMP	-cAMP	+cAMP	-cAMP	+cAMP
	Units		Units		Units	
Histone, 40	9.0	81.7	19.4	70.0	9.9	71.7
Histone, 5			19.5	73.4		
Acetylcholinesterase, 40	9.3	81.1	9.9	85.2	10.6	79.3
Horse liver alcohol dehydrogenase, 40	7.8	68.0	9.0	72.3	9.3	73.8

As was the case with the mixed histone used in most of the studies, lysine-rich histone, slightly lysine-rich histone, and arginine-rich histone were effective, at very low concentrations, in causing the activation of protein kinase. For instance, cAMP-independent protein kinase activity could be increased upon preincubation with as little as 1μg lysine-rich histone per 0.16 ml preincubation mixture. This corresponds to a histone concentration of about 3×10^{-7}M, the molecular weight of lysine-rich histone being about 21,000.

The relatively high specificity of histone in causing the dissociation and activation of protein kinase, and the extremely low concentration of histone required to bring about the activation of the enzyme, make it worth considering the possibility that substrate activation of protein kinases may be a physiological regulatory device. In this connection, we have observed that cAMP-dependent protein kinase from lobster tail muscle can also be dissociated and activated by cAMP, cyclic GMP, cyclic IMP, histone, or protamine, and, interestingly, so can cyclic GMP-dependent protein kinase from that source (14). It will be important to determine whether other natural substrates for protein kinases, e.g. phosphorylase kinase, glycogen synthetase, lipase and RNA polymerase (15-18) are able to dissociate and activate this class of enzymes. Activation of protein kinases by substrates could represent an important mechanism in the regulation of biological processes.

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