SUBUNIT STRUCTURE OF CYCLIC GMP-DEPENDENT PROTEIN KINASE FROM GUINEA PIG FETAL LUNG: DISSOCIATION OF HOLOENZYME BY CYCLIC GMP AND HISTONE

J. F. Kuo, Janice G. Patrick and Virginia L. Seery

Departments of Pharmacology and Biochemistry, Emory University School of Medicine, Atlanta, Georgia 30322

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SUMMARY: The subunit structure of cyclic GMP-dependent protein kinase from guinea pig fetal lungs was investigated. By employing Sephadex G-200 gel filtration, it was shown that a complete dissociation of the protein kinase holoenzyme (mol wt 160,000) to the catalytic subunit (mol wt 60,000) was achieved in the presence of both cyclic GMP (200 μM) and histone (200 μg/ml). The same concentration of cyclic AMP was unable to substitute for cyclic GMP. When the holoenzyme was incubated with histone and cyclic [G-^{3}H]GMP, the bound radioactivity, presumably to the regulatory subunit of protein kinase, was eluted at a position corresponding to molecular weight of 30,000. Dissociation of the cyclic GMP-dependent holoenzyme (7.3 S) into cyclic GMP-independent catalytic subunit (4.9 S) was also demonstrated by sucrose density gradient centrifugation. The phosphotransferase activity of the isolated catalytic subunit was augmented by the stimulatory modulator. The inhibitory modulator was without effect on its activity.

Several laboratories have demonstrated that adenosine 3':5'-monophosphate causes dissociation of inactive holoenzyme of cyclic AMP - dependent protein kinase into active catalytic subunit and regulatory subunit that binds cyclic AMP (1-6). Subsequently, cyclic GMP was shown to cause dissociation of cyclic GMP-dependent protein kinase from lobster tail muscle into analogous subunits, i.e., catalytic subunit and cyclic GMP-binding regulatory subunit (6). The catalytic subunit of the cyclic GMP-dependent enzyme, in contrast to that from cyclic AMP-dependent protein kinase, requires protein kinase modulator for activity (7).

It is the purpose of the present investigation to determine whether cyclic GMP-dependent protein kinase from mammalian tissues recently reported (8-12) also consists of subunits. We found that the inactive holoenzyme from guinea pig fetal lungs is made of subunits. Moreover, the isolated catalytic subunit requires stimulatory modulator

(13-16), or crude protein kinase modulator containing the stimulatory component, for activity, as in the case of the holoenzyme in the presence of cyclic GMP (8-10).

EXPERIMENTAL PROCEDURE

Cyclic GMP-dependent protein kinase was purified through the step of Sephadex G-200 (10), and cyclic AMP-dependent protein kinase was purified through the step of DEAE-cellulose (17), both enzymes from the guinea pig fetal lung. The enzyme solutions were concentrated on Amicon PM30 membrane, and were stored at -100° for about 1 to 2 months. Conditions employed for the dissociation of the cyclic GMP-dependent enzyme were indicated in the legends to the figures. Sucrose density gradient centrifugation was carried out as for the cyclic GMP-dependent holoenzyme described earlier (10), in which the sedimentation coefficients and molecular weights of the holoenzyme and the catalytic subunit were determined by the method of Martin and Ames (18). Estimation of the molecular weights by Sephadex G-200 filtration was carried out by the method of Leach and O'Shea (19). The catalytic subunit of the pulmonary cyclic AMP-dependent enzyme was prepared by incubating the holoenzyme with histone (1 mg per ml) followed by sucrose density gradient centrifugation, as described earlier for the brain enzyme (6). The crude protein kinase modulator and its stimulatory and inhibitory modulator components, all from the dog heart, were prepared by the method recently reported (10, 13-15). The conditions for assaying protein kinase activity, reagents used in the assays, and the definition of the enzyme activity were the same as described elsewhere (9,10).

RESULTS AND DISCUSSION

Dissociation of cyclic GMP-dependent protein kinase under various conditions, as visualized by filtration on Sephadex G-200 columns, is illustrated in Fig. 1. The activity peak of the holoenzyme, incubated in the absence of dissociating agents, appeared around fractions 22 and 23 (Fig. 1A). Incubation with 2 μM of cyclic GMP and mixed histone (200 μg per ml) caused a partial dissociation of the holoenzyme, as evidenced by appearance of catalytic subunit which was cyclic GMP-independent (Fig. 1B). A complete dissociation, however, was achieved when the enzyme was incubated with a higher concentration (200 μM) of cyclic GMP and the same concentration of histone, the catalytic subunit peak appearing around fraction 34 (Fig. 1C). In comparison, about 40% of the holoenzyme was dissociated in the presence of 200 μM cyclic GMP alone, whereas only a 10% dissociation was noted in the presence of

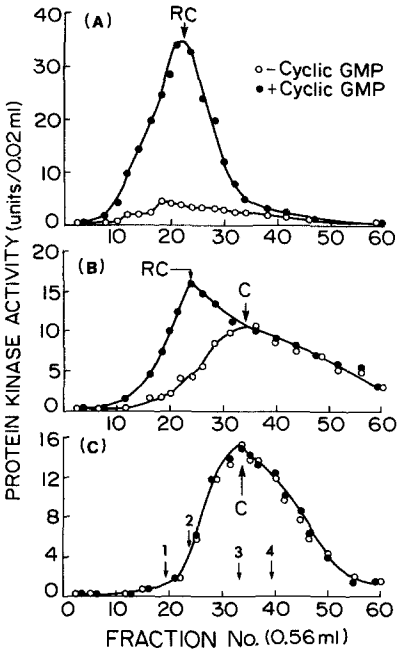

Fig. 1. Sephadex G-200 gel filtration of cyclic GMP-dependent protein kinase from guinea pig fetal lungs incubated under various dissociation conditions. (A). The holoenzyme (0.5 ml, 2.8 mg) was incubated in 50 mM potassium phosphate buffer, pH 7.0, containing 3 mM mercaptoethanol for 1 hr in ice. The enzyme solution was then chromatographed, employing the same buffer for elution. (B). Same as (A) except that 2 μM cyclic GMP and mixed histone (200 μg per ml) were included in the preincubation and elution solutions. (C). Same as (A) except that 200 μM cyclic GMP and mixed histone (200 μg per ml) were included in the preincubation and the elution solutions. The sephadex columns (1.5 x 16 cm) in all cases were previously equilibrated with the respective solutions used for elution. The fractions from (B) and (C), to which bovine serum albumin was added to a concentration of about 2 mg per ml, were individually dialyzed against 50 mM phosphate buffer containing 3 mM mercaptoethanol to remove cyclic GMP prior to assay. RC, holoenzyme; C, catalytic subunit; 1, phosphorylase b (mol wt 185,000); 2, human γ-globulin (16,000); 3, creatine kinase (80,000); 4, horseradish peroxidase (49,000).

histone (200 μg per ml) alone. Cyclic AMP (200 μM) was unable to substitute for the same concentration of cyclic GMP in all cases (data not shown).

Dissociation of cyclic GMP-dependent protein kinase was further demonstrated by means of sucrose density gradient centrifugation. The holoenzyme sedimented as a molecular species having a sedimentation co-

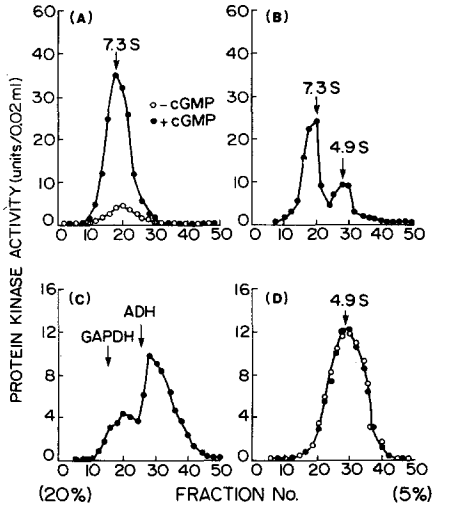

Fig. 2. Linear sucrose density gradient centrifugation of the holoenzyme and the catalytic subunit of cyclic GMP-dependent protein kinase from guinea pig fetal lungs. (A). The holoenzyme (0.2 ml, 1.1 mg) was incubated in 50 mM potassium phosphate buffer, pH 7.0, containing 3 mM mercaptoethanol (preincubation solution) for 1 hr in ice. It was then centrifuged in the sucrose density solution (5-20%) in 50 mM sodium acetate buffer, pH 6.0, containing 2.5 mM mercaptoethanol and 0.3 mM EGTA (centrifugation solution). (B). Same as (A) except that 50 μM cyclic GMP was included in both the preincubation and the centrifugation solutions. (C). Same as (A) except that 200 μM cyclic GMP and mixed histone (200 μg per ml) were included in both the preincubation and the centrifugation solutions. (D). Same as (A) except that the isolated catalytic subunit (0.2 ml, 50 μg) from Fig. 1C was used instead. GAPDH, glyceraldehyde-3-phosphate dehydrogenase (7.7 S); ADH, alcohol dehydrogenase, horse liver (5.4 S).

efficient of 7.3 S (Fig. 2A). A new 4.9 S species appeared when the enzyme was incubated and centrifuged in the presence of 50 μM of cyclic GMP (Fig. 2B). A greater dissociation was noted when a higher concentration (200 μM) of cyclic GMP and mixed histone (200 μg per ml) were included (Fig. 2C). In comparison, little or no dissociation was detected when the holoenzyme was incubated with 200 μM of cyclic AMP, either in the presence or absence of histone (data not shown). The peak fraction of the 4.9 S species (Fig. 2C) were pooled, and bovine serum albumin was added to the solution to a final concentration of about 2 mg per ml to stabilize the enzyme activity. The solution was then dialyzed

against 50 mM phosphate buffer containing 3 mM mercaptoethanol to remove cyclic GMP; its activity was found to be independent of added cyclic GMP. The isolated catalytic subunit from Sephadex column (Fig. 1C), which was concentrated on Amicon PM30 membrane, was found to sediment as the same 4.9 S species in parallel experiment (Fig. 2D).

The stimulatory modulator, or the crude modulator (a mixture of stimulatory and inhibitory modulators), augmented the activity of the isolated catalytic subunit of cyclic GMP-dependent protein kinase, either in the presence or absence of cyclic GMP (Table 1). The activity of the holoenzyme, in contrast, was augmented by the above modulator preparations only in the presence of cyclic GMP (Table 1 and Ref. 8-10). The inhibitory modulator had no effects on the catalytic subunit or the holoenzyme whether the stimulatory modulator was present or absent. The

TABLE 1. Comparison of effects of crude, stimulatory and inhibitory modulators on holoenzymes and catalytic subunits of cyclic GMP-dependent and cyclic AMP-dependent protein kinases.

Protein kinase	Cyclic nucleotide (0.5 μM)	Protein kinase activity (units) in the presence of modulator:			
		None	Crude (75 μg)	Stimulatory (50 μg)	Inhibitory (8 μg)
Cyclic GMP-dependent:					
Holoenzyme (6 μg)	None	6.1	5.0	5.9	6.1
	Cyclic GMP	6.5	35.6	28.2	6.3
Catalytic subunit (17 μg)	None	5.0	25.2	22.4	4.8
	Cyclic GMP	5.9	26.1	21.7	4.9
Cyclic AMP-dependent:					
Holoenzyme (4 μg)	None	4.0	3.6	3.6	4.1
	Cyclic AMP	28.2	5.1	26.2	8.2
Catalytic subunit (8 μg)	None	25.5	4.2	23.9	3.6
	Cyclic AMP	25.2	3.9	25.6	3.4

The modulator preparations were from the dog heart, and the protein kinases were from the guinea pig fetal lung.

activity of the catalytic subunit of cyclic AMP-dependent protein kinase, in contrast to that of cyclic GMP-dependent protein kinase, was fully active and its activity was depressed by the inhibitory modulator, as it depressed the activity of the holoenzyme in the presence of cyclic AMP. The stimulatory modulator, on the other hand, was without effect on the enzyme activity, either in the presence or absence of the inhibitory modulator (Table 1). Ashby and Walsh have shown earlier that protein inhibitor (inhibitory modulator) depressed cyclic AMP-dependent enzyme by interacting with its catalytic subunit (20,21).

When the protein kinase was incubated with both cyclic [G-^{3}H]GMP and histone followed by chromatographing on a Sephadex G-200 column using a solution containing only histone for filtration, an enzyme activity peak corresponding to the undissociated holoenzyme and an activity shoulder corresponding to the catalytic subunit, were observed (Fig. 3). In addi-

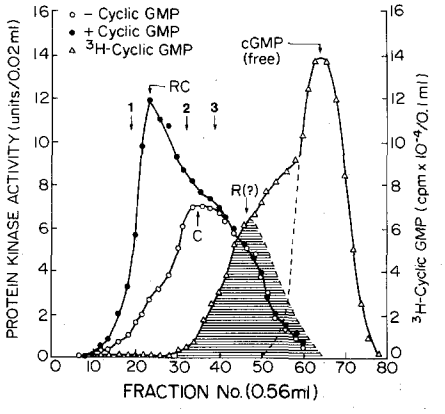

Fig. 3. Sephadex G-200 gel filtration of cyclic GMP-dependent protein kinase from guinea pig fetal lungs. The holoenzyme (0.5 ml; 2.8 mg) was incubated in 50 mM potassium phosphate, pH 7.0, containing 3 mM mercaptoethanol, 200 μM cyclic [G-3H]GMP (6.2 x 10^6 cpm) and mixed histone (200 μg per ml) for 1 hr in ice. The enzyme solution was then chromatographed on the column (1.5 x 16 cm) using 50 mM phosphate buffer, pH 7.0, containing 3 mM mercaptoethanol and mixed histone (200 μg/ml) for elution. Cyclic GMP was not included in the solution for elution. RC, holoenzyme; C, catalytic subunit; R, regulatory (cyclic GMP-binding) subunit; 1, phosphorylase b; 2, creatine kinase; 3, peroxidase.

tion, a radioactivity peak corresponding to the free radioactive cyclic GMP, and a shoulder, presumably the cyclic GMP that was bound to the regulatory subunit (mol wt 30,000), were obtained. If the protein kinase was omitted from the incubation mixture, only a symmetric peak corresponding to the free cyclic GMP was obtained (data not shown).

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