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STIMULATION BY POLYDEOXYRIBONUCLEOTIDE OF HISTONE PHOSPHORYLATION BY GUANOSINE 3':5'-MONOPHOSPHATE-DEPENDENT PROTEIN KINASE*

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SUMMARY: The phosphorylation of histone by cyclic GMP-dependent protein kinase (protein kinase G) was markedly enhanced by polydeoxyribonucleotide with the chain length of several nucleotides up to highly polymerized DNA. Such enhancement was observed for the phosphorylation of H2A, H2B, H3 and H4 histone fractions. The reaction with H1 histone was always inhibited. On the other hand, polydeoxyribonucleotide was inhibitory for the reactions of cyclic AMP-dependent protein kinase (protein kinase A) irrespective of histone fractions employed. The result raises a possibility that the histones associated with nucleosome structure may serve as preferable substrates for protein kinase G rather than for protein kinase A.

Since the first report by Kuo and Greengard (1) numerous efforts have been made to explore the role as well as the mechanism of action of cyclic GMP-dependent protein kinase (see reviews 2,3). Although physiological roles of cyclic AMP and cyclic GMP are proposed to be different and sometimes opposite (2), evidence accumulating in the literature seems to indicate that this class of enzyme possesses an intrinsic activity closely similar to the activity of cyclic AMP-dependent protein kinase when examined in purified *in vitro* systems (4-6). Recently, Shoji *et al.*(7) have isolated a homogeneous protein

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factor from dog heart, which can stimulate histone phosphorylation by cyclic GMP-dependent protein kinase. However, the physiological significance of this factor remains to be explored. The present communication will show that polydeoxyribonucleotides markedly enhance the phosphorylation of histone by cyclic GMP-dependent protein kinase, whereas inhibit the reaction by cyclic AMP-dependent protein kinase. Cyclic GMP-dependent and cyclic AMP-dependent protein kinases will be tentatively referred to as protein kinases G and A, respectively.

EXPERIMENTAL PROCEDURES

Protein kinases G and A employed for the present studies were the same preparations used earlier (4), and the specific activities were 10.0 and 2.5 units/mg of protein, respectively. One unit of enzyme was defined as that amount of enzyme which incorporated 1 nmol of phosphate from ATP into histone/min under the standard conditions (8). Five histone fractions of calf thymus were prepared by the procedures specified earlier (4). Muscle glycogen phosphorylase kinase was purified from rabbit skeletal muscle by the method of Cohen (9). Calf thymus DNA (Type I) was obtained from Sigma. Polydeoxyribonucleotides were prepared from DNA by treatment with pancreatic DNase for 5 min at 35° (DNA:DNase=100:1 (w/w); DNA concentration, 3.5 mg/ml) in 50 mM sodium acetate at pH 5.5 and 7 mM magnesium acetate. About 100 mg of DNA digest was subjected to a Sephadex G-75 column (63 x 4.8 cm) equilibrated with 50 mM ammonium bicarbonate at pH 7.5. Fractions of polydeoxyribonucleotides with various chain lengths were separately pooled, and ammonium bicarbonate was removed by lyophilization. Average chain length of the polydeoxyribonucleotide was determined by measuring the ratio of the total phosphate to the terminal phosphate which was liberated by alkaline phosphatase (10). [γ-^{32}P]ATP was prepared by the method of Glynn and Chappell (11).

Protein kinases G and A were assayed under the standard conditions described earlier (8) unless otherwise noted. The reaction was terminated by the addition of 5 ml of 25% trichloroacetic acid for H1 histone and 5 ml of 10% trichloroacetic acid for other histones. When highly polymerized DNA was employed as an effector, the reaction was terminated by the addition of an excess amount of nonradioactive ATP (0.3 μmol) and NaCl (final 1 M). The mixture was sonicated for 40 s with a Kontes ultrasonifier before being precipitated by trichloroacetic acid. Acid-precipitable material was collected on a Toyo-Roshi membrane filter (pore size, 0.45 μ; comparable to Millipore filter). The radioactivity of ^{32}P-samples was determined with a Nuclear-Chicago Geiger Muller gas flow counter, model 4338. Phosphate was determined by the method of Burton and Petersen (12) with a slight modification by Takemura and Miyazaki (13). Polydeoxyribonucleotide was determined by measuring the absorbance at 260 nm, assuming the optical density of 1 mg/ml solution as 20. Protein was determined by the method of Lowry *et al.* (14) with bovine serum albumin as a standard.

Table I

Relative rates of phosphorylation of histone fractions by protein kinases G and A in the presence and absence of polydeoxyribonucleotide

	Protein kinase G		Protein kinase A	
	minus polydeoxy-ribonucleotide	plus polydeoxy-ribonucleotide	minus polydeoxy-ribonucleotide	plus polydeoxy-ribonucleotide
	mmol ^{32}Pi/mol histone/min			
H1 histone	7.9 (100)	5.4 (68)	5.1 (100)	3.5 (69)
H2A histone	< 0.2 (2.5)	1.7 (22)	3.5 (69)	0.7 (14)
H2B histone	5.5 (70)	26.2 (332)	42.3 (829)	6.7 (131)
H3 histone	< 0.1 (1.3)	1.5 (19)	2.5 (49)	1.0 (20)
H4 histone	< 0.1 (1.3)	2.0 (25)	3.2 (63)	1.0 (20)

Protein kinases G (6 μg, 0.06 unit) and A (24 μg, 0.06 unit) were assayed under the standard conditions except that 400 μg/ml of each histone and 25 mM Mg^{2+} were employed. Where indicated, 400 μg/ml of polydeoxyribonucleotide with average chain length of about 10 was added. Incubation was carried out for 5 min at 30°. Numbers in parentheses represent percentages of each reaction velocity with that of H1 histone in the absence of polydeoxyribonucleotide as 100%.

RESULTS AND DISCUSSION

The phosphorylation of histone by protein kinases G and A in vitro was markedly influenced by the addition of polydeoxyribonucleotide. Table I shows the relative reaction velocities for five histone fractions in the presence and absence of polydeoxyribonucleotide. Under these conditions highly polymerized DNA usually precipitated histone in an insoluble aggregated form and, therefore, polydeoxyribonucleotide with average chain length of about 10 was employed (see below). With protein kinase G stimulation of phosphorylation by polydeoxyribonucleotide was observed for H2A, H2B, H3 and H4 histone fractions. In the absence of polydeoxyribonucleotide, only H1 and H2B histones served as effective substrates for this enzyme, whereas the reactions with H2A, H3 and H4 histones were extremely slow[1/]. In the presence of polydeoxyribonucleotide, the last three

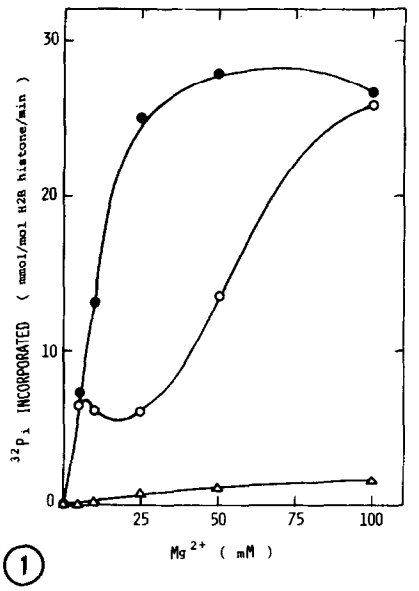
1/ A previous paper (4) has described that the relative reaction rate for H2A histone with protein kinase G varies markedly with lots of H2A histone preparation (see Table I footnote in reference 4). This was presumably due to some factors such as polydeoxyribonucleotide which contaminated the histone preparation.

histone fractions also served as phosphate acceptors. With H2B histone as substrate the reaction was enhanced also several fold. In the absence of polydeoxyribonucleotide, the phosphorylation of H2B histone showed a biphasic concentration curve for Mg^{2+}, and an extremely high concentration of this cation (100 mM) was needed to obtain the maximum reaction velocity (15). However, when polydeoxyribonucleotide was added to the reaction mixture, the concentration curve was normalized and the maximum reaction velocity was obtained at lower concentrations of Mg^{2+} (Fig. 1)[2/]. In contrast, the phosphorylation of H1 histone was inhibited irrespective of the amount and the chain length of the polydeoxyribonucleotide added. Under comparable conditions the reactions catalyzed by protein kinase A were always inhibited by polydeoxyribonucleotide (Table I).

The stimulation of reactions catalyzed by protein kinase G was observed for homopolymers of deoxyribonucleotide and, apparently, no base specificity was noted. Neither monodeoxyribonucleotides nor monodeoxyribonucleosides were stimulatory. The reaction rate of protein kinase G was enhanced progressively by increasing the chain length of polydeoxyribonucleotide added. The maximum stimulation was observed with the chain length of about 10 (Fig. 2). Highly polymerized DNA, either native or denatured, was usually less effective, presumably because it precipitated histone as an insoluble aggregated form and thus interfered with the assay. Under the same conditions the reaction of protein kinase A was inhibited sharply by increasing the chain length of polydeoxyribonucleotide added.

Another set of experiments indicated that polydeoxyribonucleotide stimulated reactions by interacting with histone rather than with enzyme. The optimum amount of polydeoxyribonucleotide varied with the amount of histone employed, and the maximum reaction rate

2/ For other histone fractions the maximum reaction velocity was obtained at less than 10 mM Mg^{2+}.



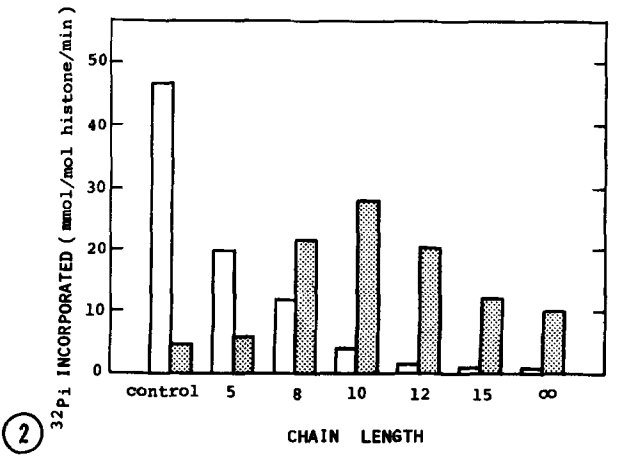


Fig. 1. Effect of varying concentrations of Mg^{2+} on phosphorylation of H2B histone by protein kinase G in the presence and absence of polydeoxyribonucleotide. Protein kinase G (6 μg, 0.06 unit) was assayed under standard conditions except that 400 μg/ml of H2B histone was employed and Mg^{2+} was varied as indicated. Where indicated 400 μg/ml of polydeoxyribonucleotide with average chain length of 10 was added. • and o, reaction rates in the presence and absence of polydeoxyribonucleotide, respectively. Δ, reaction rate in the presence of polydeoxyribonucleotide, but without cyclic GMP.

Fig. 2. Effect of chain length of polydeoxyribonucleotide on phosphorylation of H2B histone by protein kinases G and A. Protein kinases G (6 μg, 0.06 unit) and A (24 μg, 0.06 unit) were assayed under standard conditions except that 400 μg/ml of H2B histone and 25 mM Mg^{2+} were employed. Polydeoxyribonucleotide with the chain length indicated (400 μg/ml each) was added. Control and a symbol of infinite indicate the experiments in which polydeoxyribonucleotide was omitted and highly polymerized DNA was added as an effector, respectively. Open and shaded bars represent the reaction rates for protein kinases A and G, respectively.

was observed when equal amounts (w/w) of polydeoxyribonucleotide and histone were added (data not shown). The stimulation by polydeoxyribonucleotide was observed thus far only for H2A, H2B, H3 and H4 histone fractions. With muscle glycogen phosphorylase kinase as phosphate acceptor the reaction was always favorable for protein kinase A and was extremely slow for protein kinase G irrespective of the presence or absence of polydeoxyribonucleotide.

It may be noted that the stimulatory modulator protein of protein kinase G recently purified to homogeneity from dog heart by Shoji

et al.(7) is a very acidic soluble protein having a pI value of 4, and this factor appeared to interact with histone. Therefore, the stimulatory effect seems not to be specific for polydeoxyribonucleotides, and some ionic interaction between histone and polyanionic factor appears to render the basic protein more effective for protein kinase G. However, it is conceivable that this species of protein kinase has a capacity to phosphorylate preferentially the histone molecules which are associated with the nucleosome structure rather than free histones, since H2A, H2B, H3 and H4 histones are well-known constituents of the nucleosome core (16). A series of studies by Steiner and his coworkers (17), using immunofluorescence histochemical techniques, has shown that cyclic GMP as well as its binding protein, probably protein kinase G, is associated with nuclear components in many tissues and organs. It is possible to assume, therefore, that protein kinase G may be directly or indirectly concerned with gene regulation processes through phosphorylation of the histone fractions.

Several recent reports have indicated the possible existence of some phosphate acceptor proteins apparently specific for protein kinase G in crude membranes of smooth muscle (18,19) and intestinal brush border epithelium (20), and also in unfractionated cytosol of rabbit cerebellum (21), although it is uncertain whether these proteins are entirely inert as substrates for protein kinase A. Nevertheless, the results presented in this communication suggest that the topographic relationship between enzyme and substrate and/or between substrate and other macromolecules such as DNA may be an important determinant factor in the performance of specific physiological roles of this unique protein kinase.

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