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CYCLIC NUCLEOTIDE-DEPENDENT PROTEIN KINASES

VII. COMPARISON OF VARIOUS HISTONES AS SUBSTRATES FOR ADENOSINE 3',5'-MONOPHOSPHATE-DEPENDENT AND GUANOSINE 3',5'-MONOPHOSPHATE-DEPENDENT PROTEIN KINASES*

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SUMMARY

A study was made of the ability of purified histones Ib, IIb and IV to serve as phosphate acceptors in reactions catalyzed by adenosine 3',5'-monophosphate(cyclic AMP)-dependent and guanosine 3',5'-monophosphate(cyclic GMP)-dependent protein kinases. All three histones were able to serve as phosphate acceptors for all of the protein kinases tested. The relative reactivity of the histones was similar in the case of each of twenty-six cyclic AMP-dependent protein kinases obtained from diverse tissues; for all of these cyclic AMP-dependent enzymes, the reactivity of the histones decreased in the order IV > IIb > Ib. The relative reactivity of the histones exhibited a much different and more complex pattern when a cyclic GMP-dependent protein kinase was used. It is suggested that cyclic GMP-dependent protein kinases possess specificity for endogenous proteins different from that of cyclic AMP-dependent protein kinases. This difference may account for the separate and independent physiological roles believed to be mediated by cyclic AMP and cyclic GMP.

INTRODUCTION

Protein kinases which catalyze the phosphorylation of histone and of protamine by ATP, and are stimulated specifically by cyclic AMP, have been found in a variety of vertebrate and invertebrate tissues as well as in bacteria[1-9]. Recently, a protein kinase which catalyzes the phosphorylation of proteins by ATP and is activated by low concentrations of cyclic GMP rather than cyclic AMP has been found in lobster tail muscle, and separated from a cyclic AMP-dependent enzyme found in the same tissue[10]. It has been shown[9] that cyclic AMP-dependent protein kinases purified from fifteen different bovine tissues each utilized histone as phosphate acceptor in preference to any other protein (*i.e.* protamine, casein, phosvitin, bovine serum albumin) tested as a possible substrate. The cyclic AMP-dependent and cyclic GMP-dependent protein kinases purified from lobster muscle[10] have also been found (J. F.

* Papers IV, V and VI in this series are refs. 7, 9 and 10, respectively.

KUO AND P. GREENGARD, unpublished data) to utilize histone in preference to several other proteins tested as possible phosphate acceptors. LANGAN[2] has reported that a cyclic AMP-dependent protein kinase from liver phosphorylated histone f2b (*i.e.* IIb) more readily than histone f1 (*i.e.* I). With this exception, there have been no reports of the ability of pure histones to serve as substrates for cyclic nucleotide-dependent protein phosphorylation, published studies of cyclic nucleotide-dependent histone phosphorylation having been carried out with commercial batches of mixed histones. The objective of the present investigation was to compare three purified histones, namely, a lysine-rich histone (Ib), a slightly lysine-rich histone (IIb), and an arginine-rich histone (IV), as substrates for cyclic AMP-dependent and cyclic GMP-dependent histone kinases.

MATERIALS AND METHODS

Materials

Cyclic AMP-dependent and cyclic GMP-dependent protein kinases were isolated from various sources, and purified to varying degrees, as described previously[4,7,10]. Histones Ib, IIb and IV, prepared from calf thymus by procedures described elsewhere[11], were the generous gift of Professor James Bonner. Other materials used were as reported previously[7].

Assay for protein kinase

The activity of cyclic nucleotide-dependent protein kinases was assayed in an incubation volume of 0.2 ml containing: sodium glycerol phosphate buffer (pH 6.5), 10 μmoles; histone, 1–60 μg; [γ^{32}-P]ATP, 0.5 nmole, containing about $1.5 \cdot 10^6$ counts/min; magnesium acetate, 2 μmoles; NaF, 2 μmoles; theophylline, 0.4 μmole; ethylene glycol bis-(β-aminoethylether)-*N*,*N*'-tetraacetic acid, 0.06 μmole; with or without 1 mμmole of cyclic AMP or cyclic GMP. Incubations were carried out for 5 min at 30° in a shaking water bath. The reaction was terminated and the histone-bound ^{32}P was determined as described previously for mixed histones[4,10]. In control experiments, in which the cyclic GMP-dependent protein kinase from lobster muscle was used to phosphorylate the individual histones under standard incubation conditions, the overall recovery of radioactive histones Ib, IIb and IV was found to be 55, 66 and 77%, respectively, of that obtained using the procedure of LANGAN[2]. All data in the present paper have been corrected for these overall recovery values. One unit of protein kinase activity is defined as that amount of enzyme that transferred 1 pmole of ^{32}P to histone in 5 min at 30° in the assay system.

RESULTS

The ability of varying concentrations of histone Ib, histone IIb, and histone IV to serve as phosphate acceptor *in vitro* was studied for the cyclic AMP-dependent protein kinases from bovine brain (Fig. 1), rat isolated adipose cells (Fig. 2), and lobster tail muscle (Fig. 3). The pattern of results obtained was similar for all three cyclic AMP-dependent enzymes. Thus, for all three enzymes, the relative ability of the three histones to serve as substrate decreased in the order IV > IIb > Ib, at all concentrations of histone tested. In some instances, the highest concentration of the

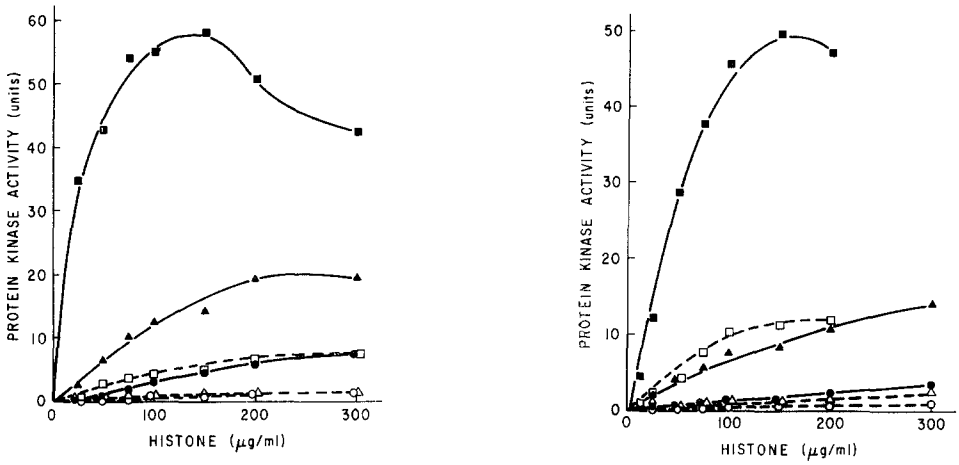


Fig. 1. Effect of histone concentration on the activity of cyclic AMP-dependent protein kinase from bovine brain. The enzyme preparation used (12 μg) was that obtained from the isoelectric focusing step[4]. Activities have been corrected for values observed in the absence of added histone, in the absence and presence of cyclic AMP, respectively. ○---○, histone Ib, −cyclic AMP; ●—●, histone Ib, +5 μM cyclic AMP; △---△, histone IIb, −cyclic AMP; ▲—▲, histone IIb, +5 μM cyclic AMP; □---□, histone IV, −cyclic AMP; ■—■, histone IV, +5 μM cyclic AMP.

Fig. 2. Effect of histone concentration on the activity of cyclic AMP-dependent protein kinase from isolated adipose cells of rats. The enzyme preparation used (250 μg) was that obtained from the $(NH_4)_2SO_4$ step[7]. Other details were as in Fig. 1.

pure histone tested (300 μg/ml) was still too low to obtain an accurate value for v_{max}. In addition, the kinetics with respect to histone concentration appeared in some cases rather complex. With these reservations, several conclusions can be drawn for these three cyclic AMP-dependent protein kinases. The v_{max} values decreased in the order IV > IIb > Ib and the apparent K_m values decreased in the order Ib > IIb > IV. The activation by the cyclic nucleotide of enzyme activity appeared to be associated in most cases with an increase in v_{max} with little or no change in the concentration of histone required for half-maximal velocity (the apparent K_m). A clear exception was found in the ability of histone IV to serve as substrate for the enzyme from bovine brain; in this case, the activation of enzyme activity by cyclic AMP was associated both with a large increase in v_{max} as well as with a decrease in the apparent K_m (from about 170 μg/ml in the absence of cyclic AMP to about 30 μg/ml in the presence of 5 μM cyclic AMP).

The data presented in Figs. 1–3 show clear differences in the ability of histones Ib, IIb and IV to act as phosphate acceptor in the reaction catalyzed by the cyclic AMP-dependent protein kinases. Although the molecular weights of the different histones vary (the approximate molecular weights being: Ib, 21 000 (ref. 12); IIb, 14 000 (ref. 13); IV, 11 000 (refs. 14–16), the same qualitative pattern of ability of the histones to serve as phosphate acceptor, *i.e.* IV > IIb > Ib, was observed when the data were compared on the basis of equimolar amounts rather than equal weights of the histones.

Histones Ib, IIb and IV were compared, at fixed concentrations, for their ability to serve as phosphate acceptors for a series of protein kinases partially purified from twenty-five vertebrate and invertebrate sources (Table I). (In this survey,

TABLE I

COMPARISON OF ABILITY OF HISTONES TO SERVE AS SUBSTRATES FOR CYCLIC AMP-DEPENDENT PROTEIN KINASES FROM VARIOUS SOURCES

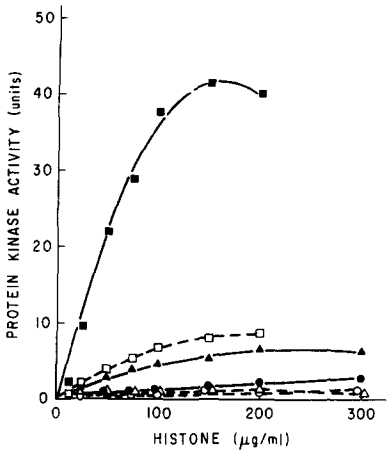
Incubation conditions were as described in MATERIALS AND METHODS, using either histone Ib (50 μg/ml), histone IIb (50 μg/ml) or histone IV (12.5 μg/ml), in the presence or absence of 5 μM cyclic AMP. To facilitate comparison of results, activities for each enzyme preparation have been expressed as percentage of the activity observed in the presence of histone IV (12.5 μg/ml) *plus* cyclic AMP (5 μM). All values were corrected for activity in the absence of added histone, in the absence and presence of cyclic AMP, respectively. Bovine enzymes were from the DEAE-cellulose step and the other enzymes were from the $(NH_4)_2SO_4$ step of purification[7].

Source of proteinkinase	*Protein kinase activity (%)*					
	Histone Ib		*Histone IIb*		*Histone IV*	
	−cyclic AMP	*+cyclic AMP*	*−cyclic AMP*	*+cyclic AMP*	*−cyclic AMP*	*+cyclic AMP*
Bovine brain	1.0	2.0	7.3	83.3	7.9	100
pancreas	8.0	22.5	8.1	82.9	14.4	100
kidney cortex	5.5	18.2	36.2	96.0	22.9	100
heart	0.6	5.8	7.9	76.5	8.6	100
lung	0.8	8.4	20.5	91.4	25.2	100
liver	5.3	9.4	16.4	79.3	13.5	100
whole thyroid	3.0	6.1	28.2	61.0	21.8	100
whole adrenal	6.6	14.4	9.4	66.2	13.6	100
skeletal muscle	1.4	6.8	7.6	63.5	10.0	100
testis	5.0	36.9	12.6	81.1	14.6	100
ovary	4.4	21.6	15.1	80.2	17.4	100
stomach	0.9	8.2	18.2	77.4	26.7	100
duodenum	7.6	15.7	25.8	75.6	30.9	100
Rabbit fat pads*	4.8	12.8	42.1	76.6	32.7	100
Rat adipose cells*	2.0	9.6	10.7	74.8	19.8	100
brown fat**	0.9	5.6	5.9	57.3	4.0	100
brain	2.7	15.1	17.5	71.3	17.7	100
testis	1.8	10.0	63.5	73.4	54.7	100
heart	8.5	12.2	55.2	92.4	55.5	100
skeletal muscle	4.3	8.5	29.7	67.0	26.9	100
Jelly fish	3.3	5.0	51.8	81.5	62.6	100
Squid	8.2	10.4	66.9	80.3	55.1	100
Star fish	15.0	63.3	23.3	83.3	56.7	100
Fish (carp)	6.5	40.3	29.0	41.9	40.3	100
Sand worm	4.0	5.7	43.3	47.7	66.3	100

* Epididymal
** Interscapular.

histone IV was tested in one-fourth the amount used for testing histones Ib and IIb both in order that the enzyme activity in the presence of histone IV would be closer to the activity in the presence of histones Ib and IIb, as well as to conserve the amount of histone IV used.) All three histones were able to serve as phosphate acceptor for every cyclic AMP-dependent protein kinase. The relative ability of the three histones to serve as phosphate acceptor was the same, namely, IV > IIb > Ib, for all of the cyclic AMP-dependent protein kinases.

A study of the ability of varying concentrations of histone Ib, histone IIb and histone IV to serve as phosphate acceptor in the reaction catalyzed by the cyclic GMP-dependent protein kinase from lobster muscle (Fig. 4) showed a pattern of substrate reactivity different from that observed with the three cyclic AMP-dependent protein



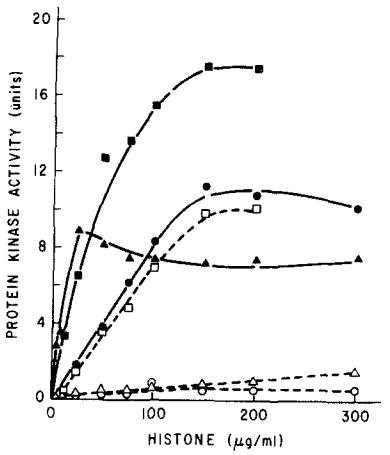


Fig. 3. Effect of histone concentration on the activity of cyclic AMP-dependent protein kinase from lobster tail muscle. The enzyme preparation used (40 μg) was that obtained from the DEAE-cellulose eluate, peak 2 (ref. 10). Other details were as in Fig. 1.

Fig. 4. Effect of histone concentration on the activity of cyclic GMP-dependent protein kinase from lobster tail muscle. The enzyme preparation used (120 μg) was that obtained from the DEAE-cellulose eluate, peak 1 (ref. 10). Other details were as in Fig. 1, except that 5 μM cyclic GMP was substituted for 5 μM cyclic AMP.

kinases (Figs. 1–3). With the cyclic GMP-dependent protein kinase as enzyme, the v_{max} values for histone Ib and histone IIb were approximately equal to each other and about half the v_{max} value observed for histone IV (Fig. 4). In addition, the apparent K_m of the cyclic GMP-dependent protein kinase for histone IIb decreased from a value greater than 150 μg/ml in the absence of cyclic GMP to a value of about 8.5 μg/ml in the presence of 5 μM cyclic GMP. Moreover, this apparent K_m value of 8.5 μg/ml for histone IIb in the presence of cyclic GMP was considerably less than that for either histone Ib or histone IV, which was not observed with any of the cyclic AMP-dependent protein kinases.

DISCUSSION

The apparent K_m values of the protein kinases for histone IIb and histone IV are impressively low in some instances. For example, the apparent K_m of the cyclic AMP-dependent protein kinase from bovine brain for histone IV was about 30 μg/ml; using a molecular weight of 11 000 for histone IV (refs. 14–16) this corresponds to an apparent K_m of 2.7 μM. Similarly, the apparent K_m of the cyclic GMP-dependent protein kinase of lobster muscle for histone IIb was about 8.5 μg/ml; using a molecular weight of 14 000 for histone IIb (ref. 13), this corresponds to an apparent K_m of 0.61 μM.

Protein kinases from rat liver[17] and fish testis[8] have been examined for their ability to phosphorylate various purified histones in the absence of added cyclic nucleotide. In both studies, slightly lysine-rich histone was found to be better than lysine-rich histone or arginine-rich histone as substrate. However, the relative ability of various proteins to serve as substrate in the protein kinase reaction can be altered

markedly by the presence of cyclic nucleotides, as shown by the data of LANGAN[2] and by the present study.

A considerable body of evidence, recently summarized[10], suggests that the cyclic GMP system may represent a second messenger system separate from that of the cyclic AMP system, *i.e.* under separate hormonal and/or metabolic control and with separate regulatory functions. The possibility has recently been suggested that all of the biochemical and physiological effects of cyclic AMP and of cyclic GMP may be mediated through regulation of cyclic nucleotide-dependent protein kinases[5,7,10]. The present results indicate that a cyclic GMP-dependent protein kinase from lobster tail muscle differs in its substrate specificity from that of a series of cyclic AMP-dependent protein kinases. In addition, it has recently been found[18] that a cyclic GMP-dependent protein kinase purified from the body wall of the cecropia silk-worm has a substrate specificity similar to that of the cyclic GMP-dependent protein kinase from lobster muscle. It seems reasonable to anticipate that other cyclic GMP-dependent protein kinases (such as those recently found[10,19] in mammalian brain, bladder and uterus, and currently being purified for purpose of characterization) will show a pattern of substrate specificity similar to that found for the cyclic GMP-dependent protein kinases from lobster muscle and cecropia silk-worm body wall. This would accord with the similarity of substrate specificity already observed with some two dozen cyclic AMP-dependent protein kinases from diverse sources. The difference in substrate specificity between the cyclic AMP-dependent group and the cyclic GMP-dependent group of protein kinases provides a tentative explanation, at the molecular level, for the differences in the biological effects of the two cyclic nucleotides.

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