Proc. Natl. Acad. Sci. USA
Vol. 74, No. 8, pp. 3239–3243, August 1977
Biochemistry

Adenosine 3′:5′-cyclic monophosphate- and guanosine 3′:5′-cyclic monophosphate-dependent protein kinases: Possible homologous proteins

(protein phosphorylation/amino acid composition/substrate specificity/protein evolution)

THOMAS M. LINCOLN AND JACKIE D. CORBIN

Department of Physiology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232

Communicated by Sidney P. Colowick, May 23, 1977

ABSTRACT **The properties of purified mammalian adenosine 3′:5′-cyclic monophosphate (cAMP)- and guanosine 3′:5′-cyclic monophosphate (cGMP)-dependent protein kinases were compared. Several physical characteristics of the two enzymes were similar, including size, shape, affinity for cyclic nucleotide binding, and K_m for ATP. In addition, the amino acid compositions of the two proteins indicated a close composition homology (70–90%). Both cyclic nucleotide-dependent protein kinases catalyzed phosphorylation of rat liver pyruvate kinase (EC 2.7.1.40) and fructose 1,6-diphosphatase (EC 3.1.3.11), rabbit skeletal muscle glycogen synthase (EC 2.4.1.11) and phosphorylase *b* kinase (EC 2.7.1.38), and calf thymus histone H_2b. The phosphorylation of several synthetic peptides and of trypsin-sensitive and trypsin-insensitive sites in glycogen synthase suggested similar recognition sites on the protein substrates for the two kinases. The cAMP-dependent protein kinase was the better catalyst with each protein or peptide substrate. The results suggest that the two enzymes evolved from a common ancestral protein.**

Adenosine 3′:5′-cyclic monophosphate (cAMP)- and guanosine 3′:5′-cyclic monophosphate (cGMP)-dependent protein kinases (cAMP kinase and cGMP kinase, respectively) have been described in several vertebrate and invertebrate tissues (1–8). Both enzymes have been purified to homogeneity and the subunit compositions characterized (9–14). While the distribution of the cAMP kinase is ubiquitous in mammalian tissues, there does not appear to be a similar distribution of the cGMP kinase since only lung, cerebellum, heart, and small intestine have appreciable amounts of specific cGMP binding activity (8). On the other hand, cGMP kinase is more widely distributed in the lower species of animals, especially in the arthropods where the enzyme was first described (3, 4). Abundant evidence has been provided that indicates that cyclic nucleotide-dependent protein kinases are the major receptors for cyclic nucleotides in eukaryotic cells.

Several reports have appeared comparing the properties of cAMP and cGMP kinases from several sources (6–9, 15). In general, these studies emphasized the differences between the two enzymes. One study by Hashimoto *et al.* (16), however, disclosed similarities in substrate specificity between the two cyclic nucleotide-dependent protein kinases. Since relatively crude enzyme preparations were used, few details concerning substrate specificity and physical nature of the enzymes could be obtained.

In this paper, we present data showing that purified mammalian cAMP and cGMP kinases have several similarities in physical properties and substrate specificity. Furthermore, we propose, on the basis of several lines of indirect evidence, that the two enzymes are homologous proteins.

MATERIALS AND METHODS

Purification of Enzymes. Homogeneous bovine liver catalytic subunit of cAMP kinase (C subunit) was prepared by the method of Sugden *et al.* (17); homogeneous bovine lung cGMP kinase was purified as described (13, 14). The regulatory subunit of cAMP kinase (R subunit) from bovine heart was prepared by a method modified from that of Dills *et al.* (12), with 8-$H_2N(CH_2)_6HN$-cAMP as the affinity ligand.

Protein Kinase Assay. The standard assay for cAMP kinase activity was performed by the method of Corbin and Reimann (18); that for cGMP kinase activity was performed as described (8). Various protein substrates were phosphorylated using C subunit and cGMP kinase at 30° in a volume of 70 μl containing 34 mM Tris·HCl (pH 7.5), 4 mM magnesium acetate, 0.4 mM [γ-^{32}P]ATP (100 cpm/pmol), 5 μl of substrate at the specified concentrations, and 10 μl of C subunit (final concentration, 0.02 μM) or cGMP kinase (final concentration, 0.2 μM) in the presence of 0.1 μM cGMP. Enzyme and substrate blanks were negligible in all cases. Reactions were terminated at the various times by spotting 10 μl of the assay mixture onto filter paper and washing in 10% trichloroacetic acid as described (18).

Various synthetic peptides were phosphorylated as described by Kemp *et al.* (19). The reactions were conducted in a volume of 0.1 ml containing 25 mM Tris·HCl (pH 7.5), 10 mM magnesium acetate, 0.3 mM [γ-^{32}P]ATP (100–300 cpm/pmol), and 0.1 mM various peptides (except where indicated otherwise). With the cGMP kinase, 0.1 μM cGMP was used in the assay. The reactions were initiated with the addition of either C subunit or cGMP kinase (final concentration, 2 nM); mixtures were incubated at 30° for 5 min. The reactions were terminated by the addition of 0.5 ml of 30% acetic acid and the peptides were isolated as described by Kemp *et al.* (19). One unit of activity is the amount of enzyme necessary for the transfer of 1 pmol of phosphate to substrate in 1 min. cAMP-dependent protein kinase and C subunit are used interchangeably.

Sodium Dodecyl Sulfate ($NaDodSO_4$) Gel Electrophoresis. Gel electrophoresis was performed by the method of Weber and Osborn in the presence of $NaDodSO_4$ (20). Molecular weights of the subunits were determined as described (14, 17).

Amino Acid Composition. Approximately 0.25–0.5 mg of purified bovine liver C subunit, bovine heart R subunit (type II), and bovine lung cGMP kinase were hydrolyzed separately

The costs of publication of this article were defrayed in part by the payment of page charges from funds made available to support the research which is the subject of the article. This article must therefore be hereby marked "advertisement" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: cAMP, adenosine 3′:5′-cyclic monophosphate; cGMP, guanosine 3′:5′-cyclic monophosphate; R subunit, regulatory subunit of cAMP-dependent protein kinase; C subunit, catalytic subunit of cAMP-dependent protein kinase; M_r, molecular weight; $NaDodSO_4$, sodium dodecyl sulfate.

Table 1. Physical properties of cAMP and cGMP kinases from animal tissues

Property	cAMP kinase	cGMP kinase
Molecular weight	140,000–180,000	130,000–190,000
Stoke's radius	50–58 Å	52–59 Å
Frictional coefficient	1.6	1.5
K_d for cyclic nucleotide	0.01–0.05 μM	0.01–0.05 μM
K_m for ATP	5–50 μM	5–100 μM

in 6 M HCl at 110° in sealed tubes for 20 hr. The hydrolysates were analyzed with a Beckman model 121 amino acid analyzer. Cysteic acid was determined by the method of Moore (21); tryptophan was determined after base hydrolysis.

Materials. Gel electrophoresis materials were from BioRad. Histones II-A (mixture) and VII (H_2b) were purchased from Sigma. The various synthetic peptides were a generous gift from B. E. Kemp, Department of Clinical Biochemistry, School of Medicine, Flinders University of South Australia. Purified rabbit skeletal muscle glycogen synthase (EC 2.4.1.11) and phosphorylase *b* kinase (EC 2.7.1.38) were kindly supplied by T. R. Soderling, Department of Physiology, Vanderbilt University. Purified rat liver pyruvate kinase (EC 2.7.1.40) and fructose 1,6-diphosphatase (EC 3.1.3.11) were generously supplied to us by J. P. Riou and S. J. Pilkis, from the Department of Physiology, Vanderbilt University.

RESULTS

Subunit Composition of cAMP and cGMP Kinases. $NaDodSO_4$ gels of the purified cAMP and cGMP kinases are shown in Fig. 1. As demonstrated by others (9, 10, 12, 17, 22) and shown in the figure, the cAMP kinase (type II) contains an R subunit with a molecular weight of approximately 55,000 and a C subunit with a molecular weight of approximately 40,000. The cGMP kinase, however, consists of only one type of subunit, having a molecular weight (M_r) of approximately 81,000 and containing both cGMP binding and cGMP kinase activities (13, 14). Based on previous studies, it has been proposed that the cAMP and cGMP kinases are activated in the following manner.*

$$\underset{M_r\ 186{,}000}{R_2C_2\ (\text{inactive})} + 2\ \text{cAMP} \rightleftharpoons \underset{M_r\ 110{,}000}{R_2\text{-cAMP}_2} + \underset{M_r\ 38{,}000}{2C\ (\text{active})} \quad [1]$$

$$\underset{M_r\ 165{,}000}{E_2(\text{inactive})} + 2\ \text{cGMP} \rightleftharpoons \underset{M_r\ 165{,}000}{E_2\text{-cGMP}_2\ (\text{active})} \quad [2]$$

where R and C are the regulatory and catalytic subunits of the cAMP-dependent protein kinase, respectively, and E is the cGMP kinase subunit.

Physical Properties of cAMP and cGMP Kinases. The physical properties of the two kinases are summarized in Table 1. The table consists of results obtained by several laboratories (6, 7, 9–11, 22–24), including our own (8, 13, 14, 25), and demonstrates that several similarities exist between the two enzymes, including molecular weight, shape of the proteins, stoichiometry of cyclic nucleotide binding, and the affinities of the two kinases for their respective cyclic nucleotides and for ATP.

The amino acid compositions of bovine lung cGMP kinase and type II cAMP kinase are shown in Table 2. The latter was determined by combining the amino acid compositions of the bovine heart (type II) R subunit and the bovine liver C subunit. The enzymes show a remarkable degree of similarity in their compositions. Using the composition divergence function of

* Recent experiments in our laboratory have indicated that the type II cAMP kinase from bovine heart binds 2 mol of cAMP per mol of R subunit monomer at equilibrium.

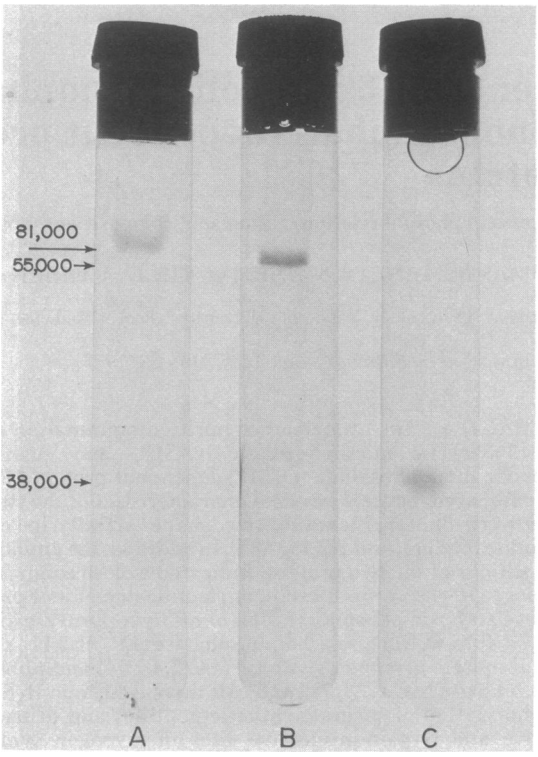

FIG. 1. $NaDodSO_4$ gel electrophoresis of purified cAMP and cGMP kinases. (*A*) Bovine lung cGMP kinase (2.5 μg); (*B*) 5.0 μg of R subunit from bovine heart type II cAMP kinase; (*C*) 2.5 μg of C subunit from bovine liver type II cAMP kinase. The molecular weights are 81,000, 55,000, and 38,000 for cGMP kinase subunit, R subunit, and C subunit, respectively.

Table 2. Amino acid compositions of bovine cAMP and cGMP kinases

Residue	Mole % of residue: Bovine heart type II R	Bovine liver type II C	RC	Bovine lung cGMP kinase
Asp	12.88	10.19	11.78	11.23
Thr	4.39	4.19	4.27	5.07
Ser	6.27	4.68	5.66	6.85
Glu	14.54	11.85	13.39	12.88
Pro	5.26	4.13	4.85	4.66
Gly	6.78	7.16	6.93	8.22
Ala	6.60	7.16	6.81	6.99
Cys*	1.55	0.82	1.50	1.37
Val	7.16	4.41	6.00	5.07
Met	2.46	1.65	2.08	2.47
Ile	4.05	4.41	4.16	3.84
Leu	7.80	9.64	8.55	8.08
Tyr	2.35	3.58	2.89	2.47
Phe	3.72	7.71	5.43	3.97
Trp†	0.30	1.65	0.92	1.23
Lys	6.86	10.19	7.85	8.08
His	1.29	2.75	1.96	1.23
Arg	5.67	4.41	5.20	6.30

Composition divergence functions are as follows: R, cGMP kinase = 0.06; C, cGMP kinase = 0.09; and RC, cGMP kinase = 0.04.
* Determined as cysteic acid.
† Determined after base hydrolysis.

Table 3. Phosphorylation of peptide sequences by C subunit and cGMP kinase

No.	Peptide	Units/ml × 10^{-3}		% peptide no. 1	
		cGMP*	cAMP†	cGMP*	cAMP†
1	Leu-Arg-Arg-Ala-Ser-Leu-Gly	1120	2840	100	100
2	Arg-Arg-Ala-Ser-Leu-Gly	1300	3060	101	108
3	Arg-Ala-Ser-Leu-Gly	110	490	10	17
4	Leu-Arg-Arg-Ala-Ser	66	312	6	11
5	Leu-*Ala*-Arg-Ala-Ser-Leu-Gly	139	497	12	17
6	Leu-Arg-*Ala*-Ala-Ser-Leu-Gly	42	150	4	6
7	Leu-*His*-Arg-Ala-Ser-Leu-Gly	166	1052	15	37
8	Leu-Arg-*His*-Ala-Ser-Leu-Gly	50	432	4	15
9	Leu-Arg-*Lys*-Ala-Ser-Leu-Gly	168	1516	15	53
10	Leu-*Lys*-Arg-Ala-Ser-Leu-Gly	508	2942	45	103
11	Leu-Arg-Arg-Ala-*Thr*-Leu-Gly	252	1232	23	40

C subunit and cGMP kinase were diluted in 3 mg of bovine serum albumin per ml in 20 mM sodium phosphate/2 mM EDTA/25 mM 2-mercaptoethanol at pH 7 immediately before use. The final concentrations were 2 nM each. Units of activity refer to the activity before dilution. *Italics* indicate the substitution in the sequence.

* cGMP, cGMP kinase.

† cAMP, cAMP kinase (or C subunit).

Harris and Teller (26), we obtained a value of 0.04, predicting a sequence homology between RC and the cGMP kinase subunit of 70–90%.

Substrate Specificities of cAMP and cGMP Kinases. C subunits from several different mammalian tissues have similar, if not identical, physical properties and substrate specificity (27, 28). The ability of the purified bovine liver C subunit and cGMP kinase to catalyze the phosphorylation of several proteins is shown in Fig. 2.† The amount of C subunit used was 0.02 μM, which was found to be a nearly maximally effective concentration of the enzyme. Since the concentration of the cGMP kinase was 0.2 μM, the results in Fig. 2 suggest that the cAMP kinase has about 10–20 times greater activity towards these substrates. By increasing the amount of the cGMP kinase, however, we have obtained similar rates of phosphorylation as when the C subunit is used (data not shown). In contrast to the cyclic nucleotide-dependent protein kinases, a partially purified cyclic nucleotide-independent protein kinase (casein kinase) from bovine liver does not catalyze significant phosphorylation of these proteins (data not shown). Skeletal muscle phosphorylase *b* kinase incorporated up to 6 mol of phosphate per mol under our assay conditions. This was somewhat unexpected since it has been shown that only 2 mol of phosphate per mol of enzyme are incorporated (29). As pointed out by Singh and Wang (30), more than 2 mol of phosphate can be incorporated under certain conditions.

Two of the above substrates (pyruvate kinase and glycogen synthase) were examined more closely for substrate specificity. Phosphorylation of these two proteins by either kinase resulted in inhibition of activity of both enzymes. For example, the activity ratio of glycogen synthase (− glucose-6-*P*/+ glucose-6-*P*) decreased from 0.62 to 0.18 in 30 min with 10 μg of purified cGMP kinase (unpublished observations). The effects of amino acid substitutions in the peptide sequence from pyruvate kinase that was phosphorylated by the cAMP kinase (31) was studied. As shown in Table 3, substitutions in the Arg-Arg position affect the activity of both enzymes dramatically. It is noteworthy that Kemp *et al.* (19, 32) demonstrated that a basic amino acid residue near the serine is an important determinant of substrate specificity for the cAMP kinase. Substitutions at the first of the two arginine residues near the serine affect the activity less than substitutions at the second one, indicating that both enzymes require basic charges close to the serine. This is supported by the fact that the substitution of neutral residues (i.e., Ala) decreases the activities more than the substitution of basic residues (i.e., Lys). Furthermore, threonine does not substitute well for serine for either kinase. In some cases, as with peptide 10, there appears to be a large difference in specificity for the two enzymes. However, as shown in Fig. 3, the K_m values of the two enzymes for both peptides 1 and 10 are quite different. At 0.1 mM peptide 10, activity of the C subunit is nearly maximal while the cGMP kinase is only about half-maximally active. Therefore, substitution of this residue decreases the activity of both enzymes similarly. These results suggest that similar, but perhaps not identical, substrate specificity determinants exist for both kinases.

Additional evidence that the cAMP and cGMP kinases are catalyzing phosphorylation of the same residues on substrate proteins was obtained using glycogen synthase. The C subunit

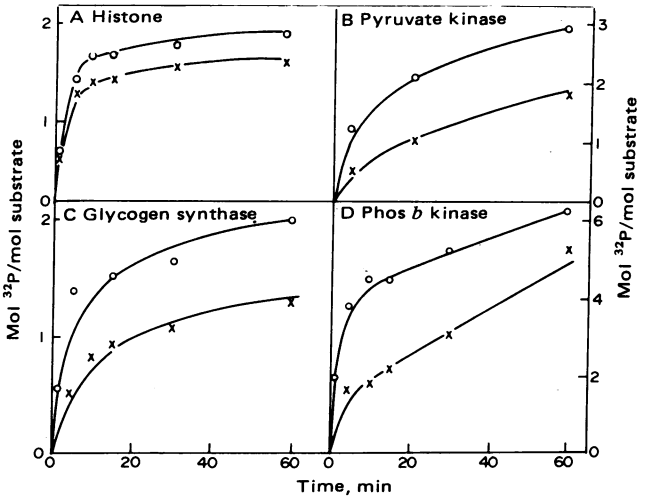

FIG. 2. Phosphorylation of protein substrates catalyzed by bovine liver C subunit (0.02 μM) (○) and bovine lung cGMP kinase (0.2 μM) (×). Reactions were performed as described in *Materials and Methods*. Protein substrate concentrations were: (*A*) H_2b histone (M_r = 30,000), 1.0 mg/ml; (*B*) pyruvate kinase (M_r = 220,000), 1 mg/ml; (*C*) glycogen synthase (M_r = 360,000), 1.56 mg/ml; (*D*) phosphorylase *b* kinase (M_r = 300,000), 1.56 mg/ml.

† It has been shown that the C subunit catalyzes the phosphorylation of rat liver fructose-1,6-diphosphatase (44). We have observed that the cGMP kinase also catalyzes the phosphorylation of this protein in a manner similar to that for the substrates shown in Fig. 2.

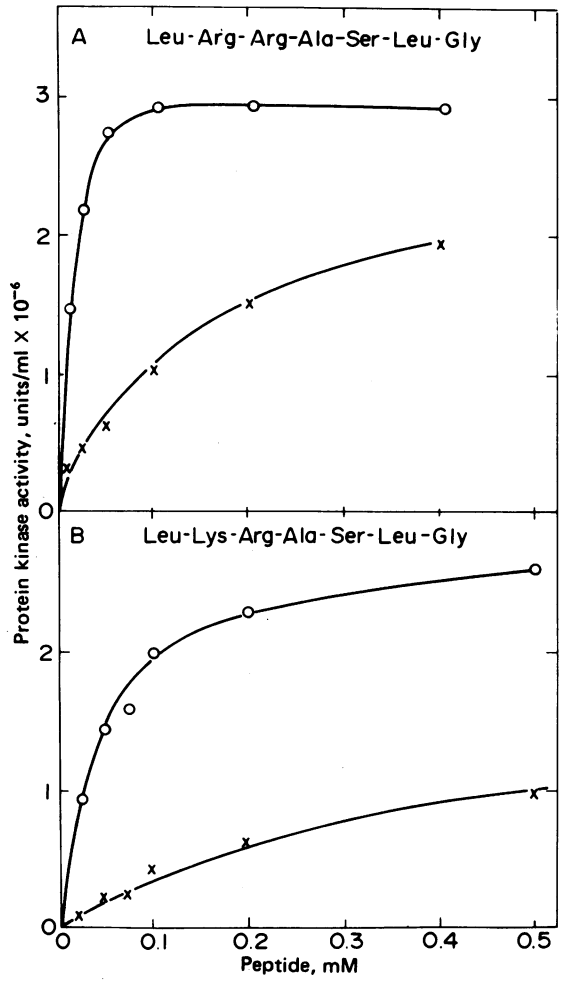

FIG. 3. Phosphorylation of peptides 1 and 10 catalyzed by bovine liver C subunit (O) and bovine lung cGMP kinase (X). The enzymes were diluted in 3 mg of bovine serum albumin per ml in 20 mM sodium phosphate/2 mM EDTA/25 mM 2-mercaptoethanol at pH 7.0 to 2 nM and used immediately. (*A*) Phosphorylation of peptide 1; (*B*) phosphorylation of peptide 10.

(33) and a cyclic nucleotide-independent glycogen synthase kinase preferentially catalyze phosphorylation of different sequences in glycogen synthase; the different phosphorylated peptides can be resolved by limited trypsin proteolysis followed by chromatographic procedures (T. R. Soderling, personal communication). As shown in Table 4, the cAMP kinase preferentially catalyzes phosphorylation of the same trypsin-sensitive site as the cGMP kinase. On the other hand, the cyclic nucleotide-independent glycogen synthase kinase catalyzes phosphorylation of the trypsin-insensitive peptide.

DISCUSSION

The results reported in this paper provide a detailed comparison of several properties of the purified cAMP and cGMP kinases. Separate binding (R) and catalytic (C) subunits exist for the cAMP kinase, while binding and kinase activities are located on the same polypeptide chain of the cGMP kinase. Thus, dissociation of active catalytic subunits is not seen for cGMP unless separate binding and kinase fragments are produced by proteolysis (13, 14, 23). In spite of these apparent differences, the two enzymes show many similarities in several physical properties. Besides size and shape, both enzymes bind their respective cyclic nucleotides with similar affinities. The cyclic

Table 4. Phosphorylation of the trypsin-sensitive and -insensitive sites of glycogen synthase by cAMP and cGMP kinases and cyclic nucleotide-independent glycogen synthase kinase (GSK)

Enzyme	% ^{32}P in trypsin-sensitive site	% ^{32}P in trypsin-insensitive site
C subunit	55	45
cGMP kinase	56	44
GSK	21	79

Average of three experiments. Reactions were incubated for 30 min as described in the legend of Fig. 2. At the end of the incubation, the reactions were terminated with 5 μl of 100 mM EDTA. To determine the percentage of total radioactivity that could be released from glycogen synthase by trypsin (trypsin-sensitive phosphorylation site), samples were incubated with 5 μl of trypsin (40 μg/ml) or water for 15 min at 30°. At this time, all of the ^{32}P that was incorporated into the trypsin-sensitive site was released. The reaction was then stopped by adding 5 μl of bovine serum albumin (5 mg/ml). Ten microliters were spotted on filter paper and immersed in 10% trichloroacetic acid. Under these conditions, 0.5–0.7 mol of phosphate per 90,000 M_r subunit was incorporated using the C subunit and cGMP kinase, while 0.2–0.4 mol was incorporated using glycogen synthase kinase.

nucleotide-dependent protein kinases are unusual in their high affinity for ATP. Several cyclic nucleotide-independent protein kinases have relatively high K_m valves for ATP (34–36). The cGMP-dependent enzyme has the unusual ability to bind ATP more tightly at high magnesium concentrations (7), although this is of doubtful physiological significance.

The most significant similarities in the two enzymes were found in substrate specificities. Both enzymes catalyzed phosphorylation of the same substrates, albeit at different affinities. Furthermore, the sites of phosphorylation of several of the substrates, including histone (16), pyruvate kinase, and glycogen synthase, appear to be the same. This is supported by our unpublished observations which indicate that the cAMP and cGMP kinases catalyze the inhibition of both pyruvate kinase and glycogen synthase similarly. While it could be argued that the large differences in affinities between the two enzymes for the various substrates indicate very little similarity, the data suggest that both enzymes contain a similar type of binding site for the various substrates, as indicated by the requirement for basic residues near serine. Most cyclic nucleotide-independent protein kinases (e.g., phosphorylase *b* kinase, pyruvate dehydrogenase kinase, and glycogen synthase kinase) are generally considered to be relatively specific for their respective substrates and will usually not catalyze phosphorylation of a wide variety of substrates (37–41). One possible explanation for the large discrepancy in the affinities of the two kinases for their substrates could be that the C subunit is a small, less sterically hindered moiety, while the cGMP kinase is a large asymmetric protein. However, it is likely that additional substrate specificity determinants exist for the cGMP kinase. It is also possible that other factors in the cell influence substrate phosphorylation. The fact that the cGMP kinase is particularly abundant in some tissues, such as lung, heart, cerebellum, and small intestine (8), suggests that specific substrates exist in these tissues. Casnellie and Greengard (42) and DeJonge (43) have shown that low concentrations of cGMP and cAMP stimulate phosphorylation of specific membrane proteins from small intestine.

The amino acid compositions of the cAMP and cGMP kinases also show striking similarities. The composition divergence function (26) indicates a significant degree of sequence homology (70–90%) between the cAMP kinase and the cGMP

kinase. The homology between the individual cAMP kinase subunits and the cGMP kinase is less evident, suggesting that one "RC" complex and one cGMP kinase subunit represent the actual homologous polypeptide chains (Table 2). As pointed out by Harris and Teller (26), however, the high composition homology is practically meaningless without supporting evidence for protein homology. Based on the similarities of the two enzymes reported here, we propose that the two cyclic nucleotide-dependent protein kinases are homologous proteins. Direct proof will only be provided by primary sequence analysis.

The difference in subunit compositions of the two enzymes has interesting evolutionary implications. The molecular weight of one R and one C subunit together is only slightly greater than that of the cGMP kinase subunit. Since the cGMP kinase seems to be the more ancestral enzyme, based on its distribution in the animal kingdom, it is possible that the R and C subunits of the cAMP kinase could have arisen from a split in the gene coding for the cGMP kinase subunit followed by gene duplication and subsequent mutation into separate R and C genes. Alternatively, it is possible that an entire R–C subunit is synthesized as one polypeptide chain in the cell and that specific proteolytic conversion occurs to produce separate R and C subunits. As mentioned above, separate cGMP-binding and kinase fragments can be obtained by proteolysis of the native cGMP-dependent protein kinase. Proteolysis of the cGMP kinase occurs spontaneously during enzyme purification (13, 14). The fact that the amino acid composition homology between the R–C complex and the cGMP kinase subunit is closer than that between the cGMP kinase subunit and either R or C alone is also evidence for such a relationship.

We thank Ms. Debbie McCarthy for excellent technical assistance. We thank Dr. T. R. Soderling for performing the glycogen synthase assays and for his criticism of the manuscript. We also thank Drs. J. P. Riou and S. J. Pilkis for performing the pyruvate kinase assays. This work was supported by Program Project Grant AM 7462 and Research Grant AM 15988 from the National Institutes of Health, U.S. Public Health Service. T.M.L. is a recipient of National Institutes of Health postdoctoral fellowship F32-CA0512 from the National Cancer Institute. J.D.C. is an investigator of the Howard Hughes Medical Institute.

1. Walsh, D. A., Perkins, J. P. & Krebs, E. G. (1968) *J. Biol. Chem.* **243**, 3763–3765.
2. Kuo, J. F. & Greengard, P. (1969) *Proc. Natl. Acad. Sci. USA* **64**, 1349–1355.
3. Kuo, J. F. & Greengard, P. (1970) *J. Biol. Chem.* **245**, 2493–2498.
4. Kuo, J. F., Wyatt, G. R. & Greengard, P. (1971) *J. Biol. Chem.* **246**, 7159–7167.
5. Corbin, J. D., Keely, S. L. & Park, C. R. (1975) *J. Biol. Chem.* **250**, 218–225.
6. Nishiyama, K., Katakami, H., Yamamura, H., Takai, Y., Shimomura, R. & Nishizuka, Y. (1974) *J. Biol. Chem.* **250**, 1297–1300.
7. Takai, Y., Nakaya, S., Inoue, M., Kishimoto, A., Nishiyama, K., Yamamura, H. & Nishizuka, Y. (1976) *J. Biol. Chem.* **251**, 1481–1487.
8. Lincoln, T. M., Hall, C. L., Park, C. R. & Corbin, J. D. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 2559–2563.
9. Erlichman, J., Hirsch, A. H. & Rosen, O. M. (1971) *Proc. Natl. Acad. Sci. USA* **68**, 731–735.
10. Brostrom, C. O., Corbin, J. D., King, C. A. & Krebs, E. G. (1971) *Proc. Natl. Acad. Sci. USA* **68**, 2444–2447.
11. Gill, G. N., Holdy, K. E., Walton, G. M. & Kanstein, C. B. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 3918–3922.
12. Dills, W. L., Beavo, J. A., Bechtel, P. J. & Krebs, E. G. (1975) *Biochem. Biophys. Res. Commun.* **62**, 70–77.
13. Lincoln, T. M., Dills, W. L. & Corbin, J. D. (1977) *Fed. Proc.* **36**, 728 abstr.
14. Lincoln, T. M., Dills, W. L. & Corbin, J. D. (1977) *J. Biol. Chem.*, **252**, 4269–4275.
15. Kuo, J. F., Kuo, W. N., Shoji, M., Davis, C. W., Seery, V. L. & Donnelly, T. E. (1976) *J. Biol. Chem.* **251**, 1759–1766.
16. Hashimoto, E., Takeda, M., Nishizuka, Y., Hamana, K. & Iwai, K. (1976) *J. Biol. Chem.* **251**, 6287–6297.
17. Sugden, P. H., Holladay, L. A., Reimann, E. M. & Corbin, J. D. (1976) *Biochem. J.* **159**, 409–422.
18. Corbin, J. D. & Reimann, E. M. (1975) in *Methods in Enzymology*, eds. Hardman, J. & O'Malley, B. W. (Adademic Press, New York), Vol. 38, pp. 287–290.
19. Kemp, B. E., Benjamini, E. & Krebs, E. G. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 1038–1042.
20. Weber, K. & Osborn, M. (1969) *J. Biol. Chem.* **244**, 4406–4412.
21. Moore, S. (1963) *J. Biol. Chem.* **238**, 235–237.
22. Gill, G. N. & Garren, L. D. (1971) *Proc. Natl. Acad. Sci. USA* **68**, 786–790.
23. Inoue, M., Kishimoto, A., Takai, Y. & Nishizuka, Y. (1976) *J. Biol. Chem.* **251**, 4476–4478.
24. Gill, G. N. & Kansten, C. B. (1975) *Biochem. Biophys. Res. Commun.* **63**, 1113–1122.
25. Sugden, P. H. & Corbin, J. D. (1976) *Biochem. J.* **159**, 423–437.
26. Harris, C. E. & Teller, D. C. (1973) *J. Theor. Biol.* **38**, 347–362.
27. Corbin, J. D., Soderling, T. R., Sugden, P. H., Keely, S. L. & Park, C. R. (1976) in *Eukaryotic Cell Function and Growth*, eds. Dumont, J. E., Brown, B. L. & Marshall, N. J. (Plenum Press, New York), pp. 231–247.
28. Yamamura, H., Nishiyama, K., Shimomura, R. & Nishizuka, Y. (1973) *Biochemistry* **12**, 856–862.
29. Cohen, P. (1973) *Eur. J. Biochem.* **34**, 1–14.
30. Singh, T. J. & Wang, J. H. (1977) *J. Biol. Chem.* **252**, 625–632.
31. Hjelmquist, G., Anderson, J., Edlund, B. & Engstrom, L. (1974) *Biochem. Biophys. Res. Commun.* **61**, 559–563.
32. Kemp, B. E., Graves, D. J. & Benjamini, E. (1976) *Fed. Proc.* **35**, 1384.
33. Soderling, T. R. (1976) *J. Biol. Chem.* **251**, 4359–4364.
34. Nimmo, H. G. & Cohen, P. (1974) *FEBS Lett.* **47**, 162–166.
35. Schlender, K. K. & Reimann, E. M. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 2197–2201.
36. Wilson, M. J. & Ahmed, K. (1975) *Exp. Cell Res.* **93**, 261–266.
37. Krebs, E. G. (1972) *Curr. Topics Cell. Regul.* **5**, 99–133.
38. Soderling, T. R. & Park, C. R. (1974) in *Advances in Cyclic Nucleotide Res.*, eds. Greengard, P. & Robison, G. A. (Raven Press, New York), Vol. 4, pp. 283–333.
39. Huang, K. P., Huang, F. L., Glinsmann, W. H. & Robinson, J. C. (1975) *Biochem. Biophys. Res. Commun.* **65**, 1163–1169.
40. Barrera, C. R., Namihira, G., Hamilton, L., Munk, P., Eley, M. H., Linny, T. C. & Reed, L. J. (1972) *Arch. Biochem. Biophys.* **148**, 343–358.
41. Davis, P. F., Pettit, F. H. & Reed, L. J. (1977) *Biochem. Biophys. Res. Commun.* **75**, 541–549.
42. Casnellie, J. E. & Greengard, P. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 1891–1895.
43. DeJonge, H. R. (1976) *Nature* **262**, 590–593.
44. Riou, J. P., Claus, T. & Pilkis, S. (1977) *Fed. Proc.* **36**, 728.