Biochimica et Biophysica Acta, 658 (1981) 191—201

BBA 69229

CYCLIC NUCLEOTIDE INDEPENDENT CASEIN KINASE (G TYPE) IN BOVINE ADRENAL CORTEX

PURIFICATION AND PROPERTIES OF TWO MOLECULAR FORMS

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(Received October 13th, 1980)

Key words Protein kinase, Casein kinase, Cyclic nucleotide, (Adrenal cortex)

Summary

Two soluble cyclic nucleotide independent protein kinase (ATP· protein-phosphotransferase, EC 2.7.1.37) activities have been purified from bovine adrenal cortex cytosol. Both purified enzymes exhibit the best affinity for acidic substrates such as casein and can use GTP as well as ATP as phosphoryl donor. They can thus be classified as casein kinase of the G type as previously proposed (Cochet C. et al., (1980) Endocrinology 106, 750—757). Whereas the two moieties could be separated using their different affinities toward a phosphocellulose resin, both purified enzymes appeared indistinguishable on the basis of several molecular and catalytic properties. Both G type casein kinase moieties have an identical sedimentation behavior (5.5 S in the presence of 0.5 M NaCl), yield similar patterns upon electrophoresis under denaturing conditions with three major protein components (42 000, 38 000 and 27 000), and show an ability to undergo self-phosphorylation mostly on the 27 000 component. Both enzymes have the same protein and nucleotide (ATP and GTP) substrate specificity, show similar increases in activity in the presence of polyamines and Mg^{2+} (optimum at 50 mM) and similar inhibition by NaCl above 0.2 M. The only difference between the two forms of casein kinase (i.e., affinity for phosphocellulose) could not be explained by a different degree of self-phosphorylation nor by a limited proteolytic process during handling and purification. These results suggest that the two active moieties may represent isoenzymatic forms of the G type casein kinase activity in bovine adrenal cortex cytosol.

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Introduction

Various types of ATP (or GTP) protein phosphotransferase (EC 2.7.1.37) activities have been characterized in mammalian tissues and criteria for their classification have been proposed [1,2]. In addition to cyclic GMP and cyclic AMP (protein kinase I and protein kinase II isoenzymes)-dependent enzymes, protein kinases independent of cyclic nucleotide (type III) have been described [1] and obtained in homogeneous form by several research groups [3–10]. Due to different substrate specificity, these various classes of protein kinases may have different physiological significance, and participate in possible cascade [11] or complementary reactions [12] leading to phosphorylation of a given specific substrate and subsequent activation-deactivation process.

The adrenal cortex is an example of hormonally-dependent differentiated tissue in which specific effectors (e.g., adrenocorticotrophic hormone ACTH) trigger a complex set of intracellular events leading to acute effects (e.g., steroidogenesis stimulation) as well as long range effects upon trophicity and growth [13]. The currently accepted mechanism of action of ACTH on adrenocortical cells lies in the concept of cyclic AMP as intracellular messenger and subsequent activation of cyclic AMP-dependent protein kinase activities [13,14]. However, the search for corresponding specific intracellular phosphorylatable substrates of key metabolic significance has proved rather inconclusive [15,16]

On the other hand, cyclic nucleotide independent protein kinases represent quantitatively important alternative phosphorylation systems [1]. We have previously described in bovine adrenal cortex cytosol the presence of a type III protein kinase activity [17]. Subsequently, this activity was separated into four active moieties, respectively named PC1 to PC4 protein kinases, according to their elution sequence from a phosphocellulose matrix [18,19]. These four activities were all characterized as cyclic AMP independent casein kinases. However, PC1 uses only ATP as the phosphoryl donor (A type), whereas PC2-4 enzymes can function with GTP as well as ATP (G type) [18,19]. The possible different biological significance of these two types of casein kinase was further strengthened by the isolation in the same tissue of an inhibitory factor, specific of the G type casein kinase [20]. In this paper, we report the purification of two bovine adrenal cortex casein kinases of the G type and some of their catalytic as well as physico-chemical properties.

Materials and Methods

[γ^{32}P]ATP (20 Ci/mmol), [γ^{32}P]GTP (25 Ci/mmol) were purchased from the Radiochemical Centre, Amersham. Phosphocellulose (P-11) was from Whatman and treated according to Keller et al. [21] before use. Acid phosphatase, type III, rabbit muscle glycogen synthetase, phosvitin, spermine, poly-L-arginine type II B, poly-D,L-ornithine type I B, trypsinogen, lysozyme and bovine serum albumin were obtained from Sigma. Poly-L-lysine was from Koch Light, ovalbumin from Calbiochem, glucose oxidase and catalase from Boehringer. Casein (Merck) was treated according to Ashby and Walsh [22]. Bovine milk caseins α_{S1}, α_{S2} and β were a generous gift from Dr. Ribadeau-Dumas (Jouy-en-

Josas, France). These caseins were dephosphorylated before use by treatment with acid phosphatase as follows: 4 mg casein were incubated (2 h at 30°C) with acid phosphatase (4 μg) in 2.8 ml 50 mM imidazole buffer, pH 6.2 1.5 mM $MgCl_2$/0.2 mM $ZnSO_4$. The reaction was stopped upon addition of 4 ml 40% trichloroacetic acid and the resulting pellet collected after centrifugation (2000 × *g*, 10 min), washed three times with distilled water, twice with ethanol/diethyl ether (1 : 4) containing 0.1 M HCl, twice with ethanol/ether (1 : 4) and finally redissolved in 10 mM Tris-HCl buffer, pH 7.5. Calmodulin was kindly supplied by Professor J. Demaille (Montpellier, France). Sepharose-casein was prepared according to Farron-Furstenthal and Lightholder [23].

Bovine adrenal cortex cytosol. This was prepared as previously described [24] in ice cold 10 mM Tris-HCl buffer, pH 7.5/0.5 mM dithiothreitol/2% glycerol (buffer A) with 0.1 M NaCl.

Protein kinase activity Measurements were performed following the incubation conditions of Corbin and Reiman [25] and the trichloroacetic acid precipitation procedure of Sandoval and Cuatrecasas [26]. The standard reaction mixture (80 μl) contained 0.1 mM [γ^{32}P]ATP or [γ^{32}P]GTP (spec. act. 100 cpm/pmol)/600 μg casein/50 mM $MgCl_2$ unless otherwise indicated. The reaction was initiated by addition of the enzymatic preparation (50 μl) and run at 30°C, under linear conditions with regard to time and enzyme concentration. 1 enzyme unit was defined as the amount of enzyme incorporating 1 pmol ^{32}P/min into casein under the standard assay conditions.

Self phosphorylation. Phosphorylation of the purified enzymatic preparations was examined after incubation (30°C) in the presence of 0.01 mM [γ^{32}P]ATP (30 000 cpm/pmol) in buffer A/50 mM $MgCl_2$/0.1% bovine serum albumin, for the indicated time. Recovery of the labeled proteins was carried out as described for protein kinase activity measurement.

Polyacrylamide slab gel electrophoresis. This was performed using 0.1% sodium dodecyl sulfate (SDS) and 15% polyacrylamide gels, according to Laemmli and Favre [27]. Bovine serum albumin (M_r 68 000), ovalbumin (M_r 45 000), trypsinogen (M_r 25 000), calmodulin (M_r 16 500), and lysozyme (M_r 14 500) were used as molecular weight calibration standards.

Sedimentation studies were carried out in linear (5—20%) sucrose density gradients prepared in buffer A containing 0.1 or 0.5 M NaCl and spun at 127 000 × g_{av} in a MSE SW-50 rotor at 4°C for 16 h. Sedimentation coefficients were determined according to Martin and Ames [28] using glucose oxidase ($S_{20,w}$ = 7.8 S) and bovine serum albumin ($S_{20,w}$ = 4.3 S) as calibration standards.

Gel filtration. Gel filtration for apparent molecular weight determination was performed using a 1.6 × 92.5 cm Sephacryl S-300 column equilibrated and eluted with buffer A/0.4 M NaCl/250 μg/ml bovine serum albumin. Glucose oxidase, bovine serum albumin, ovalbumin and trypsinogen were used as calibration standards.

Protein measurement was performed using the method of Lowry et al. [29] using bovine serum albumin as the standard and the technique of Kalckar [30] for low protein levels.

Radioactivity was counted in Bray's solution [31] with a Nuclear Chicago liquid scintillation spectrometer. Conductivity measurements were performed with a Radiometer instrument.

Purification of PC3 and PC4 casein kinases Two different protocols were followed in the course of this work. All procedures were carried out between 0—4°C. Adrenal cortex cytosol (500 ml) was brought to 70% saturation by addition of $(NH_4)_2SO_4$ under continuous stirring. After overnight precipitation, the pellet was collected after centrifugation (20 000 × *g*, 30 min), redissolved in 200 ml buffer A/0.5 M NaCl and dialysed against 50 vol. of the same buffer

Protocol 1. The extract was applied onto a first (2 × 11 cm) phosphocellulose column previously equilibrated with buffer A. After a 15 vol. wash with the same buffer containing 0.5 M NaCl, the enzymatic activities were eluted with 2.5 col. vol. buffer A/0.9 M NaCl. The eluate was immediately brought to 0.4 M NaCl and 0.1% bovine serum albumin and transferred onto a second (2 × 5.5 cm) phosphocellulose column previously equilibrated with bufferA/0.4 M NaCl After a 5 col. vol. wash with buffer A/0.6 M NaCl, a 0.6—1 M linear NaCl gradient in buffer A/0.025% bovine serum albumin was applied to the column (15 vol.). Fractions containing PC3 (0.70—0.78 M NaCl) and PC4 (0.78—0.85 M NaCl) were separately pooled and concentrated. Each preparation was dialysed overnight against buffer A/20% glycerol. The flocculate occurring in the dialysis bag was recovered after centrifugation (20 000 × *g*, 10 min) and dissolved in 1 ml buffer A/0.5 M NaCl. $MgCl_2$ concentration was adjusted to 50 mM and the mixture was immediately transferred onto a 0.5 × 4.7 cm casein-Sepharose column previously equilibrated in buffer A/0.1 M NaCl. After 1 h standing at 4°C the column was washed with 5 col. vol. buffer A/0.2 M NaCl and eluted with buffer A/0.7 M NaCl. The preparation was either used immediately or brought to 0.1% bovine serum albumin and 10% glycerol, frozen and stored for further study.

Protocol 2 was identical to protocol 1 up to the loading and washing of the first phosphocellulose column. PC3 and PC4 enzymes were then separately recovered from this column upon elution with the 0.6—1 M linear NaCl gradient. Each active fraction was carried through the second phosphocellulose step of protocol 1 except that the column was washed with 15 vol. buffer A/0.6 M NaCl before elution with the linear NaCl gradient. After dialysis, during which no flocculation occurred, further steps including casein-Sepharose chromatography were the same as in protocol 1.

Results

Isolation of two casein kinases from adrenal cortex cytosol

The purification protocols used in this work make extensive use of the differential affinity of various types of protein kinases for phosphocellulose matrix, as previously emphasized by several research groups [4—10]. After adsorption of a crude adrenal cortex cytosol onto a phosphocellulose column it has been previously observed that the bulk of cyclic AMP-dependent histone kinase activity was washed off at low salt concentrations (i.e., ⩽ 0.4 M NaCl), whereas subsequent elution with a 0.4—1 M NaCl gradient yielded a separation between several cyclic AMP independent casein kinase activities [18,19]. The major active moieties have been previously [19] termed PC1 (eluted at 0.5 M NaCl), PC2 (0.6 M NaCl), PC3 (0.75 M NaCl) and PC4 (0.81 M NaCl). The goal of this work was the purification of PC3 and PC4, previously characterized as

TABLE I

PURIFICATION SCHEME OF PC3 AND PC4 CASEIN KINASE ACTIVITIES FOLLOWING PROTOCOL 1

Due to the presence of added BSA (see Methods and Materials) at several purification steps, protein concentration and specific activity were not determined (n d) in these cases

Step	Protein (mg)	Casein kinase activity (units 1 10^{-4})	Specific activity (units/mg protein)	Purification factor
Cytosol	9 500	46	48	1
$(NH_4)_2SO_4$ precipitate	4 080	48	118	2 4
First phosphocellulose	9 2	24	26 000	537
Second cellulose				
PC3		9	n d	n d
PC4		21		n d
Flocculate				
PC3		5 7	n d	n d
PC4		13	n d	n d
Casein-Sepharose				
PC3	0 110	6 6	600 000	12 500
PC4	0 250	5 5	220 000	4 545

the major components of the G type casein kinase activity.

The $(NH_4)_2SO_4$ precipitation step yielded an approx. 2-fold enrichment in enzymatic activity and the first phosphocellulose step increased this enrichment factor about 300-fold (Table I). Isolated PC3 and PC4 activities tended to be irreversibly lost upon handling and in a dilute form, however, this loss was greatly reduced when serum albumin was added to the purified preparations.

In both protocols, adsorption of the enzymatic preparations onto the second phosphocellulose column required rather high ionic strength (0.5 M NaCl) as already observed in the case of a reticulocyte casein kinase [7]. However, adsorption under these conditions did not modify the subsequent elution behavior of the enzymes. This is illustrated in Fig. 1 showing that PC3 and PC4 isolated by phosphocellulose chromatography and NaCl gradient elution could be analysed on a second phosphocellulose column without change in their respective elution behavior. This also demonstrated that no PC3-PC4 interconversion occurred under these conditions and suggests that both enzymatic activities are present in native cytosolic extract and are not the result of artifactual events during handling and purification.

During dialysis against buffer A/glycerol buffer preparation resulting from protocol 1 led to the occurrence of a flocculate containing the bulk of the casein kinase activity. This phenomenon was of practical interest since it resulted in concentrated enzymatic preparations essentially devoid of bovine serum albumin with an excellent yield (Table I).

The Sepharose-casein affinity chromatography step allowed removal of the remaining detectable protein contaminents, as judged by the absorbance at 280 nm. The samples were transferred onto the affinity support in the minimum buffer volume (i.e., 1 ml) in order to optimize the casein-enzyme interaction.

Disregarding contamination by added bovine serum albumin, purified PC3 and PC4 preparations obtained by both protocols appeared homogeneous upon

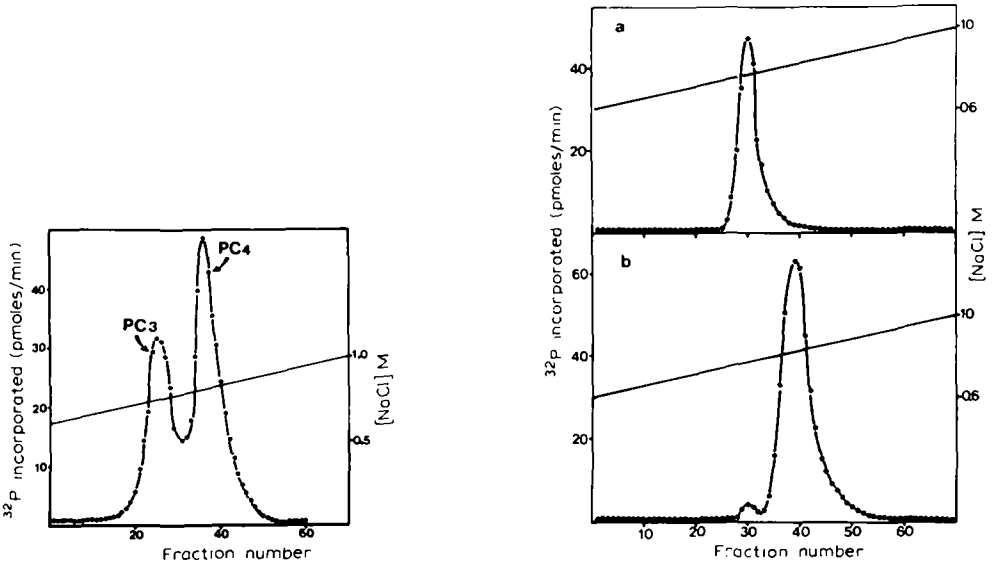


Fig 1 Separation of PC3 and PC4 casein kinase activities by chromatography on a phosphocellulose column Elution was carried out with a 0 4—1 M NaCl gradient, as described under Materials and Methods Hand-trace PC3 and PC4 resolution by the second phosphocellulose column of protocol 1 Hand-trace the same phosphocellulose system was than used to analyse idependently the isolated activities PC3 (a) and PC4 (b) Collected fractions were diluted 1 5 with buffer A/0.1% bovine serum albumin before assay.

gel filtration through a Sephacryl S-300 column, as judged by absorbance at 280 nm (with 30—50 μg protein) and exhibited similar composition upon denaturating polyacrylamide gel electrophoresis (see below). As shown in Table I, the final specific activity was higher for PC3 than for PC4, with overall purification factors of similar magnitude.

Molecular properties of purified PC3 and PC4

Fig. 2 illustrates the results of polyacrylamide gel electrophoresis analysis in the presence of 0.1% sodium dodecyl sulfate (SDS) for PC4 obtained by protocol 1 (trace a), PC3 (trace b) and PC4 (trace c) resulting from protocol 2. Disregarding the high bovine serum albumin contamination resulting from protocol 2, the pattern revealed after Coomassie blue staining was similar in all cases. Each enzymatic preparation yielded three major components corresponding to protein moieties of M_r 27 000, 38 000 and 42 000, respectively. In all preparations, the 38 000 and 42 000 components were obtained as doublet bands regardless of the preparation protocol used.

Determination of an apparent molecular weight by gel filtration through a Sephacryl S-300 column using catalase (250 000), glucose oxidase (152 000), bovine serum albumin (68 000), ovalbumin (45 000) and trypsin (25 000) as protein calibration standards yielded a value of 140 000 for PC3 and PC4, regardless of their preparation protocol Together with the aforementioned electrophoretic pattern under denaturing conditions (Fig. 3), this suggests that both PC3 and PC4 enzymes have a multimeric molecular structure. Although a limited number of major components could be detected under denaturing conditions (Fig. 2), in both PC3 and PC4, the homogeneity of the preparations

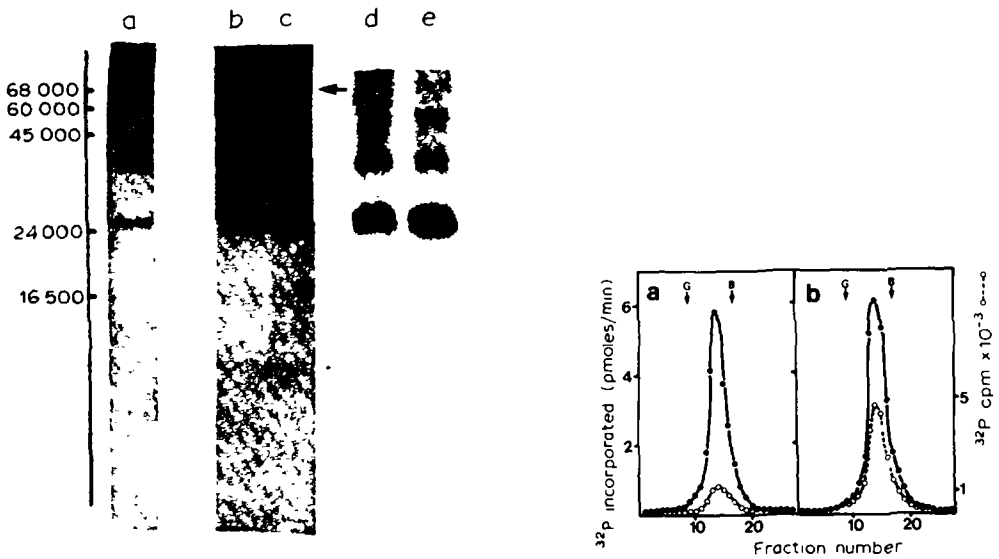


Fig 2. SDS-polyacrylamide gel electrophoresis of purified PC3 and PC4 casein kinases. Lane a PC3 obtained by protocol 1 Lanes b and c PC3 and PC4 final preparations resulting from protocol 2 Lanes d and e autoradiography of PC3 (d) and PC4 (e) after self phosphorylation The arrow indicates the position of bovine serum albumin present in the preparations from protocol 2 Molecular weight scale obtained by analysis of protein markers is given on the left

Fig. 3. Sucrose density gradient analysis of purified PC3 (a) and PC4 (b) kinases. Each enzyme preparation (2800 units) was self phosphorylated and layered on a linear 5—20% sucrose density gradient prepared in buffer A/0 5 M NaCl/20 mM $MgCl_2$ Casein kinase activity (●———●) and content in ^{32}P (○ - - - - - - ○) were measured in collected fractions Arrows indicate the positions of protein markers glucose oxidase (G) and bovine serum albumin (B)

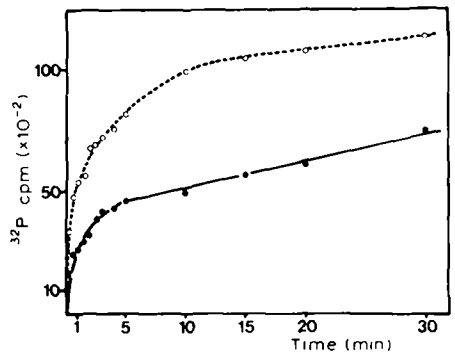
remains to be more fully assessed before subunit stoichiometry can be determined.

The purified enzymatic preparations were subjected to velocity sedimentation analysis in 5—20% linear sucrose density gradients in buffer A/0.5 M NaCl. As illustrated in Fig. 3, PC3 and PC4 both exhibited a sedimentation coefficient of 5.5 S under these conditions. At lower ionic strength, both purified casein kinases showed a propensity to aggregate into heavier molecular forms, whereas salt concentrations higher than 0.5 M NaCl had no further effect upon the enzyme sedimentation.

Although clearly different by their affinity for phosphocellulose matrix, PC3 and PC4 thus appeared indistinguishable with regard to their molecular size and major protein components under denaturing conditions. Further experiments were undertaken to examine in more detail possible functional differences between the two enzymatic moieties.

Self phosphorylation

Purified PC3 and PC4 appeared able to incorporate radioactive phosphate when incubated with [γ^{32}P]ATP in the presence of Mg^{2+}. This self phosphorylation process was found to be associated with the purified casein kinase activ-



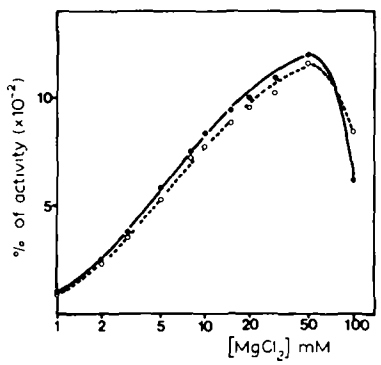


Fig 4 Kinetics of self phosphorylation of purified PC3 and PC4 casein kinases PC3 and PC4 (3000 units) were incubated with [γ^{32}P]ATP as described under Materials and Methods At different time intervals aliquots were withdrawn for determination of ^{32}P incorporation after trichloracetic acid precipitation Each point is the mean of four replicates in the case of PC3 (●——●) and PC4 (○ - - - - - ○)

Fig 5. Effect of Mg^{2+} on purified PC3 and PC4 casein kinase activities Increasing concentrations of $MgCl_2$ were introduced in the standard assay mixture Casein kinase activities at various Mg^{2+} concentrations were plotted with regard to the activity at 1 mM Mg^{2+}, taken as 100% Each point for PC3 (●——●) and PC4 (○ - - - - - ○) activities is the mean of triplicate determinations

ities upon gel filtration, phosphocellulose chromatography and density gradient centrifugation (see Fig. 3). Autoradiography of self phosphorylated PC3 and PC4 preparations after electrophoresis under denaturing conditions is illustrated in Fig. 2. For both enzymes, the radioactivity was found associated mostly with the 27 000 component. A low level of phosphate incorporation was associated with the 38 000 band, although almost negligible in the case of PC4 as compared to PC3. Self phosphorylation kinetics of PC3 and PC4 were followed as illustrated in Fig. 4 and revealed a rapid process in both cases. However, PC4 incorporated almost twice as much phosphate as PC3 after 30 min incubation. This quantitative difference is in agreement with visual inspection of Figs. 2 and 3.

The behavior of purified PC3 and PC4 on phosphocellulose was compared with that of the same preparations after maximal self phosphorylation on one hand, and after treatment with acid phosphatase on the other hand· these treatments did not modify the enzyme affinities for the ion-exchange matrix. Thus, a quantitative difference in phosphorylation state is not likely to be the origin of PC3-PC4 separation. Similarly, addition of protease inhibitor (e.g., diisopropyl fluorophosphate) to the cytosol or incubation (30°C, 30 min, with or without addition of $CaCl_2$) of the cytosol prior to the purification procedure did not modify the PC3-PC4 quantitative ratio as obtained by phosphocellulose chromatography [19].

Catalytic activities of purified PC3 and PC4

Phosphoryl donors. Kinetic parameters of purified PC3 and PC4 were determined under standard assay conditions using either ATP or GTP as the source of phosphate and casein as substrates. The corresponding values, calculated from Lineweaver-Burk plots, are given in Table II. Both casein kinases appeared to use ATP and GTP, with a similar apparent K_m value. The only noticeable dif-

TABLE II

KINETIC PARAMETERS (K_m, V) OF PURIFIED PC3 AND PC4 CASEIN KINASE WITH ATP AND GTP AS THE SOURCE OF PHOSPHATE

Phosphoryl donor	K_m (μM)		V (pmol ^{32}P incorporated/min per mg protein)	
	ATP	GTP	ATP	GTP
PC3	6 3	12	2 9 10^3	1 8 10^3
PC4	7 2	8 9	2 0 10^3	0 8 10^3

ference was found in the ratio V GTP/V ATP which was 40% for PC4 and about 60% for PC3. However, both purified PC3 and PC4 were confirmed to be casein kinases of the G type (i.e., using GTP as well as ATP) as previously defined [18,19].

Inhibitory effect of NaCl. Increasing NaCl concentration in the assay medium progressively inhibited the casein-kinase activity of both PC3 and PC4 (data not shown). This observation agrees with the marked inhibitory effect of NaCl above 0.2 M concentration previously reported with crude G type casein kinase preparations [18,19]. No significant difference appears between the behavior of the isolated PC3 and PC4 with regard to their NaCl sensitivity.

Effect of magnesium and polycations. Fig. 5 illustrates the effect of increasing $MgCl_2$ concentration upon PC3 and PC4 casein kinase activities. A maximal (about 10-fold) stimulation of both enzymatic activities was obtained for about 50 mM $MgCl_2$ in the assay medium and purified PC3 and PC4 exhibited similar behavior in these experiments. Polyamines which have been found to be similar potent activators of crude G type casein kinase [18,19] can also be very effective in increasing purified PC3 and PC4 activities (Table III). A 7—10-fold stimulation was observed for both preparations with 2 mM spermine in the assay medium. As shown in Table III, basic polyaminoacids such as polylysine and polyornithine were also potent activators, whereas polyarginine had a clear inhibitory action. However, PC3 and PC4 were indistinguishable with regard to their polycation susceptibility.

TABLE III

EFFECT OF ADDITION OF POLYCATIONIC COMPOUNDS ON PURIFIED PC3 AND PC4 CASEIN KINASE ACTIVITIES (^{32}P INCORPORATION)

Values are the mean ± S D of quadruplicate experiments

Enzyme preparation addition	Casein kinase activity (pmol ^{32}P incorporated/min)	
	PC3	PC4
None (control)	1 48 ± 0 03	1 61 ± 0 03
Spermine (2 mM)	10 86 ± 0 08	11 81 ± 0 07
Poly-lysine (2 4 10^{-5} M)	3 65 ± 0 11	3 58 ± 0 05
Poly-arginine (2 7 10^{-5} M)	0 96 ± 0 01	1 08 ± 0 03
Poly-ornithine (8 10^{-5} M)	6 40 ± 0 21	6 55 ± 0 07

TABLE IV

SUBSTRATE SPECIFICITY OF PURIFIED PC3 AND PC4 PREPARATIONS

Values of ^{32}P incorporation are the mean ± S D of quadruplicate experiments

Enzyme preparation, substrate	Casein kinase activity (pmol ^{32}P incorporated/min)	
	PC3	PC4
Whole casein	12 58 ± 0 11	13 34 ± 0 11
α_{S1} Casein	5 51 ± 0 52	5 91 ± 0 37
α_{S2} Casein	0 82 ± 0 09	0 64 ± 0 04
β Casein	8 35 ± 0 28	5 31 ± 0 30
Glycogen synthase	0 45 ± 0 02	0 54 ± 0 01
Phosvitin	5 05 ± 0 15	6 04 ± 0 21
Histone II A	0 24 ± 0 02	0 19 ± 0 01

Substrate specificity. As shown in Table IV, the casein kinase nature of PC3 and PC4 [18,19] was confirmed using the purified preparations, whereas histone phosphorylation was negligible. Several casein substrates were used with the aim of a detailed comparison of PC3 and PC4 activities. As illustrated in Table IV, both purified enzymes exhibited similar patterns of substrate specificity.

Discussion

Two cyclic AMP independent casein kinases purified from bovine adrenal cortex cytosol, during this work, could not be distinguished one from the other on the basis of several molecular and functional properties Based on the present results, it could be suggested that PC3 and PC4 represent two isoenzyme forms of a G type casein kinase in adrenal cortex, as previously defined [18,19].

PC3 and PC4 appear similar to casein-phosvitin kinases described in ascites tumor cells [4], liver nuclei [5], bovine brain [8,9], rat liver chromatin [32] and human erythrocyte [10] and they closely resemble the rabbit reticulocyte CK II [7] These casein-phosvitin kinases have an apparent molecular weight between 100 000 and 200 000 in common, a propensity to aggregate at low ionic strength and are eluted from a phosphocellulose resin above 0.5 M NaCl concentration. When purified, these preparations exhibit a multimeric structure and demonstrate autophosphorylation on a small subunit [4,7–9]. Another characteristic feature of this class (G type) of enzymes is the ability to use GTP as well as ATP as phosphate donor [7,8,10,18,19,33]. The G type casein kinase appears similar to the 'TS' casein-phosvitin kinase as described by Meggio et al. [32]. These properties clearly distinguish G type from the lower molecular weight, monomeric A type (using only ATP as phosphate source), as previously described in adrenal cortex cytosol [18,19] and resembling the rabbit reticulocyte CK I [7], and the 'S' type casein kinase of Meggio et al. [32]. However, there is a discrepancy with regard to the G type enzyme subunit composition, which has been reported to be made up of two [9,10] or three [4,7,8] moieties, it has been suggested that a limited proteolytic process might be the origin of this phenomenon [8,9].

One of the characteristic properties of our PC3 and PC4 preparations was the striking enhancement of their activity in the presence of Mg^{2+} and polycations such as polyamines. These features have been previously shown to be a character of the adrenal cortex G type casein kinase [18,19]. Similar sensitivity to Mg^{2+} has been reported for cyclic nucleotide independent protein kinases from other sources [4,5,9,10] and polyamine stimulation has also been described [9], although not generally examined. However, this direct stimulating effect of naturally occurring polyamines might be of interest in view of a possible regulation of these casein kinase (G type) activities in the intact cell. In addition, it has been shown that polyamines can modulate G type casein kinase activity in adrenal cortex tissue extracts through release of the inhibition due to the presence of an endogenous specific G type casein kinase inhibitor [20,34]. This working hypothesis will be examined using purified preparations of PC3 and PC4, in order to define the mechanism of interaction of the enzymes with this inhibitor and the possible implication of the G type casein kinase system in the regulation of adrenocortical cell functions.

Acknowledgements

This work was possible thanks to the support of the INSERM (FRA No. 54, ATP 79-114 and 77-84), the CNRS (ERA 478) and the Fondation pour la Recherche Médicale Française.

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