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MULTIPLE FORMS OF CASEIN KINASE FROM RABBIT ERYTHROCYTES

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Summary

Two rabbit erythrocyte casein kinases, GTP:casein kinase I and GTP:casein kinase II, have been purified 29 000- and 47 000-fold, respectively. Studies employing sucrose density gradient centrifugation indicate that kinase I has a molecular weight of about $9.5 \cdot 10^5$ (25 S) and kinase II about $1.4 \cdot 10^6$ (32 S). These enzymes can utilize either ATP or GTP as the phosphoryl donor. Among various protein substrates examined, these kinases catalyze the phosphorylation of casein >50% dephosphorylated phosvitin ≃50% dephosphorylated casein >phosvitin. Histones, protamine and bovine serum albumin are poor phosphoryl acceptors. Kinetic data indicate that both enzymes are inhibited by high casein substrate concentrations which may be partially relieved by NaCl.

Both phosphotransferases require Mg^{2+} for activity and are optimally active at pH 9.0. The enzymes have apparent K_m values of $2.5 \cdot 10^{-5}$ M for GTP, $2 \cdot 10^{-5}$ M for ATP, and 0.4—0.6 mg/ml for casein. The incorporation of the terminal phosphate of GTP into casein as catalyzed by these enzymes is inhibited to varying degrees by ATP, ITP, ADP, and GDP but not by UTP, CTP, GMP, adenosine 3′:5′-cyclic monophosphate, and guanosine 3′:5′-cyclic monophosphate. In addition, NaF and 2,3-diphosphoglyceric acid are also found to inhibit the activity of both kinases. The effect of 2,3-diphosphoglycerate is interesting and suggests that this metabolite may regulate the activity of the casein kinases in the red blood cells.

Introduction

Protein phosphokinases, particularly those regulated by adenosine 3′:5′-cyclic monophosphate (cyclic AMP), and the attendant phosphorylation reactions in relation to hormone action, have elicited considerable interest and attention in recent years [1,2]. The cyclic AMP-dependent protein kinases specifically utilize ATP to phosphorylate a variety of proteins [1—4]. However, protein

kinases which are not regulated by cyclic nucleotides have also been isolated from a number of sources [1,5—7]. Interestingly, several of these enzymes have been reported to exhibit little specificity with respect to the phosphoryl donor and can utilize both ATP and GTP to phosphorylate protein substrates such as casein, phosvitin, etc. [5,7,8]. More recently, a protein kinase which specifically employs GTP in the phosphorylation of certain rat liver ribosomal proteins has been identified [9]. The function of many of these kinases remains unknown but conceivably they may play an important role in metabolic regulatory processes.

During the course of our investigation of the cyclic AMP-dependent protein kinases of rabbit red blood cells, we have found two other protein kinase activities in these cells. Unlike the cyclic AMP-regulated enzymes, these kinases seem to prefer casein as the phosphoryl acceptor and can utilize both ATP and GTP as phosphoryl donors. Since the specific function of these enzymes in the red cells is at present unknown, they will be tentatively identified as casein kinases. Recently, Traugh and Traut [7] have detected a similar enzymic activity in both rabbit reticulocytes and erythrocytes. This communication describes the partial purification and characterization of these casein kinases and further extend the studies of Traugh and Traut [7].

Materials and Methods

[γ-^{32}P] ATP and [γ-^{32}P] GTP were purchased from New England Nuclear. Casein (Hammersten) was a product of Mann Research Laboratories. Phosvitin, histones, bovine serum albumin, and protamine were obtained from Sigma Chemical Co. Cyclic AMP, guanosine 3′:5′-cyclic monophosphate (cyclic GMP), and all other nucleotides were acquired either from Sigma Chemical Co. or P-L Biochemicals, Inc. Beef spleen acid phosphatase was isolated by the procedure described by Campbell and Zerner [10]. Dephosphorylated casein was prepared according to Bingham et al. [11] and dephosphorylated phosvitin, according to Rabinowitz and Lipmann [12].

Assay of protein kinase activity

Protein kinase was assayed by measuring the incorporation of ^{32}P from [γ-^{32}P] GTP into casein. The reaction mixture (0.2 ml) contained 50 mM of Tris · HCl (pH 8.5), 10 mM of $MgCl_2$, 2 mg/ml of casein, 0.2 mM of [γ-^{32}P]-GTP (specific activity of 20—30 cpm/pmol) and an appropriate dilution of the enzyme prepared in 1 mg/ml of bovine serum albumin. Incubation was performed at 37° C for 5 min; and the reaction terminated by the addition of 2 ml of 10% (w/v) trichloroacetic acid. Bovine serum albumin (0.5 mg) was added as a co-precipitant when phosvitin was used as a substrate. The precipitate was collected on Whatman GF/C glass fiber filter, washed 10 times with about 2-ml portions of 10% trichloroacetic acid, and counted in 5 ml of Bray's solution [13]. One unit of kinase activity is defined as that amount which will catalyze the incorporation of one nmol of ^{32}P into casein or other proteins per min. Specific activity of the enzyme is expressed as units/mg of protein. Protein concentration was determined by the method of Lowry et al. [14] using bovine serum albumin as the standard.

Cyclic AMP-dependent protein kinases were assayed by determining the amount of ^{32}P transferred from [γ-^{32}P]ATP into histones in the presence of cyclic AMP as described previously [3].

Sucrose density gradient centrifugation

The method of Martin and Ames [15] was used in determining the sedimentation coefficients of the kinases, with the modification that the enzymes were layered onto 5 ml of a 20—40% linear sucrose gradient. They were then subjected to centrifugation in a SW65L swinging bucket rotor in a Beckman L2-65B ultracentrifuge at 2° C. At the conclusion of the run, 10-drop fractions were collected from the bottom of the tube and assayed for enzyme activity.

Results

Purification of casein kinases

Unless noted otherwise, all operations were performed at 0-4° C. Centrifugations were carried out in a Sorvall RC-2B centrifuge.

Crude lysate. Young rabbit red blood cells were purchased from Pel-Freez Biologicals, Inc. and received at the laboratory frozen in dry ice. Two liters of red blood cells were thawed and lyzed in 8 volumes of 2.5 mM $MgCl_2$. Unlyzed cells and cell membranes were removed by centrifugation at 17 000 × *g* for 30 min.

Ammonium sulfate fractionation. Solid ammonium sulfate (30 g/100 ml) was added to the crude lysate with stirring. After 30 min, the precipitate was collected by centrifugation, dissolved in and dialyzed overnight against 4 l of 0.02 M Tris · HCl, pH 7.5, containing 1 mM dithiothreitol (Buffer A).

DEAE-cellulose chromatography. The dialyzed ammonium sulfate fraction was applied to a DEAE-cellulose column (5 × 28 cm, Cellex D, Bio-Rad Laboratories) equilibrated with Buffer A. The column was washed with Buffer A until no more reddish substance emerged from the column. Subsequent elution was carried out with 2.5 l of a 0—0.6 M linear KCl gradient. Fractions of 22 ml were collected and assayed for kinase activities. The conductivity of each fraction was also measured using a Radiometer conductivity meter. The

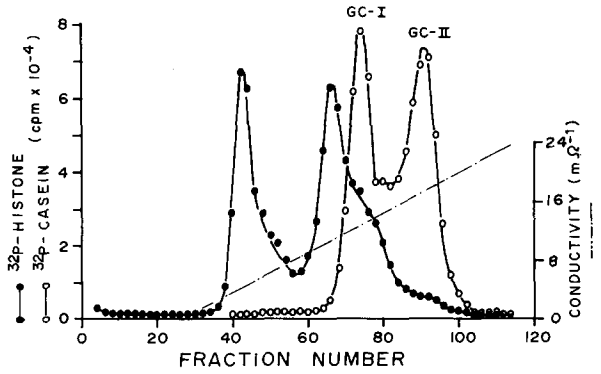


Fig. 1. Resolution of rabbit erythrocyte protein kinases by DEAE-cellulose chromatography. Experimental details are described in the text.

salt concentration of each fraction was ascertained from a conductivity calibration curve for KCl solutions.

As shown in Fig. 1, several protein kinase activity peaks were eluted from the column. The two activity peaks which emerged at KCl concentrations of 0.08 M (3.8 $m\Omega^{-1}$) and 0.21 M (10.3 $m\Omega^{-1}$) correspond to the cyclic AMP-dependent protein kinases. The properties of these enzymes have been described in detail in an earlier communication [3]. The cyclic AMP-dependent protein kinases catalyzed the transfer of ^{32}P from [γ-^{32}P]ATP into histones. GTP was a poor phosphoryl donor and casein an inefficient substrate for these enzymes [3].

Two additional activity peaks (GTP:casein-I, and GTP:casein-II) representing transfer of ^{32}P from [γ-^{32}P]GTP into casein were also eluted from the column. GTP : casein I (kinase I) activity emerged at about 0.28 M KCl (12.8 $m\Omega^{-1}$) whereas GTP:casein II (kinase II), at about 0.38 M KCl (18.5 $m\Omega^{-1}$). The active fractions corresponding to each peak were pooled and concentrated by ammonium sulfate (30 g/100 ml) precipitation. The precipitates were collected by centrifugation and dissolved in 0.05 M potassium phosphate buffer, pH 6.8, containing 1 mM dithiothreitol (Buffer B) and dialyzed against this buffer.

Phosphocellulose chromatography. The dialyzed kinase-I and kinase-II fractions were each applied to a phosphocellulose column (2.5 × 31 cm, Cellex P, Bio-Rad Laboratories) equilibrated with Buffer B. The column was washed with 2 bed volumes of the same buffer and then eluted with a 1-liter linear gradient of 0.05 to 0.6 M potassium phosphate buffer, pH 6.8, containing 1 mM dithiothreitol. The effluent was collected in 14-ml fractions.

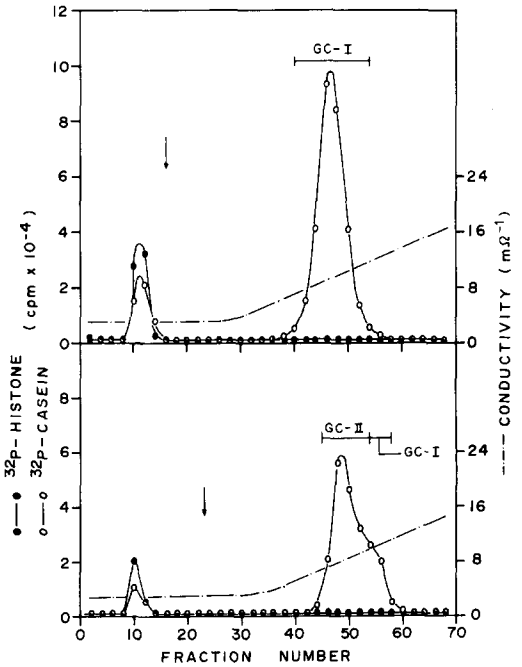


Fig. 2. Phosphocellulose chromatography of protein kinases I and II (GC-I and GC-II). Experimental details are presented in the text. The arrows denote the application of the salt gradient.

The elution profiles of kinase-I and kinase-II from the phosphocellulose column are shown in Fig. 2. A major portion of their activities was retarded by the column but was eluted when the salt gradient was applied. A shoulder on the trailing edge of the kinase-II peak probably resulted from a minor contamination by kinase-I. Neither kinase-I nor kinase-II catalyzed the phosphorylation of histones when assayed under the conditions employed for cyclic AMP-dependent protein kinases. A small amount of activity representing both histones and casein phosphorylation was not retained by the phosphocellulose. The histones kinase activity could be due to contamination of these preparations with cyclic AMP-dependent protein kinases. These kinases did not adsorb to phosphocellulose (unpublished observation). The casein kinase activity that emerged at the breakthrough from either column was not due to overloading since reapplication of these unadsorbed fractions on phosphocellulose columns exhibited the same chromatographic behavior. The nature of these unadsorbed casein kinase activities remains to be determined. The active fractions under each of the various peaks (Fig. 2) were pooled, concentrated by DIAFLO-ultrafiltration (PM-10 membrane) and dialyzed against Buffer B.

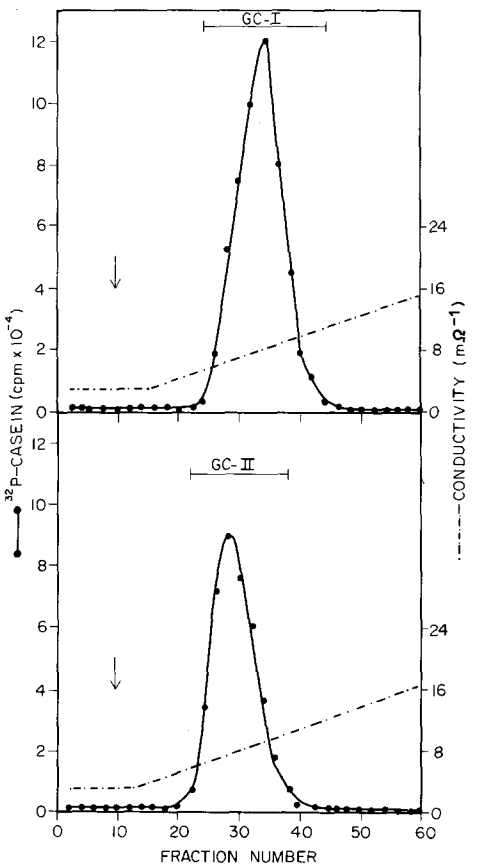


Fig. 3. Hydroxylapatite chromatography of kinases I and II (GC-I and GC-II). Experimental details are presented in the text. The arrows denote the application of the salt gradient.

Hydroxylapatite chromatography. Further purification of kinase-I and kinase-II was carried out employing hydroxylapatite ion exchanger (Bio-Gel HTP, Bio-Rad Laboratories). Each of the dialyzed enzyme samples was applied to the column (1.8 × 9 cm) which had been previously equilibrated with Buffer B. After washing the column with 2 bed volumes of Buffer B, the enzyme was eluted with a 400 ml linear gradient of 0.05 to 0.4 M potassium phosphate buffer, pH 6.8, containing 1 mM dithiothreitol. Fractions of 6.5 ml were collected and assayed for phosphotransferase activity. A single peak of kinase activity was obtained for both GTP:casein kinases as shown in Fig. 3. The peak fraction of kinase-I was eluted at a phosphate concentration of 0.15 M, whereas the peak of kinase-II was at 0.14 M. These salt concentrations were derived from conductivity measurements.

The fractions containing the bulk of the activity were pooled, concentrated by DIAFLO-ultrafiltration and dialyzed against Buffer A. The enzyme solutions were stored in 50—100 μl aliquots in liquid nitrogen. After 3 months, kinase-I lost about 50% of its activity while kinase-II was fully active. The above purification method is modified from our own procedure employed for the fractionation of cyclic AMP-dependent protein kinases [3] and that of Traugh and Traut [7], for cyclic AMP-dependent and -independent protein kinases.

Table I summarizes the overall purification for both kinases. The final preparation of kinase-I was enriched by approximately 29 000-fold, and kinase-II, 47 000-fold. The total recovery of enzyme activity of 42% was comprised of 27% of kinase-I and 15% of kinase-II. In spite of the high degree of purification achieved, polyacrylamide gel electrophoresis revealed that neither enzyme preparation was homogeneous.

General properties

Both kinases rapidly lost their activity in dilute solutions. At a concentration of about 10 μg/ml, approximately 70% of both enzyme activities were lost within 30 min at 0°C. However, the presence of 1 mg/ml of bovine serum albumin in the diluted enzyme solutions completely prevented such an inactiva-

TABLE I

PURIFICATION OF CASEIN KINASES GTP:CASEIN I (GC-I) AND GTP:CASEIN II (GC-II)

Casein kinases were purified as described in the text.

Fraction	Volume (ml)	Protein (mg/ml)	Specific activity (units/mg)	Purification (fold)
Crude lysate	8840	98	0.01	1
Ammonium sulfate	193	78	0.57	57
DEAE-cellulose, GC-I	19	70	2.62	262
Phosphocellulose, GC-I	7.6	2.4	148	14 800
Hydroxylapatite, GC-I	6.2	1.3	288	28 800
DEAE-cellulose, GC-II	15.2	40	6.6	660
Phosphocellulose, GC-II	7.8	1.0	223	22 300
Hydroxylapatite, GC-II	2.5	1.1	465	46 500

tion. Both enzymes were optimally active at pH 9.0 and Mg^{2+} (3 mM) was required to realize their maximal activities (data not shown).

Substrate specificity

Table II shows the relative degree of phosphorylation of various protein substrates by the two kinases. Of a number of protein substrates tested, casein was by far the most effective substrate for both enzymes. When casein was partially dephosphorylated, its phosphoryl acceptor activity was reduced by about half. Bingham et al. [16] have similarly demonstrated that dephosphorylated casein is a relatively poor substrate compared to native casein for a cytoplasmic casein kinase from rat mammary gland. In contrast, the partially dephosphorylated form of phosvitin was a better substrate than the native form. However, this may depend upon the degree of dephosphorylation of phosvitin. As shown by Rabinowitz and Lipmann [12], dephosphorylation greater than 60% also results in the reduction of the acceptor activity of phosvitin. Histones, protamine and bovine serum albumin were poor phosphoryl acceptors.

As shown in Table II, neither kinase is specific with respect to the phosphoryl donor and both can utilize ATP and GTP as the phosphorylating agent. However, ATP appeared to be a relatively superior substrate than GTP in the phosphorylation reaction. This observation is in agreement with that reported by Traugh and Traut [7] for a similar enzyme found in rabbit reticulocytes. Fig. 4 shows the effect of varying concentrations of GTP and ATP on the activities of kinase-I and kinase-II. The apparent Michaelis constant calculated from the Lineweaver-Burk plots for GTP ($2.5 \cdot 10^{-5}$ M) and ATP ($2.0 \cdot 10^{-5}$ M) are identical for both enzymes. Although ATP was a better phosphate donor, we chose to use GTP in the characterization of these enzymes. This is to avoid interference by the cyclic AMP-dependent protein kinases and possibly other enzyme(s) which specifically utilize ATP as the substrate.

TABLE II

GTP:CASEIN KINASE I AND GTP:CASEIN KINASE II CATALYZED PHOSPHORYLATION OF VARIOUS PROTEIN SUBSTRATES BY EITHER ATP OR GTP

Assays were conducted as described under Materials and Methods in the presence of 0.2 mM of either [γ-^{32}P] ATP or [γ-^{32}P] GTP as the phosphoryl donor and 2.0 mg/ml of each of the protein substrates as acceptor. Each of the reaction mixtures contained either 3.3 μg/ml of kinase I or 2.2 μg/ml of kinase II.

Acceptor	^{32}P incorporated (pmol)			
	Kinase-I		Kinase-II	
	ATP	GTP	ATP	GTP
Casein	817	432	1075	722
50% dephosphorylated casein	472	292	377	237
Phosvitin	246	158	237	154
50% dephosphorylated phosvitin	419	312	374	310
Histones	18	43	27	49
Protamine	4	11	25	16
Bovine serum albumin	2	3	0	0

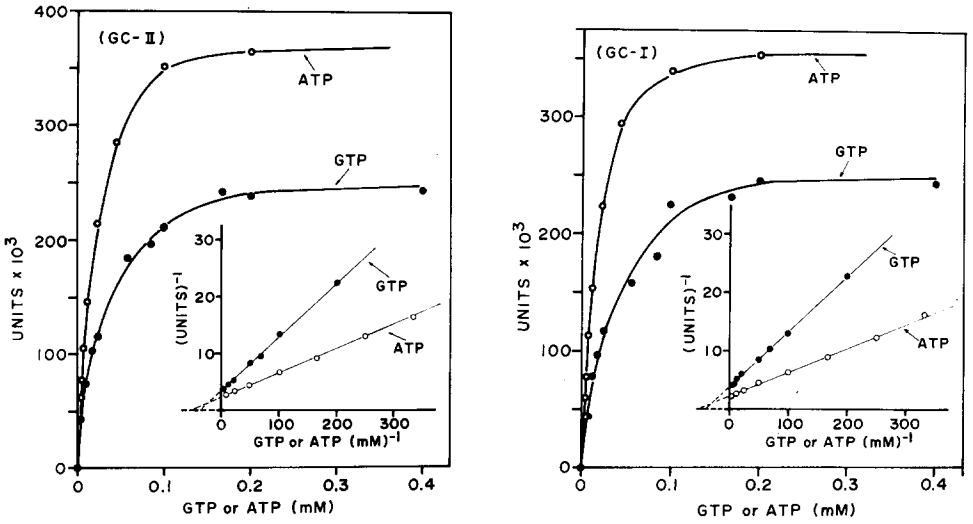


Fig. 4. Effect of GTP or ATP concentration on the rate of casein phosphorylation by the kinases (GC-I and GC-II). Incubation mixtures were as described in Materials and Methods, except that varied concentrations of GTP or ATP were used. The reaction mixture contained either 4 μg/ml of GC-I or 3 μg/ml of GC-II. The insets are double-reciprocal plots of the same data.

Effect of nucleotides

Table III shows the effect of various nucleotides on the phosphorylation of casein by GTP. The addition of 0.2 mM of either ATP or GTP to the reaction mixture caused an inhibition of approximately 70% or 50% respectively of the phosphotransferase activity of both kinases. At 0.2 mM, cyclic AMP

TABLE III

EFFECT OF NUCLEOTIDES ON THE PHOSPHOTRANSFERASE ACTIVITY OF KINASE I AND KINASE II

Each reaction mixture was incubated at 37°C for 5 min under the standard assay conditions and contained either 4 μg/ml of kinase I or 3 μg/ml of kinase II. Each nucleotide was added at a final concentration equal to (0.2 mM) or 10 times (2 mM) that of [γ-^{32}P] GTP as indicated in the table.

Addition	% control	
	Kinase-I	Kinase-II
None (control)	100	100
ATP, 0.2 mM	29	36
2.0 mM	4	5
ADP, 2.0 mM	6	8
GTP, 0.2 mM	54	52
2.0 mM	13	12
GDP, 2.0 mM	18	18
GMP, 2.0 mM	104	98
ITP, 2.0 mM	20	24
CTP, 2.0 mM	101	92
UTP, 2.0 mM	84	90
Cyclic AMP, 0.2 mM	111	109
Cyclic GMP, 0.2 mM	102	101

and cyclic GMP did not significantly affect the activity of either kinase. In view of the fact that certain protein kinases are activated by low concentrations of either cyclic AMP [1—3] or cyclic GMP [17], the effect of these nucleotides on the kinase-I and kinase-II catalyzed reaction has been investigated in more detail at concentrations ranging from $2 \cdot 10^{-8}$ M to $5 \cdot 10^{-4}$ M. There was no positive indication of regulation of either kinase activity by either of these cyclic nucleotides.

At 10 times the substrate concentration, ATP, ADP, GTP, GDP and ITP inhibited more than 75% of the activity of the kinases while GMP, CTP and UTP had only a slight effect, if any. ITP has not been examined for its ability to serve as phosphoryl donor.

Effect of NaCl and NaF

NaCl is known to inhibit the activity of cyclic AMP-dependent protein kinase from rabbit skeletal muscle [18] and to activate certain protein kinases which are not regulated by cyclic AMP [7,8]. In our studies, we find that the effect of NaCl is dependent on the casein concentration. As shown in Fig. 5, the phosphorylation of casein by either kinase-I (Fig. 5A) or kinase-II (Fig. 5B) has the general appearance of substrate inhibition kinetics. However, near-normal kinetics were observed in the presence of 0.1 M NaCl, indicating a reversal by NaCl of the inhibitory effect of high casein concentrations. The apparent K_m values for casein as determined in the presence (K_m = 0.6 mg/ml) and absence (K_m = 0.4 mg/ml) of 0.1 M NaCl were very similar for both enzymes.

Fig. 6 shows the effect of NaF on the activity of both kinases. Each of the enzyme samples was preincubated with NaF at 0°C for 10 min, following

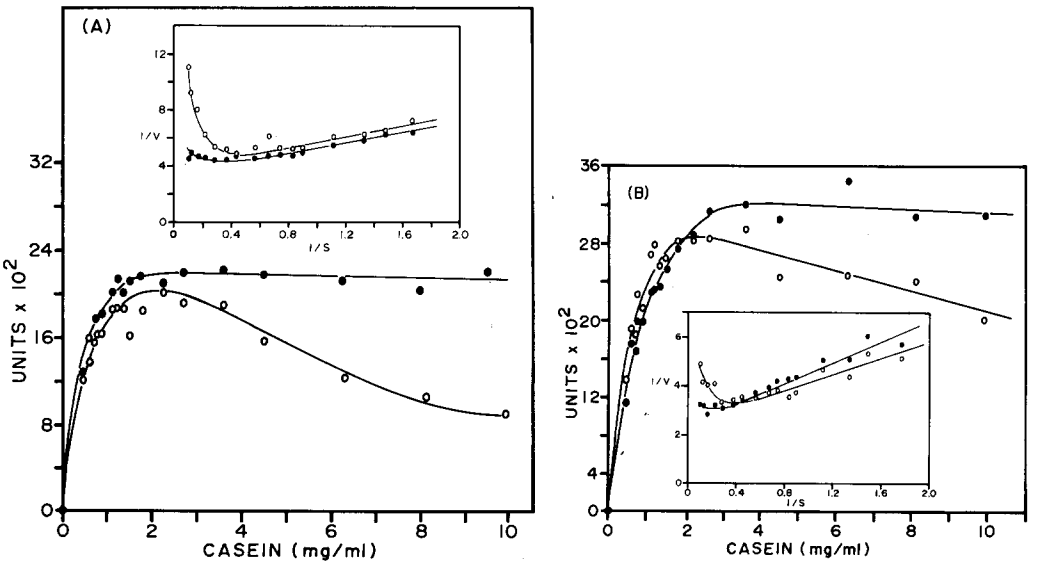
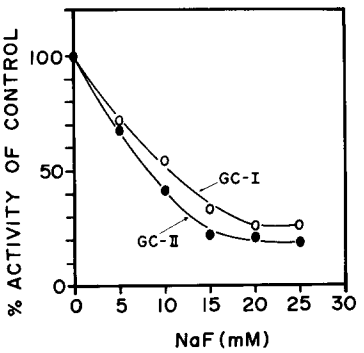


Fig. 5. Effect of NaCl on the phosphorylation reaction at varying concentrations of casein. Each incubation mixture contained 3 μg/ml of either kinase (GC-I or GC-II). Closed circles represent assays in the presence of 0.1 M NaCl. (A) GC-I; (B) GC-II. The insets are double reciprocal plots of the same data.



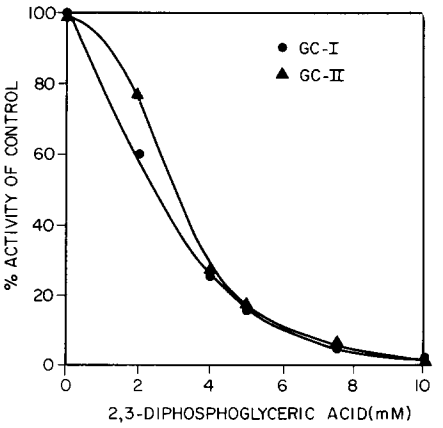


Fig. 6. Inhibition of casein phosphorylation by NaF. The enzymes were preincubated at 0°C for 10 min with varying concentrations of NaF, following which the assays were performed as indicated in Materials and Methods, but in the presence of 40 mM $MgCl_2$.

Fig. 7. Effect of 2,3-diphosphoglyceric acid on casein phosphorylation. The enzymes (2.5 μg/ml) were preincubated with varying concentrations of the compound at 0°C for 10 min following which kinase assays were performed as described in Materials and Methods.

which the other components of the reaction mixture were added. These experiments were performed in the presence of 40 mM $MgCl_2$. The high concentration of $MgCl_2$ used was to rule out the possibility that the effect observed might be due to the tying up of the divalent cation by the fluoride added. Both enzyme reactions were inhibited by NaF. At NaF concentrations of 20 mM or higher, inhibition of enzyme activities of up to 80% was observed. NaF does not appear to affect the cyclic AMP-dependent and cyclic GMP-dependent protein kinases and has been included by several investigators in the assay of these enzymes [17,18].

Effect of 2,3-diphosphoglyceric acid

In view of the fact that the erythrocyte contains unusually large amounts of 2,3-diphosphoglyceric acid [19], we have also investigated the effect of this metabolite on kinase-I and kinase-II activities. On preincubation at 0°C for 10 min with 10 mM 2,3-diphosphoglyceric acid, both enzymes were virtually completely inhibited (Fig. 7). This effect was observed even at 40 mM Mg^{2+} in the assay mixture, thereby overiding the possibility of depletion of Mg^{2+} by 2,3-diphosphoglycerate as the cause for inhibition. On the other hand, neither 3-phosphoglycerate under similar conditions, nor 40 mM Mg^{2+} by itself, interfered with the kinase reaction.

Sedimentation in sucrose density gradient

We have examined the sedimentation behavior of both kinase-I and kinase-II in a 20—40% linear sucrose density gradient. Using thyroglobulin (19.3 S, M_r 669 000) as the marker protein, kinase-I was estimated to have a sedimentation coefficient of 25 S (mol.wt, $9.5 \cdot 10^5$) and kinase-II, 32 S (mol.wt, $1.4 \cdot 10^6$).

Discussion

A number of protein phosphokinases have been found in rabbit red blood cells. As reported previously, three of these enzymes are characterized as cyclic AMP-dependent protein kinases [3]. The present investigations report the partial purification and characterization of two additional protein kinases from rabbit erythrocytes. These enzymes are tentatively identified as GTP:casein kinase I and GTP:casein kinase II and have properties different from those of cyclic AMP-dependent protein kinases reported in red cells [3,7]. Both kinases catalyze the phosphorylation of the acidic proteins, casein and phosvitin, employing either ATP or GTP as the phosphoryl donor. This is in contrast to the cyclic AMP-dependent protein kinases which utilize ATP, but not GTP, preferentially to phosphorylate histones [3,7]. Neither cyclic AMP nor cyclic GMP can stimulate the activity of either kinase. The enzyme activities are also not affected by the heat-stable inhibitor of cyclic AMP-dependent protein kinase (unpublished observation). Furthermore, both kinase-I and kinase-II are excluded by Sephadex G-200 and have molecular weights of about $9.5 \cdot 10^5$ and $1.4 \cdot 10^6$, respectively, when determined by sucrose density gradient centrifugation. These values are much higher than those reported for all the known cyclic AMP-dependent protein kinases of rabbit erythrocytes [3].

Kinase-I and kinase-II are clearly separable by DEAE-cellulose chromatography. Except for a difference in their molecular weights, both enzymes are very similar with respect to their kinetic parameters and substrate specificities. The possibility that one form of the enzyme is derived from the other should not be overlooked, although repeated sedimentation of kinase-I and kinase-II in a sucrose density gradient failed to reveal any suggestion of interconversion. Traugh and Traut [7] have reported the presence of only one form (probably kinase-I) of casein kinase in rabbit reticulocyte following DEAE-cellulose chromatography. This observation may be attributed to incomplete elution since a lower salt concentration (0—0.3 M) is employed in the gradient elution by these workers as compared to 0—0.6 M KCl reported here. Kinase-II is eluted at a KCl concentration of about 0.38 M. It is interesting to note that multiple forms of phosvitin kinase differing in electrical charge and molecular weight have been found in rat liver cytosol [20].

Our kinetic data suggest that NaCl may counteract the inhibitory effect of high substrate (casein) concentrations. A similar stimulation by KCl and/or NaCl in the presence of relatively high protein substrate concentration has been reported for a cerebral phosvitin kinase [8] and a rabbit reticulocyte protein kinase [7]. Although no explanation has been provided by these investigators regarding the effect of salts, their data are amenable to an interpretation similar to that propounded by us. The nature of the inhibition of these kinases by NaF is presently unknown but does not appear to be a consequence of the depletion of the effective Mg^{2+} concentration. It is also interesting that the concentration at which both the enzymes are inhibited by 2,3-diphosphoglycerate is close to the physiological level (5.7 mM) of the metabolite found in the human red blood cell [19]. However, the significance of this observation remains to be explored.

Both kinases are capable of utilizing either ATP or GTP in the phosphoryla-

tion of various protein substrates. This property is similar to the rooster liver [5] and ox brain [8] phosvitin kinases.

The function of these protein kinases in rabbit red blood cells is at present unclear. A recent report by Traugh et al. [21] suggests that one or both of these enzymes may play a role in the regulation of ribosome activity by modification of certain ribosomal proteins in the reticulocyte system. Since we have isolated these enzymes from mature red blood cells, erythrocytes, it appears that they may have additional function other than ribosomal phosphorylation. On the other hand, it is conceivable that these are vestigial activities carried over during differentiation of the red cells and may not have a specific role in erythrocytes. The isolation and characterization of endogenous protein substrate(s) of these enzymes should enable us to ascertain their significance in the mature red cells.

Acknowledgements

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References

1 Langan, T.A. (1973) in Advances in Cyclic Nucleotide Research (Greengard, P. and Robison, G.A., eds) Vol. 3, pp. 99—154, Raven Press, New York
2 Walsh, D.A. and Krebs, E.G. (1973) in The Enzymes (Boyer, P.D., ed.), Vol. 8, pp. 555—581, Academic Press, New York
3 Tao, M. and Hackett, P. (1973) J. Biol. Chem. 248, 5324—5332
4 Labrie, F., Lemaire, S. and Courte, C. (1971) J. Biol. Chem. 246, 7293—7302
5 Goldstein, J.L. and Hasty, M.L. (1973) J. Biol. Chem. 248, 6300—6307
6 Bingham, E.W. and Farrell, Jr., H.M. (1974) J. Biol. Chem. 249, 3647—3651
7 Traugh, J.A. and Traut, R.R. (1974) J. Biol. Chem. 249, 1207—1212
8 Rodnight, R. and Lavin, B.E. (1964) Biochem. J. 93, 84—91
9 Ventimiglia, F.A. and Wool, I.G. (1974) Proc. Natl. Acad. Sci. U.S. 71, 350—354
10 Campbell, H.D. and Zerner, B. (1973) Biochem. Biophys. Res. Commun. 54, 1498—1503
11 Bingham, E., Farrell, J.r., H.M. and Carroll, R.J. (1972) Biochemistry 11, 2450—2454
12 Rabinowitz, M. and Lipmann, F. (1960) J. Biol. Chem. 235, 1043—1050
13 Bray, G.A. (1960) Anal. Biochem. 1, 279—285
14 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
15 Martin, R.G. and Ames, B.N. (1961) J. Biol. Chem. 236, 1372—1379
16 Bingham, E.W., Farrell, Jr., H.M. and Basch, J.J. (1972) J. Biol. Chem. 247, 8193—8194
17 Kuo, J.F. and Greengard, P. (1970) J. Biol. Chem. 245, 2493—2498
18 Reimann, E.M., Walsh, D.A. and Krebs, E.G. (1971) J. Biol. Chem. 246, 1986—1995
19 Jacobasch, G., Minakami, S. and Rapoport, S.M. (1973) in Cellular and Molecular Biology of Erythrocytes (Yoshikawa, H. and Rapoport, S.M., eds) pp. 55—92, University Park Press, Baltimore
20 Baggio, B. and Moret, V. (1971) Biochim. Biophys. Acta 250, 346—350
21 Traugh, J.A., Mumby, M. and Traut, R.R. (1973) Proc. Natl. Acad. Sci. U.S. 70, 373—376