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PROTEIN KINASES OF RABBIT AND HUMAN ERYTHROCYTE MEMBRANES

SOLUBILIZATION AND CHARACTERIZATION

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Summary

Two protein kinases (EC 2.7.1.37) from rabbit and one from human erythrocyte membranes have been solubilized with 0.5 M NaCl. These enzymes have been partially purified by $(NH_4)_2SO_4$ fractionation and gel filtration. The rabbit membrane enzymes have apparent M_r values of 100 000 and 30 000, as determined in the presence of 0.4 M NaCl. In the absence of salt, these enzymes aggregate into high molecular weight species. The kinase from human erythrocyte membranes has an apparent M_r of 30 000 and appears to have properties similar to those of the 30 000-dalton rabbit kinase. All three enzymes catalyze the phosphorylation of casein and phosvitin in salt-stimulated reactions. None of these enzymes appears to be related to cyclic AMP-dependent protein kinases.

Introduction

Both rabbit [1] and human [1—3] erythrocyte membranes contain an endogenous protein phosphorylating system. Several lines of indirect evidence indicate that multiple protein kinase (EC 2.7.1.37) activities are present in these membrane preparations. That human erythrocyte membranes contain both cyclic AMP-dependent and -independent protein kinases was initially suggested by Fairbanks and Avruch [2,3] based on their observations that cyclic AMP and mono- and divalent cations differentially affected the phosphorylation of membrane proteins. Using a different approach, we have similarly detected the presence of multiple phosphorylation reactions in red cell membranes. This is revealed by comparing the phosphorylation patterns of erythrocyte membranes phosphorylated in the presence of ATP and of GTP. From the differences in

the phosphorylation patterns, we suggested that both human and rabbit erythrocyte membranes contain a protein kinase which specifically utilizes ATP as its phosphoryl donor and another protein kinase which can utilize either ATP or GTP to phosphorylate membrane proteins [1]. Our studies also indicate that human erythrocyte membranes differ from that of the rabbit in that cyclic AMP-dependent protein kinase activity is absent in the latter preparation [1,4]. In this report, we describe the solubilization, partial purification and characterization of the cyclic AMP-independent erythrocyte membrane protein kinases.

Experimental procedures

Preparation of membranes. Hemoglobin-free ghosts of fresh rabbit and human red blood cells were prepared according to Dodge et al. [5] as previously described [6]. Ghosts were also prepared from packed frozen rabbit red blood cells, however, in this instance the preliminary washings with isotonic buffer were necessarily omitted. Frozen cells were thawed and directly lysed in 20 mOsm sodium phosphate or Tris · HCl buffer, pH 7.5, and processed further according to the Dodge procedure. Ghosts obtained from frozen cells did not become pearly white, rather they remained rosy pink in color. The final ghost pellets were either frozen in liquid nitrogen or processed further immediately.

Enzyme extraction. Washed ghosts were extracted with an equal volume of 0.5 M NaCl for 10 min at 0°C and centrifuged at 16 300 × *g* for 30 min. The supernatant was saved and the membranes re-extracted as above. The supernatants were combined, dialyzed overnight against 0.02 M Tris · HCl, pH 7.5, containing 1 mM dithiothreitol (buffer A), and concentrated on a Diaflo PM-10 ultrafiltration membrane. The concentrated extract was brought to 50% saturation with ammonium sulfate and allowed to stand at 0°C for 30 min. The pellet obtained after centrifugation was resuspended and dialyzed overnight against buffer A.

Protein phosphorylation. The phosphotransferase activities of membrane extracts were determined by measuring the phosphorylation of casein using the trichloroacetic acid-precipitation method previously described [7]. The incorporation of ^{32}P from [γ-^{32}P]ATP or [γ-^{32}P]GTP into casein was assayed at 37°C for 10 min in a reaction mixture containing (in a final volume of either 0.1 or 0.2 ml): 50 mM buffer (pH as specified), 5 mM $MgCl_2$, 150 mM KCl, 0.2 mM [γ-^{32}P]ATP or 0.2 mM [γ-^{32}P]GTP (20—60 cpm/pmol), 2 mg/ml casein, ± 1 μM cyclic AMP, and membrane extracts. The following buffers were used: pH 6.0, Tris/acetate; pH 6.5—8.0, Tris · HCl; and pH 8.5, glycine/NaOH. In experiments where histone, protamine, phosvitin or bovine serum albumin were substitued for casein, their concentrations were 2 mg/ml. The reactions were initiated with the addition of labelled nucleotide and terminated by the successive additions of 15 μl of 12 mg/ml of bovine serum albumin and 2 ml of 10% trichloroacetic acid. Samples were processed further according to Tao and Hackett [7].

Materials. [γ-^{32}P]ATP and [γ-^{32}P]GTP were obtained from either New England Nuclear or Amersham/Searle. ATP and GTP were obtained from P-L Biochemicals. Cyclic AMP, histone (Type II-A), protamine (free base), bovine

serum albumin, and dithiothreitol were obtained from Sigma Chemical Co. Frozen rabbit red blood cells (young, type II) were purchased from Pel-Freez. Casein was obtained from Mann Research Laboratories.

Results

Characterization of salt extract

Preliminary characterization of the salt extract from frozen rabbit red blood cells indicated that it contained protein kinase activity which catalyzed the phosphorylation of casein in the presence of either [γ-^{32}P]ATP or [γ-^{32}P]GTP. Furthermore, the kinase activity appeared to be affected by the concentration of KCl present in the reaction mixture. However, the effect of KCl was dependent on the phosphoryl donor used. In the presence of ATP, maximum stimulation of casein phosphorylation occurred at 0.15—0.25 M KCl. When GTP was used as the phosphoryl donor, a slight increase in phosphorylation was observed at 0.1—0.15 M KCl. Higher concentrations of KCl (0.5 M) completely inhibited the GTP:casein phosphotransferase (GTP-kinase) activity but not the ATP:casein phosphotransferase (ATP-kinase) activity.

These results suggested that the solubilized enzyme preparation contained multiple kinase activities. This possibility was born out by studies of the sedimentation properties of the solubilized enzyme preparation in sucrose density gradients. As shown in Fig. 1, a single peak of GTP-kinase activity which sedimented at about 9.7 S was observed. On the other hand, the sedimentation profile of the kinase activity assayed in the presence of ATP appeared to be less discrete. In addition to an activity peak found coincident with the GTP-kinase peak, ATP-kinase activity was also detected throughout the sucrose gradient.

The sedimentation profile of kinase activity in the presence of ATP suggests that one is dealing with an association-dissociation phenomenon. To explore this possibility, the solubilized enzyme preparation was sedimented in a 5 to 15% sucrose density gradient containing 0.4 M NaCl in order to minimize protein-protein interaction (Fig. 2). Under these conditions, two distinct activity peaks are obtained. Both kinases utilize ATP as the phosphoryl donor. However, only one of these enzymes (the fast sedimenting minor component) exhibits significant activity in the presence of GTP. These kinases are tentatively identified as membrane kinases I and II corresponding to the 6.1 S and 2.8 S species, respectively.

In a comparative study, the human erythrocyte membrane extract was similarly analyzed using sucrose density gradient centrifugation. In contrast to the pattern observed with the rabbit erythrocyte membrane extract, only one peak of casein kinase activity was apparent (in gradients containing 0.4 M NaCl). This kinase has properties similar to the rabbit membrane kinase II: it prefers ATP as a phosphoryl donor, has an apparent M_r of 29 500 and an s value of 2.9 S. If salt was omitted from the sucrose gradients, the human erythrocyte membrane kinase activity was distributed throughout the gradient. Gradients of human extracts were also assayed using histone as substrate in the presence or absence of cyclic AMP. As seen in Fig. 3, a small amount of histone kinase activity is extracted along with casein kinase. The histone kinase activity

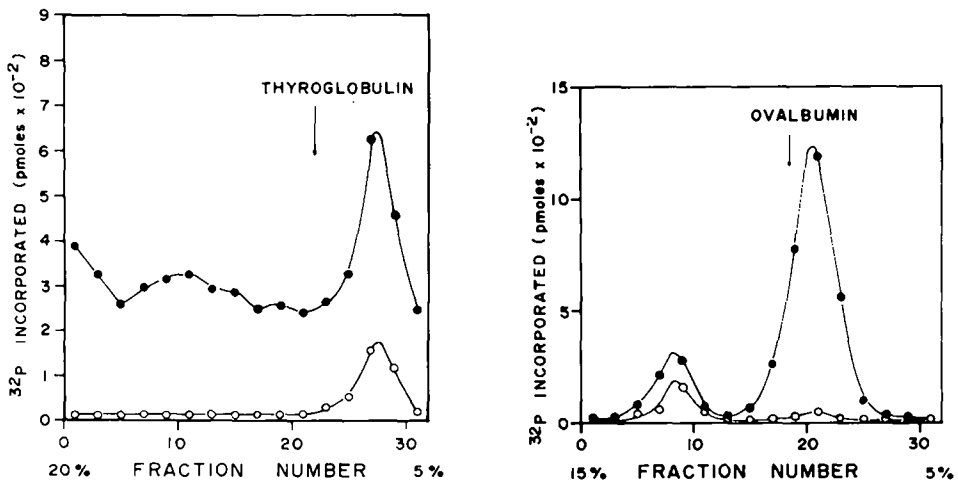


Fig. 1. Sucrose density gradient centrifugation of solubilized membrane kinase from rabbit erythrocytes. A 100 μl sample of enzyme was layered onto 5 ml of a 5—20% sucrose density gradient made in 0.02 M Tris · HCl, pH 7.5, and 1 mM dithiothreitol. The gradient was centrifuged at 320 000 × *g* for 90 min. Twelve-drop fractions were collected and 50 μl of every other fraction were assayed for kinase activity at pH 8.0 under the conditions described in Experimental Procedures using [γ-^{32}P]ATP (●———●) and [γ-^{32}P]GTP (○———○).

Fig. 2. Sucrose density gradient centrifugation of solubilized rabbit erythrocyte membrane kinase in the presence of NaCl. The enzyme sample (120 μl of enzyme extract plus 30 μl of 2 M NaCl, incubated for 5 min at 0°C) was layered onto 5 ml of a 5—15% sucrose density gradient containing 0.4 M NaCl, 0.02 M Tris · HCl, pH 7.5, and 1 mM dithiothreitol. Ovalbumin (120 μl of 10 mg/ml solution plus 30 μl of 2 M NaCl) was layered onto an identical gradient. The gradients were centrifuged for 15 h at 320 000 × *g*. Twelve-drop fractions were collected and 50 μl of every other fraction were assayed at pH 8.0 for kinase activity with [γ-^{32}P]ATP (●———●) and [γ-^{32}P]GTP (○———○) as described under Experimental Procedures. Since the gradient contained NaCl, the amount of salt in the assay mixture was adjusted accordingly.

probably represents that of the free catalytic subunit of the cyclic AMP-dependent protein kinase which is partially released from the membranes with high salt concentrations [8]. The majority of the cyclic AMP-dependent protein kinase activity remains associated with the membranes. Rubin [9] has shown that a more complete solubilization of this activity can be achieved with Triton X-100.

The fact that human erythrocyte membrane extracts contained only one kinase which phosphorylated casein made us question whether the two kinases obtained in extracts of frozen rabbit red blood cells were artifacts resulting from the use of frozen vs. fresh cells. As mentioned earlier, ghosts prepared from frozen cells retained more hemoglobin than fresh cells, thus it seemed possible that they might also have retained other soluble proteins. Therefore, extracts were prepared from fresh rabbit red blood cells. The sedimentation profiles of protein kinases from extracts of fresh cells are identical to those of the frozen cells shown in Fig. 2. (data not shown).

Separation of solubilized membrane kinases by gel filtration

In order to facilitate the study of membrane kinase I and membrane kinase II, it was necessary to resolve these enzymes into two separate fractions. This was achieved by Sephadex gel filtration in the presence of 0.4 M NaCl (Fig. 4).

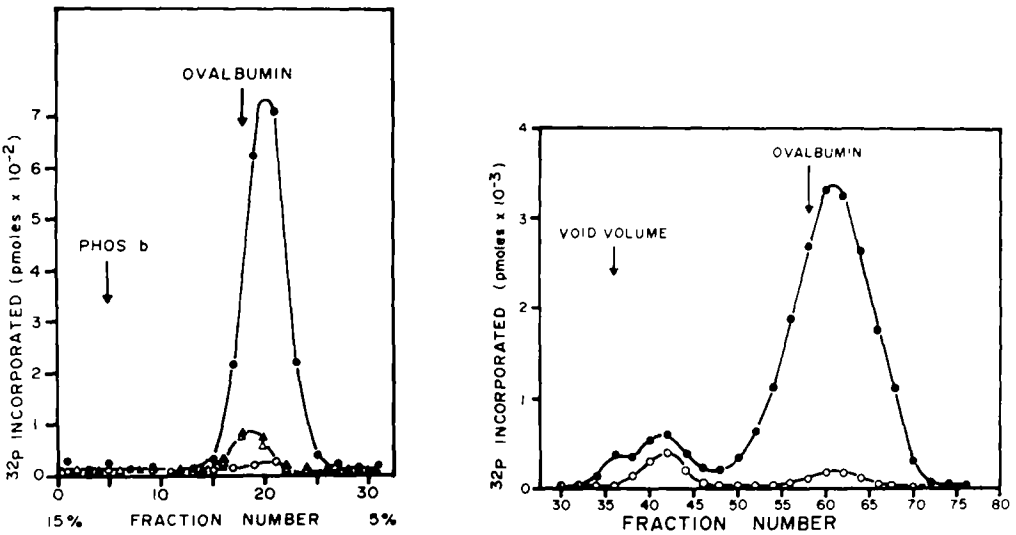


Fig. 3. Sucrose density gradient centrifugation of solubilized human erythrocyte membrane kinase in the presence of NaCl. Experimental conditions are as described in Fig. 2. The fractions were assayed for casein kinase activity with [γ-^{32}P]ATP (●———●) and with [γ-^{32}P]GTP (○— —○) and for histone kinase activity with [γ-^{32}P]ATP and with (▲———▲) or without (△———△) cyclic AMP.

Fig. 4. Separation of membrane kinase I from membrane kinase II by Sephadex G-150 gel filtration. Approximately 5 ml of rabbit membrane extract (after ammonium sulfate precipitation) in 0.4 M NaCl was applied to Sephadex G-150 column (90 × 2.6 cm) and eluted with buffer A containing 0.4 M NaCl. The void volume was 182 ml as calibrated with blue dextran. Each fraction contained 4.9 ml. Kinase activity was measured at pH 7.5 as described in Experimental Procedures with [γ-^{32}P]ATP (●———●) and [γ-^{32}P]GTP (○———○).

Using either Sephadex G-100 or G-150, membrane kinase I was eluted at or near the void volume, while membrane kinase II was eluted at a volume (after ovalbumin) with an apparent M_r of 30 000—40 000. The extracted casein kinase from human erythrocyte membranes was also further purified using Sephadex gel filtration in the presence of 0.4 M NaCl. The human erythrocyte membrane casein kinase activity was eluted from the column slightly ahead of histone kinase activity. The active fractions under each peak were individually pooled, dialyzed against buffer A, concentrated and stored in liquid nitrogen. All further characterizations of the enzymes were carried out using these fractions.

Attempts to determine the molecular weights of membrane kinases I and II by sucrose density gradient centrifugation in the absence of salt after gel filtration have yielded variable results. However, in the presence of 0.4 M NaCl, the molecular weights of these enzymes have been estimated: membrane kinase I, 95 000—100 000; membrane kinase II and human membrane kinase, 29 000—40 000.

Substrate specificity

The substrate specificities of membrane kinase I and II were determined in the presence and absence of cyclic AMP. As shown in Table I both kinases phosphorylate casein and phosvitin but not histone, protamine or bovine serum albumin. Neither kinase appears to be stimulated by cyclic AMP.

TABLE I

SUBSTRATE SPECIFICITY OF MEMBRANE KINASES I AND II

Phosphorylation was carried out in the presence of 36 μg of membrane kinase I or 8.5 μg of membrane kinase II as described in the Experimental Procedures. Results are expressed as pmol ^{32}P incorporated/10 min.

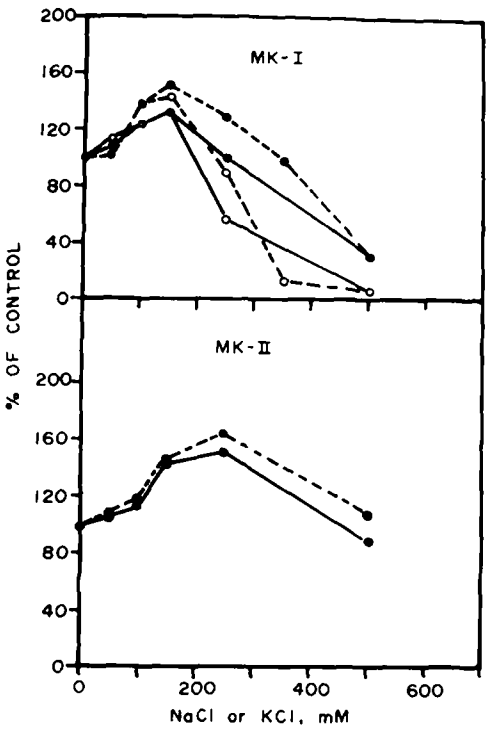
	Membrane kinase I				Membrane kinase II	
	[γ-^{32}P]ATP		[γ-^{32}P]GTP		[γ-^{32}P]ATP	
	Control	+ Cyclic AMP	Control	+ Cyclic AMP	Control	+ Cyclic AMP
Casein	154	154	55	65	662	713
Phosvitin	131	147	69	73	502	557
Histone	34	38	15	14	18	22
Protamine	37	36	14	15	14	12
Bovine serum albumin	14	7	6	8	5	2

pH activity profile

The effect of pH on the phosphorylation of casein by membrane kinase I and II was determined (data not shown). In the presence of ATP, membrane kinase I is more or less equally active between pH 6.5—9, whereas with GTP, greater activity is observed at higher values. The pH activity profile of membrane kinase II is bimodal with the highest activity occurring at pH 7.5. The pH activity profile of casein phosphorylation by the solubilized enzymes is somewhat different from that of membrane autophosphorylation [1]. The rate of endogenous membrane phosphorylation in the presence of ATP exhibits a pH optimum between 6 and 6.5, whereas in the presence of GTP a slight activity peak occurs at about pH 8.5. These results are similar in nature to that found with cyclic AMP-dependent protein kinases. The pH activity profile for histone phosphorylation by these kinases differs from that for membrane phosphorylation [4].

Effect of KCl and NaCl

Avruch and Fairbanks [2] have reported that there is a monovalent cation stimulated phosphorylation reaction in human erythrocyte membranes. Therefore, the effects of KCl and NaCl on membrane kinases I and II were determined and the results are shown in Fig. 5. While the activity of membrane kinase I is enhanced by 0.10—0.15 M KCl or NaCl, concentrations of 0.5 M significantly inhibit the enzyme (Fig. 5, upper panel). The activity of membrane kinase I in the presence of GTP appears to be slightly more sensitive to the inhibitory effects of high concentrations of Na^+ or K^+ than it is with ATP. The activity of membrane kinase II is also stimulated by NaCl and KCl, with concentrations between 0.15—0.25 M (Fig. 5, lower panel). However, in contrast to that observed for membrane kinase I, high concentrations of NaCl or KCl do not inhibit membrane kinase II. The pattern of activity observed with membrane kinase II reflects that obtained with ATP and the membrane extract before gel filtration while that obtained with membrane kinase I agrees with that observed with GTP.



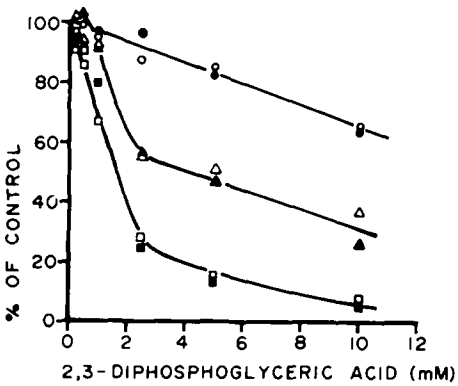


Fig. 5. Effect of varying concentrations of KCl and NaCl on the phosphorylation of casein by membrane kinase I and by membrane kinase II. Kinase activity was assayed at pH 7.5 in the presence of [γ-^{32}P]ATP (●) or [γ-^{32}P]GTP (○) as described in Experimental Procedures except that the standard assay concentration of KCl was omitted in lieu of varying concentrations of NaCl (———) or KCl (- - - - -). The concentration of membrane kinase I was 24 μg and of membrane kinase II was 8.5 μg.

Fig. 6. Effect of 2,3-diphosphoglyceric acid on the activities of membrane kinase I and membrane kinase II. Kinase activity was assayed at pH 7.5 in the presence of varying concentrations of 2,3-diphosphoglyceric acid. Conditions were as described in Experimental Procedures except that experiments designated with open symbols contained 25 mM Mg^{2+}. ●———●, ○———○, membrane kinase II (8.5 μg) with [γ-^{32}P]ATP; ▲- - - - -▲, △- - - - -△, membrane kinase I (36 μg) with [γ-^{32}P]ATP; ■- - - - -■, □- - - - -□, membrane kinase I (36 μg) with [γ-^{32}P]GTP.

Effect of divalent cations

Both enzymes have an absolute requirement for Mg^{2+}. The optimum Mg^{2+} concentration for membrane kinase I is 5 mM, using either ATP or GTP as phosphoryl donor. Similarly, the optimum Mg^{2+} concentration for membrane kinase II is between 2—5 mM. High concentrations of Mg^{2+} are not inhibitory (data not shown).

It has been reported that the autophosphorylation of erythrocyte membranes is stimulated by Ca^{2+} [2]. Therefore, we determined the effects of varying concentrations of Ca^{2+} on the activities of membrane kinases I and II. Both membrane kinase I and membrane kinase II are inhibited by concentrations of Ca^{2+} greater than 1 mM; concentrations ranging between 0.5 mM through 1 mM are with little effect (data not shown).

Effects of 2,3-diphosphoglyceric acid and 3-phosphoglyceric acid

It is of interest to determine whether red cell metabolites can regulate the

activities of the solubilized kinases. Fig. 6 illustrates the effects of varying concentrations of 2,3-diphosphoglyceric acid on casein phosphorylation by membrane kinases I and II. In view of the known chelating properties of 2,3-diphosphoglyceric acid, these experiments were carried out at two different Mg^{2+} concentrations in order to eliminate chelation of the divalent ion as a cause of enzyme inhibition. 2,3-Diphosphoglyceric acid appears to inhibit both kinases; however, this effect is much more pronounced for membrane kinase I. At 5 mM 2,3-diphosphoglyceric acid, less than 20% inhibition is observed with membrane kinase II, while greater than 50% inhibition occurs with membrane kinase I. The sensitivity of membrane kinase I to 2,3-diphosphoglyceric acid is somewhat dependent on the phosphoryl donor used. As seen in Fig. 6, membrane kinase I appears to be inhibited to a greater extent in the presence of GTP than with ATP. The reasons for these results are not known. In all instances, 3-phosphoglyceric acid was without effect on the activities of membrane kinases I and II (data not shown).

Effect of nucleotide concentration on membrane kinases I and II

The effects of varying concentrations of phosphoryl donor on casein phosphorylation by membrane kinases I and II was determined (data not shown). The apparent K_m of membrane kinase I for ATP and GTP does not appear to be significantly different (62 μM and 48 μM, respectively), however, the V value for ATP (4.3 nmol/mg protein per min) is approximately three times greater than that for GTP (1.3 nmol/mg protein per min). Membrane kinase II has an apparent K_m for ATP (65 μM) similar to that of membrane kinase I, however, the V of membrane kinase II (22 nmol/mg protein min) is 4—8 times that of membrane kinase I with ATP.

Effect of cyclic AMP-dependent protein kinase regulatory subunit on membrane kinase I, membrane kinase II and human membrane kinase

Since none of the casein kinases phosphorylate histone, it appears unlikely that these enzymes represent the free catalytic subunit of soluble or membrane-bound cyclic AMP-dependent protein kinases. This hypothesis is supported by the finding that a separate histone kinase, that is presumably derived from the

TABLE II

EFFECT OF REGULATORY SUBUNIT OF CYCLIC AMP-DEPENDENT PROTEIN KINASE I ON THE PHOSPHORYLATION OF CASEIN BY MEMBRANE KINASE I, MEMBRANE KINASE II AND HUMAN MEMBRANE KINASE IN THE PRESENCE OF ATP

The phosphorylation reactions were performed as described in Experimental Procedures using 36 μg of membrane kinase I, 8.5 μg of membrane kinase II, or 2.4 μg of human membrane kinase. The amount of ^{32}P incorporated in 65 μg of regulatory subunit alone was 22 pmol

	pmol ^{32}P incorporated/10 min		
	Control	+25 μg regulatory subunit	+65 μg regulatory subunit
Membrane kinase I	677	602	653
Membrane kinase II	1216	1147	1189
Human membrane kinase	182	209	218

cyclic AMP-dependent protein kinases, is present in human erythrocyte membranes. However, to further substantiate our argument that these enzymes are distinct from the cyclic AMP-dependent enzymes, we tested the effect of the regulatory subunit of the soluble cyclic AMP-dependent protein kinase I from rabbit erythrocytes on the activity of membrane kinases I and II and human membrane kinase. The regulatory subunit was prepared by heating purified cyclic AMP-dependent protein kinase I at 53°C in the presence of cyclic GMP [10]. Under these conditions, the catalytic subunit is preferentially inactivated. As shown in Table II, the regulatory subunit of cyclic AMP-dependent protein kinase has no inhibitory effect on membrane kinase I and membrane kinase II or human membrane kinase when ATP was used as the phosphoryl donor. Similarly, the regulatory subunit did not alter the activity of membrane kinase I in the presence of GTP (data not shown).

Discussion

This report confirms our previous studies [1,2,4] which suggested that erythrocyte membranes contain multiple protein kinases. The protein kinases solubilized from both human and rabbit erythrocyte membranes are distinct from the membrane-bound and the cytosolic cyclic AMP dependent enzymes. They catalyze the phosphorylation of casein but not histone and are neither regulated by cyclic nucleotides nor by the regulatory subunit of purified cyclic AMP-dependent protein kinase I of rabbit erythrocyte (Table II).

Although two protein kinases (membrane kinase I and membrane kinase II) were extracted from the rabbit erythrocyte membranes, using the same extraction procedure, only one kinase (human membrane kinase) was solubilized from the human erythrocyte ghosts. The properties of the human kinase appear to resemble those of membrane kinase II derived from the rabbit. The cyclic AMP-dependent protein kinase associated with the human erythrocyte membranes is not appreciably extracted under the conditions described.

The effect of 2,3-diphosphoglyceric acid on the activity of membrane kinase I is puzzling. 2,3-Diphosphoglyceric acid (5 mM) inhibited about 50% of the membrane kinase I activity when measured with ATP but more than 80% when GTP was used as the phosphoryl donor. The explanation for this observation remains unknown. Whether the inhibition by 2,3-diphosphoglyceric acid is via competition for the substrate, Mg:ATP or Mg:GTP, also awaits to be determined. The susceptibility of membrane kinase I to inhibition by 2,3-diphosphoglyceric acid is similar to that observed for the soluble casein kinases isolated from rabbit erythrocyte lysates [11]. Under similar conditions, membrane kinase II appears to be less sensitive to 2,3-diphosphoglyceric acid, retaining greater than 80% of its activity.

That certain reactions in human erythrocyte membrane autophosphorylation are stimulated by monovalent and divalent cations has been demonstrated [2]. The phosphorylation of casein by either membrane kinase I or membrane kinase II is stimulated by physiological (150 mM) concentrations of NaCl or KCl. However, high concentrations of NaCl or KCl (0.5 M) inhibit membrane kinase I but not membrane kinase II (Fig. 5). Furthermore, the phosphorylation of casein by membrane kinase I or membrane kinase II is inhibited by

Ca^{2+} concentrations greater than 1 mM. Low concentrations of Ca^{2+} are without effect. While the effects of cations on casein phosphorylation differ in some respects from membrane autophosphorylation, it is possible that the stimulatory or inhibitory effects of ions and other agents may be related to the nature of their interaction with the protein substrate rather than with the enzyme directly.

Acknowledgements

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References

1 Hosey, M.M. and Tao, M. (1976) Biochemistry 15, 1561—1569
2 Avruch, J. and Fairbanks, G. (1974) Biochemistry 13, 5507—5514
3 Fairbanks, G. and Avruch, J. (1974) Biochemistry 13, 5514—5521
4 Hosey, M.M. and Tao, M. (1977) J. Biol. Chem., in the press
5 Dodge, J.T., Mitchell, C. and Hanahan, D.J. (1963) Arch. Biochem. Biophys. 100, 119—130
6 Hosey, M.M. and Tao, M. (1975) Biochem. Biophys. Res. Commun. 64, 1263—1269
7 Tao, M. and Hackett, P. (1973) J. Biol. Chem. 248, 5324—5332
8 Rubin, C.S., Erlichman, J. and Rosen, O.M. (1972) J. Biol. Chem. 247, 6135—6139
9 Rubin, C.S. (1975) J. Biol. Chem. 250, 9044—9052
10 Tao, M. (1972) Biochem. Biophys. Res. Commun. 47, 361—364
11 Kumar, R. and Tao, M. (1975) Biochem. Biophys. Acta 410, 87—98