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PURIFICATION, PROPERTIES AND MECHANISM OF INTERCONVERSION OF KIDNEY PHOSPHORYLASE

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SUMMARY

A procedure is described for the purification of glycogen phosphorylase (α-1,4-glucan:orthophosphate glucosyl transferase, EC 2.4.1.1) from rat and beef kidneys. The Michaelis constants of the components of the phosphorylase-catalyzed reaction and other characteristics of the enzyme are presented. Phosphorylase kinase (ATP: phosphorylase phosphotransferase, EC 2.7.1.38) and phosphatase (phosphorylase phosphohydrolase, EC 3.1.3.17) (free of phosphorylase) also have been partially purified from kidney extracts. Kidney phosphorylase kinase can be substituted for muscle phosphorylase kinase in the phosphorylase activation reaction.

From the characteristics of the enzyme, as well as from the results of the activation and inactivation reactions it is concluded that kidney phosphorylase is a form of the enzyme which is different from both liver and muscle phosphorylase.

INTRODUCTION

Kidney preparations are being used with increasing favor for the study of the control mechanisms in gluconeogenesis and glycolysis. The presence of glycogen in kidney has been known for some time, as well as the presence of the enzymes involved in its synthesis and degradation[1,2].

Recently, papers reporting the effects of vasopressin[3] or diabetes[4] on kidney phosphorylase activity have appeared. However, no study of the properties of kidney glycogen phosphorylase (α-1,4-glucan:orthophosphate glucosyl transferase, EC 2.4.1.1) or of the enzymes which mediate its interconversion, namely, phosphorylase kinase (ATP: phosphorylase phosphotransferase, EC 2.7.1.38) and phosphatase (phosphorylase phosphohydrolase, EC 3.1.3.17) has been reported.

In this paper, a method for the purification of kidney phosphorylase and procedures for the partial purification of kidney phosphorylase kinase and phosphatase are described. Properties of kidney phosphorylase and characteristics of the reactions of activation and inactivation are presented.

Abbreviation: MES, 2(*N*-morpholine)ethanosulfonic acid.

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METHODS

Chemicals

Rabbit liver glycogen was purchased from Nutritional Biochemicals Corp. and was further purified by passage (8–10% solutions) through a column of mixed-bed ion-exchange resin (Amberlite MB-3) (50 ml bed volume per g of glycogen) and reprecipitation with ethanol, in the presence of traces of LiBr. Amylopectin was prepared from starch by the method of LANSKY, KOOI AND SCHOCH[5]. Glucose 1-phosphate was obtained from Nutritional Biochemicals Corp. AMP, ATP, NADPH 3′, 5′ cyclic adenylic acid, L-epinephrine bitartrate and Tris were obtained from Sigma Chemical Co. 2(*N*-Morpholine)ethanosulfonic acid (MES) was purchased from Calbiochem. Amorphous pork insulin (glucagon free) and amorphous glucagon were a gift from Eli Lilly and Co. Calcium phosphate gel was prepared by the method of KEILIN AND HARTREE[6]. 2-Mercaptoethanol was purchased from Eastman.

Assay Procedures

Phosphorylase activity was measured by a modification of the method of DANFORTH, HELMREICH AND CORI[7]. The assay mixture contained 75 mM glucose 1-phosphate, 1.2% glycogen, 75 mM potassium fluoride, adjusted to pH 6.1 with HCl. To 0.1 ml of test mixture, 0.05 ml of enzyme were added and the mixture was incubated at 30° for 5 to 30 min. The reaction was terminated by the addition of 8.65 ml of ice-cold acetate buffer (100 mM, pH 4.0). When testing the activity of preparations with a high concentration of protein, as in crude extracts, the reaction was stopped by addition of 1 ml of ice-cold trichloroacetic acid (5%); after removing the protein precipitate by centrifugation, 1 ml of supernatant fluid was diluted with 7.8 ml of 150 mM acetate buffer, pH 4.0. Controls were prepared by adding the acetate buffer (or trichloroacetic acid) to the test mixture before adding the enzyme.

For the development of color, 1 ml of 2.5% ammonium molybdate in 0.025 M H_2SO_4 and 0.2 ml of Fiske-SubbaRow reducing reagent were mixed to these solutions and the absorbance (660 mμ) was determined after incubating for at least 45 min at room temperature. The color was found to be stable in these conditions for over 12 h.

Purified preparations of phosphorylase were reactivated before the assay by incubating at 30° for 10 to 15 min after mixing with a solution containing 50 mM MES, 0.1% glycogen, 100 mM KF, 50 mM mercaptoethanol, pH 6.1, with or without 5 mM 5′-AMP.

In the direction of glycogen degradation, phosphorylase activity was measured by a spectrophotometric method previously described[8]. Protein concentration was determined by a modification of the biuret method[9].

The unit of activity is defined as that amount of enzyme which catalyzes the formation of one μmole of P_i (or glucose 1-phosphate) per min at 30° under the conditions of the assay. Specific activities are expressed as units per mg of protein.

RESULTS

(1) Purification of kidney phosphorylase

Kidneys from the rat, rabbit, hog, dog and cow were tested in order to find the most convenient source of the enzyme. Rat kidneys showed the highest activity per g

TABLE I

PHOSPHORYLASE ACTIVITY IN KIDNEYS FROM DIFFERENT SPECIES

The extracts were obtained by homogenizing fresh kidneys with two volumes (w/v) of 100 mM KF–50 mM Tris–0.05% glycogen (pH 7.8), followed by centrifugation of the homogenates at 16 000 × *g* for 15 min. Assays were carried out on the supernatant fractions which had about twice the specific activity found in the crude homogenates.

Species	*Phosphorylase (units/g wet wt.) + AMP*	*Protein extracted (mg/g tissue)*	*Specific activity of the extracts (milliunits/mg protein) + AMP*
Rat	1.520	173	8.8
Beef	0.470	69	6.8
Dog (cortex)	1.104	160	6.9
(medula)	0.640	52	12.3
Rabbit	0.800	147	5.4
Pork (cortex)	0.450	95	4.7

wet wt. (Table I) and yielded extracts with higher specific activity than those from the other species tested. For large-scale preparations, beef kidneys were preferentially used. Except in the case of the rabbit, the kidney medulla was found to have less phosphorylase activity than the cortex. For the preparation of phosphorylase from bovine kidney only the cortex was used. The whole organ was used for the purification of the enzyme from rat kidney. Except for this difference, the same method applied for the purification of rat and bovine kidney phosphorylases. The procedure used for the purification of bovine kidney phosphorylase was as follows:

(I) Fresh beef kidneys, obtained at the slaughterhouse, were immediately chilled in ice and dissected free of fat and medullary tissue. Portions of the cortex were homogenized for 1 min in a Waring blendor with 2 volumes (w/v) of ice-cold 100 mM KF, 50 mM Tris–HCl, 0.05% glycogen, pH 7.8. The homogenates were centrifuged at 16 000 × *g* for 15 min and the resulting supernatant fluids filtered through glass wool.

(II) The pH of the extracts was adjusted to 6.0 with 2 M acetic acid, and calcium phosphate gel (50 mg/ml) was added to a final concentration of 0.2 mg of gel per mg of protein. After 15 min at 3°, the suspension was centrifuged at 16 000 × *g* for 15 min. The gel precipitate retained the phosphorylase-activating and -inactivating enzymes. The supernatant fluid, which contained the phosphorylase activity, was adjusted to pH 7.0 with 2 M Tris base.

(III) Glycogen solution (8%) was added to the supernatant fluid to a final concentration of 1 mg per ml. The temperature of the solution was lowered to 0° in an ice–salt bath, and, to each l, 250 ml of acetone (−80°) were added rapidly with constant stirring while the temperature was decreased to −5°. The inactive precipitate was removed by centrifuging at −10° for 15 min at 16 000 × *g*. Glycogen solution was again added to the supernatant fluid to a final concentration of 1 mg per ml, the temperature of the suspension was brought to −5°, and cold acetone added to a final concentration of 35% (−5 to −10°). The precipitate, collected by centrifugation as above, was suspended by means of a motordriven glass homogenizer in 100 mM KF, 50 mM Tris–HCl, 0.05% glycogen (pH 7.8), using 1/10 the volume of the original extract.

(IV) To the suspension, glycogen solution (8%) was added to a final concentration of 5 mg per ml, the temperature was lowered to 0°, and 250 ml of cold acetone added per l to the suspension. Following centrifugation as described in step (III), the precipitate was suspended in 100 mM KF, 50 mM Tris–HCl, 0.05% glycogen (pH 7.8), to a final volume one-half that of the preceding step.

(V) Ethanol (−80°) was added rapidly to the suspension to a final concentration of 25% while the temperature was maintained between 0 and −5°. The precipitated enzyme was collected by centrifugation at −10° and resuspended in 2/3 the volume of the same buffer as in Step (IV). To obtain the soluble phosphorylase, the suspension was centrifuged for 30 min at 36 000 × *g*.

(VI) An equal volume of saturated $(NH_4)_2SO_4$ solution (5°) was added to the supernatant obtained in the preceding step. After 1 h at 5°, the precipitate was removed by centrifugation at 30 000 × *g* for 15 min. To the supernatant solution, solid $(NH_4)_2SO_4$ was added to bring the solution to 75% saturation (175 g/l). After 1 h at 5°, the precipitate was collected by centrifugation, dissolved in a small volume of 30 mM Tris–HCl (pH 7.5), and dialyzed against several liters of the same buffer.

Attempts to purify the enzyme further by means of diethylaminoethyl- or carboxymethyl cellulose columns resulted in loss of enzyme activity; gel filtration with Sephadex G-200 columns was also unsuccessful, although this treatment did not inactivate the enzyme.

In order to obtain phosphorylase in the (partially) active form, rat kidney extracts were treated as follows: to each 100 ml of the filtered supernatants obtained after step I, 2 ml of 50 mM ATP, 50 mM $MgCl_2$, 20 mM Tris–HCl (pH 7.8), were added and the temperature was raised to 15° for 20 min. The incubated mixtures were then cooled in an ice bath and, after adjusting the pH to 6.0, the calcium phosphate gel treatment described in step II was applied. The phosphorylase in beef kidney extracts was not activated by this treatment. Typical results of purifications of bovine and rat kidney phosphorylase are summarized in Tables II and III.

(2) Properties of kidney phosphorylase

The purified enzyme was stable at −80° for several weeks. Preincubation at 30°

TABLE II

PURIFICATION OF BEEF KIDNEY PHOSPHORYLASE

Fraction No. and Step	*Volume (ml)*	*Total units + AMP*	*Protein (mg/ml)*	*Specific activity + AMP (units/mg protein)*	*Yield (%)*
I. Extract	1000	260	42	0.007	100
II. Calcium Phosphate gel	990	296	28.7	0.0104	114
III. First acetone fraction	142	317	47.8	0.0467	122
IV. Second Acetone fraction	100	308	27.7	0.111	118
V. Ethanol supernatant	90	277	20.2	0.153	107
VI. $(NH_4)_2SO_4$ fraction	9.4	118	17.7	0.707*	45

* After activation by the kinase, the specific activity (+AMP) was increased to 3.8 units/mg.

TABLE III

PURIFICATION OF RAT KIDNEY PHOSPHORYLASE

Fraction No. and Step	*Volume (ml)*	*Total units +AMP*	*Protein (mg/ml)*	*Specific activity +AMP (units/mg protein)*	*Yield (%)*
I. Extract	150	28.2	13.5	0.009	100
II. Calcium phosphate gel	170	35.9	8.75	0.0241	127
III. First acetone fraction	38	23.6	5.85	0.167	84
IV. Second acetone fraction	18.5	11.9	0.737	0.869	42
V. Ethanol supernatant	9.3	10.9	0.700	1.56	37
VI. $(NH_4)_2SO_4$ fraction	5.7	3.3	0.135	4.28*	12

* DEAE-cellulose column fractions have been obtained with a specific activity of 9.9 units/mg.

with freshly prepared diluting solution was required to restore full enzymic activity. In Fig. 1 the stability of rat kidney phosphorylase at 3° and different pH values is shown. The enzyme was found to be relatively heat labile, even in the presence of glycogen and AMP (Fig. 2). Maximal activity in the direction of glycogen degradation (Tris–maleate buffer) was found at pH values near 6.0 (Fig. 3).

The activity of purified rat kidney phosphorylase was determined at different temperatures, in the presence of 5 mM AMP and at the concentrations of other components present in the usual test mixture. The results are presented in Fig. 4. The Arrhenius plot of the data results in a curve, instead of a straight line. No inflexion

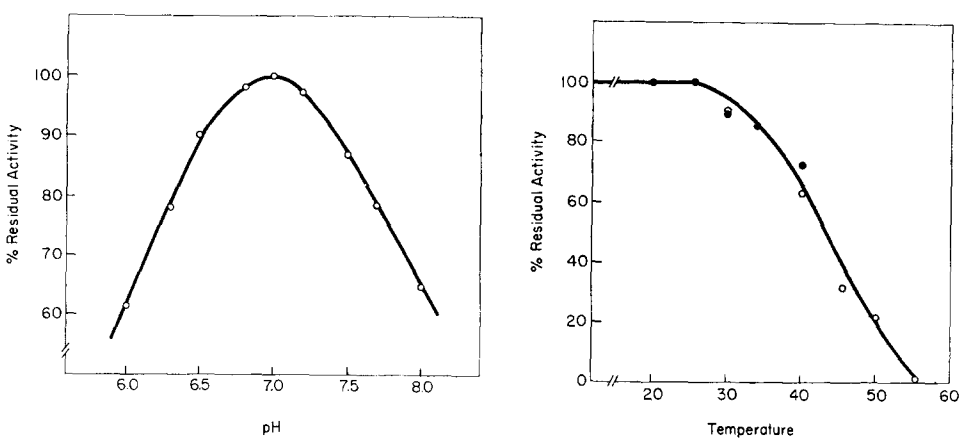
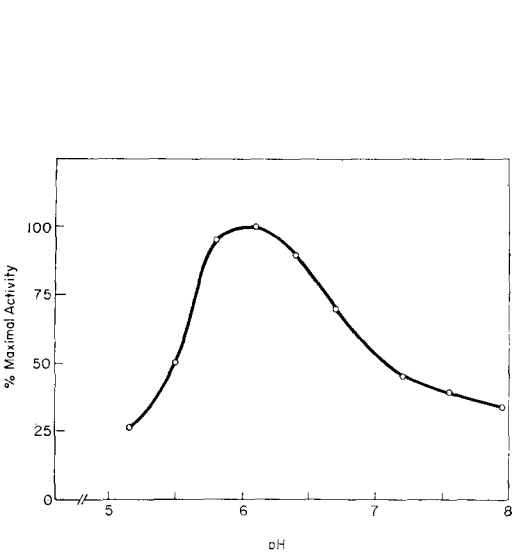


Fig. 1. Stability of kidney phosphorylase as a function of pH. Samples of rat kidney phosphorylase, containing 1 mM AMP and 0.4 mg glycogen/ml, were brought to the indicated pH with either 1 M acetic acid or 1 M NH_4OH and incubated at 0° (ice bath) for 30 min. The samples were then diluted with the preincubation mixture described in METHODS at pH 6.1 and tested.

Fig. 2. Heat stability of kidney phosphorylase. Rat kidney phosphorylase (containing AMP and glycogen (pH 6.4) as in the experiment of Fig. 1) was incubated at temperatures between 0° and 60° for 30 min and, after dilution with preincubation mixture containing AMP, were tested at 30° for 10 min.



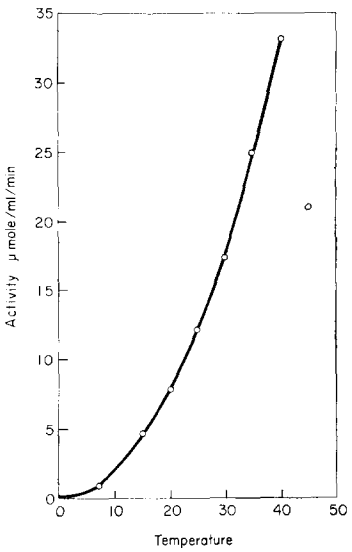


Fig. 3. Activity–pH curve. Test mixture, containing 0.2 M Tris–maleate buffer and 2.5 mM AMP, was brought to the pH values indicated. After addition of the enzyme, the mixture was incubated for 15 min and activity determined by the release of P_i.

Fig. 4. Activity of kidney phosphorylase as a function of temperature. Purified rat kidney phosphorylase was incubated with the text mixture (pH 6.1), at the designated temperatures for 10 to 90 min and the P_i released was determined.

point was observed in the present experiments. The calculated energies of activation vary continuously from 26 150 cal. for the interval of temperatures 7 to 15° to 11 850 cal. between 35 and 40°. These values are comparable to the ones reported by HELMREICH AND CORI[10] for muscle phosphorylase in the same temperature intervals (about 20 000 and 10 000 cal., respectively).

The Michaelis constants of the purified rat kidney enzyme for glycogen, amylopectin, glucose 1-phosphate and P_i were determined in the presence of AMP. The results are presented in Table IV. The K_m for P_i was determined by the spectrophotometric measurement of glucose 1-phosphate; the others were determined by the formation of inorganic phosphate. The activity of bovine kidney phosphorylase, as obtained by the purification method described, was stimulated about 20-fold by 2 mM AMP. The K_a of this form of the enzyme for AMP was found to be 2.8×10^{-4} M at pH 6.1. As illustrated in Fig. 5, glucose inhibited kidney phosphorylase. The inhibition

TABLE IV

EFFECT OF SUBSTRATE CONCENTRATION ON THE ACTIVITY OF PHOSPHORYLASE

Substrate	K_m *(mM)*
Glycogen	5
Amylopectin	15.6
P_i	2.5
Glucose 1-phosphate	5.0

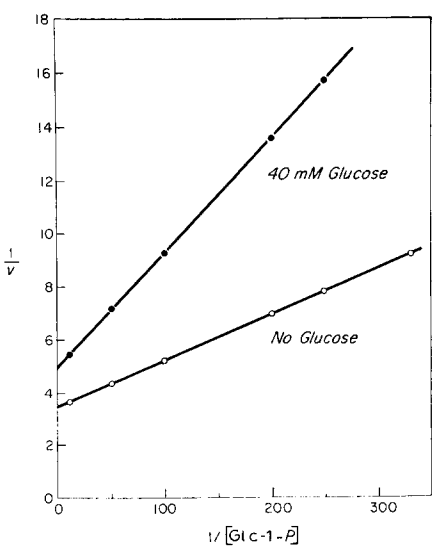
was apparently not of a competitive nature with glucose 1-phosphate, but, as has been pointed out[11], the evaluation of this point is difficult. The calculated K_i for glucose was 50 mM.

The absorption spectrum of the most highly purified beef enzyme showed absorption maximums at pH 7.0 and pH 4.0 suggesting the presence of pyridoxal 5'-phosphate in the preparations.

Interconversion reactions in homogenates

Kidneys from male, fed rats, killed while under seconal anesthesia (40 mg/kg, intraperitoneal injection), were homogenized in ground-glass homogenizers with two volumes (w/v) of ice-cold 100 mM Tris–HCl, 5 mM EDTA (pH 7.6), containing 1 mg glycogen/ml. Unhomogenized tissue segments were removed by filtration through cheese cloth.

The homogenates were incubated at 37° and phosphorylase activity determined in the synthetic direction by measuring the appearance of P_i from glucose 1-phosphate. In homogenates incubated without any additions, phosphorylase *a* activity (−AMP) decreased rapidly and after 10 to 20 min was undetectable (Fig. 6). Total activity (+AMP) also decreased, but stabilized after 10 to 20 min at a final level of about 40% of the initial activity. Addition of 50 mM KF to the homogenates prevented the loss of activity (±AMP) for up to 90 min. If 5 mM ATP, 10 mM Mg^{2+} and 50 mM KF were



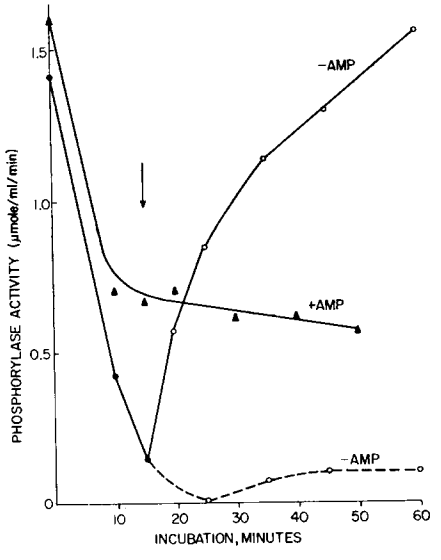


Fig. 5. Inhibition of rat kidney phosphorylase by glucose. Determined with test mixture containing varying concentrations of glucose 1-phosphate, no AMP, and with and without 50 mM glucose. In no case was the utilization of glucose 1-phosphate larger than 15% of the initial concentration.

Fig. 6. Rat kidney homogenates, prepared as described, were incubated at 37° and, at the times indicated, 0.3-ml aliquots were removed and mixed with 0.15 ml of cold 50 mM sodium-maleate, 50 mM KF, 20 mM EDTA, ± 3 mM AMP, pH 5.7 (final pH 6.1), and phosphorylase activity measured. ●—●, no addition, −AMP; ▲—▲, no addition, + AMP; ○—○, 5 mM ATP, 10 mM Mg^{2+}, 50 mM KF (pH 7.8), added after 15 min of incubation, −AMP; ○— — —○, 50 mM KF added after 15 min of incubation, − AMP.

added after 15 min of incubation, a rapid reactivation took place so that, after 45 to 60 min, phosphorylase activity was equal to or greater than that measured at zero time. ATP or Mg^{2+} alone was ineffective, both being required for the reaction. Occasionally, an apparent activation was produced by KF alone after a delay of 5 to 10 min, probably because of an inhibition of the phosphatase activity.

The following compounds, added together with the ATP, Mg^{2+} and KF, did not significantly affect the reactivation in this system: epinephrine (50 μg/ml), glucagon (250 μg/ml), insulin (5 units/ml) and 3',5' cyclic adenylate (0.5 mM).

Purification of phosphorylase kinase and phosphatase from kidney

The following method was used for the preparation of the kinase and phosphatase from rat and beef kidney:

The calcium phosphate precipitate obtained in step (II) of the purification of kidney phosphorylase was found to contain both phosphorylase kinase and phosphatase. The precipitated gel was washed twice, first at pH 8, then at pH 9, by resuspension in a volume of 20 mM Tris–HCl, 1 mM $MgCl_2$ equal to that of the initial kidney extract. The phosphorylase phosphatase and kinase were then both eluted by suspending the gel in a volume of 50 mM Tris–HCl, 300 mM Na_2SO_4, 1 mM $MgCl_2$ at pH 9.0 equal to 1/2 the weight of the starting tissue. The suspension was stirred at room temperature for 10 min and then centrifuged at 16 000 $\times$ g for 15 min. The supernatant fluid obtained was cooled in ice and solid $(NH_4)_2SO_4$ (243 g/l) added slowly, stirring until the salt was dissolved. After 1 h at 3°, the suspension was centrifuged at 16 000 $\times$ g for 20 min, the precipitate dissolved in a minimum volume of 50 mM Tris–HCl, 5 mM EDTA (pH 7.8), and dialyzed for 3 to 5 h against a large volume of the same buffer (Fraction 0–40% sat.).

To the supernatant fluid, solid $(NH_4)_2SO_4$ (205 g/l) was again added to increase the concentration to 70% saturation and, after 1 h at 3°, the precipitate was collected, dissolved and dialyzed as above (Fraction 40–70% sat.).

The first fraction (0–40% sat.) contained the phosphorylase phosphatase, occasionally contaminated with traces of the kinase; the second fraction (40–70% sat.) had most of the phosphorylase kinase and no detectable phosphatase. Both fractions were free of detectable phosphorylase and ATPase activities. By this procedure, both activities were unstable and further attempts to purify the enzymes have been unsuccessful.

Interconversion reactions in partially purified systems

Purified rat kidney phosphorylase (−AMP/+AMP activity ratio, 0.88; specific activity (+AMP), 3.7 units/mg) was prepared by the method previously described. When incubated at 37° with the phosphatase (0–40% sat. fraction), the phosphorylase activity (−AMP) became undetectable after 10 to 45 min, the time depending on the ratio of phosphorylase to phosphatase. The total phosphorylase activity (+AMP) also decreased, but as in the homogenate, leveled off at about 40% of the initial activity. If the phosphatase fraction was heated at 100° for 3 min, or if the reaction was run in the presence of 50 mM KF, there was no loss of phosphorylase activity.

When kidney phosphorylase kinase (Fraction 40–70% sat.) together with 7.5 mM ATP, 15 mM Mg^{2+} was added to purified beef kidney phosphorylase at pH 8.2 (−AMP/+AMP activity ratio, 0.15; specific activity, 0.62 units/mg) and the complete

reaction mixture was incubated at 30°, a considerable activation of phosphorylase took place; after 10 min, the −AMP/+AMP activity ratio had increased to 0.77 and the specific activity to 1.02 units/mg. Neither the kinase nor the ATP and Mg^{2+} alone had any effect. Addition of 3',5' cyclic adenylate to the complete reaction mixture did not increase the rate of activation.

When muscle phosphorylase kinase (0.56 units), purified by the method of KREBS *et al.*[12], was substituted for the kidney phosphorylase kinase fraction, the specific activity of the kidney phosphorylase (+AMP) increased from 0.37 to 1.5 units/mg after 15 min of incubation, the −AMP/+AMP activity ratio increasing during the same time from 0.06 to 0.87.

To test the reversibility of the phosphorylation and dephosphorylation, a mixture of 1 ml of purified rat kidney phosphorylase, 0.25 ml of kidney phosphorylase phosphatase and 0.25 ml of kidney phosphorylase kinase was prepared. To allow the phosphatase to inactivate the phosphorylase, this mixture was incubated at 30° for 15 min without addition. At this point, 5 mM ATP and 10 mM Mg^{2+} were added, and the incubation continued for an additional 20 min to permit the kinase to reactivate the inactivated phosphorylase (Fig. 7). It was apparent that all the phosphorylase

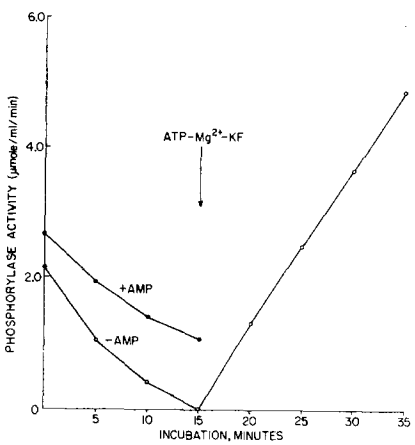


Fig. 7. Purified rat kidney phosphorylase, diluted 1/2.5 with 50 mM Tris, 50 mM mercaptoethanol, 0.2% glycogen (pH 7.8), was preincubated at 0° for 15 min. To 1-ml aliquots of the phosphorylase, 0.5 ml of partially purified kidney phosphorylase kinase and phosphatase were added, and the tubes incubated at 30°. After 15 min of incubation, 0.5 ml of 20 mM ATP, 40 mM Mg^{2+}, 100 mM KF (pH 7.8), were added, and the incubation continued for 20 additional min. At the times indicated, aliquots of 0.1 ml were removed, mixed with 0.2 ml of cold 50 mM 2(*N*-morpholino)ethanosulfonic acid, 50 mM KF, 20 mM EDTA, 0.2% glycogen, ± 1 mM AMP (pH 5.7), and phosphorylase activity tested.

converted by the action of the phosphatase as well as some inactive or partially active enzyme originally present before phosphatase action can be activated by the kinase-catalyzed reaction.

DISCUSSION

From these results, it appears that phosphorylase activity in kidney is regulated allosterically by AMP, as well as by interconversion reactions catalyzed by a kinase

and a phosphatase. Bovine kidney phosphorylase, purified by the method described here, was obtained in a form essentially similar to muscle phosphorylase *b* in regard to the large activation produced by AMP. Purified rat kidney phosphorylase preparations were largely independent of AMP, corresponding to the *a* form of muscle phosphorylase. As indicated, there was no activation by ATP–Mg of bovine kidney phosphorylase in crude extracts (step II of purification), while the phosphorylase in rat kidney extracts was partially activated. The similarity between muscle and kidney phosphorylases, however, did not extend to other characteristics. The K_a of activation by AMP was 10-fold greater for the beef kidney enzyme than for muscle phosphorylase *b* (ref. 13). In addition, the concentrations of $(NH_4)_2SO_4$ at which each enzyme precipitates were also different.

Purified kidney phosphorylase, in the presence of ATP and Mg^{2+}, was activated by a protein fraction obtained from kidney extracts. The fact that the effect of this kidney fraction could be reproduced with purified muscle phosphorylase kinase, an enzyme which has been found to be specific for the incorporation of phosphate from ATP into serine groups of phosphorylase and some serine-containing peptides, supports the hypothesis that the kidney kinase catalyzes a similar process.

In kidney homogenates, as well as in more purified systems, phosphorylase activation and inactivation reactions were readily reversible. Since the inactivated enzyme is reactivated by a phosphorylation reaction, the inactivation appears to be a dephosphorylation of the phosphorylase molecule. The observation that inactivation was completely prevented by fluoride also supports this contention.

When the kinetics of activation and inactivation of kidney phosphorylase are compared with those of the muscle and liver phosphorylases, similarities to and difference from both are apparent. The action of kidney phosphorylase phosphatase on kidney phosphorylase resulted in the appearance of a form of phosphorylase which was totally dependent on AMP for activity, as is muscle phosphorylase *b*; this extensive activation by AMP is not found in liver dephospho-phosphorylase[14,15]. On the other hand, total (+AMP) kidney phosphorylase activity increased upon reactivation by phosphorylase kinase more than 5-fold over the initial activity. Large increases in total activity are observed in the activation of liver dephospho-phosphorylase[14], but not in the interconversion of muscle phosphorylase *b* to *a*, where the increase in activity has been reported as 37% (ref. 16) and 82% (ref. 17).

Kidney phosphorylase has properties of both and, therefore, appears to be a type intermediate between muscle and liver phosphorylase.

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