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ISOLATION OF AN INACTIVE COMPONENT FROM PIG HEART PHOSPHOPROTEIN PHOSPHATASE AND ITS REASSOCIATION WITH AN ACTIVE COMPONENT

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Summary

Treatment of a pig heart phosphoprotein phosphatase (phosphoprotein phosphohydrolase, EC 3.1.3.16) of M_r 224 000 with 40% ethanol followed by gel-filtration on Sephadex G-150, dissociated the enzyme into an active component of M_r 31 000 and an inactive component of M_r 80 000. The inactive component reassociated with the active component, resulting in the formation of an enzyme form of M_r 123 000. A large excess of either component in the reassociation produced only this enzyme form. The ability of the inactive component to associate with the active component was lost by treatment of the inactive component with trypsin and heat (60°C, 2 min) but not with DNAase and RNAase. Effects of the inactive component on the activities of the active component by the association were as followings. The inactive component: (1) stimulated slightly the ^{32}P-H2B histone phosphatase activity in the presence of either NaCl or $Mg(CH_3COO)_2$ but inhibited strongly in the absence of the salts; (2) stimulated the ^{32}P-H1 histone phosphatase activity in the presence of the salts; (3) inhibited the phosphorylase *a* phosphatase activity in the presence and absence of the salts; (4) enhanced the response to the stimulatory effects of the salts on the dephosphorylation of ^{32}P-histone; and (5) protected the phosphorylase *a* phosphatase activity from inhibition by the salts.

Introduction

Phosphorylase phosphatase, in crude extracts of various rat tissues, can be markedly activated by treatment with 80% ethanol with the concomitant con-

version of the enzyme from multiple molecular weight forms to a single form of M_r 30 000—35 000 [1]. The single form of phosphorylase phosphatase has been purified from rabbit liver homogeneously and has shown to dephosphorylate not only phosphorylase *a* but also glycogen synthetase D, phosphorylase *b* kinase and other proteins phosphorylated by cyclic AMP-dependent protein kinase [2,3].

In a previous paper [4], we reported the dissociation, by treatment with 40% ethanol, of a pig heart phosphoprotein phosphatase of M_r 224 000 into an active component of M_r 31 000 and other components of higher molecular weight. Although the preparation of other components was quite heterogenous and had some endogenous phosphoprotein phosphatase activity, the mixing of the preparation with the active component produced an enzyme form of M_r 188 000 with a concomitant increase in ^{32}P-H1 histone phosphatase activity [4].

In this paper, we report the isolation of an inactive component of M_r 80 000 from the preparation of other components and its reassociation with the active component. The inactive component associated with the active component stoichiometrically and formed an enzyme form of M_r 123 000. The association of the two components stimulated the ^{32}P-H1 histone phosphatase activity and inhibited the phosphorylase *a* phosphatase activity of the active component. The present data indicate that the original large form of phosphoprotein phosphatase is composed of at least a catalytic subunit and an inactive subunit which is able to change the substrate specificity and response to salts of the catalytic subunit by association.

Materials and Methods

Materials. H2B histone and H1 histone were prepared from calf thymus as described previously [5]. Cyclic AMP-dependent protein kinase was purified from pig heart by the method of Rubin et al. [6]. [γ-^{32}P]ATP with a specific activity of 10—300 cpm/pmol was prepared by the method of Glynn and Chappell [7]. Rabbit muscle phosphorylase *b* (3 times crystallized) was prepared by the method of Fischer and Krebs [8]. Phosphorylase *b* kinase was purified as described by Cohen [9]. Bovine serum albumin, horse heart cytochrome *c*, beef liver catalase, phenylmethylsulfonyl fluoride, soybean trypsin inhibitor, bovine pancreas DNAase and RNAase were obtained from Sigma. Yeast alcohol dehydrogenase was purchased from Boehringer. Trypsin was a product of Worthington. Leupeptin was provided by Research Resources Program for Cancer Research, Ministry of Education, Science and Culture, Japan. Other chemicals were obtained from commercial sources.

Preparation of substrates. ^{32}P-H2B histone (63 nmol alkali-labile phosphate/mg H2B histone) and ^{32}P-H1 histone (17 nmol alkali-labile phosphate/mg H1 histone) were prepared with [γ-^{32}P]ATP and cyclic AMP-dependent protein kinase as described before [4]. [^{32}P]Phosphorylase *a* (8 nmol alkali-labile phosphate/mg phosphorylase *a*) was prepared by phosphorylating phosphorylase *b* with [γ-^{32}P]ATP and phosphorylase *b* kinase as described by Krebs et al. [10]. All substrate concentrations represent the concentration of the alkali-labile phosphate moiety of the substrate protein.

Enzyme assay. Phosphoprotein phosphatase was assayed by measuring [^{32}P]orthophosphate which was released from ^{32}P-labelled substrates. The reaction mixture (0.1 ml), containing 50 μM ^{32}P-H2B histone, 50 mM Tris-HCl (pH 7.2), 50 mM $Mg(CH_3COO)_2$ and 0.5 mM dithiothreitol, was incubated for 10 min at 30°C and [^{32}P]orthophosphate released was determined as described previously [11]. When ^{32}P-H1 histone (25 μM) was used as a substrate, $Mg(CH_3COO)_2$ was replaced by 200 mM NaCl. When 16 μM [^{32}P]phosphorylase *a* was used as a substrate, $Mg(CH_3COO)_2$ was omitted. One unit of the enzyme was defined as the amount of enzyme which catalyzed the release of 1 nmol of $^{32}PO_4^{3-}$ per min. Protein was determined by the method of Lowry et al. [12] with bovine serum albumin as a standard.

Purification of phosphoprotein phosphatase. Phosphoprotein phosphatase was purified from pig heart as described previously [11] except that buffer A contained 0.1 mM phenylmethylsulfonyl fluoride and the first $(NH_4)_2SO_4$ precipitation was dialyzed for 15 h and the second $(NH_4)_2SO_4$ precipitation was omitted. The purified enzyme had a molecular weight of 224 000 and a specific activity of 113 units/mg protein. The active component of the phosphoprotein phosphatase was prepared by either 40 or 80% ethanol treatment of the enzyme as described before [4,11]. The active component had a molecular weight of 31 000 and a specific activity of about 2000 units/mg protein.

Assay for inactive components. Samples were mixed with 1 unit of the active component in a total volume of 100—140 μl containing 50 mM Tris-HCl (pH 7.4) and 1 mM dithiothreitol (protease inhibitors in sample solution did not interfere with the assay). After standing for 30 min at 1°C, ^{32}P-H1 histone phosphatase activities of the mixtures were measured in a reaction mixture of 200 μl containing 50 mM Tris-HCl (pH 7.2), 1 mM dithiothreitol, 200 mM NaCl and 25 μM ^{32}P-H1 histone. Incubations were started by the addition of ^{32}P-H1 histone and carried out for 10 min at 30°C. $^{32}PO_4^{3-}$ released during the incubation was measured as described previously [11]. Control incubations without samples and without the active component were run simultaneously. The sum of these two control values was subtracted from the complete value. One unit of inactive components was defined as the amount of the components which stimulated the release of 1 nmol $^{32}PO_4^{3-}$ per min. The measurable unit of inactive components was within 0.13 unit under the assay conditions.

Determinations. Molecular weights and frictional ratios (f/f_0) of proteins were determined by the method of Siegel and Monty [13] from sedimentation coefficients ($s_{20,w}$) and Stoke's radii. Sedimentation coefficients were determined by sucrose gradient centrifugation analysis by the method of Martin and Ames [14]. The Stoke's radii were estimated by gel-filtration with a Sephadex G-150 column as described previously [4].

Results

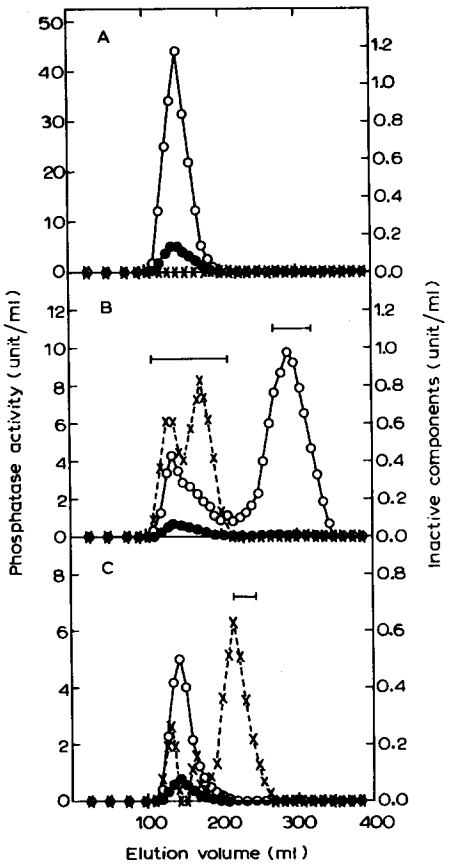
Isolation and properties of an inactive component

A partially purified phosphoprotein phosphatase of M_r 224 000 was dissociated into an active component of M_r 31 000 and other components of higher molecular weights by treatment with 40% ethanol followed by gel-filtration on a Sephadex G-150 column (Fig. 1A and B). Since the other components com-

bine with the active component and stimulate P_i release from ^{32}P-H1 histone catalyzed by the active component in the presence of 200 mM NaCl [4], the other components in each fraction eluted from a Sephadex G-150 column were assayed by measuring P_i release from ^{32}P-H1 histone in the presence of 200 mM NaCl and saturating amounts of the active component (see Materials and Methods). No other component was detected in the original enzyme of M_r 224 000 before ethanol treatment (Fig. 1A). By treatment of the enzyme with 40% ethanol, other components emerged with concomitant dissociation of the active component which was eluted at elution volume of 275—300 ml (Fig. 1B). The other components were eluted as two peaks in fractions with a Stoke's radius range higher than 4.3 nm (Fig. 1B). The first peak was eluted near the void volume of the column and the second peak was eluted at a Stoke's radius of 5.8 ± 0.1 nm (values were mean ±S.D. of data from three separate experiments). 104-ml fractions of the other components, as shown by solid bar in Fig. 1B, were pooled and concentrated to 2.4 ml in an Amicon ultra-filtration cell equipped with a YM-10 filter membrane and applied to a Sephadex G-150 column. The gel-filtration of the other components resulted in a significant shift of a main peak of the components to fractions with a lower Stoke's radius range (Fig. 1C). This shift could not be prevented in the presence of protease inhibitors (1 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride and 0.01% leupeptin) during concentration and gel-filtration of the fractions. Fractions of other components, as shown by solid bar in Fig. 1C, were essentially free from phosphatase activity. Rechromatography of the concentrated fractions through a Sephadex G-150 column did not result in a further shift of the elution profile of the inactive component. However, when the inactive component was treated with 40% ethanol and subjected to gel-filtration on a Sephadex G-150 column under the same conditions as employed for the treatment of phosphoprotein phosphatase of M_r 224 000, significant amounts of the inactive component was eluted in fractions near the void volume of the column (data not shown). These results suggest that the change in the elution profile of the other components might be caused in part by the effects of ethanol on the inactive component and/or the Sephadex G-150 column. Sucrose density gradient analysis of the concentrated fractions of other components in Fig. 1B showed that most of the components were sedimented as a single peak of sedimentation coefficient of 4.7 S and was dissociated from the phosphatase activity found at a sedimentation coefficient of 7.7 S (Fig. 2). The Stoke's radius of the inactive component was estimated to be 4.0 nm by gel-filtration on Sephadex G-150. The molecular weight of the inactive component was estimated to be 80 000 from the sedimentation coefficient and the Stoke's radius (Table I). The calculated frictional ratio (f/f_0) of the component was 1.41 showing its asymmetry.

The ability of the inactive component to stimulate ^{32}P-H1 histone phosphatase activity of the active component was completely suppressed by treatment of the inactive component with trypsin or heat (60°C, 2 min), but not with RNAase or DNAase (Table II) indicating that the inactive component was heat labile protein but not nucleic acid.

The half life of the inactive component (5 units, 0.14 mg protein/ml in 50 mM Tris-HCl (pH 7.4) containing 1.0 mM dithiothreitol) was about 2 days either on ice-water slurry or in a frozen state at —20°C.



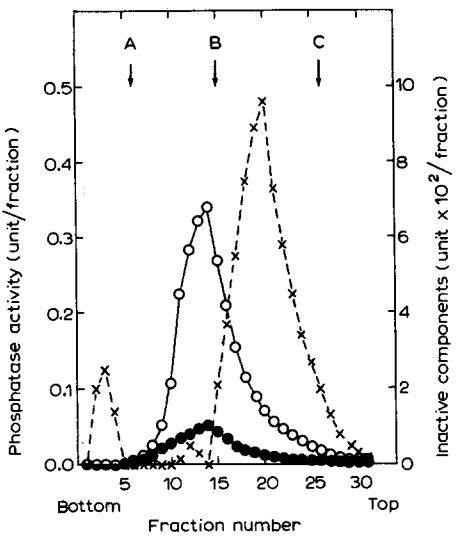


Fig. 1. Sephadex G-150 gel filtration of pig heart phosphoprotein phosphatase and its components. Pig heart phosphoprotein phosphatase (1697 units, 15 mg protein) before (A) and after (B) 40% ethanol treatment and other components in fractions (45.8 units, 7.4 mg protein) as shown by solid bar in B (C), were applied to a Sephadex G-150 column (2.5 × 80 cm) equilibrated with 50 mM Tris-HCl (pH 7.4) containing 1 mM dithiothreitol, 1 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride and 0.01% leupeptin. Elution was carried out upward with the same buffer and fractions of 4.0 ml each were collected at a flow rate of 16 ml/h. 40% ethanol treatment was performed on ice-water slurry by adding 0.73 ml of 95% ethanol (—30°C) gradually to one ml of pig heart phosphoprotein phosphatase with gentle stirring within 5 min. The mixture was immediately centrifuged for 5 min at 17 000 × *g* at —20°C. The supernatant solution was diluted with the same volume of the equilibration buffer and applied directly to a Sephadex G-150 column. Inactive components (X) and phosphatase activity with ^{32}P-H1 histone (•), or with ^{32}P-H2B histone (○) as substrate were determined.

Fig. 2. Sedimentation profiles of the other components in a sucrose density gradient. The fractions as shown by solid bar in Fig. 1B (1.34 units, 0.15 mg protein) were layered onto a linear gradient (4 ml) of 5—20% sucrose in 50 mM Tris-HCl (pH 7.4) containing 1 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride. The tube was centrifuged at 40 000 rev./min in a Hitachi rotor RPS 56T for 13.5 h at 4°C. Fractions (3 drops each) were collected and assayed for inactive components (X) and phosphatase activity with ^{32}P-H1 histone (•), or with ^{32}P-H2B histone (○) as a substrate. These activities are plotted along with the positions of markers: catalase (11.3 S), A; alcohol dehydrogenase (7.4 S), B; and cytochrome *c* (1.9 S), C.

TABLE I

MOLECULAR PARAMETERS OF THE ORIGINAL AND RECONSTITUTED PIG HEART PHOSPHOPROTEIN PHOSPHATASES AND THEIR COMPONENTS

Enzyme	$s_{20,w}$ (S)	Stoke's radius (nm)	Molecular weight	f/f_0
Original form	7.7 ± 0.1 *	6.8 ± 0.3 *	224 000 ± 8000 *	1.70 *
Active component	3.5 ± 0.1 *	2.1 ± 0.1 *	31 000 ± 2000 *	1.01 *
Inactive component	4.7 ± 0.1 **	4.0 ± 0.1 **	80 000 ± 2000	1.41
Reconstituted form	5.5 ± 0.2 **	5.2 ± 0.2 **	123 000 ± 5000	1.58

* Values are taken from the previous report [4].

** The mean ± S.D. of data of experiments from more than six separate analyses are given.

Reassociation of the inactive and active components

Various proportions of the inactive and active components were mixed in 3 ml 50 mM Tris-HCl (pH 7.4) containing 1 mM dithiothreitol. After standing for 30 min at 1°C, the mixtures were separately subjected to gel-filtration on a Sephadex G-150 column. Fig. 3A shows an elution profile of a mixture of one unit of the inactive component and 3.5 units of the active component (unit ratio = 0.29). All of the added active component was reassociated with the inactive component and formed a new active peak which was eluted in fractions of Stoke's radius of 5.2 nm. Recoveries of ^{32}P-H2B histone and ^{32}P-H1 histone phosphatase activities were 130 and 770%, respectively. This may indicate that the inactive component stimulated both ^{32}P-histone phosphatase activities of the active component by the association. Fig. 3B and C show elution profiles of the active component alone and the inactive component alone, respectively. Exposure of the inactive component to 60°C for 2 min before mixing with the active component produced an elution profile similar to Fig. 3B indicating that the ability of the inactive component to associate with the active component was heat labile. Sucrose density gradient analysis of the newly formed enzyme revealed that the sedimentation coefficient of the enzyme was 5.5 S. The molecular weight of the enzyme was estimated to be 123 000 from the Stoke's radius and the sedimentation coefficient, suggesting that the enzyme form consisted of 1 mol of the inactive component (M_r 80 000) and 1 mol of the active component (M_r 31 000) (Table I).

When 1 unit of the inactive component was mixed with 17.5 units of the active component (unit ratio = 0.057), a new active peak was eluted in fractions of Stoke's radius of 5.2 nm in addition to a peak of the excess active component (Fig. 3D). The amount and the molecular weight of the newly formed enzyme were identical with those in Fig. 3A.

Large excess of the inactive component (unit ratio = 2.0) or higher concentration of both components (20 units each) did not produce a higher molecular weight form other than the enzyme form of M_r 123 000 (data not shown). These results indicate that even in the presence of a large excess of either component during the reassociation, the molar ratio of the two components in the newly formed enzyme was constant.

No dissociation of the reconstituted form was observed during gel-filtration

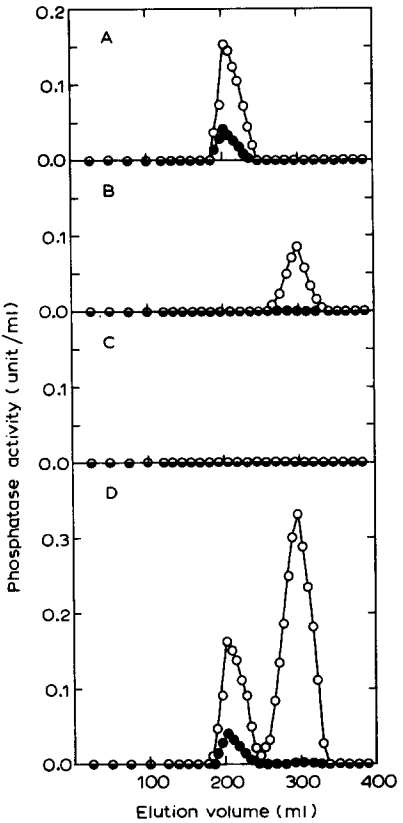


Fig. 3. Sephadex G-150 gel-filtration of the inactive and active components. The active component (3.5 units with ^{32}P-H2B histone, 0.09 unit with ^{32}P-H1 histone, 40 μg protein) and the inactive component (1 unit, 93 μg protein) (A); the active component alone (B); the inactive component alone (C); the active component (17.5 units with ^{32}P-H2B histone, 0.45 unit with ^{32}P-H1 histone, 200 μg protein) and the inactive component (1 unit, 93 μg protein) (D); in 3 ml 50 mM Tris-HCl (pH 7.4) containing 1 mM dithiothreitol were left standing for 30 min at 1°C and applied to a Sephadex G-150 column (2.5 × 80 cm), separately. Gel-filtration was performed as described in the legend to Fig. 1. Phosphatase activity with ^{32}P-H1 histone (●) or with ^{32}P-H2B histone (○) as substrates was assayed.

or sucrose density gradient centrifugation. Further, the dilution of the reconstituted form did not change the linear relationship between the concentration and ^{32}P-H1 histone phosphatase activity of the enzyme. These results indicate that the two components formed a tight complex in 50 mM Tris-HCl (pH 7.4) containing 1 mM dithiothreitol.

Effect of the inactive component on activities of the active component with various substrates

Phosphatase activities of the active component toward various substrates were changed differently by the association with the inactive component (Table III). The ^{32}P-H2B histone phosphatase activity was slightly stimulated in the presence of 50 mM $Mg(CH_3COO)_2$ but strongly suppressed in the absence of the salt. The ^{32}P-H1 histone phosphatase activity was stimulated about

TABLE II

NATURE OF THE INACTIVE COMPONENT FROM PIG HEART PHOSPHOPROTEIN PHOSPHATASE

The preincubation mixture (200 μl) contained 50 mM Tris-HCl (pH 7.2), 1 mM $Mg(CH_3COO)_2$, 1 mM dithiothreitol, 0.086 unit (12 μg protein) of the inactive component, and when applicable, 2 μg of RNAase, DNAase, or trypsin. For heat treatment, the reaction mixture was heated at 60°C for 2 min. After preincubation at 30°C for 30 min, 40 μl of the mixture were withdrawn and ^{32}P-H1 histone phosphatase activity was measured in the presence or absence of 1 unit of the active component as described in Methods. To a mixture (40 μl) which contained trypsin, 0.2 mg of trypsin inhibitor was added before measuring its ^{32}P-H1 histone phosphatase activity.

Treatment	^{32}P-H1 histone phosphatase activity (unit × 10^3)			Inactive component (unit × 10^3)
	Inactive + active	Inactive	Active	
None	20.3	0.9	13.0	6.4
RNAase	20.3	0.9	12.5	6.9
DNAase	20.5	0.8	12.8	6.9
Trypsin	13.2	0.1	13.1	0
Heat	13.2	0.2	13.0	0

2-fold in the presence of 50 mM $Mg(CH_3COO)_2$ but insignificantly changed in the absence of the salt. The phosphatase activity was stimulated about 6-fold in the presence of 200 mM NaCl with the saturating amount (unit ratio >0.5) of the inactive component. The phosphorylase *a* phosphatase activity was inhibited 30% in the presence of 50 mM $Mg(CH_3COO)_2$ and 50% in the absence of this salt.

Comparison of activity ratios for ^{32}P-H1 histone and ^{32}P-H2B histone (activity ratio H1/H2B), and for phosphorylase *a* and ^{32}P-H2B histone (activity ratio phosphorylase *a*/H2B) of the active component and the reconstituted form also shows that ^{32}P-H1 histone phosphatase activity was increased and phosphorylase *a* phosphatase activity was decreased by the association of the inactive and active components (Table IV). The opposite changes in the activity ratios were

TABLE III

EFFECT OF THE INACTIVE COMPONENT ON ACTIVITIES OF THE ACTIVE COMPONENT WITH VARIOUS SUBSTRATES

P_i release from various substrates was measured as described in Methods with 0.14 unit of the active component and/or 0.01 unit of the inactive component. When ^{32}P-H1 histone was used as substrate, 200 mM NaCl was replaced by 50 mM $Mg(CH_3COO)_2$.

Substrate	P_i release (pmol/min)					
	Plus 50 mM $Mg(CH_3COO)_2$			No $Mg(CH_3COO)_2$		
	Active	Inactive	Active + inactive	Active	Inactive	Active + inactive
^{32}P-H2B histone	69.3	3.4	80.0	60.0	0.5	16.8
^{32}P-H1 histone	1.7	0.4	3.6	5.2	0.4	4.8
[^{32}P]Phosphorylase *a*	10.0	0.0	7.0	40.2	0.0	20.4

TABLE IV

SUBSTRATE SPECIFICITIES OF THREE FORMS OF PIG HEART PHOSPHOPROTEIN PHOSPHATASE

^{32}P-H2B histone, ^{32}P-H1 histone and [^{32}P]phosphorylase *a* phosphatase activities were measured as described in Methods.

Enzyme	Activity ratio (X 100)	
	H1/H2B	Phosphorylase *a*/H2B
Active component	2.5	45.5
Reconstituted form	19.2	27.8
Original form	25.0	12.2

observed by the dissociation of the original form of phosphoprotein phosphatase to the active component. However, the reconstituted form had an intermediate value of the activity ratio between those of the active component and the original form of the enzyme.

Effect of the inactive component on response of the active component to salts

With ^{32}P-H2B histone as a substrate, the activities of the active component, the reconstituted form and the original form of phosphoprotein phosphatase were stimulated 1.6-, 4.4- and 8.8-fold, respectively, by $Mg(CH_3COO)_2$ with optimal concentration of 30, 75 and 75 mM, respectively (Fig. 4). Although the reactions catalyzed by the active component were inhibited by $Mg(CH_3COO)_2$ at concentrations higher than 75 mM, the reactions by the reconstituted form and the original form were not inhibited by the salt at concentrations up to 200 mM. Similar effects were observed with similar ionic strengths of NaCl (Fig. 4). These observations indicate that the transformation of the active component to the reconstituted form increased the responses to these salts as well as the concentrations of the salts needed for greatest stimulation.

The activity of the reconstituted form towards ^{32}P-H1 histone was slightly stimulated by $Mg(CH_3COO)_2$ at 10 mM but inhibited by the salt at concentrations higher than 30 mM (Fig. 4) whereas the activity of the active component was not stimulated even at low concentrations of $Mg(CH_3COO)_2$ and inhibited by the salt at a concentration range between 10 and 100 mM. The original large form of the enzyme was more responsive than the reconstituted form to the stimulatory effect of $Mg(CH_3COO)_2$ and required a higher concentration (20 mM) of the salt to get maximum activity. NaCl had the same effect on the dephosphorylation of ^{32}P-H1 histone by the three forms of phosphoprotein phosphatase but double the ionic strength of $Mg(CH_3COO)_2$ was needed to exert the same effect as $Mg(CH_3COO)_2$ (Fig. 4).

Using phosphorylase *a* as a substrate, the three forms of phosphoprotein phosphatase were inhibited by $Mg(CH_3COO)_2$ as well as NaCl (Fig. 4). $Mg(CH_3COO)_2$ at 50 mM (ionic strength, 0.15 M) inhibited the reaction of the active component, the reconstituted form and the original form of the enzyme by 78, 56 and 48%, respectively. Under the same conditions, NaCl at 150 mM

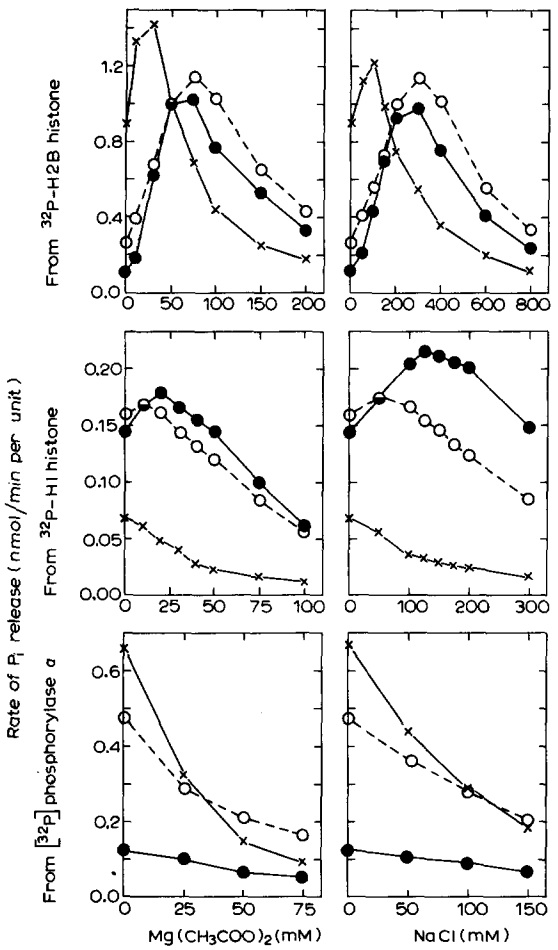


Fig. 4. Effect of $Mg(CH_3COO)_2$ and NaCl on the dephosphorylation of ^{32}P-H2B histone, ^{32}P-H1 histone and [^{32}P]phosphorylase *a* by three forms of pig heart phosphoprotein phosphatase. The rates of P_i release from various substrates were measured with 1.09 units/ml of the original form of the enzyme (•), 0.42 unit/ml of the reconstituted form (○) and 0.79 unit/ml of the active component (X); $Mg(CH_3COO)_2$ or NaCl at the indicated concentration was included instead of 50 mM $Mg(CH_3COO)_2$ or 200 mM NaCl.

(ionic strength, 0.15 M) inhibited the reaction of these forms of the enzyme by 72, 57 and 44%, respectively. These results indicate that the inactive component buffered the inhibitory effect of $Mg(CH_3COO)_2$ and NaCl on the activity of the active component towards phosphorylase *a*.

Discussion

In this paper, an inactive component of M_r 80 000 and an active component of M_r 31 000, were isolated from a pig heart phosphoprotein phosphatase of M_r 224 000. The inactive component was a heat-labile protein and associated instantaneously at 1°C with the active component, in 50 mM Tris-HCl (pH 7.4)

containing 1 mM dithiothreitol, and formed a tight complex of M_r 123 000. Although the inactive component inhibited the phosphorylase *a* phosphatase activity of the active component by the association, the inactive component was apparently different from the heat-stable inhibitors of phosphorylase phosphatase which have been isolated from various mammalian tissues [15—17]. These inhibitors were heat-stable and had molecular weights of not more than 30 000 [18,19] and did not form tight complexes with phosphoprotein phosphatase [19].

As previously reported [4], the active component combined with other components of higher molecular weights and formed a phosphoprotein phosphatase of M_r 188 000. As shown in this paper, the active component and the inactive component could only form a complex of M_r 123 000 in 50 mM Tris-HCl (pH 7.4) containing 1 mM dithiothreitol. Whether this difference in molecular weights is due to the absence of unknown components or to the alteration of the other components at the second peak (Fig. 1B) to the inactive component, remains obscure. The possibility of proteolysis of the other components by sulfhydryl-activated proteases was not completely eliminated even in the presence of protease inhibitors.

Recently, Khandelwal [20] reported that PP_i caused an apparent conversion of a 4.1 S form of phosphoprotein phosphatase to a 7.8 S form in rat liver crude extracts. The addition of 0.25 mM PP_i in a mixture of the inactive component (2.7 units) and the active component (12 units), however, did not produce an enzyme which had a molecular weight higher than 123 000.

Irreversible changes in the conformations and properties of each component of the phosphoprotein phosphatase during 40% ethanol treatment must also be taken into account in order to compare physical and catalytic properties of the original and the reconstituted forms of the phosphoprotein phosphatase.

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