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Interconvertible forms of muscle phosphorylase phosphatase

Phosphorylase *a* phosphatase (phosphorylase phosphohydrolase, EC 3.1.3.17) catalyzes the conversion of phosphorylase *a* (α-1,4-glucan:orthophosphate glucosyltransferase, EC 2.4.1.1) to phosphorylase *b* (refs. 1 and 2). The present paper reports preliminary evidence that skeletal muscle phosphorylase phosphatase exists in two interconvertible forms.

Phosphorylase *a* phosphatase was obtained from pigeon breast muscle. The tissue was homogenized with two volumes of 250 mM sucrose containing 50 mM glycylglycine buffer (pH 7.2) and adjusted to pH 7.0. Aliquots of the homogenate (2 ml) were incubated for different periods of time at 37° without any addition. The samples were then passed through Sephadex G-25 columns (1 cm × 20 cm) equilibrated with a solution containing 250 mM sucrose and 50 mM glycylglycine buffer (pH 7.2), and the first 1.5 ml of the colored effluent were collected. Reactivation or further inactivation of the phosphatase was performed at 37° by incubating 0.25-ml aliquots of the eluate with the indicated additions in a total volume of 0.3 ml. The final concentrations of the additions were as follows: mercaptoethanol, 10 mM; theophylline, 20 mM; ATP–$MgCl_2$, 2.5 mM; phosphocreatine–$MgCl_2$, 5 mM; $MgCl_2$, 5 mM; cyclic 3′,5′-AMP, 0.017 mM; ATP, 2.5 mM and phosphocreatine, 5 mM. Reactions were stopped by the addition of 2.7 ml of a cold solution containing 10 mM mercaptoethanol, 5 mM EDTA and 40 mM glycerophosphate buffer (pH 6.8). The diluted samples were assayed for phosphatase activity. The assay mixture containing 0.02 ml of the enzyme sample and 0.01 ml of ^{32}P-labeled phosphorylase *a* (48 Cori units; 1000–3000 counts/min per Cori unit) was incubated at 30° for 5 min, and the radioactive phosphate liberated was determined as previously indicated[3]. Phosphatase activity was expressed as the rate of consumption of phosphorylase *a*. ^{32}P-labeled

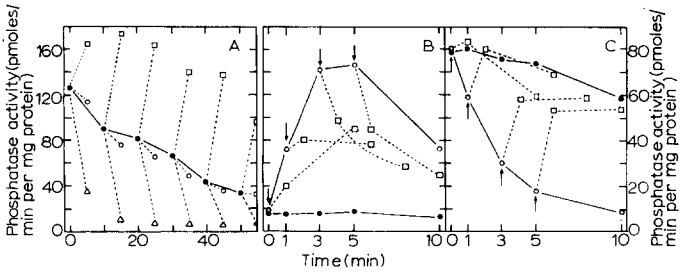

Fig. 1. Inactivation and reactivation of phosphorylase *a* phosphatase. (A) The pigeon breast muscle homogenate was incubated at 37° and passed through a Sephadex column. The phosphatase activity in the Sephadex eluate was measured after dilution and addition of theophylline, as described in the text (●—●). Aliquots of this eluate were further incubated for 5 min at 37° in the presence of mercaptoethanol and theophylline *plus* the following additions: (○— —○) none; (△— —△) ATP; (□— —□) ATP–$MgCl_2$ and phosphocreatine–$MgCl_2$. After dilution, the phosphatase activity was assayed as described above. (B) Reactivation of the phosphorylase phosphatase activity. Inactivation was carried out for 40 min. Reactivation mixtures contained mercaptoethanol and theophylline (●—●) or mercaptoethanol, theophylline, ATP–$MgCl_2$ and phosphocreatine–$MgCl_2$ (○—○); cyclic AMP was added at the times indicated by the arrows (□— —□). (C) Inactivation of phosphorylase phosphatase. An active preparation of phosphorylase phosphatase was incubated with mercaptoethanol and theophylline in the presence (○—○) or absence (●—●) of ATP; phosphocreatine–$MgCl_2$ and $MgCl_2$ were added at the times indicated by the arrows (□— —□). Other conditions were as described in the text.

TABLE 1

REQUIREMENTS FOR DIFFERENT SUBSTANCES IN THE ACTIVATION AND INACTIVATION OF MUSCLE PHOSPHORYLASE PHOSPHATASE

Conditions were as described in the text.

	Reactivation	*Activity (pmoles/min per mg protein)*	
Time (min)	*Additions*	*Expt. I*	*Expt. II*
0	None	4.3	25.0
0	Mercaptoethanol, theophylline		45.0
5	None	2.2	20.0
5	Mercaptoethanol, theophyline	12.2	48.0
5	Mercaptoethanol, theophylline, ATP–Mg^{2+}	60.0	72.0
5	Mercaptoethanol, theophylline, phosphocreatine–Mg^{2+}	43.5	89.0
5	Mercaptoethanol, theophylline, ATP–Mg^{2+}, phosphocreatine–Mg^{2+}	80.0	115.0
5	Mercaptoethanol, theophylline, Mg^{2+}	23.5	62.0
5	Mercaptoethanol, theophylline, ATP	0.1	37.0
5	Theophylline	12.5	
5	Theophylline, ATP–Mg^{2+}	64.0	
5	Theophylline, phosphocreatine–Mg^{2+}	49.0	
5	Theophylline, Mg^{2+}	30.0	
5	Theophylline, ATP	0.1	
5	ATP–Mg^{2+}	19.3	
5	Phosphocreatine–Mg^{2+}	26.0	
5	ATP–Mg^{2+}, phosphocreatine–Mg^{2+}	28.0	
5	Mg^{2+}	8.0	
5	ATP	0.1	
5	Phosphocreatine	1.6	

crystalline phosphorylase *a* was obtained as described by HURD *et al.*[4] using a γ-^{32}P-labeled ATP prepared according to LOWENSTEIN[5]. Protein determinations were carried out by the method of LOWRY *et al.*[6].

As can be seen in Fig. 1A, the incubation of the homogenate at 37° resulted in a time-dependent decrease in phosphatase activity. This effect is not an irreversible inactivation of the enzyme, since the activity of the Sephadex eluate was restored by reincubation with ATP, phosphocreatine and Mg^{2+}. The opposite effect, *i.e.*, a decrease in activity, was produced by reincubation with ATP in the absence of Mg^{2+}.

A partially inactive phosphorylase *a* phosphatase was obtained by incubation of the homogenate for 40 min at 37°. Fig. 1B shows the time course for the phosphatase reactivation in the presence of ATP, phosphocreatine and Mg^{2+}. Cyclic AMP decreases the level of the active enzyme when it is added either at the beginning of or during the reactivation.

Fig. 1C shows that the incubation of an active phosphatase preparation with ATP (in the absence of Mg^{2+}) resulted in a time-dependent inactivation of the enzyme. The ATP effect was reversed by the addition of phosphocreatine and Mg^{2+}.

The requirements for different substances in the activation and inactivation of muscle phosphorylase phosphatase were studied in two types of enzymatic preparations. In experiment I of Table I, the enzyme preparation was a Sephadex eluate obtained from a homogenate partially inactivated by incubation at 37° for 40 min. In experiment II, the Sephadex eluate was precipitated by the addition of satd. $(NH_4)_2SO_4$ solution to obtain 0.6 satn. After centrifugation at 10 000 $\times$ *g* for 10 min the precipitate was resuspended in 250 mM sucrose containing 50 mM glycylglycine buffer (pH 7.2), and the suspension was passed through a Sephadex G-25 column as described above. It can be seen in Table I that theophylline stimulated the phosphatase and that the effect of this substance was exerted in the reaction assay of the enzyme. This observation corroborates the results obtained by WOSILAIT AND SUTHERLAND[7] with the liver enzyme. The presence of mercaptoethanol is not a requirement for either the inactivation or the reactivation of phosphorylase phosphatase. In the presence of Mg^{2+}, ATP and phosphocreatine both activated the phosphatase to a similar extent. The maximal rate of activation was observed in the presence of ATP, phosphocreatine and Mg^{2+}. On the other hand, Mg^{2+} alone also activated the enzyme, but more slowly.

From these experiments it can be concluded that phosphorylase phosphatase in pigeon breast muscle has at least two interconvertible forms. Conversion to the more active form is associated with the presence of ATP and phosphocreatine. This type of conversion provides an adequate mechanism for the regulation of glycogen deposition according to the levels of "high energy phosphates" and Mg^{2+}. The regulation of phosphorylase phosphatase in pigeon breast muscle shows a striking resemblance to that observed in bovine adrenals by MERLEVEDE AND RILEY[8]. In the muscle preparation, however, it was found that phosphorylase *b* kinase (ATP:phosphorylase phosphotransferase, EC 2.7.1.38) can be activated by ATP-Mg^{2+} and cyclic AMP as described in rabbit skeletal muscle[9,10]. Therefore, the pigeon breast muscle preparation seems to be the first system in which it has been possible to demonstrate a coordinated effect of cyclic AMP on the two enzymes, phosphorylase phosphatase and kinase, that regulate the levels of active phosphorylase.

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Instituto de Investigaciones Bioquímicas
"Fundación Campomar" and Facultad de
Ciencias Exactas y Naturales,
Obligado 2490, Buenos Aires (Argentina)

CÉSAR A. CHELALA*
HÉCTOR N. TORRES**

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* Fellow of the Consejo Nacional de Investigaciones Cientificas y Técnicas.
** Career investigator of the Consejo Nacional de Investigaciones Cientificas y Técnicas.