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Effects of Nucleoside Phosphates and Salts on the Activity of a Heart Phosphoprotein Phosphatase and Its Catalytic Subunit

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A phosphoprotein phosphatase (phosphatase B, M_r = 161000) isolated from canine heart extract can be dissociated by ethanol into a catalytic subunit (phosphatase S, M_r = 34800). The dissociation of phosphatase B into phosphatase S is accompanied by an increase in the enzymic activity toward phosphohistone, phosphocasein and phosphorylase *a* and by a pronounced change in catalytic properties. Phosphatases B and S exhibit different substrate saturation kinetics, pH profile and respond differently to nucleoside phosphates and salts.

The increased activity toward phosphohistone accompanying the dissociation of phosphatase B reflects loss of substrate inhibition. Preincubation of either phosphatase B or S with ATP results in an inactivation of its activity and phosphatase S is much more susceptible to ATP inactivation than phosphatase B. ATP and various ionic compounds, however, can greatly stimulate the activity of phosphatase B by interacting with phosphohistone, indicating the modification at the substrate level represents an important regulatory mechanism. By contrast, the activity of phosphatase S is slightly stimulated by relatively low concentrations of salts, and is inhibited by nucleoside phosphates. The present data indicates that the activity of phosphatase B is more sensitive to the modification of the substrate, phosphohistone, by nucleoside phosphates and salts than is phosphatase S. These observations suggest that the regulatory properties of phosphatase B are lost following its dissociation.

The interconversion of enzymes between active and inactive forms through cycles of phosphorylation and dephosphorylation represents an important mechanism for metabolic control. Several laboratories have reported that phosphoprotein phosphatases, which catalyze dephosphorylation reactions, can exist in multiple forms [1–12] and exhibit a broad substrate specificity [2–6,8,11–17]. Multiple molecular forms of phosphoprotein phosphatases can be dissociated by treatment with ethanol [9,10] or 2-mercaptoethanol [5,6] to yield a catalytic subunit with concomitant activation of the enzyme.

In the previous communication [1], we have reported that phosphohistone (*P*-histone) phosphatase activity in the 0–55% $(NH_4)_2SO_4$ fraction of canine heart extract can be resolved by DEAE-cellulose chromatography into four major fractions which were arbitrarily designated as phosphatases A, B, C and U. The enzymic activity of each fraction shows a different response to nucleoside triphosphates and metal ions. The activity of phosphatase B toward *P*-histone is markedly stimulated by millimolar concentrations of ATP whereas its catalytic subunit, phosphatase S, is inhibited by this nucleotide [8]. In the present communication, we present detailed studies of changes in catalytic properties associated with the conversion of phosphatase B to phosphatase S. Nucleoside triphosphates and other ionic compounds appear to stimulate phosphatase B activity by interacting with *P*-histone rather than with the enzyme *per se*, and the activity of the putative catalytic subunit, phosphatase S, is less sensitive to the modification of the phosphoprotein substrate than that of phosphatase B.

Abbreviations. *P*-histone, phosphohistone; *P*-casein, phosphocasein; cyclic AMP, adenosine 3′:5′-monophosphate; cyclic GMP, guanosine 3′:5′-monophosphate; AMP(CH_2)P, (α,β-methylene)-adenosine 5′-diphosphate; AMP(CH_2)PP, (α,β-methylene)adenosine 5′-triphosphate; AMP-P(CH_2)P, (β,γ-methylene)adenosine 5′-triphosphate.

Enzymes. Phosphoprotein phosphatase or phosphoprotein phosphohydrolase (EC 3.1.3.16); phosphorylase (EC 2.4.1.1); phosphorylase kinase or ATP : phosphorylase phosphotransferase (EC 2.7.1.38); protein kinase or ATP : protein phosphotransferase (EC 2.7.1.37).