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Purification and Properties of Phosphoprotein Phosphatases with Different Substrate and Divalent Cation Specificities from Canine Heart

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Three phosphoprotein phosphatases have been isolated from canine heart by a procedure involving treatment of the enzyme preparation with 80% ethanol at room temperature. They are a divalent-cation-independent, nonspecific enzyme ($M_r = 35000$) referred to as phosphatase S, an Mg^{2+}-activated enzyme ($M_r = 49300$) with activity predominately toward phosphocasein and an Mn^{2+}-activated enzyme ($M_r = 61000$) with activity predominately toward phosphohistone. Phosphatase S has been purified over 10000-fold and the two divalent-cation-dependent enzymes have been purified over 1000-fold from the crude extract. The purified preparation of phosphatase S showed a single broad protein stain band which coincided with the enzymic activities when examined with polyacrylamide gel electrophoresis. Sodium dodecyl sulfate gel electrophoresis of the enzyme preparation also showed a single protein band corresponding to a molecular weight of 35000. Phosphatase S exhibited a broad specificity and was about equally active toward phosphorylase *a*, phosphorylated histone, casein and cyclic-AMP-dependent protein kinase. Phosphatase S showed an optimum between pH 6.5–8 and was inhibited by ATP, other nucleoside phosphates, P_i, PP_i and NaF. The K_m values of phosphatase S for phosphorylase *a*, phosphorylated histone, and casein are 4, 27, and 16 μM, respectively. Evidence indicates that phosphatase S is a catalytic subunit of several nonspecific, divalent-cation-independent enzymes reported previously [*Biochim. Biophys. Acta*, *483*, 337–347 (1977)].

The present findings indicate that at least three basic catalytic entities of phosphoprotein phosphatase can be isolated from canine heart extract. These phosphatases which differ from one another in substrate specificity and divalent cation dependence may play different roles in the regulations of protein dephosphorylation in the cell.

The covalent modification of enzymes and regulatory proteins through a cycle of phosphorylation and dephosphorylation reactions is an important control mechanism involved in the regulation of many biological processes. Several laboratories have reported that phosphoprotein phosphatases, which catalyze the dephosphorylation reaction, exist in multiple forms [4–15] and exhibit a broad substrate specificity [6–20]. Several larger molecular forms of phosphoprotein phosphatases can be activated and dissociated into a catalytic subunit of $M_r = 31000–40000$ by treatment with either ethanol [4,8] or 2-mercaptoethanol [12,13]. Recently, phosphoprotein phosphatases of $M_r = 31000–35000$ have been purified from rabbit liver [1,2] and bovine heart [3]. These highly purified enzymes are nonspecific and have been suggested to be the catalytic entities of the larger molecular forms of phosphoprotein phosphatases [1,3].

The objectives of this study were to purify and identify the basic catalytic entities in canine cardiac muscle. To achieve these objectives, the ethanol treatment technique [1,4] was employed as a step in the purification scheme for dissociating the larger-molecular-weight phosphoprotein phosphatases. As a result, a divalent-cation-independent phosphoprotein

A preliminary report has been presented before the American Society of Biological Chemists [35].

Abbreviations. Cyclic AMP, adenosine 3′:5′-monophosphate; cyclic GMP, guanosine 3′:5′-monophosphate.

Enzymes. Phosphoprotein phosphatase or phosphoprotein phosphohydrolase (EC 3.1.3.16); phosphorylase (EC 2.4.1.1); phosphorylase kinase or ATP:phosphorylase-*b* phosphotransferase (EC 2.7.1.38); protein kinase or ATP:protein phosphotransferase (EC 2.7.1.37); glycogen synthetase or UDPG:glycogen α-4-glucosyltransferase (EC 2.4.1.11).