

Isolation and Properties of a Mn^{2+} -Activated Phosphohistone Phosphatase from Canine Heart

HENG-CHUN LI AND KWANG-JEN HSIAO

Department of Biochemistry, Mount Sinai School of Medicine of the City University of New York, New York, New York, 10029

Received March 24, 1976

A Mn^{2+} -activated phosphohistone phosphatase has been isolated from canine heart. The $s_{20, w}$ for the enzyme is 3.8. Using this value and the value for Stokes radius (39 Å), the molecular weight for the enzyme was calculated to be 61,000. The enzyme is inactive in the absence of divalent cations, among which Mn^{2+} is the most effective activator. Co^{2+} and Mg^{2+} are less effective than is Mn^{2+} . Zn^{2+} , Fe^{2+} , and Cu^{2+} are inhibitory. The enzyme has a pH optimum between 7 and 7.5 and has an apparent K_m for phosphohistone and Mn^{2+} of about 17 μM and 0.5 mM, respectively. The enzyme is inhibited by nucleoside triphosphate, ADP, AMP, phosphate, and pyrophosphate, but is not affected by cyclic AMP or cyclic GMP. The dephosphorylation of phosphohistone is stimulated by salts. Kinetic studies reveal that KCl and other salts greatly affect both the rate of hydrolysis and the K_m for either Mn^{2+} or phosphohistone by interacting with the substrate. The data suggest that modification at substrate level is an important regulatory mechanism for the enzyme. The enzyme preparation also dephosphorylates phosphorylase α and phosphocasein. Evidence suggests that one enzyme possesses both phosphohistone and phosphorylase phosphatase activities and that a different enzyme catalyzes the Mg^{2+} - and Mn^{2+} -activated dephosphorylation of phosphocasein.

The reversible phosphorylation and dephosphorylation of both enzymes and regulatory proteins is an important metabolic control mechanism. Several enzymes are known to be regulated by a phosphorylation reaction catalyzed by protein kinases, which are in turn under the control of hormonally directed formation of cyclic AMP¹ (1-5). In comparison to protein kinases, much less is known about phosphoprotein phosphatases, which catalyze the corresponding dephosphorylation reaction.

Phosphoprotein phosphatases of various tissues have been reported to exist in multiple forms (6-13) and exhibit a broad substrate specificity (8-10). The multiple forms of the enzyme can be dissociated by

treatment with either β -mercaptoethanol (9, 10) or ethanol (11) into a lower molecular weight species with a concomitant increase in the enzyme activity. Brandt *et al.* (11) have proposed that the multiple forms of phosphoprotein phosphatase exist in an inactive form consisting of an enzyme-inhibitor complex which contains a single, common catalytic subunit with a molecular weight of 32,000. Subsequently, isolation of a heat-stable inhibitory protein (14) and purification of a catalytic subunit of phosphorylase phosphatase to homogeneous state (15) have been reported. This homogeneous preparation of phosphorylase phosphatase is inhibited by Mn^{2+} , Mg^{2+} , and Ca^{2+} (15). On the other hand, phosphoprotein phosphatases of brain (7), skeletal muscle (8, 16, 20), adrenal cortex (17), liver (10), heart (13, 18), and sperm (19) have been shown to be stimulated by Mn^{2+} to various extents.

In a preliminary communication (13), we reported that phosphohistone phosphatase

¹ Abbreviations used: cyclic AMP, adenosine 3',5'-monophosphate; cyclic GMP, guanosine 3',5'-monophosphate; AMP(CH₂)PP, (α,β -methylene)-adenosine 5'-triphosphate; AMP-P(CH₂)P, (β,γ -methylene)-adenosine 5'-triphosphate; DEAE, diethylaminoethyl; TES, *N*-tris[hydroxymethyl]-methyl-2-aminoethanesulfonic acid.