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# Characterization of a Novel Alkaline Phosphatase Activity Which Co-purifies with a Phosphorylase (Phosphoprotein) Phosphatase of $M_r$ = 35,000 Cardiac Muscle*

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**In a previous communication (Li, H.-C., Hsiao, K.-J., and Chan, W. W. S. (1978) *Eur. J. Biochem.* 84, 215–225) we reported the purification of a divalent cation-independent, nonspecific phosphoprotein phosphatase (phosphatase S, $M_r$ = 35,000) from canine cardiac muscle to apparent homogeneity. It was found that the homogeneous enzyme preparation exhibited significant activity toward *p*-nitrophenyl phosphate. Since the enzymic activity had an optimum pH around 8.5, it was termed alkaline phosphatase S. Further studies demonstrated that the major alkaline phosphatase activity in the soluble fraction of the canine heart homogenate was co-purified with the phosphoprotein phosphatase S throughout the purification procedure. The alkaline and the phosphoprotein phosphatase activities were found to co-migrate on polyacrylamide gel electrophoresis, ion exchange, and gel filtration chromatographies and sucrose density gradient ultracentrifugation. These two activities, however, exhibited distinct thermostability, pH activity profile, and metal ion specificity, and could be partially separated by hydrophobic interaction chromatography. Discussions concerning whether these two activities reside in the same enzyme protein or in two different yet very similar polypeptide chains have been presented.**

**The properties of alkaline phosphatase S appear to be different from other known mammalian alkaline phosphatases, and it may represent a new isozyme species of the hydrolytic enzyme. In contrast to other well studied alkaline phosphatases of membrane origin purified from various tissues, the cardiac muscle alkaline phosphatase S was of cytosolic origin and required the simultaneous presence of $Mg^{2+}$ and a sulfhydryl compound for activity. The close association of alkaline phosphatase S activity with phosphoprotein phosphatase S suggests that it may play a role in the regulation of protein phosphorylation-dephosphorylation reaction.**

Several laboratories have reported that highly purified preparations of phosphoprotein phosphatase from various tissues do not show activity toward low molecular weight phosphoesters (1–5). These data suggest that phosphoprotein phosphatases are specific for phosphoproteins and are different from other known mammalian phosphatases which catalyze the hydrolysis of nonprotein phosphoesters.

In a previous communication, we reported the purification of a divalent cation-independent, nonspecific phosphoprotein phosphatase of $M_r$ = 35,000 (phosphatase S) from cardiac muscle (1) by a procedure involving ethanol treatment as described by Brandt *et al.* (6). The enzyme preparation appeared to be homogeneous as judged by polyacrylamide gel electrophoresis and represented the major phosphorylase phosphatase activity in cardiac muscle. In this communication, we report that, by the same purification scheme, a *p*-nitrophenyl phosphate phosphatase activity with an optimum pH of 8.5 (arbitrarily designated as alkaline phosphatase S) is co-purified with phosphoprotein phosphatase S to apparent homogeneity. Some of the catalytic and physical properties of alkaline phosphatase S have been examined. The results indicate that the catalytic properties of alkaline phosphatase S are distinctly different from those of phosphoprotein phosphatase S. These two activities, however, are closely associated physically.

## EXPERIMENTAL PROCEDURES

*Materials*—*p*-Nitrophenyl phosphate, *p*-nitrophenol, α- and β-naphthyl phosphate, phosphoserine, phosphothreonine, glucose 1-phosphate, glucose 6-phosphate, 2-mercaptoethanol, rabbit skeletal muscle phosphorylase *b* (twice crystallized), and phosphorylase kinase were from Sigma. Dithiothreitol, ATP, L-homoarginine, and L-phenylalanine were from Calbiochem. $MgCl_2$, $MnCl_2$, $CoCl_2$, $ZnCl_2$, and $CaCl_2$ were from Fisher Scientific Co. All chemicals used were of reagent grade.

Phosphorylase *b* was converted to [$^{32}$P]phosphorylase *a* by a modified method of Torres and Chelala (7). The [$^{32}$P]$P_i$ content of phosphorylase *a* was 8 to 10 nmol per mg of protein. The specific radioactivity of [$^{32}$P]phosphorylase *a* used in the assay of phosphatase activity varied from 25 to 400 cpm per pmol of phosphate.

*Enzyme Assay*—Alkaline phosphatase activity was routinely assayed by measuring the release of *p*-nitrophenol. The standard assay mixture contained 50 mM Tris·HCl, pH 8.6, 1 mM dithiothreitol, 20 mM $MgCl_2$, and 20 mM *p*-nitrophenyl phosphate in a total volume of 0.5 ml. The reaction was initiated by the addition of the enzyme. Incubation was at 30°C for 10 min; 0.5 ml of 1 M $Na_2CO_3$ was added, and the absorbance at 410 nm of the mixture was then measured spectrophotometrically by using a control lacking enzyme as a blank (the extinction coefficient for *p*-nitrophenolate anion: $1.75 \times 10^4$ $M^{-1}$ $cm^{-1}$). A unit of alkaline phosphatase was defined as the amount of enzyme catalyzing the release of 1 nmol of *p*-nitrophenol per min. Acid phosphatase activity was measured by the same method except that 50 mM sodium acetate, pH 5.0, was used instead of 50 mM Tris·HCl, pH 8.6, as the buffer in the assay mixture. In studies of divalent cation specificity, the assay mixture contained 50 mM Tris·HCl, pH 8.6, 0.1 mM dithiothreitol, 2.5 mM divalent cation, and 20 mM *p*-

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