Isolation of ^{32}P-labeled Phosphoserine from a Preparation of Intestinal Alkaline Phosphatase Incubated with Radioactive Inorganic Phosphate

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In a previous paper it was demonstrated that ^{32}P-labeled phosphoserine could be isolated from a yeast hexokinase preparation incubated with radioactive ATP or glucose 6-phosphate [1]. This was taken as evidence of the formation of an intermediate enzymephosphate in the hexokinase reaction. In the reaction a P—O linkage is opened [2] which means that according to our result a phosphoryl group is transferred to the enzyme. The actual site of the phosphoryl group on the enzyme has so far not been investigated. Stein and Koshland [3] have shown that a P—O linkage also is opened when alkaline phosphatase acts on glycerol phosphate, phenyl phosphate and 3′-adenosine monophosphate. The formation of an intermediate enzymephosphate in the reaction of this enzyme would be of considerable interest.

In our experiments we have used a commercial preparation *. Since the enzyme reaction is reversible it should be possible to use radioactive inorganic phosphate as a substrate. 500 mg of enzyme dissolved in 25 ml of water was incubated with 2 mC ^{32}P. After half a minute the protein was precipitated with trichloroacetic acid. The precipitate was hydrolyzed and radioactive phosphoserine could be isolated as previously described [1]. Alkaline phosphatase activity is completely inhibited by a 0.05 M solution of versene [5]. During these conditions no radioactive phosphoserine could be isolated.

The enzyme was purified by ethanol fractionation at −5°C. In the presence of 0.05 M magnesium acetate 75 % of the phosphatase activity was recovered between 37 and 50 % ethanol. The incorporation of activity in the phosphoserine phosphorus ran parallel with the increase of enzyme activity. To exclude an unspecific reaction between inorganic phosphate and protein-bound serine a number of proteins were incubated with a 2-fold increase of ^{32}P-labeled phosphate but otherwise during the same conditions as in the enzyme experiments. Crystalline serum albumin, egg albumin, pepsin, chymotrypsin, insulin and a γ-globulin preparation were used. No trace of radioactive phosphoserine could be revocered from the hydrolyzed proteins.

In a series of experiments 500 mg samples of enzyme and 2 mC ^{32}P were incubated at different pH for 5 min at room temperature. The total radioactivity of the isolated phosphoserine fractions measured in a L.K.B. Robot Scaler was the following:

pH 5	24 600 cpm (counts per min)	pH 8	13 200 cpm
pH 6	45 000 cpm	pH 9	7 900 cpm
pH 7	34 400 cpm	pH 10	2 200 cpm

At pH 5 the preparation could not be completely dissolved. If the reaction between enzyme and substrate is formulated as follows:

$$\text{Enzyme} + [H_2PO_4^-] \rightleftharpoons \text{Enzymephosphate} + [OH^-]$$

a decrease of hydroxyl ions should drive the reaction to the right. This should be accompanied by an increase of the activity of the phosphoserine isolated from the enzyme-phosphate which was actually found.

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1. Ågren, G. and Engström, L. *Acta Chem. Scand.* **10** (1956) 489.
2. Cohn, M. *Biochim. et Biophys. Acta* **20** (1956) 92.
3. Stein, S. S. and Koshland, D. E. *Arch. Biochem. and Biophys.* **39** (1952) 229.
4. Hofstee, B. H. J. *Arch. Biochem. and Biophys.* **51** (1954) 139.
5. Hofstee, B. H. J. *Arch. Biochem. and Biophys.* **59** (1955) 352.

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* Worthington intestinal alkaline phosphatase with an activity of 400—500 units [4] per mg of enzyme.