

**Isolation of  $^{32}\text{P}$ -labeled  
Phosphorylserine from a Purified  
Preparation of Alkaline Bone  
Phosphatase Incubated with Radio-  
active Inorganic Phosphate**

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In a previous paper from this laboratory it was reported that  $^{32}\text{P}$ -labeled phosphorylserine could be isolated from an acid hydrolysate of a commercial preparation of intestinal alkaline phosphatase<sup>1</sup>. This seemed to indicate that an intermediate enzyme-phosphate was formed in the enzyme reaction, as we had earlier suggested for the hexokinase reaction<sup>2</sup>. The results with intestinal alkaline phosphatase have recently been confirmed with a highly purified preparation of the enzyme<sup>3</sup>. Meanwhile similar results have also been obtained with preparations of alkaline bone phosphatase. Bone tissue is a poor source of phosphatase when compared with intestinal tissue<sup>4</sup>.

The enzyme was first prepared from calf foreleg bones as described by Volkin<sup>5</sup> and for subsequent purification the method of Portmann<sup>6</sup> was followed. The fraction corresponding to step 14 was further purified by chromatography on a diethylaminoethyl cellulose column<sup>3</sup>. The buffer used was Tris-hydrochloric acid buffer pH 7.8. The column was loaded with 1 mg of protein per ml of column volume. The elution was performed with a stepwise increase of magnesium acetate concentration. The enzyme appeared in a protein peak at a magnesium acetate concentration of 0.04 M as determined by its ultraviolet absorbancy at 2800 Å. The specific activity of the most active enzyme preparation, calculated according to Portmann, was 70 000 PU (Portmann units). In this way the enzyme was purified about 300 times as compared with the five-fold increase in purity obtained by Martland and Robison<sup>7</sup>.

6 300 PU with the specific activity of 70 000 PU/mg N in Tris-buffer pH 7.0 was incubated with 0.75 mC of  $^{32}\text{P}$  in the presence of 0.03 M magnesium acetate. After

5 min the enzyme was precipitated by adding 50 % trichloroacetic acid to a final concentration of 10 % and acetone was added to complete precipitation. The enzyme was hydrolyzed with 2 N HCl and radioactive phosphorylserine isolated as previously described<sup>3</sup> and identified by scanning of paper strips of electropherograms.

In a series of experiments 600 mg samples of enzyme with a specific activity of 420 PU/mg N and 5 mC  $^{32}\text{P}$  were incubated at different pH for 5 min at room temperature. The total radioactivity of the isolated phosphorylserine fractions measured in L.K.B. Robot Scaler was the following:

pH 5 839 000 cpm	pH 8 629 000 cpm
(counts per min)	
pH 6 885 000 cpm	pH 9 126 000 cpm

In a second series of experiments similar samples of enzyme were incubated for 3, 6, 12, 24, and 48 sec. with 5 mC  $^{32}\text{P}$ . The total activities of the isolated phosphorylserine fractions were the same in all cases. The results indicate that inorganic phosphate is rapidly incorporated into the bone phosphatase molecule by covalent linkage. Calculations showed that the affinity of radioactive inorganic phosphate to bone alkaline phosphatase was of the same size as that of intestinal alkaline phosphatase. The incorporation of phosphate into the bone enzyme also increased within the same pH range as previously found with the intestinal enzyme<sup>1</sup>. The rapid incorporation and the high affinity of phosphate to the bone enzyme may be of interest with regard to its possible rôle as a transphosphorylating agent in bone tissue.

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