The Phosphotransferase Activity of Alkaline Phosphatases. Studies with Dephosphorylated Phosphopeptone as Phosphate Acceptor

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In a recent paper it was reported that alkaline bone phosphatase catalysed the synthesis of phosphorylated amino acids and amines when labeled glycerophosphate was used as phosphate donor [1]. The present report describes similar experiments with dephosphorylated phosphopeptone as phosphate acceptor.

Radioactive glycerophosphate was obtained by use of AT^{32}P and glycerokinase prepared according to Bublitz and Kennedy [2]. Dephosphorylated phosphopeptone was obtained from a caseinphosphopeptone preparation made according to Mellander [3] and further separated in a four cell electrodialysing apparatus [4]. Material from cell 2 with a phosphorus content of 5.5 % was dephosphorylated with phosphoprotein-phosphatase purified in this institute [5] and at pH 5.8 in the presence of thioglycolic acid [6]. Dephosphorylated phosphopeptone was separated from phosphopeptone on a Dowex 1 formate column. Dephosphorylated phosphopeptone eluted from the column with water appeared in two fractions, one containing considerable amounts of sodium phosphate and a second rather saltfree fraction. This second fraction, II, was used in the following. It was not homogeneous. Electropherograms carried out at pH 3 showed the presence of at least 6 fractions.

A comparison was made between the transphosphorylating activity of alkaline phosphatases from milk [7] and bone [8]. 16 mg of milk phosphatase with a total activity of 1 120 Portmann units (PU)[9], and 23 mg of bone phosphatase with a total activity of 1 960 PU were each incubated with 25 mg of dephosphorylated phosphopeptone in bicarbonate buffer of pH 9.5 for 10 min at room temperature and in a total volume of 2 ml. To each solution 25 mg of glycerophosphate labeled with 1 mC of radioactivity had also been added. The heat-inactivated solutions were separated by gradient elution with 0 → 1 M formic acid on small Dowex 1 formate columns. Only in the experiment with milk phosphatase was it possible to isolate phosphopeptides where radioactivity moved parallel with ninhydrin colour on electropherograms. One of the 4 fractions gave sufficient amount of material for amino acid analysis and the one-dimensional chromatogram gave spots corresponding to aspartic acid, serine, glycine, glutamic acid, alanine and valine.

While the detailed sequence has not been established, it is to be noted that the composition of the peptide is in agreement with the composition of phosphopeptides obtained from the phosphate-catching "active sites" in chymotrypsin and phosphoglucomutase, respectively [10]. In a further attempt to elucidate Koshland's hypothesis [10] 90 mg of dephosphopeptone II were incubated in the presence of 0.01 M magnesium acetate with 5 mC of ^{32}P at pH 6 for 10 min [8]. The hydrolysed portion was separated on a Dowex 50 column. After inorganic phosphate three small radioactive peaks were obtained. The last one ran parallel with unlabeled phosphorylserine on electropherograms. The first fraction after a second hydrolysis with 2 N HCl for 20 h at 120°C showed an identical pattern of amino acids when run parallel on the chromatogram with the hydrolysate of the phosphopeptide isolated from the enzymatic transphosphorylation. The result supports Koshland's hypothesis [10] of a more common sequence of amino acids around serine making the otherwise inert CH_3OH side chain reactive to the phosphoryl group and a second amino acid sequence responsible for the enzyme specificity.

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