On the Phosphorus Linkage in a Muscle Phosphorylase Preparation

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Recently we reported the isolation of ^{32}P-labeled phosphoserine from a yeast hexokinase preparation [1] incubated with radioactive adenosinetriphosphate or glucose-6-phosphate. It was concluded that hexokinase was a phosphoprotein functioning as a phosphotransferring enzyme with an incorporation of phosphate from the substrate to the enzyme molecule or an exchange between substrate and enzyme phosphate.

This paper deals with the isolation of ^{32}P-labeled phosphoserine from a muscle phosphorylase preparation incubated with glycogen and radioactive ^{32}P. The following different types of experiments were carried out with the same amount of enzyme in each experiment.

100 mg of a commercial muscle phosphorylase preparation (Delta Chemical Works, Inc.) and 150 mg of glycogen (Merck) were dissolved in 5 ml of a freshly prepared cysteine-glycerophosphate buffer [2]. 5 mC carrierfree ^{32}P was added. The solution was kept at room temperature. After 30 minutes the enzyme was precipitated with trichloroacetic acid to a final concentration of 10 %. The protein was hydrolyzed according to Lipmann [3]. ^{32}P-labeled phosphoserine was identified as previously described [1]. The specific activity calculated as cpm per μg P was 72 186. This is the highest value so far recorded by us for any phosphorylated amino acid isolated from animal or plant tissue proteins. When glycogen was present the addition of 5 mg of adenosinemonophosphate (AMP) did not seem to increase the incorporation of radioactive phosphate into the enzyme molecule. The specific activity of the isolated phosphoserine was 32 977.

Next the enzyme was incubated with ^{32}P without the addition of glycogen. The phosphoserine was isolated in the usual way. It was only slightly labeled. The specific activity was 182. Finally when the enzyme was incubated with ^{32}P and AMP the specific activity of the isolated phosphoserine was 2 626 cpm per μg P.

The results seem to indicate that muscle phosphorylase is a phosphoprotein according to our definition, since we have been able to isolate phosphoserine from the partially hydrolysed enzyme. In the enzyme reaction there is probably an exchange between enzyme and substrate phosphate. Glycogen appears to play an essential role in this mechanism. The small exchange which takes place when no polysaccharide is added to the system may depend on traces of glycogen present in the enzyme preparation.

The bearing of our results to those obtained by Sutherland and coworkers [4] is difficult to discuss at present time. The possibility of an exchange with glucose-1-phosphate without addition of glycogen will be examined later. When possible, the results will also be controlled by repeated experiments with a more purified enzyme preparation.

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On the Presence of Phosphoproteins in Baker's Yeast

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In previous reports from this laboratory it has been demonstrated that phosphoproteins are present in several microorganisms [1,2]. It has now been possible to demonstrate the presence of phosphoproteins in yeast.

The cells were washed twice in distilled water and cultured in the medium of Juni *et al.*[3] for different times. Non-labeled inorganic phosphate was excluded from the