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Isolation of ^{32}P-Labeled Phosphoserine from a Yeast Hexokinase Preparation, Incubated with Labeled ATP or Glucose-6-Phosphate

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In earlier communications from this laboratory we reported the incorporation of radioactive inorganic phosphate into phosphoserine isolated from the Schneider protein residues of several rat organs [1]. The maximum incorporation for all organs investigated occurred at approximately three hours after injection of radioactive phosphate [2]. This seemed to infer that some intracellular phosphoproteins may function as transphosphorylating enzymes.

The incorporation of radioactive inorganic phosphate into a new phosphorylated amino acid [3], prepared from the protein of *Lactobacillus casei*, was also compared with the phosphorus uptake of nucleotide and nucleic acid fractions in an attempt to trace the source and the destination of the amino acid phosphorus [4]. However, all these measurements can only give an average value for presumably different kinds of phosphoproteins of different loci within the cell.

As an alternative it seemed of interest to study pure phosphoproteins, as hexokinase or phosphoglucomutase, which are known to be involved in the transfer of phosphorus. The possibility that this transfer might include the formation of an enzyme-phosphate intermediate has been suggested by many authors [5]. Recently this hypothesis received support from the demonstration by Najjar and Pullman [6], that phosphoglucomutase exists in two states, a phosphoenzyme and a dephosphoenzyme. The former transfers phosphate to glucose-1-phosphate or glucose-6-phosphate. Glucose-1,6-diphosphate is formed in the process.

In the present study we have shown the formation of an enzyme -phosphate intermediate in the hexokinase reaction by isolating phosphoserine from the acid hydrolysate of the enzyme, incubated with ^{32}P-labeled ATP [7] in the presence of glucose. In another experiment we also obtained phosphoserine from the enzyme incubated with glucose-6-phosphate isolated from the reaction mixture of the enzyme and AT^{32}P.

The enzyme was purified according to the first six steps of the methods of Berger *et al.*[8] and contained one per cent of glucose *. About 20 mg of this protein was incubated for a few seconds with 1—2 mg ATP, containing about 1—2 mC of phosphorus activity and in the presence of 0.01 M $MgCl_2$. The enzyme was precipitated with 10 % trichloroacetic acid. The protein was hydrolysed and fractionated on a Dowex 50-column according to Ågren *et al.*[1]. The peak eluted after the inorganic phosphate in the expected position of phosphoserine weighed less than 0.1 mg. A part of this material was run through a Dowex 1 formate column according to Busch *et al.*[9] with the addition of non-labeled phosphoserine. The ninhydrin colour from the non-labeled phosphoserine and the activity from the isolated fraction were eluted in the same column volume. Other parts of the activity together with non-labeled phosphoserine were analyzed with one-dimensional paper chromatography using *iso*butyric acid-ammonia and benzene-propionic acid systems. After development of the ninhydrin colour, the paper strips were scanned in the apparatus previously described [2]. The ninhydrin colour from the non-labeled phosphoserine and the activity from the isolated fraction completely agreed.

The trichloroacetic acid centrifugate from the reaction mixture of enzyme and AT^{32}P was run through a Dowex 1 formate column according to Siekevitz and Potter[10], and the column volume containing the radioactive glucose-6-phosphate was incubated with more enzyme solution, con-

* The enzyme was kindly furnished by professor O. Lindberg, Wenner-Gren Institute, Stockholm.

taining glucose. $MgCl_2$ was added as before. From the hydrolysed enzyme phosphoserine was identified in the same manner as previously described.

Our present method to show that the phosphorus atom is linked to the protein molecule consists in the isolation of a phosphorylated amino acid as phosphoserine from the hydrolysed protein. It therefore seems that the transfer of phosphorus in the hexokinase system is at least a two-step reaction. The phosphorus atom is first transferred from the donator molecule to the enzyme and secondly donated from the enzyme to the acceptor. We therefore consider that our results suggest that an enzyme-phosphate is an intermediate in the hexokinase reaction. Hexokinase and phosphoglucomutase are examples of phosphoproteins functioning as transphosphorylases. It is highly possible that other phosphoprotein-enzymes may be engaged in the transfer of phosphorus in a similar way. This assumption is in line with the previously published high values for the incorporation of radioactive phosphate in the proteins from different cells [3].

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An Absolute Determination of the Rate of the Exchange Reaction between Cadmium Amalgam and Cadmium Cyanide Solutions

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The kinetics and mechanism of the heterogeneous exchange reaction at equilibrium electrode potential between cadmium amalgams and cadmium cyanide solutions containing sodium cyanide in excess was investigated by a radioactive tracer method in a previous work [1]. Preliminary measurements showed that the rate of exchange in this system was not controlled by the diffusion of cadmium in the solution phase, as was the case in perchlorate solutions [2] or in solutions containing cadmium complexes of moderate strength [3].

In order to attain sufficiently high accuracy in the isotopic exchange determinations, the main measurements were performed according to a procedure where the amalgam and the solution were shaken under reproducible conditions. For an activation-controlled exchange process a relationship of the following form should be obtained

$$r = k\, c^{1-\alpha}\, q^{\alpha}\, [CN^-]^{-j(1-\alpha)} \qquad (1)$$

where r denotes the rate of exchange, c the total concentration of cadmium in the solution, q the total concentration in the amalgam and k a constant. The exponent α is also a constant, fulfilling the condition $0<\alpha<1$, and j is the number of cyanide ligands in the complex that predominates in the exchange process. Actually an expression of the form (1) for r was obtained in the investigation with the values $\alpha = 0.34 \pm 0.02$ and $j = 2.2 \pm 0.1$. Thus the measurements indicated that under the existing concentration conditions the exchange reaction is activation-controlled and that the predominating reversible transfer reaction is the following one:

$$Cd(CN)_2 + 2e^-(\text{electrode}) \rightleftharpoons Cd(\text{amalgam}) + 2CN^- \qquad (2)$$