On the Migration of the Phosphoryl Group in Phosphoproteins

Gunnar Ågren and
Carl-Henric de Verdier

Institute of Medical Chemistry, University of Uppsala, Sweden

In a previous paper [1] on the phosphorus-containing proteins of cells we discussed the possible migration of phosphorus from a phospho-amide linkage in serine to the hydroxyl groups. Plapinger and Wagner-Jauregg [2] have shown this to occur, when N-di*iso*propylphosphorylserine is hydrolysed with aqueous hydrochloric acid. Elliott [3] also found a transfer of approximately 60 % of the peptide chains linked to the nitrogen atoms of serine residues to the hydroxyl groups of these residues. Possible migration within the serine molecule when the corresponding phenylthiohydantoin is formed has been discussed by Edman and Lauber [4].

Müller *et al.*[5] recently published an interesting report where they demonstrated that aminophosphate (AP) at 25° and pH 7.2 phosphorylated the imidazole group of histidine. They also isolated a phosphorylated insulin containing 1 % of phosphorus when the protein was incubated with AP. They suggested that the phosphoryl group was linked to histidine in the protein molecule. This compound offered an unique opportunity to investigate a possible phosphorus migration within a phosphoprotein with known amino acid sequences, probably only containing nitrogen-bound phosphorus.

AP was synthesized according to Stokes [6] as modified by Müller *et al.*[5] 7 g of bovine crystalline insulin * was incubated at pH 8.0 and 25° with 4.37 g AP, divided in five equal parts and added with intervals of 2 h. Samples of the incubation mixture containing 120 mg of insulin were purified from AP by electrodialysis for 1 h at pH 8. pH was kept constant by adding ammonia. The yield was 6 g of lyophilised material containing 0.48 % P. After partial acid hydrolysis and repeated purification on Dowex-50 colums it was possible to isolate a ninhydrin-positive substance which on paper chromatograms with different solvents and on paper-electrophoresis strips at different pH moved as O-phosphoserine. It contained about 0.4 % of the phosphorus present in phosphoinsulin. The partial hydrolysis used always gives a mixture of phosphopeptides [1,7]. In a similar manner the partial hydrolysis of phosphoinsulin resulted in several phosphopeptides containing serine and threonine.

Model experiments with several basic and neutral amino acids including serine, threonine and cysteine are in agreement with the hypothesis that the phosphoryl group is primarily attached to the imidazole- and ε-amino groups of histidine and lysine, respectively. The isolation of O-phosphoserine seems to demonstrate a nitrogen to oxygen migration of the phosphoryl-group from one amino acid to another within the molecule. Perlmann [8] recently showed that the phosphate in some phosphoproteins can be N-, O-, and pyrophosphate bound. The amide-bound phosphorus, as representing high-energy linked phosphorus, is of special interest in metabolic reactions. Recent results from this laboratory [1,9-11] seem to demonstrate that several of the intracellular phosphoproteins must function as transphosphorylating enzymes. It is likely that many of these phosphoproteins contain the nitrogen-linked type of phosphate. The O-phosphoserine and O-phosphothreonine isolated from such proteins by our standard procedure originally may have been nitrogen-linked as in phosphoinsulin and then by the partial acid hydrolys migrated to the O-position in serine and threonine.

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