## Special cases of non-enzymic transphosphorylation

The results reported here are obtained from experiments at 25° C and pH 7.20. Under these conditions, aminophosphate (AP), the monoamide of phosphoric acid, is hydrolyzed in aqueous solution according to a first order reaction with a half-life time of about 13 hours.

The hydrolysis is strongly catalyzed by pyridine and its derivatives. With 0.1 *M* nicotinic acid, *e.g.*, the half-life time of AP is about 1 hour, the reaction still being of the first order.

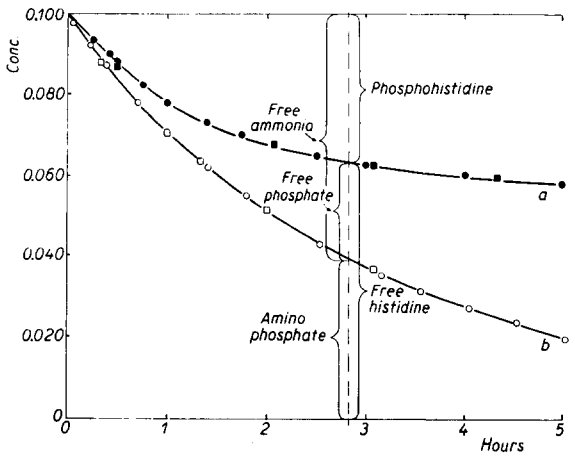


Fig. 1. Time course of the reaction between aminophosphate and histidine in aqueous solution at 25° C and pH 7.20. Circles: in the presence of KCl; squares: in the presence of $NH_4Cl$. Curve a: histidine concentrations; curve b: aminophosphate concentrations.

With derivatives of imidazole, the splitting of AP was found to be a complex process, including phosphorylation of a nitrogen in the ring and catalysis by the phosphorylated product both of the hydrolysis of AP and of its own hydrolysis. These reactions were studied in some detail with L-histidine. Aliquots were taken at different intervals from solutions containing phosphate buffer, potassium chloride, histidine, and potassium aminophosphate, maintained at a constant pH during the reaction. Hydrolysis and transphosphorylation were followed by determination of free ammonia and free histidine. Ammonia was distilled from an alkaline solution, in which AP is stable, and titrated as usual. Histidine was determined spectrophotometrically by a modification of the procedure described by MACPHERSON[1], where the phosphohistidine yields no color.

As an example, Fig. 1 shows the results of experiments in which the original concentrations of AP and of histidine are both 0.1 *M*. At the beginning of the experiment the transphosphorylation far exceeds the hydrolysis of AP, the splitting of AP by histidine being about 6 times faster than by water. Since however the first reaction produces a catalyst for the second, the rate of hydrolysis increases at the expense of transphosphorylation. At the end of the experiment, about 40% of the histidine is phosphorylated. Substitution of potassium chloride by ammonium chloride has no influence, indicating that the back reaction (phosphorylation of ammonia by phosphohistidine) does not take place to a measurable extent under these conditions.

The transphosphorylation also seems to occur, although with a rather low yield, when histidine is bound as a component amino acid of a protein. In experiments with insulin, the protein was phosphorylated to contain about 1% bound phosphorus by repeated treatment with AP.

The phosphohistidine formed by phosphorylation with AP was isolated as a calcium salt following the procedure of ZEILE AND FAWAZ[2] for the isolation of phosphocreatine, as indicated by GUSTAFSON AND WAGNER-JAUREGG[3], who prepared phosphohistidine from histidine and phosphorus oxychloride. The isolated salt contains histidine and phosphate in a molar ratio of 1:1, and usually is contaminated by small amounts of free histidine and inorganic phosphate and by calcium carbonate. In paper-chromatographic studies (Whatman No. 1, propanol-ammonia-water solvent) the phosphohistidine migrates without being split as is evident from the coincidence of the spots for phosphate and for histidine.

With the isolated product, the catalytic effect of phosphohistidine on the hydrolysis of AP was confirmed and the value of the catalytic constant determined. The autocatalytic hydrolysis of phosphohistidine is indicated by the fact that the reciprocal concentration of phosphohistidine in aqueous solution varies linearly with time.

For the simultaneous splitting of AP by histidine and water, the following expression fits well with all experimental results:

$$-\frac{dap}{dt} = k_1 \cdot h \cdot ap + k_2' \cdot ap + k_2'' \cdot hp \cdot ap + k_2''' \cdot h \cdot ap$$

Here

| | | |
|---|---|---|
| $k_1$ | (constant for transphosphorylation) | = 2.94 |
| $k_2'$ | (constant for spontaneous AP-hydrolysis) | = 0.049 |
| $k_2''$ | (constant for AP-hydrolysis catalyzed by phosphohistidine) | = 1.67 |
| $k_2'''$ | (constant for AP-hydrolysis catalyzed by histidine) | = 0.14 |

In the above equation, $t$, $ap$, $hp$, and $h$ represent the time in hours, and the molar concentrations of AP, phosphohistidine and histidine resp.

A detailed report of these studies will be published elsewhere[4]. We are indebted to Professor Linderstrøm-Lang, Carlsberg Laboratory, for stimulating discussions.

TH. MÜLLER
*Biochemistry Department, Nordisk Insulinlaboratorium, Gentofte (Denmark)* T. RATHLEV
TH. ROSENBERG

[1] H. T. MACPHERSON, *Biochem. J.*, 36 (1942) 59.
[2] K. ZEILE AND G. FAWAZ, *Z. physiol. Chem.*, 256 (1938) 193.
[3] C. GUSTAFSON AND TH. WAGNER-JAUREGG, *Federation Proc.*, 13, No. 738 (1954).
[4] Reports of the Steno Mem. Hospital and the Nordisk Insulinlaboratorium.

Received December 14th, 1955

## The production of β-linked glucose saccharides from cellobiose by *Chaetomium globosum*

BUSTON AND JABBAR[1] have recorded an enzymic synthesis of cellotriose by the action of enzyme preparations from *Chaetomium globosum;* they noted also that under certain circumstances a mixture of other saccharides was produced. We now report that if the organism from which the enzyme preparation was made had been grown on a medium containing cellobiose only as the source of carbon, the production of a variety of saccharides was rapid and extensive when the enzyme acted upon cellobiose.

The digest of the enzyme preparation with 5 % aqueous cellobiose was held at 30° for 9 days, and samples were removed for chromatographic examination at suitable intervals. After 2 hours, cellotriose and a second trisaccharide could be detected and the amount of these steadily increased up to 24 hours; by this time a third trisaccharide had also appeared. The first disaccharide detected was laminaribiose which was very faintly visible on the chromatogram at 4 hours; this was followed by gentiobiose at 12 hours and sophorose at 22 hours. During the next 48 hours the quantities of trisaccharides remained constant, and those of the newly-appearing disaccharides increased slightly at first and then remained constant; cellobiose decreased steadily and glucose correspondingly increased. In the later stages (after 96 hours) the trisaccharides disappeared while the disaccharides were still present, gentiobiose being predominant among these and being the only disaccharide detectable at the end of 9 days. Cellotriose in particular was more rapidly removed than any other trisaccharide, none being detectable after 96 hours.

The process thus evidently resembles that reported by BARKER, BOURNE AND STACEY[2] working with *Aspergillus niger*. These authors suggested that the three new disaccharides were formed by the coupling of glycosyl residues liberated from cellobiose with glucose molecules already present, and while this process may undoubtedly take place we are of the opinion that the disaccharides may also have been produced through the formation in the first place of trisaccharides (*e.g.*, Glu-1:6-Glu-1:4-Glu) which cleaved subsequently at the 1:4 linkage. This view is supported by the observation that at least two trisaccharides were detectable in the earliest stages of the digestion before the appearance of any disaccharide. This would imply also that addition of a glycosyl unit to cellobiose and the subsequent release of another glycosyl unit from the trisaccharide would take place at "opposite sides" of the cellobiose molecule, thus:

Glu-1:4-Glu + Glu-1:4-Glu →
Glu-1:6-Glu-1:4-Glu + Glu → Glu-1:6-Glu + Glu
or Glu-1:4-Glu-1:6-Glu + Glu → Glu + Glu-1:6-Glu

The more rapid disappearance of cellotriose might be due to the presence in the molecule of two 1:4 linkages which were apparently more readily attacked by the enzymes present than any of the other arrangements.

It may be noted that the aniline-diphenylamine-phosphate reagent of BUCHAN AND SAVAGE[3] gives very distinctive colours with the isomeric β-disaccharides of glucose. GIRI AND NIGAM[4] reported that cellobiose gave a blue colour and gentiobiose a brown; laminaribiose gives a light olive-brown and sophorose a very distinct pinkish yellow. One of the trisaccharides found here gave a distinctly pinkish yellow tone, suggesting a structural relation to sophorose.

*Biochemical Laboratories, Imperial College of Science and Technology, London (England)* H. W. BUSTON
A. H. KHAN