Cellobiose Phosphorylase in *Fomes annosus*

THE basidiomycete *Fomes annosus* (Fr.) Cooke causes root and stem rot in various conifers. It is economically one of the most damaging fungi in the forestry of the temperate zones of the northern hemisphere. It is not able to compete in soils with other microorganisms but lives as a facultative biotrophic parasite in wood¹. Adaptations for a saprophytic and pathogenic life are to be expected. One possible adaptation is the presence in *Fomes* of cellobiose phosphorylase (EC 2.4.1.20). This enzyme has not yet been described for any other fungus.

We found that *F. annosus* grows three times better on cellobiose as the sole carbon source than on glucose in a salts-glucose medium² supplemented with vitamin B₁ and aspartic acid³. Such growth stimulation on supplying di- or oligosaccharides instead of glucose is typical of bacteria which have enzymes that cleave the glycosidic bonds phosphorolytically rather than hydrolytically. The energy of the bond is conserved in glucose-1-phosphate (Glc-1-P) and this gives the system a much higher energy yield.

The presence of phosphorolytic activity in extracts of *F. annosus* was demonstrated enzymatically. Extracts obtained by ultrasonication of the mycelia grown under submerged conditions in a cellobiose-containing medium were incubated with cellobiose in 0.1 M phosphate buffer, pH 7.0. The reaction was stopped by heating at 100° C for 2 min and any glucose-1-phosphate (Glc-1-P) produced was determined by conversion to Glc-6-P with phosphoglucomutase and subsequently to 6-phosphogluconate with glucose-6-phosphate dehydrogenase. The increase in A_{340} in the latter reaction was taken as a measure of the enzymatic activity, compared with a control lacking substrate.

Glc-1-P was further identified as the reaction product by TLC using ³²Pi (New England Nuclear GmbH, Dreieichenhain)

in the incubation mixture and Sugino's method⁴ for the removal of the non-reacted inorganic phosphate. Subsequent chromatography of the reaction products revealed that most of the phosphate was incorporated into a compound which in two separation systems had R_F values identical to Glc-1-P (Fig. 1). The formation of this compound was also detected in the chromatograms by spraying with 1,3-dihydroxy-naphthalene phosphoric acid. In some experiments, labelled Glc-6-P as well as Glc-1-P was found in the chromatograms, which indicates the presence of phosphoglucomutase in the extracts from *F. annosus*.

This detection of Glc-1-P and its reaction product Glc-6-P represents the first evidence for the presence of cellobiose phosphorylase in a fungus.

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A. HÜTTERMANN

Forstbotanisches Institut,
der Universität Göttingen

C. VOLGER

Institut für Waldbau,
der Universität Göttingen,
34 Göttingen

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⁵ Randerath, K., *Dünnschichtchromatographie* (Verlag Chemie GmbH, Weinheim, 1965).

Reference Abbreviations

ALL abbreviations of references in *Nature* should now conform to the style of the *World List of Scientific Periodicals*, fourth ed. (Butterworth, 1963-65). Authors submitting manuscripts are asked to ensure that the references are written appropriately.

Fig. 1 Autoradiogram of a chromatogram of the reaction product of cellobiose phosphorylase of *Fomes annosus*. Two separation systems were used. In the first the mobile phase comprised *n*-propanol : ethyl acetate : water in the proportions 7 : 1 : 4, and the layer phase MN cellulose 300 activated for 10 min at 100° C. In the second the mobile phase comprised methyl-ethylketone : acetic acid : water (3 : 1 : 1), and the layer phase silica gel G (Merck) in 0.1 N boric acid⁵. I, U-¹⁴C-Glc-1-P and U-¹⁴C-glucose; II, ³²Pi; III, reaction product of the cellobiose phosphorylase assay with *Fomes annosus* extract after removal of the non-reacted inorganic phosphate. I and II acted as controls.

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