Transglycosylation reactions catalysed by two β -mannanases

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By using [^3H]mannobiose as a labelled acceptor, it was possible to demonstrate transfer reactions catalysed by two β -mannanases, with mannotetraose and mannopentaose as substrates. The enzyme from *Streptomyces* transfers one mannose unit from the oligosaccharides, whereas the enzyme from fenugreek (*Trigonella foenum-graecum*) seeds is able to transfer oligomannose residues.

Endo-1,4- β -D-mannanases (EC 3.2.1.78) are known to catalyse random hydrolysis of 1,4- β -D-mannosidic linkages in mannans, galactomannans and glucomannans.

Previous studies on the mode of action of two β -mannanases, one from fenugreek (*Trigonella foenum-graecum* L.) seedlings (Clermont-Beaugiraud & Percheron, 1968), and one from a soil *Streptomyces* strain (Coulombel *et al.*, 1976), led us to report the unusual hydrolysis of some oligomannosides. These enzymes hydrolyse randomly the 1,4- β -mannan from ivory nut (*Phytalephas macrocarpa*) as well as oligomannosides with a degree of polymerization higher than 5, according to the classical mechanisms for endopolysaccharidases, but the hydrolysis of tetramer and pentamer substrates is not of this type. Neither enzyme hydrolyses mannotriose or mannobiose. The rate of hydrolysis of higher substrates increases with the chain length of these substrates.

The β -mannanase from *Streptomyces* degrades mannotetraose to mannobiose and mannotriose, without any liberation of mannose; the enzyme of fenugreek seeds also produces mannobiose and mannotriose with only traces of mannose. These patterns could be explained by the intervention of transfer reactions associated with the hydrolysis.

Transglycosylation activities have been demonstrated for two cellulases from *Trichoderma viride* (Okada & Nisizawa, 1975), and postulated for a xylanase from *Ceratocystis paradoxa* (Dekker & Richards, 1975).

No studies have been made of transglycosylation by β -mannanases; although some results suggest such a mechanism (Villarroya & Petek, 1976), no demonstration was afforded.

We previously proposed two hypothesis for the action pattern of the two above-cited mannanases.

The utilization of a radioactively labelled acceptor, namely [^3H]mannobiose, allows us to demonstrate transglycosylation reactions by these enzymes.

Experimental

The enzymes were prepared as previously described for *Streptomyces* (Coulombel *et al.*, 1976), and fenugreek seeds (Courtois & Percheron, 1961; Clermont-Beaugiraud & Percheron, 1968). The *Streptomyces* mannanase was the more purified enzyme, but we have verified that the two preparations were devoid of mannobiase and β -mannosidase activities.

The oligomannosides used as substrates or as reference products were isolated from a partial hydrolysate (0.5 M-oxalic acid, 1 h at 100°C) of ivory-nut mannan, by preparative t.l.c. on silica-gel plates. Chromatograms were developed with the following solvent system, which gave a good resolution of these oligosaccharides: propan-1-ol/ethyl acetate/ethanol/acetic acid/pyridine/water (7:3:3:2:2:4, by vol.). The same system was used for the identification of reaction products.

The [^3H]mannobiose was prepared by C.E.N. (Saclay, France) by catalytic exchange with pure $^3\text{H}_2$ (Evans *et al.*, 1974) (1.83 mCi $\cdot$ ml $^{-1}$ in 2 mM aqueous solution).

Enzymic assays

Routine reaction medium containing 0.1 M-citrate/0.2 M-phosphate buffer, pH 5.5 (50 μ l), 0.04 M-mannotetraose (or mannopentaose) (20 μ l), [^3H]mannobiose (80 μ l) and enzyme preparation (100 μ l). The incubation was performed at 37°C, and the reaction was monitored by t.l.c. (silica-gel plates) of portions removed at 15–240 min intervals, and then at 24 h. Radioactive products were detected by

autoradiography (Ultrafilm ^3H ; LKB). Non-radioactive oligosaccharides were detected with 5% (v/v) H_2SO_4 in acetone, followed by heating at 100°C for 10 min. Authentic samples 1,4- β -oligomannosides, from mannobiose to mannopentaose, were used as chromatographic references for the identification of products.

Fig. 1. T.l.c. diagram of incubation mixtures of mannanases with mannotetraose in the presence of [^3H]mannobiose [revealed by autoradiography on Ultrafilm ^3H (LKB)]

1 and 2, [^3H]mannobiose (1, 1 h; 2, 24 h); 3 and 4, reaction of *Streptomyces* enzyme (3, 30 min; 4, 1 h); 5, reference products, from mannose (top) to mannohexaose (bottom); 6 and 7, reaction of fenugreek enzyme (6, 1 h; 7, 24 h).

Results and discussion

Streptomyces β -mannanase

In order to explain the accumulation of mannotriose without any release of mannose in the hydrolysates of mannotetraose by the mannanase, we postulated the transfer of one mannose unit, from the tetramer to the major product of hydrolysis, namely the mannobiose.

This hypothesis was confirmed by using radioactively labelled mannobiose as acceptor. In the early stages of incubation of the enzyme with mannotetraose, in the presence of labelled mannobiose, radioactive mannotriose appears. Both the chemical and autoradiographic locations of the oligosaccharides on the chromatograms show that the reaction products are only mannobiose and mannotriose, even after a 24 h incubation (Fig. 1). The whole reaction scheme is summarized in Scheme 1.

In a similar way, incubation of mannopentaose with the mannanase, in the presence of radioactive mannobiose, leads to labelled mannotriose.

Trigonella β -mannanase

The analysis of the reaction mixture, when incubating this enzyme with mannotetraose, reveals large quantities of mannobiose, accompanied by mannotriose and only a trace amount of mannose.

To explain this apparently unusual behaviour for a polysaccharidase, different pathways may occur: (a) the major attack on mannotetraose produces mannobiose; (b) the small amount of mannose may

Scheme 1. Reaction pattern of *Streptomyces* β -mannanase on mannotetraose
Man-, mannose residue involved in transglycosylation; *Man, [^3H]mannose residue.

Scheme 2. Reaction pattern of *fenugreek* β -mannanase on mannotetraose
Man-Man-, mannobiose residue involved in transglycosylation; *Man, [^3H]mannose residue.

be produced by a minor attack on mannotetraose at an extremity of the molecule: (c) the presence of large amounts of mannotriose may be produced from the transfer of one mannose unit to the major hydrolysis product, namely the mannobiose. Another mechanism can be postulated: some mannotetraose molecules may be used by the enzyme as donors of mannobiose residues, which are accepted by other mannotetraoses, leading to mannohexaose, which is in turn hydrolysed into two mannotrioses. A similar hypothesis was proposed to explain the activity of a xylanase towards xylo-tetraose (Dekker & Richards, 1975).

As radioactive mannotetraose was not available to verify the last hypothesis, we performed the same experiments as those reported for the *Streptomyces* enzyme. If the hypothesis concerning the formation of mannohexaose is correct, the radioactive mannobiose would not be an acceptor, and no labelled higher oligomannosides would appear.

Unexpectedly, after the first 1 h of incubation of the enzyme with mannotetraose, in the presence of [^3H]mannobiose, radioactive mannotetraose can be detected. This suggests the transfer of a mannobiose residue from the tetrameric substrate to the radioactive acceptor, as represented in Scheme 2.

Similar experiments were performed with mannopentaose as substrate. In this case the appearance of radioactive mannopentaose was observed after 15 min, proceeding from the transfer of a mannobiose residue from the substrate to the [^3H]mannobiose. In the following hours, this radioactive pentamer is further hydrolysed, and radioactive mannotriose appears.

Thus the β -mannanases of *Streptomyces* and *Trigonella* behave as true endopolysaccharidases. They do not hydrolyse mannotriose or mannobiose, but only higher oligomannosides. Both these enzymes are able to catalyse transglycosylation reactions in connection with their hydrolytic activity. Transfer reactions from mannotetraose and mannopentaose by the *Streptomyces* enzyme involve only a single mannose unit, whereas β -mannanase from fenugreek seedlings is able to transfer oligomannose residues: a mannobiose group from mannotetraose, and a mannotriose group from mannopentaose; a slow transfer of a mannose residue is not excluded. The occurrence of such transfer reactions may explain the complex pattern commonly observed with endopolysaccharidases.

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