HYDROLYSIS OF PLANT MANNANS BY RUMEN PROTOZOAL ENZYMES*

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Mannans or heteromannans (gluco- or galactoglucomannans) are commonly considered to be present in plants only in wood or associated with seeds. The present authors (Gaillard and Bailey 1968) have, however, recently isolated from the leaves and stems of red clover (*Trifolium pratense*) a polysaccharide fraction giving on hydrolysis galactose, glucose, mannose, and xylose (approximate ratios 1 : 4·0 : 2·0 : 1·3), and which may, therefore, contain a mannan or heteromannan. This polysaccharide is designated "clover mannan" in the present work. Although apparently absent from grasses, such mannans may be common as minor constituents of pasture legume leaves and stems; for example, 1–2% of polymer mannose was reported present in lucerne (Hirst, MacKenzie, and Wylam 1959). Ivory nut (*Phytelephas macrocarpa*) mannan has been reported to be digested by ruminants (Beals and Lindsey 1916) and these pasture-plant mannans are probably also digested, presumably after hydrolysis by mannanases secreted by the rumen microflora. The only study of the action of rumen microorganisms on plant mannans appears to be that of Williams and Doetsch (1960), who isolated from the rumens of cows fed guaran (soluble galactomannan) several bacteria which could grow on this polysaccharide and which secreted extracellular mannanase.

Rumen protozoa also play an important part in digesting plant polysaccharides in the rumen. On disruption they yield cell extracts which readily hydrolyse, for example, plant hemicelluloses *in vitro* (Bailey and Gaillard 1965). We have therefore examined the action on plant mannans of extracts prepared from protozoa isolated from cattle feeding on red clover.

Ordinary digests containing protozoal extract and ivory nut mannan, guaran, or clover mannan were incubated and analysed at hourly intervals by paper chromatography for liberated sugars. The chromatograms showed that the protozoal extracts, from several batches of protozoa from different cows, rapidly (1–2 hr) liberated mannose and the other constituent monosaccharides from clover mannan, guaran, and ivory nut mannan. With the first two polymers galactose and mannose appeared to be released at the same rates. No oligosaccharides were detected at any stage in any of these simple digests. Other digests showed that, in agreement with previous results (Bailey and Gaillard 1965), the extracts also hydrolysed clover

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hemicellulose pentosans. When the protozoal–mannan digests were incubated with continuous concurrent dialysis, chromatograms of the dialysates showed rather different results to those from the ordinary digests. With ivory nut mannan, during the first 45 min of incubation no free mannose but only oligosaccharides with a degree of polymerization > 3 were detected in the dialysates. Subsequent dialysates (1–2 hr of incubation) were rich in these higher oligosaccharides and also mannose and possibly mannobiose. With guaran the first dialysates, removed after incubation for 15 and 30 min, contained only galactose. Later dialysates contained galactose, oligosaccharides, and finally free mannose. A dialysis digest containing clover mannan gave only monosaccharides in the dialysates, possibly because it was a more complex polysaccharide.

The combined results from the ordinary and dialysed enzyme digests indicate the presence of two types of β-mannanase. These are, firstly, endopolysaccharase which randomly breaks the mannan chain to oligosaccharide fragments, and secondly, oligosaccharase which hydrolyses these fragments to mannose. The results with guaran suggest that the accompanying α-galactosidase activity must first remove sufficient of the galactose side units before the β-mannanase action can be effective.

Mixed protozoa were used in the present work so that it is not possible to assign the mannanase to particular protozoal species. Of the rumen protozoa the holotrichs do not ingest plant particles and are unlikely, therefore, to contain the mannanase, whereas the oligotrich protozoa do ingest small plant fragments so that some of these latter species could contain the enzyme.

The fact that rumen protozoal extracts contain β-mannanase suggests that these organisms can contribute to the overall action of the rumen microflora in hydrolysing ingested plant mannan or heteromannan. Any work on rumen digestion of plant mannans must, therefore, consider both the bacteria and the protozoa.

Experimental

Washed mixed protozoa (Oxford 1958) isolated from the rumen contents of cows fed fresh red clover for several weeks were disrupted at 0°C in citrate buffer (0·1M, pH 6·0) by grinding for 1 min with Ballotini beads in a Lourdes Omnimix high-speed grinder. Centrifuged cell-free preparations, 10 ml from 5–10 g of wet organisms, were dialysed at 2°C against the same buffer before use.

Freeze-dried red clover stems were extracted with dilute alkali (10% KOH, w/v) to remove hemicellulose pentosans, and the insoluble plant residue was then extracted with potassium hydroxide (24%, w/v)–boric acid (4%, w/v) solution. Treatment of this latter extract with barium hydroxide by the method of Timell (1965) yielded the clover mannan polysaccharide. Ivory nut mannan was prepared from ivory nuts (Klages 1934) and guaran (galactomannan) was a commercial product.

Ordinary enzyme digests contained protozoal extract (0·2 ml), citrate buffer (0·8 ml), and polysaccharide (2–5 mg), and were incubated under toluene at 37°C. At hourly intervals, portions (50–100 μl) were removed for paper chromatographic analysis. Dialysis digests contained protozoal extract (1 ml), the citrate buffer (1 ml), and polysaccharide (10 mg). These digests were incubated at 37°C and dialysed continuously against water (10 ml) with stirring as described by Bailey and Gaillard (1965). At intervals (15 min during the first hour and then hourly) the dialysate was removed and replaced by fresh water. Dialysates were concentrated for paper chromatographic analysis by freeze-drying and redissolving in water (1 ml).

Paper chromatograms were developed with either ethyl acetate–pyridine–water (2 : 1 : 2) or ethyl acetate–acetic acid–formic acid–water (9 : 1·5 : 0·5 : 2), and sugars were located with aniline hydrogen phosphate spray reagent.

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