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Note

α-Anomeric configuration of the saccharides produced by the action of debranching enzymes on glycogen and pullulan

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We have reported on the purification and characterisation of a rice debranching-enzyme that hydrolyses (1→6)-α-D-glucosidic linkages in amylaceous polysaccharides[1–4]. However, the anomeric configuration of the sugars released by the enzyme has not been studied. The anomeric form of the products of glycosidase action has generally been studied by polarimetric[5–7] and g.l.c.[8] techniques. Quantitative determination of the anomers produced by amylases has also been reported[9–12]. However, it is difficult to use these methods to determine the anomeric forms of oligosaccharides formed during enzymic hydrolysis of polysaccharides, but ^{1}H-n.m.r. spectroscopy can be applied[13]. We now describe the application of 400-MHz, ^{1}H-n.m.r. spectroscopy to determine the anomeric configuration of the saccharide(s) produced by rice and bacterial debranching-enzymes.

Debranching enzyme (28 U/mg of protein) was highly purified from non-glutinous rice seeds (*Oryza sativa* L. cv. "Yukara") as previously described[4]. *Enterobacter aerogenes* pullulanase (crystalline, 40 U/mg of protein) and *Pseudomonas amyloderamosa* isoamylase (crystalline, 59,000 U/mg of protein) were purchased from Seikagaku Kogyo Co. Ltd. A solution of each enzyme protein in D_2O was dialysed against D_2O. Solutions of pullulan (Hayashibara Biochemical Laboratories Inc.) and oyster glycogen (Nakarai Chemical Industries Ltd.) in D_2O were lyophilised. This procedure was repeated three times.

The rice debranching-enzyme (40 U in 0.2 mL of D_2O) and pullulan (40 mg in 0.5 mL of 0.01M sodium acetate buffer in D_2O, pH 5.6) were incubated in an n.m.r. spectrometer tube at 20°, and ^{1}H-spectra were taken at intervals of 0, 3, 5, 17, and 1200 min; the HOD signal was reduced by the homogenated decoupling technique. Sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS) was used as internal standard. The spectrum of pullulan at zero time is shown in Fig. 1. Three signals attributed to the α linkages were separated. The resonances at δ 5.35 ($J_{1,2}$ 3.91 Hz)

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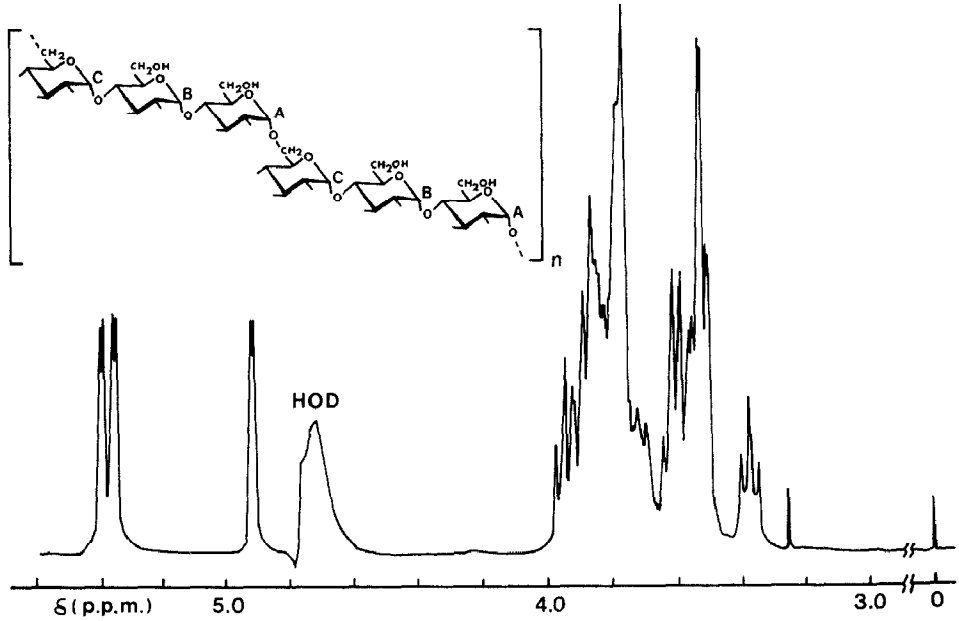

Fig. 1. ^{1}H-N.m.r. spectrum of pullulan in D_2O at 400 MHz.

and 5.39 ($J_{1,2}$ 3.42 Hz) were tentatively assigned to H-1^C and H-1^B by comparison with the corresponding resonances of maltotriose[14]. The upfield position of the signal for H-1^C reflects the shielding effect of the glucose residues linked to position 6 of glucosyl residue C. The H-1^A resonance assigned to glucosyl residue A involved in a (1→6)-α linkage occurred at δ 4.92 ($J_{1,2}$ 3.93 Hz)[14].

The signals for H-1 of the anomers released by the enzyme are shown in Fig. 2a. After 3 min, a strong doublet (δ 5.21, $J_{1,2}$ 3.42 Hz)[14] due to H-1α was observed accompanied by almost complete disappearance of the H-1 signal associated with the (1→6)-α-D-glucosidic linkage in pullulan. No signal for H-1β was observed during this phase of the reaction. A signal (δ 4.64, $J_{1,2}$ 8.30 Hz)[14] for H-1β was detected after 5 min and increased thereafter. Also, a signal associated with (1→4)-α-D linkages in maltose was found at δ 5.38 ($J_{1,2}$ 3.42 Hz). After reaction for 5 min, >90% of the (1→6)-α-D linkages in pullulan had been hydrolysed, and, after 17 min, almost all of the other linkages. The increase of the H-1β signal appeared to be due to mutarotation, since the increase was accompanied by a decrease of the signal of H-1α during incubation for 1200 min.

These findings indicate that the products released from pullulan by the debranching enzyme had the α configuration only.

E. aerogenes pullulanase (20 U) and pullulan (50 mg) were also incubated in a similar manner. The results (Fig. 2b) show that the products released had the α configuration only.

Isoamylase hydrolyses the (1→6)-α-D linkages of glycogen, but not those of

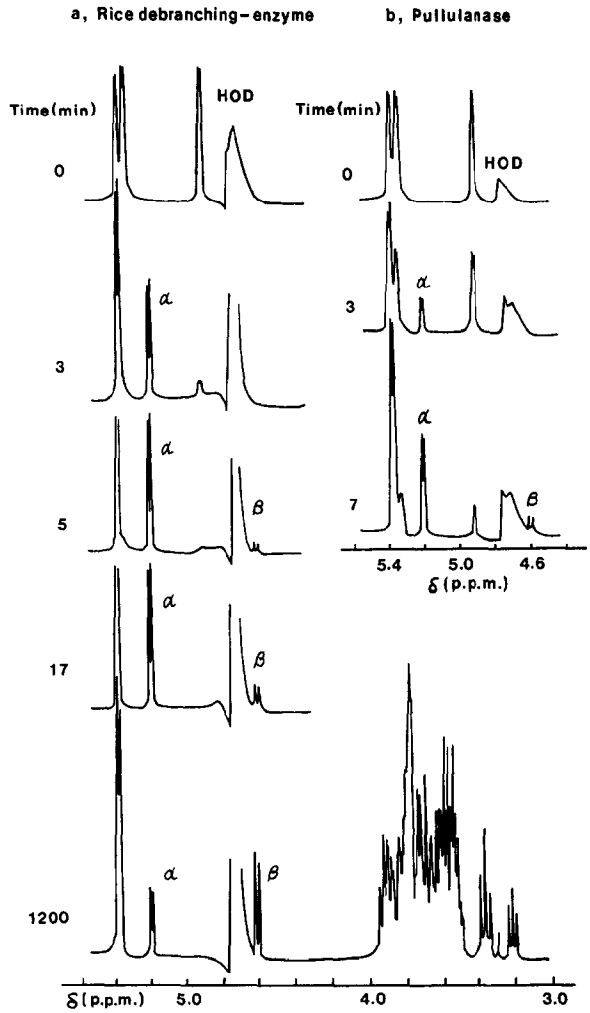

Fig. 2. ^{1}H-N.m.r. spectra of anomeric protons of sugars released from pullulan by rice debranching-enzyme and bacterial pullulanase.

pullulan. *P. amyloderamosa* isoamylase (5900 U in 0.2 mL of D_2O) and glycogen (40 mg in 0.5 mL of 0.01M sodium acetate buffer in D_2O, pH 4.5) were incubated in an n.m.r. spectrometer tube at 20°, and the ^{1}H spectra were recorded at intervals of 0, 5, 30, 60, and 1200 min (Fig. 3). A doublet (δ 5.21) attributed to H-1α of malto-oligosaccharides was found after 5 min, but no signal due to H-1β was detected. The H-1β signal appeared as mutarotation occurred after prolonged reaction. Thus, the malto-oligosaccharides produced from glycogen by bacterial isoamylase have the α configuration.

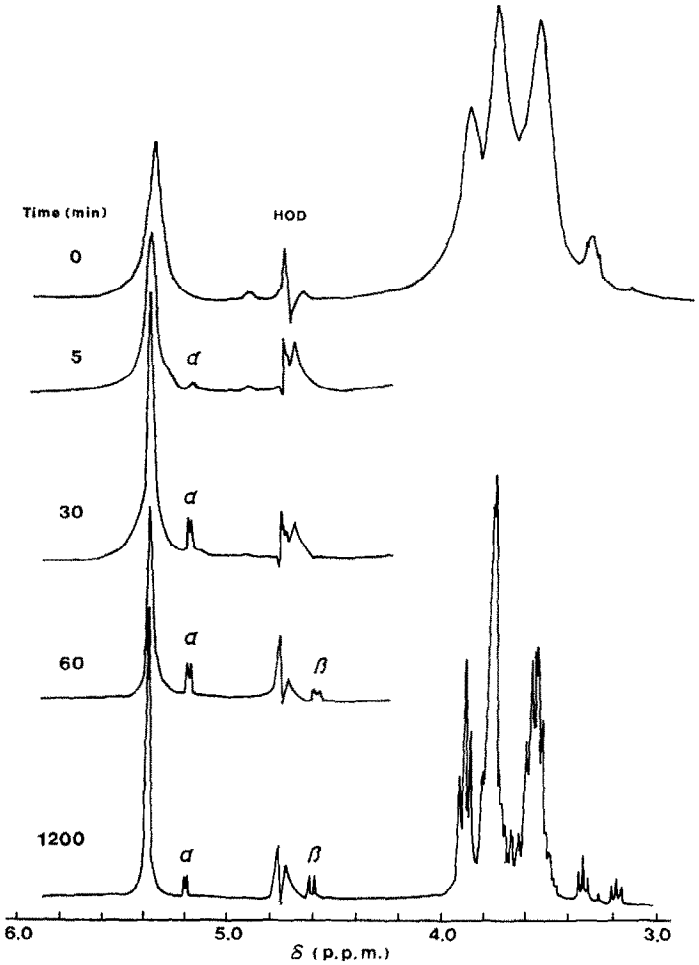

Fig. 3. ^{1}H-N.m.r. spectra of anomeric protons of sugars released from oyster glycogen by bacterial isoamylase.

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