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A STUDY OF THE ACTION OF LAMINARINASES FROM *SPISULA SACHALINENSIS* BY THE USE OF NUCLEAR MAGNETIC RESONANCE

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SUMMARY

The anomeric configuration of sugars obtained with enzymatic hydrolysis of laminarin with laminarinases III and IV from *Spisula sachalinensis* was determined by NMR-^{1}H spectroscopy. Laminarinases III and IV yielded products in which the anomeric configuration of the substrate was retained. Hence, laminarinases III and IV belong to endoenzymes.

The study by the use of NMR of the anomeric configuration of products resulting from the enzymatic hydrolysis of D-glycosides and D-glucans with glycosidases and glucanases shows that enzymes with an endo-type activity do not change the anomeric configuration of their substrates, whereas enzymes with exo-type activity invert their substrates[1]. The highly characteristic values of the chemical shifts and spin–spin interaction constants of anomeric proton signals in the NMR spectra of carbohydrates[2] allows one to consider the NMR spectroscopy data resulting from the study of enzyme action on carbohydrate chains as reliable.

Two laminarinases (β-1,3-glucan glucanohydrolase, EC 3.2.1.6) were isolated from the sea mollusc *Spisula sachalinensis*[3]. One of these, denoted by the authors as "laminarinase IV" was homogeneous, and the other, denoted as "laminarinase III", heterogeneous. In this work, we have carried out an NMR spectroscopic analysis for the enzymatic hydrolysis products of laminarin in order to establish the type of activity of the said laminarinases in addition to previously cited data[4].

The spectra were recorded on a Varian HA-IOOD instrument at 45° (optimum pH of enzymatic activity) after definite time intervals during 6 h and more. 40 mg of laminarin were dissolved in 400 μl of ^2H_2O with subsequent addition to the resultant solution of 10 mg of lyophilic powder of laminarinases IV and III (enzymatic activity 0.1 and 0.05 unit, respectively).

As apparent from the NMR spectra (Figs. 1a and 1b), on hydrolysing laminarin with laminarinase IV, the 30th min of the reaction witnessed the appearance of proton signals of β-anomers of oligosaccharide and glucose reducing ends; these signals were overlapped by a broad proton signal ($\delta = 4.96$ ppm) at the glycoside

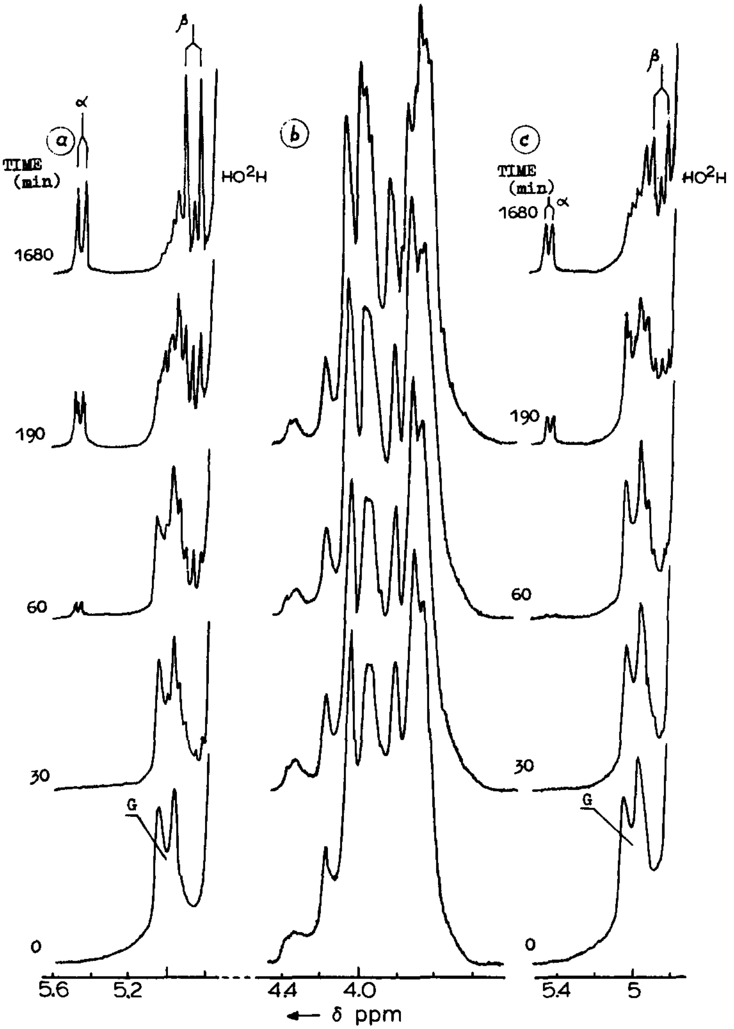

Fig. 1. Partial PMR spectra in deuterium oxide, recorded during assay of laminarinases IV and III. (a) and (b) are laminarinase IV with laminarin at 45°; and (c) is laminarinase III with laminarin at 45°. The HO^2H signal is due to residual water; the α-signal is due to H-1 of the α-D-anomer released; the β-signal is due to H-1 of the β-D-anomer released; the G signal is due to H-1 of the glycosyl unit of laminarin.

carbon atom. At this stage of hydrolysis (30–50 min), the proton signal intensity at the C-1 atom of glucose β-D-anomer (δ = 4.82 ppm, J_{12} = 7.9 Hz) indicated an insignificant proton content. This was likewise corroborated by the signal range (δ = 3.40–4.20 ppm) of the ring protons (Fig. 1b). Further hydrolysis stages showed the appearance of a glucose α-D-anomer signal (δ = 5.41 ppm, J_{12} = 3.5 Hz) caused by mutarotation of the β-D-anomer, and led to the accumulation of liberated glucose.

The increased intensity of the β-D-anomer signal and the redistribution in the intensities of the ring protons indicate an increased quantity of free glucose (Figs. 1a and 1b). The fact that hydrolysis of laminarin with laminarinase IV proceeds in such a manner shows that the quantity of reducing sugars being formed surpasses the quantity of liberated glucose, and hydrolysis does not lead to changes in the anomeric configuration of the substrate. Similar regularities were observed in the spectra on hydrolysing laminarin with an enzymatic preparation from laminarinase III (Fig. 1c).

Thus, the NMR spectroscopy data showed that on hydrolysing laminarin with laminarinases III and IV optical inversion does not occur. Hence, it is possible to assign the said laminarinases to enzymes with endo-type activity.

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