Alginate Lyase in the Brown Alga *Laminaria digitata* (Huds.) Lamour

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Recent studies on alginic acid's block-like [1] structure have been directed towards the use of enzymes [2-4] which may show specificity for the different linkages of uronic acid residues in this polysaccharide. Although a decade has elapsed since the demonstration of alginate lyases,[5-8] they have only been prepared from non-algal sources.[2-8]

In this communication evidence is presented for the occurrence of an alginate-degrading enzyme system in the peripheral tissues of *Laminaria digitata*. A partially purified extract of this brown algae effected rapid reduction in the viscosity of sodium alginate with a concomitant rise in thiobarbituric acid reactive material. Preparation of the active fraction was facilitated by pretreatment of tissues with acetone at subzero temperatures.

Material and methods. L. digitata stipes were freshly harvested from Trondheimsfjord (Flakk), at low tide on 23.10.72, packed in dry ice and stored at $-15°C$ until used. Epiphyte free outer layers, (*ca.* 0.1 mm thick; 7.96 g dry wt.), were scraped directly into a dry ice acetone bath (400 ml) in a mortar, and ground to small particles. The temperature of the solution was allowed to rise, (*ca.* $-10°C$), and the acetone filtered off under vacuum. The disintegrated tissues were then rinsed with diethyl ether, (10 sec) and dried in an air draft (20°C, 10 min). The pale green residue was reground as finely as possible and extracted with water for 18 h at 7°C. This brei was centrifuged in the cold and the supernatant freeze-dried (260 mg). The off-white powder was resuspended (2.4 mg/ml) in phosphate buffer, (pH 7.8, 0.05 M), and treated with ammonium sulphate, (85 % saturation at 0°C). Resulting flocculate, collected by centrifugation, (4°C), was dissolved in tap water, dialysed, (18 h, 7°C), with stirring, and freeze-dried (102 mg). Enzyme assays were carried out in collidine buffer (pH 7.1) at 21°C. The incubate contained calcium chloride (0.025 M), freeze-dried dialysate (1 mg/ml), and sodium alginate (ex. *Ascophyllum nodosum*, 93 % mannuronic acid) (0.83 mg/ml). The thiobarbituric acid reaction, TBA, was according to Weissbach and Hurwitz [9] and viscosity was measured with a pipette viscometer.

Results. Fig. 1 shows the time course of alginate degradation in terms of decreasing relative viscosity, η_r, and increasing TBA

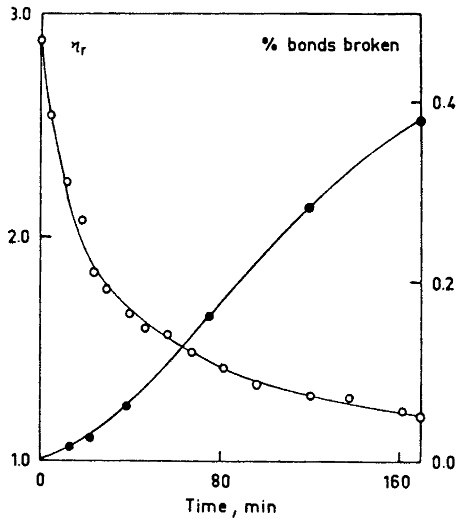

Fig. 1. Time course of enzymic degradation of alginate, (93 % mannuronic acid). O: Relative viscosity, η_r. ●: Bonds broken, TBA.

reactivity. Boiled extracts gave no increase in TBA, but had a small decrease in viscosity. A further control with no added alginate also gave no increase in TBA over the incubation period.

Fig. 2 shows the viscosity drop, as function of time, expressed as the change in reciprocal intrinsic viscosity, $[\Delta(1/[\eta])]$, which is proportional to the number of bonds broken during depolymerisation. Straight lines were obtained which showed that the reaction occurred at a constant rate in the time interval studied. The slight decrease in viscosity in the boiled enzyme preparation was probably caused by the presence of small amounts of phenols.[10]

Fig. 3 shows the linear correlation between the simultaneous viscosity drop, [expressed as $\Delta(1/[\eta])$], and the rise in un-

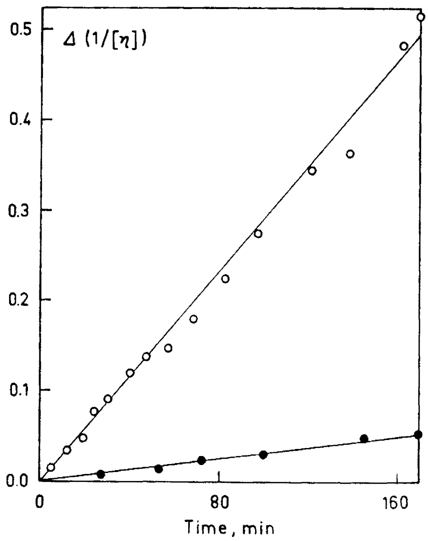

Fig. 2. Change in reciprocal intrinsic viscosity, $\Delta(1/[\eta])$, as a function of time for the enzymic breakdown of alginate by a *L. digitata* lyase preparation. O: Enzymic degradation. ●: Boiled enzyme extract.

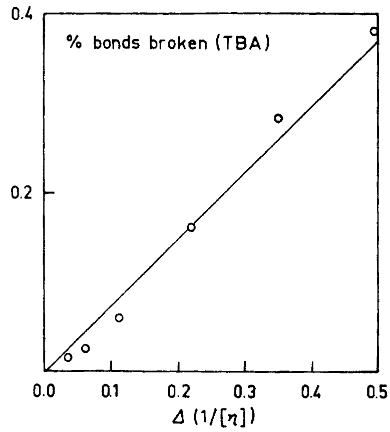

Fig. 3. Correlation between % bonds broken (as calculated from TBA reactivity), and decrease in intrinsic viscosity, $\Delta(1/[\eta])$, during degradation of alginate by an enzymic extract from *L. digitata.*

saturated uronic acid derivatives, (% bonds broken), and was interpreted as the result of a random scission of alginic acid by an alginate lyase [EC 4.2. 99].

A lyase was also prepared from a particulate fraction, (5 % total stipe protein), of the interior layers in *L. digitata*. Enzymic activity, per protein content, was similar for this preparation and for that from peripheral tissue described above. Demonstration of a lyase inside the plant was important because it indicated enzymic acitivity was a property of the algae and not that of microorganisms on the surface of the plants. Moreover, during enzyme preparations, care was taken to avoid the possibility of bacterial growth which might lead to formation of non-algal lyases. Studies on the characteristics and possible functions of this algal alginate lyase are in progress.

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