ENZYMIC HYDROLYSIS OF ALGINIC ACID

by

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INTRODUCTION

Alginic acid is known to be a difficultly hydrolysable substance: acid hydrolysis can only be achieved by vigorous treatment, *e.g.* 12 hours' boiling with 2.5% H_2SO_4[1], or 10 hours' boiling with 90% formic acid[7]; hydrolysis is accompanied by extensive decomposition.

Under physiological conditions also, alginic acid is very stable. According to OSHIMA[6], there are no higher animals and only a few lower animals that can digest alginic acid: OSHIMA found that a watery extract of intestine of *Haliotis giganteus* (a marine Gastropoda) and of *Sphaerechinus pulcherrimus* (an Echinoderm) hydrolyzed alginic acid, as measured by a decrease of viscosity and by an increase in reducing power of the alginate solution. In pancreatin, shark-intestine extracts, crab-liver extract and starfish extract no activity could be demonstrated.

OSHIMA found no activity in *Aspergillus oryzae*. WAKSMAN *et al.*[9] tried several fungi, but none showed more than limited activity as measured by CO_2-production, by decrease of uronic acid in the substrate and by decrease of the Ca-alginate precipitate. This limited activity is perhaps due to impurities in the alginic acid preparations that were used.

However, WAKSMAN *et al.*[10] isolated some kinds of bacteria from soil and from seawater which were able to grow upon alginic acid. Enzyme (alginase) preparations prepared from autolyzed culture media by precipitation with alcohol were able to hydrolyze alginic acid into smaller molecules, part of which formed soluble Ca salts in contrast to alginic acid itself.

Though in a few instances WAKSMAN *et al.* found reduction of Fehling's solution with the hydrolysate, the authors concluded that "the enzyme can split alginic acid into smaller groups of mannuronic acid units, but not into the simple units".

Recently, THJÖTTA and KÅSS *et al.*[4] isolated a number of alginase-producing bacteria from soil and seawater. Alginase production was detected by viscosimetry of alginate solutions and by the liquefication of Ca-alginate gels.

It is to be expected that bacteria that can utilize alginic acid as a sole carbon source have enzymes capable of hydrolyzing alginic acid to mannuronic acid, in a way analogous to the hydrolysis of pectic acid. The existence of such an enzyme, however, has never been demonstrated and therefore a preliminary study of alginase-action was undertaken.

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MATERIALS AND METHODS

Sodium alginate used throughout the experiments was a specimen of Manucol II manufactured by Alginate Industries Ltd., London.

Isolation of alginase-producing bacteria was accomplished in the following way. A 2% sodium alginate solution in distilled water was supplemented with inorganic salts ($(NH_4)_2SO_4$, 1 g; KH_2PO_4, 1 g; $MgSO_4$, 0.5 g per l alginate solution). This nutrient solution (pH 7–7.5) was inoculated with soil suspension and incubated at 35° C. Liquefaction of the viscous solution indicated the presence of alginase-producing organisms. Six pure cultures were obtained using alginate-agar plates (1% alginate, 1% agar). All these formed dark red-brown colonies on alginate-agar, excreted a reddish brown colouring substance when cultured in salt-supplemented alginate-solutions and grew in tough, slimy threads. Presumably all the strains were of one type.

In addition a different kind of bacteria was obtained in an enrichment culture which could not be isolated in pure culture and which was a strong alginase producer. None of these kinds of bacteria was identical with one of the bacteria described by WAKSMAN *et al.* and by THJÖTTA *et al.*

The experiments were carried out with the first mentioned bacteria in pure culture, unless otherwise stated.

The bacteria were cultivated in 2% alginate solutions, supplied with the salts mentioned before (pH of the medium 7–7.5) at 35° C during 7–10 days; the cultures were then centrifuged and the filtrates supplied with toluene.

Enzyme preparations were prepared from centrifuged toluene-treated culture liquids by adding 3 volumes of alcohol, centrifuging the precipitate, dehydrating the precipitate with alcohol and drying in vacuo over silica gel.

Viscosity measurements were carried out with an Ostwald-viscosimeter at 25° C. 5 ml 0.25% sodium alginate solution (or l.m. pectin, or C.M.C.) adjusted to pH 7–7.5 were placed into the viscosimeter, 1 ml of enzyme solution (1% filtered solution of the dry preparation in distilled water or 1 ml culture filtrate or 1 ml of a commercial fungal enzyme preparation) was added and mixed by bubbling air through the solution. Addition of a buffer solution was not necessary.

Circular paper chromatography was applied with *iso*butyric acid (300 ml) — water (205 ml) as a solvent (JERMYN AND TOMKINS[3]). The rings on the paper chromatograms were detected by spraying with anisidine phosphate[5] or with benzidine-trichloroacetate[2].

As reference substances were used: mannuronic acid (prepared by acid hydrolysis of alginic acid), glucuronic acid, galacturonic acid (the three uronic acids move at the same speed) and enzymic hydrolysates (prepared with a commercial fungal enzyme preparation, purified by reprecipitation with alcohol) of an apple h.m. pectin and an apple l.m. pectin.

RESULTS AND DISCUSSION

A series of viscosity measurements with culture filtrate shows alginase activity (Fig. 1, curve 1).

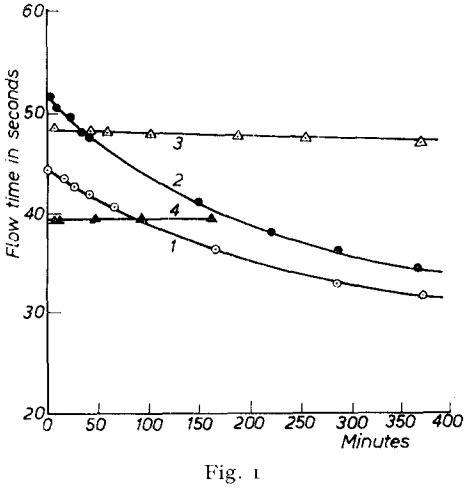

Fig. 1

An enzyme preparation (alcohol precipitate) of this culture filtrate likewise liquefies alginate (Fig. 1, curve 2).

After enzymic hydrolysis the alcohol precipitate of the substrate is much less gelatinous than the alcohol precipitate of original alginate solution.

The activity of the enzyme preparation towards C.M.C. was negligible (curve 3); towards l.m. pectin the activity was nil (curve 4). Several fungal enzyme preparations (Pektolyt, Pektinol, Luizym) were tested; all proved to be inactive.

After two weeks' incubation the culture filtrates showed strong reduction of Fehling's solution. Paper chromatograms of the culture filtrates showed a series of rings, all correspond-

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ing in colour and in location with those obtained with the pectin hydrolysates; in both, the ring that had run fastest corresponded with the rings of uronic acid.

These experiments were repeated with an enzyme preparation of an impure strain of bacteria which produces much alginase. On paper chromatographic analysis both the culture liquid and an alginate solution which had been incubated for some days with an enzyme preparation (alcohol precipitate) showed the same series of rings. Chromatograms from cultures that had been incubated for longer periods *e.g.* 4 weeks, showed only the mannuronic acid ring.

These results justify the conclusion that mannuronic acid and a series of di-, tri-, tetra-, etc. mannuronides are formed by the action of alginase. Obviously the oligomannuronides are intermediates and mannuronic acid is the ultimate end product of the hydrolysis. So far there is complete analogy between the action of polygalacturonase (pectinase) and polymannuronase (alginase).

SUMMARY

An alginic acid hydrolyzing bacterium has been isolated which excretes an enzyme capable of degrading alginic acid into mannuronic acid. A series of intermediary oligomannuronides has been demonstrated by paper chromatography to be present during enzymic hydrolysis.

RÉSUMÉ

L'auteur a isolé une bactérie qui hydrolyse l'acide alginique et qui excrète une enzyme dégradante l'acide alginique en acide mannuronique. La chromatographie sur papier a permis de démontrer l'apparition, au cours de l'hydrolyse enzymatique, d'une série d'oligomannuronides intermédiaires.

ZUSAMMENFASSUNG

Es wurde ein Alginsäure hydrolysierendes Bakterium isoliert, das ein Enzym abscheidet, das fähig ist Alginsäure bis zu Mannuronsäure abzubauen. Während der enzymatischen Hydrolyse treten, wie durch papierchromatographische Untersuchungen gezeigt wurde, eine Reihe von Oligomannuroniden als Zwischenprodukte auf.

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Received November 12th, 1953