Alginic Acid Degradation by Eliminases from Abalone Hepatopancreas*

HENRY I. NAKADA AND PATRICIA C. SWERNY

From the Department of Biological Sciences, University of California at Santa Barbara, Santa Barbara, California 93106

SUMMARY

Two separate alginic acid eliminases have been purified from abalone hepatopancreas. Alginase I appeared to be specific for \( \beta-1,4 \) bonds involving \( \alpha \)-mannuronic acid units and apparently acts at random on internal bonds. Alginase II appeared to cleave \( 1,4 \) bonds involving \( L \)-guluronic acid and to work at or near chain ends. A combination of both enzymes could not degrade alginic acid completely, indicating the presence of other types of bonds. An ionic concentration of about 50 to 75 mM was required for maximum activity. It appears that the high ionic strength was necessary to remove bound water from the substrate, alginic acid, and to neutralize the negative charges on the polyanion in order to render the molecular configuration more suitable for enzyme action.

Although an extensive literature exists on alginic acid, its structure has not yet been unequivocally elucidated. Reduction and partial hydrolysis have indicated that \( 4-O-\beta-D \)-mannuronosyl-\( D \)-mannuronate and \( 4-O-\beta-D \)-mannuronosyl-\( L \)-guluronate linkages are present in alginic acid with some evidence for possible \( 1,3 \) linkages (1-3). Alginates are variable in their \( D \)-mannurionate and \( L \)-guluronate contents, and mannuronate-rich and guluronate-rich fractions can be separated by salt fractionation procedures (4, 5). Because of the relative simplicity of algin and the excellent previous work done on this polymer, it was chosen as a model for structural studies with the use of a combination of enzymic and chemical procedures. Enzymes capable of degrading alginic acid have been reported in lower animals and some microorganisms (6-11). Partial purification of an alginic acid eliminase has been accomplished from *Pseudomonas* extracts (11), but most other "alginases" have been reported in a crude state. This paper presents the purification and separation of two alginic acid eliminases from abalone hepatopancreas (*Haliotus rufescens* and *Haliotus corrugata*). Tsujino and Saito (10) have reported that algin degradation by an extract from abalone hepatopancreas proceeded by an eliminase reaction. Although the methods used for preparation of their extract and enzymatic degradation procedures did not preclude bacterial contamination, our work confirms the enzyme-catalyzed elimination reaction from this animal source. The properties of the two alginic eliminases are discussed.

MATERIALS AND METHODS

Sodium alginate (Kelco-Gel HV) was obtained as a generous gift from the Kelco Company, Los Angeles, California. Manuronate acid- and guluronic acid-rich fractions of alginic acid were prepared by the potassium chloride method of Haug (4), repeating the procedure three times to obtain fractions designated as \( K_1 \) and \( K_2 \). The potassium chloride-soluble fractions were contaminated by KCl and were further purified by dissolving in water and precipitating with ethanol. This procedure was repeated three times. Chondroitin sulfate was isolated and purified by the method of Einbinder and Schubert (12). All other chemicals were obtained from commercial sources.

The enzymes were isolated from the hepatopancreas of the Pacific abalones, *H. rufescens* and *H. corrugata*. The animals were killed as soon as possible after gathering or were maintained in a healthy state in large tanks at the Marine Biology Laboratory at the University of California at Santa Barbara.

Ultraviolet Absorption Assay

This method, based on that described by Nakada et al. (13), takes advantage of the marked absorption at 235 nm of unsaturated uronides produced by the enzymic hydrolysis of algin. The reaction was followed in a Beckman DK-2A recording spectrophotometer with quartz cuvettes (1-cm light path). Substrate blanks were usually employed. This assay was the method of choice because of its rapidity and sensitivity.

Reducing Group Assay

The Nelson adaptation of the Somogyi method was used (14). A curve obtained with galacturonic acid was used as a standard as the closest approximation to a hypothetical curve produced by a mixture of the two uronic acids in algin. The results obtained from this assay were lower than those from the ultraviolet absorption assay.

Thiobarbituric Acid Assay

This modification of the Weisbach and Hurwitz method (15) detects \( \beta \)-formyl pyruvate produced by periodate oxidation of 4-deoxy-5-keto compounds. The test is specific for this group-
Intact alginic acid did not react, but the reaction products were highly positive.

Viscosity Measurements

Viscosity was measured with the Brookfield Synchroelectric viscosimeter at 25°.

Optical Rotation

This was measured at five wave lengths by the Perkin-Elmer polarimeter at 30°.

Protein

Protein was determined by the method of Warburg and Christian (16).

Separation of Products

Column Chromatography—A partial separation of the products of Alginase I on alginic acid was achieved by passage through a Sephadex G-25 column (3 × 110 cm) with 0.2 M NaCl or 1 M acetic acid as the eluant at a flow rate of approximately 3 ml per min (17). Fractions were assayed at 235 nm and concentrated by flash evaporation below 50°.

Descending Paper Chromatography—The solvent used was n-butyl alcohol-acetic acid-water (50:12:25); irrigation time was 48 hours (11). The chromatograms were developed by spraying with periodate followed by thiobarbituric acid (18).

An estimate of the sizes of the products in different fractions was made by comparing the values obtained with the reducing group assay, ultraviolet absorption assay, and the Dische modification of the carboxazole test which measures total uronic acid (19). The standard curve used for the carboxazole test was obtained from the relative values given by n-mannuronic and L-guluronic acids to galacturonic and glucuronic acids as shown by Haug (20).

Specific activity was defined as millimicromoles of glycoside bonds cleaved per min per mg of protein.

RESULTS

Purification of Enzymes—Preliminary studies on the comparative biochemical aspects of marine organisms revealed that a crude extract from the hepatopancreas of the Pacific abalones, H. rufescens and H. corrugata, possessed high activity against alginic acids, as measured by the release of reducing sugars with time. It became apparent that the mechanism of the reaction was very similar to, or identical with, that described by Preiss and Ashwell (11) with their preparation from a Pseudomonad, when partial purification of the abalone enzyme made it possible to use the ultraviolet absorption assay at 235 nm to measure the appearance of unsaturated product.

Precautions were taken in the preparation of this enzyme to exclude the possibility of bacterial contamination. After washing the exposed hepatopancreas with cold KCl solution, it was dissected away from the surrounding gonadal tissue, taking care to avoid cutting into the stomach or the gut. The minced tissue was immediately placed in 4 volumes of a buffered solution composed of 0.3 M KCl, 0.15 M MgSO₄, and 0.1 M phosphate buffer (pH 7.0; 85:5:10), and was homogenized for 30 sec at high speed in a Waring Blender. This crude homogenate was then chilled and centrifuged for 30 min at 16,000 rpm in a refrigerated Servall RC-2 centrifuge. All subsequent operations were carried out at 0–5°. The large amount of fat in the supernatant was partially removed by filtration through layers of cheesecloth.

Early work utilizing ammonium sulfate fractionation (where the activity was usually found in the 40 to 60% saturated fraction) and centrifugation at 97,000 × g for 1 hour followed by treatment with calcium phosphate gel achieved a small degree of purification. However, passage of the supernatant from the high speed centrifugation of the 40 to 60% saturated ammonium sulfate fraction through a DEAE-Sephadex column with 0.02 M Tris-succinate, pH 5.6, as the eluant produced an enzyme with a specific activity of 2444, representing an approximately 257-fold purification. This enzyme appeared in the very early fractions from the column, although occasionally a small amount of activity was noted in the later fractions.

Reactions with this enzyme were essentially complete in 15 to 25 min. Further incubation, up to 24 hours, failed to achieve any significant increase in the products as measured by all three assays. This inability to degrade the substrate completely was not due to an inactivation of the enzyme, as addition of more enzyme to a reaction which had leveled off had no effect. However, the reaction proceeded anew in the presence of added algin.

One or more additional enzymes seemed to be a requisite for the complete breakdown of algin by abalone. The discovery and isolation of an enzyme, capable of attacking both algin and the products produced by the first enzyme, was finally achieved by a modification of the procedure described above.

To the supernatant from the crude homogenate was added slowly and with stirring an amount of protein sulfate, pH 8.0, equal to 60% of the amount of the nucleic acids in the preparation, followed by centrifugation at 15,000 rpm for 20 min. The cloudy supernatant fluid was then spun at 97,000 × g. The resultant clear brown supernatant fluid was equilibrated with 0.02 M Tris-succinate buffer, pH 5.6, by passage through a Sephadex G-25 column equilibrated with the same buffer. The volume at this point was 72 ml. The protein concentration was 16 mg per ml.

This solution was put onto a DEAE-Sephadex column (27 × 4 cm) and eluted with 2 liters of Tris succinate buffer, pH 5.6, on a gradient of 0.02 to 0.2 M in a refrigerated fraction collector. The fractions were assayed against algin at pH 7.3 and at pH 4.0, the latter being the second optimum found by Eppley and Lasker (8) with preparations from the gut wall of the sea urchin. Fig. 1 shows the results. Fractions with high activity at pH 7.3, but none at pH 4.0, were found after an eluant volume of about 70 ml. After an eluant volume of approximately 500 ml, a peak with activity only at pH 4.0 was revealed. These enzymes were called, in order of their elution, Alginase I and Alginase II. Intervening fractions had no effect at all on the substrate at either pH.

Substrate Specificity Purified Alginase I was totally ineffective against hyaluronic acid, chondroitin sulfate, fucoidan, and sodium pectate (Table I). Chromatograms developed to detect unsaturated products (18) clearly showed that its action on algin produced a small amount of a monosaccharide and a series of oligosaccharides.

Alginase II was slightly active against algin, but exhibited increased rates of up to 4-fold when the substrates were fractions from the passage of Alginase I products obtained either by passage of the products through a Sephadex G-25 column or by heat-inactivated Alginase I reaction mixtures (heated products).
Fig. 2 represents a combined run with the two enzymes assayed by measuring the increase in reducing power. Alginase I was allowed to react with algin until tests indicated that no further breakdown had occurred. At this point the reaction mixture was heated for 10 min in a boiling water bath.

The appropriate conditions for the action of Alginase II were provided with an aliquot of the heated reaction products used as substrate. Note the clear increase in released reducing groups (also checked separately by the ultraviolet assay method with similar results) in sharp contrast to a control experiment in which Alginase I was incubated with the same products and no further degradation of the oligosaccharides could be detected. When both enzymes had been allowed to act on alginic acid to completion, approximately 50% of the theoretical number of glycosidic linkages was cleaved (see below). Since further additions of enzyme resulted in no further degradation, it was apparent that the two alginases were specific for two types of bondings, and that one or more additional enzymes would be required to cleave alginic acid to monosaccharides. A search for additional alginases is in progress.

An indication of the bond cleavage specificity was obtained by enzyme action on mannanuronic acid- or guluronic acid-rich fractions (Table I). Alginase I showed a more rapid rate with mannanuronic acid-rich fractions and degraded a greater percentage of the mannanuronic acid-rich fraction than it did with the guluronic acid-rich fraction or the original alginic acid. Alginase II, an enzyme that seemed to act on end groups (see "Discussion"), had moderate activity on the guluronic acid-rich fraction but had practically no effect on the mannanuronic acid-rich fraction.

Stability—The enzymes were stable if kept in a frozen state. After 4 months at -15°C, the purified alginases retained their original activity, but repeated freezing and thawing caused a decrease in activity. At pH values above 8, a rapid destruction of Alginase I occurred.

Effect of Enzyme Concentration—Both enzymes exhibited a linear relationship as the concentration of the enzyme was increased (Fig. 3). Alginic acid was the substrate for Alginase I and Sephadex G-25 products were the substrate for Alginase II. Alginase I was about 65 times more active than Alginase II. For most experiments, considerably more Alginase II was used to obtain easily measured rates.

Effect of Substrate Concentration—Fig. 4 shows the effect of substrate concentration on Alginase I assayed at 235 μm. No attempt was made to ascertain a Michaelis-Menten constant because of the impossibility of assigning any meaningful units to the substrate.

Substrate concentration studies were not carried out with Alginase II. The substrates used so far in our work with this enzyme have all contained a variety of molecules, and it was not clear what constituted a true substrate or substrate concentration.

Effect of pH—The pH rate profile for Alginase I appears on Fig. 5. Ionic strength was maintained at over 50 mM in Na+ and K+ ions in order to eliminate ionic strength effects (see Fig. 7). Optimal pH for enzyme action was between pH 7.4 and 7.6.

Alginase II exhibited a sharp peak at pH 4.0 in acetate buffer.
Alginase I

FIG. 3. Effect of enzyme concentration. The reaction mixtures for Alginase I contained per ml: algin, 0.5 mg; phosphate buffer (pH 7.3), 25 μmoles; KCl, 50 μmoles; and enzyme as indicated. The reaction mixtures for Alginase II contained per ml: Sephadex G-25 products, 0.1 mg; acetate buffer (pH 4.1), 25 μmoles; MgCl₂, 50 μmoles; and enzyme as indicated.

FIG. 4. Alginase I: effect of substrate concentration. The reaction mixture contained per ml: algin, 0.05 to 2 mg; Tris-HCl buffer (pH 7.8), 25 μmoles; KCl, 50 μmoles; and enzyme, 0.01 mg.

FIG. 5. Alginase I: pH rate profile. The reaction mixtures contained per ml: algin, 0.5 mg; sodium phosphate buffers, 25 μmoles; KCl, 50 μmoles; and enzyme, 0.0002 mg. Indicated pH values were measured on the complete reaction mixture.

FIG. 6. Alginase II: pH rate profile. The reaction mixtures contained per ml: Alginase I products, 0.1 mg; sodium acetate buffer, 25 μmoles; MgCl₂, 50 μmoles; and enzyme, 0.06 mg. The indicated pH values were measured on the complete reaction mixture.

FIG. 7. Alginase I: effect of cations and ionic strength. Each reaction vessel contained: algin, 0.5 mg; Tris-Cl buffer (pH 7.6), 25 μmoles; enzyme, 0.0120 mg; and the various salts in indicated quantities in a total volume of 1 ml.
The effect of some ions on Alginase II is shown on Table II. The buffer used most often for these studies was sodium acetate at pH 4.1. Increasing sodium acetate concentration gave results similar to those found with NaCl on Alginase I, with maximal activity at about 0.05 M of added salt.

The activity of Alginase II without added ions was low, but real when compared to both enzyme and substrate controls. In acetate buffer, the addition of magnesium chloride at a final concentration of 0.02 M gave the greatest enhancement. Cobalt and nickel had lesser effects. The monovalent ions of potassium and sodium both increased the activity, but at higher concentrations than needed with the divalent ions. Calcium ions inhibited the reaction. The effect of manganous ions could not be assessed in acetate buffer because of the formation of the insoluble manganous acetate. In the less efficient formate buffer, the enhancement conferred by magnesium and manganous ions was about the same.

In order to check the effect of compounds that might disrupt organized water structure, the effect of dimethylsulfoxide and dimethylformamide, in the presence or absence of KCl, was tested on Alginase I activity (Table III). Both compounds were capable of stimulating enzyme activity, but reaction velocities were greater in the presence of added KCl.

Effect of Alginases on Algin Viscosity—The effect of alginases on algin viscosity is shown in Fig. 8. With Alginase I, the viscosity dropped rapidly, whereas almost no viscosity change was noted with Alginase II. The results with Alginase I can be interpreted as the cleavage of internal glycoside bonds resulting in a rapid decrease in viscosity. The initial drop in viscosity occurred much more rapidly than the appearance of either reducing groups or unsaturation. Note that, in the viscosity studies, the concentration of Alginase I was about one-tenth of that usually used in the ultraviolet assay procedure.

The negligible change in viscosity with Alginase II may be due to the possibility that this enzyme acts on specific glycoside bonds that appear at the ends of the polymer; that is, we believe Alginase II to be an exoglycoside eliminase that was specific for one of the possible L-guluronic acid bonds. Such an explanation would be consistent with our finding that Alginase II was far more active on Sephadex G-25 products than on alginic acid, and the extent of reaction was greater on the oligo saccharides than on the intact polymer.

Separation and Preliminary Characterization of Reaction Products—Large scale Alginase I reaction mixtures were allowed to run to completion. All solutions had been made up fresh with boiled water and the incubation was carried out in sterilized flasks with cotton plugs. The course of the reaction was followed by measuring the increase in reducing groups (14). The reaction was complete after 1 hour, but additional enzyme was added at 1 and 2 hours to ensure against enzyme inactivation. The reaction mixtures were reduced to about 5 ml in a flash evaporator at 50° or below. This was placed on a Sephadex G-25 column as described under “Materials and Methods,” and eluted with 0.2 M NaCl or 1 M acetate. The fractions were assayed for unsaturated compounds at 235 mp (13), for reducing groups (14), and for total uronic acid content by the carbazole method (19). Paper chromatograms were run as described under “Materials and Methods” and developed with periodate and thiobarbituric acid (18). Ultraviolet absorption spectra of the products indicated an absorption maximum under acid conditions at 235 mp, as compared to 232 mp for disaccharides produced by bacterial hyaluronidas (21) or chondroitinases (13).

As far as a clear-cut separation of the products was concerned, the results of the fractionation were disappointing. Most of the products appeared in a single large peak, but the ratio of total uronic acid to amount of unsaturated material became...

---

**Table II**

<table>
<thead>
<tr>
<th>Salt</th>
<th>Activity</th>
<th>% maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>MgCl₂ (50 μmoles)</td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>MnCl₂ (50 μmoles)</td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>NaCl (50 μmoles)</td>
<td></td>
<td>70</td>
</tr>
<tr>
<td>KCl (50 μmoles)</td>
<td></td>
<td>59</td>
</tr>
<tr>
<td>CoCl₂ (50 μmoles)</td>
<td></td>
<td>43</td>
</tr>
<tr>
<td>NiCl₂ (50 μmoles)</td>
<td></td>
<td>39.2</td>
</tr>
<tr>
<td>CaCl₂ (50 μmoles)</td>
<td></td>
<td>13.5</td>
</tr>
<tr>
<td>None added</td>
<td></td>
<td>30</td>
</tr>
</tbody>
</table>

**Table III**

<table>
<thead>
<tr>
<th>Additions</th>
<th>Optical density at 4 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>5</td>
</tr>
<tr>
<td>KCl (20 μmoles)</td>
<td>8.5</td>
</tr>
<tr>
<td>Dimethylsulfoxide (0.1 ml)</td>
<td>8.0</td>
</tr>
<tr>
<td>KCl (20 μmoles) and dimethylsulfoxide (0.1 ml)</td>
<td>13.8</td>
</tr>
<tr>
<td>KCl (50 μmoles)</td>
<td>14.0</td>
</tr>
<tr>
<td>Dimethylformamide (0.1 ml)</td>
<td>7.5</td>
</tr>
<tr>
<td>KCl (20 μmoles) and dimethylformamide</td>
<td>13.0</td>
</tr>
</tbody>
</table>
FIG. 9. Paper chromatogram of products of Alginase I and Alginase II on alginic acid. Reaction I shows the products obtained from the complete reaction of Alginase I on alginic acid. Reaction II shows the products obtained from complete reaction of Alginase II on an aliquot from Reaction I. The remaining chromatograms shows the partial separation of Reaction I products on a Sephadex G-25 column.

FIG. 10. Optical rotation changes during algin degradation by Alginase I. The reaction mixture contained: algin, 2.5 mg per ml; KCl, 50 μmoles per ml; Tris-Cl buffer (pH 7.6), 25 μmoles per ml; and Alginase I, 0.012 mg in a total volume of 5 ml. Optical rotations were measured at 30°.

smaller as the eluant volume increased, indicating that there had been some retention of the smaller molecules. This was confirmed in paper chromatograms of the various fractions which showed a progressive increase in the faster moving components in the later fractions, culminating in well defined spots in the last three tubes with R_f values identical with that found by Preiss and Ashwell (11) in the same solvent to be an unsaturated monosaccharide (Fig. 9). Although no monosaccharide appeared on the reproduced chromatogram for Reaction I, similar chromatograms usually showed evidence for small amounts of material that has been tentatively identified as a 5-ketouronic acid.

Fig. 9 also shows the results when actual reaction mixtures of both enzymes were chromatographed. The substrate for the second reaction was heated Alginase I products.

Alginase II also produced three main spots, but the R_f values of the first two were higher than the corresponding ones produced by the first enzyme. Both sets of spots, however, showed the same relationship to each other, the second one moving about twice as fast as the first. Nothing remained at the origin following the second reaction and a larger amount of monosaccharide was formed.

Effect of Alginase I on Optical Rotation of Alginic Acid—Early evidence for a β-1,4 bond in alginic acid was based on its negative rotation. On this basis, rupture of β bonds should result in a positive rotation. This was checked with the results shown on Fig. 10. Although only the values at 589 μm are shown, similar results were obtained at all five wave lengths tested. A definite positive rotation was observed during Alginase I degradation of alginic acid.

DISCUSSION

Tsujino and Saito (10) reported that a crude extract of abalone hepatopancreas was capable of degrading alginic acid to an α,β-unsaturated disaccharide that appeared to be formed from 2 mannuronic acid residues. Their methods of enzyme extraction and extended incubation period (20 hours) with no stated precautions against microbial contamination made their results questionable. We have confirmed the work of Tsujino and Saito on the β elimination mechanism for abalone hepatopancreas alginases. Care was exercised to prevent contamination, and the rapid reactions of Alginases I and II (reactions could be completed in a few minutes) made it unlikely that the growth of bacteria could account for our observed activities. The α,β-unsaturated nature of the end products of alginase action on algin was shown by ultraviolet absorption and by reaction with the thioarbituric acid reagent after periodate oxidation. However, the products of algin degradation by the two partially purified alginases were mixed oligosaccharides with very little, if any, disaccharide appearing on paper chromatograms. These findings extend into the animal kingdom the polysaccharidases that catalyze the elimination reaction, a type of reaction that had previously been considered an exclusive property of microorganisms.

Alginic acid is a heteropolysaccharide made up of β-mannuronic acid and α-guluronic acid and is probably a family of polymers containing varying amounts of the two acids. Present evidence indicates that alginic acid is linear and two of the possible glycosidic linkages have been identified. Strong evidence exists for a β-1,4-β-mannuronosyl-β-mannuronate bond, and other evidence suggests the presence of a 1,4-β-mannuronosyl-α-guluronate linkage (1, 3). There also may be a 1,3 linkage between two β-mannuronate units (1, 2).

The enzyme designated as Alginase I has been highly purified from abalone hepatopancreas and is believed to be an endotranseliminase that attacks the 1,4-β-mannuronosyl-β-mannuronate bonds to form unsaturated oligosaccharides plus small amounts of unsaturated monosaccharides. The β bond was assumed from the work of Hirst, Percival, and Wold (1) and from the
positive optical rotation observed during alginic acid degradation by Alginase I (Fig. 10). The formation of \(\alpha,\beta\)-unsaturated products confirmed the presence of 1,4 linkages. The formation of a family of oligosaccharides and the very rapid decrease in alginic acid viscosity during enzymic attack showed that Alginase I acted on internal glycoside bonds. Furthermore, Alginase I reacted more rapidly on, and degraded to a greater extent, the \(\beta\)-mannuronate-rich algin fractions as compared to unfractionated algin or to the \(\alpha\)-guluronate-rich fraction.

Alginase II is believed to cleave a 1,4 bond involving \(L\)-guluronic acid either at or near the end of alginic acid. The formation of \(\alpha,\beta\) unsaturation indicated the 1,4 bond. Specificity for chain ends was inferred by the enhanced effect of Alginase II on oligosaccharides produced by Alginase I action on algin; that is, greater numbers of end groups promoted reactivity. The probable specificity for \(L\)-guluronic acid was inferred from the activity of Alginase II on \(L\)-guluronate-rich fractions and the almost complete lack of activity on \(\beta\)-mannuronate-rich fractions.

The two alginases differed not only in apparent specificities, but also in properties. Alginase I had a pH optima at about 7.6 but Alginase II had a sharp optimum at 4. Both enzymes required salt concentration of about 0.05 to 0.075 M for maximum activation.

The viscosity of polyanions, including alginic acid, decreased with increased ionic strength (22). The effect of ionic strength on enzyme activity paralleled the effect of ionic strength on decreasing the viscosity of alginate. Because of the polyanionic character of alginic acid, the high ionic strengths were possibly required to maintain the uronic acid units at a minimal interunit period for proper fit on the enzyme.

Another possible function of high salt concentrations could have been the disruption of bound or organized water surrounding the alginic acid molecule (23). It appears that polymers that can form hydrogen bonds readily can stabilize the surrounding water structure. This organized water could inhibit the rate of formation of an enzyme-substrate complex. On this assumption, it follows that other substances that can interfere with water structure should be capable of accelerating the rate of alginic acid degradation by Alginase I. Dimethylsulfoxide and dimethylformamide in low concentrations were capable of accelerating Alginase I activity, with a greater activity noted at higher salt concentrations. It would appear that high salt concentrations were necessary to give alginic acid the proper conformation for enzyme activity and to disrupt the organized water structure around the alginate molecule. Physical studies are in progress to determine the extent of water associated with alginate. Specific metal ions were probably not required by the enzyme because of the nonspecificity of the various salts tested. Although not shown, \(\text{NH}_4\text{Cl}\) and various amine hydrochlorides were as effective as the metal salts.

REFERENCES

16. Warburg, O., and Christian, W., Biochim. Z., 310, 384 (1941).
Alginic Acid Degradation by Eliminases from Abalone Hepatopancreas
Henry I. Nakada and Patricia C. Sweeny


Access the most updated version of this article at http://www.jbc.org/content/242/5/845

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at
http://www.jbc.org/content/242/5/845.full.html#ref-list-1