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Degradation of Alginate in the Presence of Reducing Compounds

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Alginate has been shown to be more or less rapidly degraded in the presence of a number of different reducing compounds. The autoxidation of the reducing compound leads to the formation of hydrogen peroxide. The degradation of the alginate is caused by free radicals, probably hydroxyl radicals, formed by the reaction between the reducing compound and the hydrogen peroxide. The difference in the effect of the various reducing compounds is most probably due to a difference in ability to form and destroy radicals. In the presence of the most active reducing compounds, *e.g.* ascorbic acid, the intrinsic viscosity of alginate decreases from 20 dl/g to 2.5 in about 3 h, under conditions where pure alginate solutions are stable.

In 1940 Säverborn [1] reported that high molecular weight pectin was rapidly degraded in apple and lemon juice under conditions unfavourable for the well-known hydrolysis reactions. At the same time several workers observed that vitreous humour was degraded in the presence of ascorbic acid [2] or other reducing substances such as diazonium compounds and phenylhydrazine.[3,4] The degrading action was greatly accelerated by the presence of hydrogen peroxide. Robertson *et al.*[4] found that ascorbic acid and hydrogen peroxide also degraded other polysaccharides such as starch, pectin, flaxseed mucilage and capsular polysaccharides of pneumococci. The same authors demonstrated that addition of catalase prevented the degrading action of ascorbic acid.

In 1943 Deuel [5] published a detailed investigation of oxidative degradation of pectin. He confirmed the oxidative degradation of pectin in the presence of ascorbic acid, and the acceleration of the process by hydrogen peroxide. Among the additional polysaccharides degraded by this system he mentions alginic acid and cellulose derivatives such as methylcellulose. Deuel concluded that the autoxidation of ascorbic acid leads to the oxidation of pectin, either by formation of an active oxidation product of ascorbic acid, or by the formation of hydrogen peroxide "*in statu nascendi*".

In a series of works, von Euler *et al.*[7] investigated the oxidation of various high molecular weight substances by ascorbic acid and reductones. In 1957

Gilbert *et al.*[6] found a decrease in the viscosity of desoxyribonucleic acid and alginic acid in the presence of glutathione, particularly at high oxygen pressure. The degradation of desoxyribonucleic acid by ascorbic acid has recently been reported by Berneis.[8]

During an investigation of the stability of alginate solutions we discovered [9] that the rate of degradation was markedly influenced by the reducing phenolic compounds ("fucosan") present in brown algae. These compounds are present in varying amounts in the different species of brown algae,[10] and account for the difference in the stabilities of alginate preparations from different raw materials.[9] A number of other reducing substances were found to have a similar effect on the alginate. In view of the considerable importance this degradation reaction may have in the preparation and use of alginates, an investigation of the influence of reducing substances on alginate solutions was undertaken. In the present work the effect of various reducing substances are compared. The particular effects of this degradation reaction in the preparation of alginates will be discussed in a later publication.*

EXPERIMENTAL

The preparation of alginate from seaweed samples has been described earlier.[11] With the exception of one experiment, alginate prepared from *Laminaria digitata*, harvested at Kråkvågøy, 3/7—59, $[\eta] = 25$ dl/g, has been used throughout this work.

The degradation experiments were carried out by placing alginate solutions in constant temperature water baths and removing samples at various time intervals by means of a pipette. In all cases, flasks of a size of ten times the volume of liquid have been used, thus assuring a sufficient amount of oxygen present. The alginate concentration was 0.3 %. The following buffer systems were used:

pH 6: McIlvain buffer, adjusted to ionic strength 0.2 by means of sodium chloride.

pH 9.8: Carbonate buffer, ionic strength 0.2.

In one experiment phthalate and phosphate buffers of various strengths were used. The acidity of the solution was determined after addition of the reducing compound, and if necessary adjusted.

In the degradation experiment with DPNH and methylene blue, 0.05 mg methylene blue in 10 ml 0.01 M DPNH solution were used.

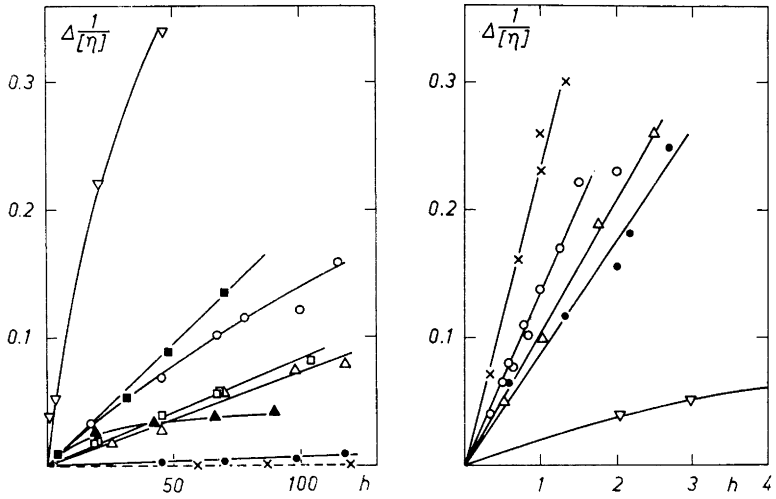
Viscosity was measured at 20°C in pipette viscometers calibrated against Ubbelohde viscometers, and the intrinsic viscosity determined by using empirical curves of the type described earlier.[12]

The catalase used was a crystalline commercial preparation (Boehringer).

RESULTS

The degradation of alginate at room temperature (20°C) and pH 6 in 0.01 M solutions of various reducing agents is given in Figs. 1 and 2. The degradation is expressed as $\Delta(1/[\eta]) = (1/[\eta]_t) - (1/[\eta]_0)$ as a function of time.[13] Pure alginate is very stable under the conditions used, and solutions may be stored for several weeks without a significant decrease of viscosity. The degrading effect is very different for the various reducing compounds tested and the rate

* Note added in proof: Pigman *et al.* (*Arthritis Rheumat.* **4** (1961) 240) investigated the decrease in viscosity of solutions of hyaluronic acid in the presence of reducing reagents. They proposed the name oxidative-reductive depolymerization (ORD) for reaction responsible for the viscosity decrease.



Figs. 1 and 2. Effect of 0.01 M of various reducing compounds on the rate of degradation of alginate. 20°C, pH 6.0.

Fig. 1.:

× No addition
● Hydroquinone
○ Sulphite
△ Phloroglucinol
▲ Sodium hydrogen sulphide
[-] Dithionite
■ Cystein
▽ Thioglycolic acid

Fig. 2.:

▽ Thioglycolic acid
○ Phenylhydrazine
△ Ascorbic acid
× DPNH + methylene blue
● Dihydroxymaleic acid

of degradation with the most active reagents is remarkable. Ferrous ions were also used as reducing compound, but due to the tendency of alginate to form gels with ions of heavy metals, it was necessary to use concentrations as low

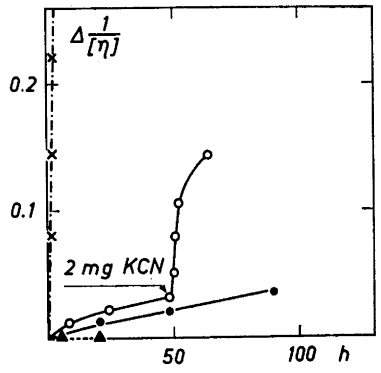


Fig. 3. Action of catalase on the degradation in the presence of ascorbic acid. 20°C, pH 6.0.

× 0.001 M ascorbic acid
○ 0.001 M » » + 0.2 mg catalase
● 0.001 M » » + 2.0 » »
▲ 0.001 M » », oxygen excluded.

as 10^{-4} M. With this concentration, the rate of degradation of alginate is about twice that obtained with 10^{-4} M ascorbic acid.

The effect of catalase on the action of the reducing agents is demonstrated in Fig. 3 for ascorbic acid. The presence of catalase very markedly inhibits the degrading effect of ascorbic acid, while the inhibitory effect of the enzyme is destroyed by the addition of potassium cyanide. A similar effect of catalase has been demonstrated for the other reducing compounds tested. For some of the sulphur containing compounds the inhibitory effect of catalase is less marked, probably due to a poisoning of the enzyme by the reducing compound. The effect of catalase demonstrates the oxidative nature of the degradation caused by the reducing compounds. This is confirmed by an experiment with ascorbic acid where oxygen was excluded. The degradation was in this case insignificant as shown in Fig. 3.

The action of catalase also demonstrates that peroxides are essential for the degradation process. Earlier observations have shown that the effect of ascorbic acid is greatly increased by the addition of hydrogen peroxide. We have determined the rate of degradation of alginate with mixtures of hydrogen peroxide and varying amounts of reducing compounds. The amount of hydrogen peroxide was kept constant at 0.01 M in these experiments. In Fig. 4 the degradation after 2.5 h at 20°C with ascorbic acid as reducing compound is given. The same figure gives the degradation with various amounts of ascorbic acid alone. The degrading effect of ascorbic acid passes through a marked maximum both when used alone and in the presence of hydrogen peroxide. Ascorbic acid markedly increases the effect of hydrogen peroxide at all concentrations tested.

The corresponding experiment with hydroquinone was conducted at 55° and the results are given in Fig. 5. In this case, also, a slight increase in the

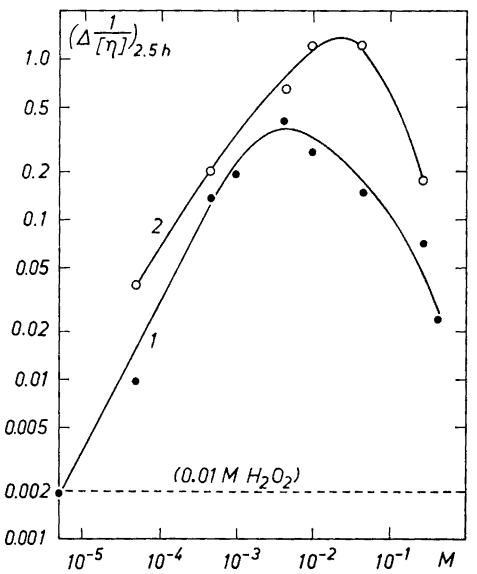


Fig. 4. Degradation of alginate in the presence of hydrogen peroxide and varying amounts of ascorbic acid. 20°C, pH 6.0. 1 = Ascorbic acid; 2 = Ascorbic acid, 0.01 M hydrogen peroxide.

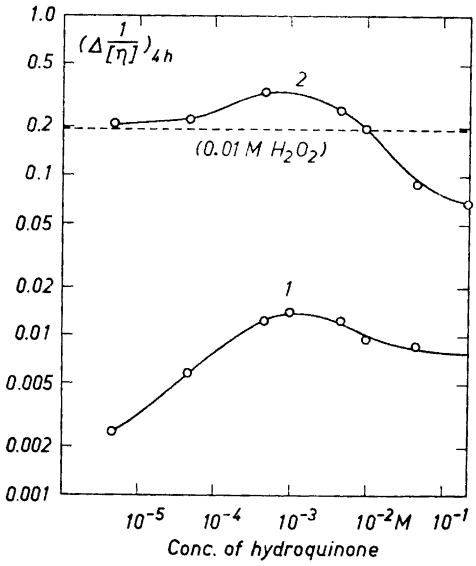


Fig. 5. Degradation of alginate in the presence of hydrogen peroxide and varying amounts of hydroquinone. 55°C, pH 6.0. 1 = Hydroquinone; 2 = Hydroquinone, 0.01 M hydrogen peroxide.

effect of hydrogen peroxide is observed at low concentrations of hydroquinone, while at higher concentrations a pronounced retarding effect is obtained.

Besides hydrogen peroxide, benzoyl peroxide and sodium persulphate are commonly used in polymerization processes as oxidation catalysts. If one of the two latter substances were substituted for hydrogen peroxide in degradation experiments with 0.02 M ascorbic acid and hydroquinone, ascorbic acid was found to increase and hydroquinone to decrease the rate of degradation, as was the case with hydrogen peroxide (Figs. 4 and 5).

The effect of temperature on the rate of degradation has been investigated for hydroquinone and ascorbic acid at pH 6. When $\Delta(1/[\eta])$ was plotted as a function of degradation time, straight lines were obtained as shown in Figs. 1

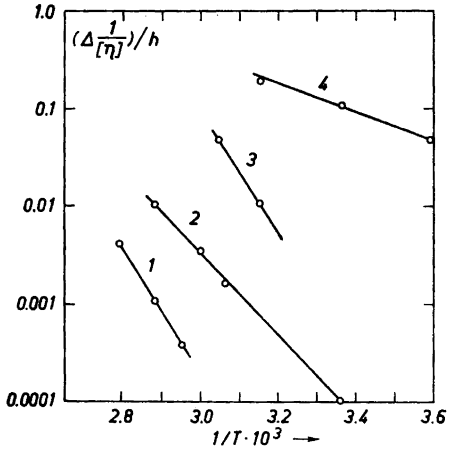


Fig. 6. Temperature dependence for different types of degradation of alginate. 1 = No addition, pH 6; 2 = 0.01 M hydroquinone, pH 6; 3 = 1 N hydrochloric acid; 4 = 0.01 M ascorbic acid, pH 6.

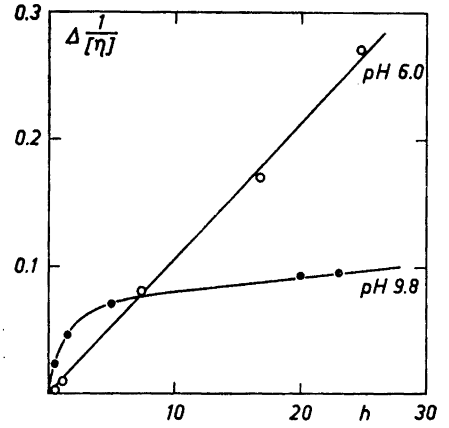


Fig. 7. Degradation in the presence of hydroquinone at two different values of pH. 0.001 M hydroquinone, 68°C.

and 2. In Fig. 6 the slopes of these curves are given as a function of $1/T$. For comparison the same curves have been determined for ordinary thermal degradation of the alginate at pH 6 in the absence of reducing compounds and for degradation with 1 N acid. The activation energy for the thermal and acid degradation was found to be 29 kcal/mole. In the presence of hydroquinone the activation energy was 19 kcal/mole and with ascorbic acid it was found to be as low as 7 kcal/mole.

The oxidative nature of the degradation makes it reasonable to expect that conditions which influence the rate of autoxidation of the reducing compounds also influence the rate of degradation of alginate in the presence of reducing compounds. It is well known that the rate of autoxidation generally increases with increasing pH. In Fig. 7 the degradation with hydroquinone at 68° at

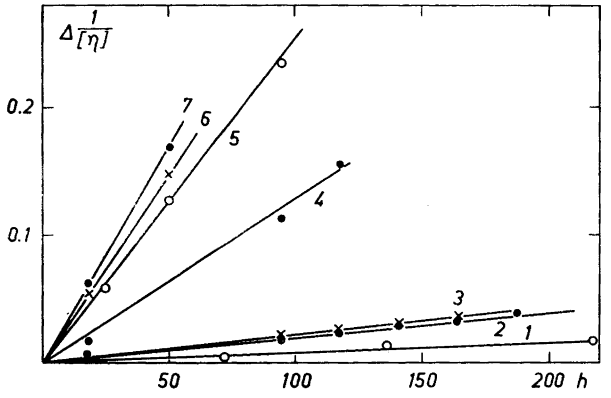


Fig. 8. Degradation in the presence of 0.01 M of different phenolic compounds. 55°C, pH 6.0.
1 = No addition; 2 = β-Naphthol, E_0 = 1.153; 3 = Resorcinol, E_0 = 1.179; 4 = α-Naphthol, E_0 = 0.933; 5 = Pyrocatechol, E_0 = 0.810; 6 = Hydroquinone, E_0 = 0.715; 7 = Toluhydroquinone, E_0 = 0.653.

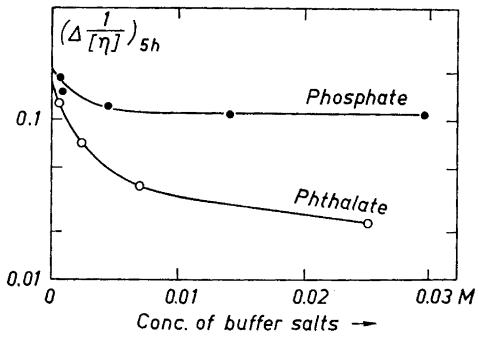


Fig. 9. Degradation of alginate, containing small amounts of "fucosan", in two different buffer solutions of varying strength. 68.5°C, pH 5.5.

pH 6.0 and 9.8 is shown. The initial rate of degradation is much higher at pH 9.8 than at pH 6.0. After 4–5 h, however, the rate of degradation at pH 9.8 decreases to the same rate as in the absence of reducing compounds, as the hydroquinone has been completely destroyed.

Within a group of chemically related compounds, such as the phenolic compounds, the rate of autoxidation is in some cases closely correlated to the reducing power, as expressed by the E_0 value.[14] Fig. 8 shows the degradation of alginate at 55.5°C in the presence of different phenolic compounds at a concentration of 0.01 M. The rate of degradation of alginate increases with decreasing values of E_0.

The degradation rate of alginate in the presence of the phenolic compounds of brown algae ("fucosan") has been found to be markedly influenced by the nature and amounts of the buffer ions present in the solution. Fig. 9 shows the degradation of alginate prepared from *Ascophyllum nodosum*, containing a small amount of the phenolic compounds naturally occurring in this plant, in solutions of two different buffer salts at varying concentration. The pH was 5.5 and the ionic strength was kept constant at 0.2 by sodium chloride. The rate of degradation of the alginate is much higher in phosphate buffer than in phthalate buffer. When the concentration of the buffer salts decreases, the rate of degradation increases.

DISCUSSION

When the degree of polymerization is above 10 and α in the modified Staudinger equation is 1, we can write for a first order, random degradation reaction:

$$kt = \frac{1}{[\eta]_t} - \frac{1}{[\eta]_0} = \Delta \frac{1}{[\eta]} \text{ (Ref.[15])}$$

When $\Delta(1/[\eta])$ is plotted as a function of time, straight lines should be obtained for a uniform rate, and the slope of the lines may be used as an expression of the rate of degradation. Figs. 1 and 2 show that the degradation of alginate in

the presence of reducing compounds in most cases gives straight lines. If we express the rate of degradation as $\Delta(1/[\eta])$ per hour, we find that while the degradation of a pure alginate solution at 20°C and pH 6 is too slow to be measured, the degradation in the presence of 0.01 M hydroquinone proceeds at a rate of 6×10^{-4} and in the presence of 0.01 M ascorbic acid at a rate of 0.1. The latter rate of degradation can be illustrated by the following example: A solution of 1.0 % alginate ($[\eta] = 20$) in phosphate buffer of pH 6 and ionic strength 0.1 N has a viscosity of 3000 cp. After 3 h at 20°C in the presence of 0.01 M ascorbic acid the viscosity will be reduced to 9 cp.

The oxidative nature of the degradation of polysaccharides in the presence of reducing compounds has been demonstrated earlier, and is confirmed by the experiments with catalase and exclusion of oxygen (Fig. 3). The catalase experiment also demonstrates that the formation of peroxide is essential.

The rate of degradation with dilute solutions of the most active reducing agents (*e.g.* ascorbic acid; Fig. 4) is, however, much higher than degradation with hydrogen peroxide of the same molarity. A marked increase in the rate of degradation with hydrogen peroxide has been found when reducing compounds are present. This acceleration is very pronounced with ascorbic acid, but significant acceleration is also observed with small amounts of hydroquinone (Fig. 5). These observations clearly indicate that a reaction between the reducing compounds and the peroxide takes place, leading to compounds which are very active in the degradation of alginate. Most probably these compounds are free radicals.

Figs. 4 and 5 demonstrate that the rate of degradation has a pronounced optimum at a certain concentration of reducing compounds. With hydrogen peroxide alone as the degrading agent no such optimum is observed: the rate of degradation increases steadily with increasing concentration of hydrogen peroxide. The most probable explanation of this optimal concentration seems to be that a reaction between the active radical and the reducing compound occurs, leading to the destruction of the active radicals. This inhibiting reaction must then be of a higher order with respect to the reducing compound than the reaction leading to the formation of the radical.

The observations discussed so far seem best explained by assuming that the following reactions take place:

1. Oxidation of reducing compounds leading to formation of peroxide.
2. Reaction between peroxide and reducing compound leading to formation of active radical.
3. Reaction between radical and reducing compound, leading to destruction of radical.
4. Reaction between radical and alginate, leading to degradation of alginate.

The differences among the reducing agents with respect to rate of degradation of alginate should thus be due to a different reactivity of the reducing compounds in one or more of the reactions 1, 2, and 3. If we compare the degradation in the presence of 0.01 M hydrogen peroxide and low concentration of reducing substances (*e.g.* 0.001 M or lower), the difference in rate of degradation is probably not due to a difference in ability to form peroxide (reaction 1) but rather due to a difference in ability to form and destroy radicals (reactions 2

and 3). Figs. 4 and 5 illustrate the pronounced difference between ascorbic acid and hydroquinone in the presence of hydrogen peroxide, indicating that the ascorbic acid has a high ability to form active radicals with hydrogen peroxide (reaction 2), while with hydroquinone the retarding effect (reaction 3) is most pronounced.

The difference in activation energy (Fig. 6) between degradation in the presence of hydroquinone (19 kcal/mole) and in the presence of ascorbic acid (7 kcal/mole) is of considerable interest in this respect. The activation energy of degradation of alginate with pure hydrogen peroxide has been found to be 25 kcal/mole, while hydrogen peroxide and 10^{-4} M ferric ions give an activation energy of 7 kcal/mole. It is well known that the decomposition of hydrogen peroxide leads to the formation of radicals, *e.g.* hydroxyl radicals. The presence of ferric ions greatly accelerates the decomposition of hydrogen peroxide and thus the formation of radicals. The results are thus in agreement with the assumption that the main difference between the degrading effect of ascorbic acid and of hydroquinone is due to a difference in the amounts of radicals present in the solutions.

In the preceding discussion we have assumed that the same radicals are formed with each of the different reducing agents. With ferrous ions as the reducing compound, the only radicals present are those formed by decomposition of hydrogen peroxide. We have found that the rate of degradation of alginate is higher in the presence of 10^{-4} M ferrous chloride and 0.01 M hydrogen peroxide than with the same amount of hydrogen peroxide and 10^{-4} M of the most active organic reducing compounds. This observation shows that the degradation rates observed may be explained without postulating the formation of radicals other than those formed by decomposition of hydrogen peroxide.

The rapid decrease in viscosity clearly indicates that the reaction between the radicals and alginic acid leads to chain rupture. The reaction between hydrogen peroxide and polysaccharides has not been very extensively investigated. Whistler and Schweiger [16] investigated the oxidation of amylopectin with hydrogen peroxide at room temperature, and found that the initial effect was depolymerization which was followed by an extensive oxidation, presumably of end units, to produce mainly carbon dioxide and formic acid. O'Colla *et al.*[17] found that a mixture of hydrogen peroxide and ferric acetate rapidly degraded alginic acid and other polysaccharides containing uronic acids, while neutral polysaccharides were not attacked. The end products of the reaction were not determined. We have so far not investigated the reaction products of the degradation of alginate in the presence of reducing compounds, but work is in progress on this point.

The earlier work in this field indicates that the oxidative degradation in the presence of reducing compounds is a general reaction for polysaccharides. Accordingly we have found a decrease of viscosity in the presence of ascorbic acid for all the polysaccharides we have tested; methyl cellulose, carboxymethyl cellulose, carrageenin, chondroitin sulphate, hyaluronic acid and pectin. Apart from the obvious practical importance of this degradation reaction in the pre-

paration and application of polysaccharides, we should also like to point out, as already done by Robertson *et al.*, the possible biological significance of this reaction.

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