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Alginates

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ABSTRACT

This short review covers some developments in the understanding of the structure and properties of alginates. Particular emphasis is given to discussing analytical methods for determining alginate composition, the modification and degradation of alginates by enzymes and the differences between bacterial and seaweed alginates. Alginate biosynthesis is also considered.

INTRODUCTION

A process for the extraction of 'algin' from brown seaweeds was patented originally by Stanford (1881). Over the last century, and particularly the last 50 years, the alginate industry has grown steadily so that approximately 15 000 tonnes dry weight of polysaccharide is processed annually from 400 000 tonnes wet weight of seaweed (Waaland, 1981). In this article the structure, analysis, biosynthesis and enzymic modification of both algal and bacterial alginates will be reviewed and some possible future developments will also be considered.

Most of the alginate is currently extracted from just three of the 265 reported genera (Wynne, 1981) of the marine brown algae (*Phaeophyceae*). *Macrocystis* is the major genus used and is harvested off the west coast of the USA whereas in Northern Europe *Laminaria* and *Ascophyllum* are the principal raw materials. Much smaller quantities of other genera are also used, e.g. *Durvillea* (Australia, Chile) and *Sargassum* (India, Philippines), although the latter produces alginate of low viscosity (Waaland, 1981). These plants are all harvested from naturally growing stands and it is only in China where large-scale

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attempts have been made to cultivate seaweed for the alginate industry. Here *Laminaria japonica* has been introduced from Japan and cultivated on rope rafts in beds that cover an area of approximately 19 000 hectares (McHugh, 1984) which is sufficient to satisfy the requirements of the local alginate industry.

Several microbial sources of alginate have also been discovered although at present these are not used commercially. Originally, two species of bacteria, *Azotobacter vinelandii* (Gorin & Spencer, 1966) and the opportunistic pathogen *Pseudomonas aeruginosa* (Linker & Jones, 1966) were identified as alginate producers. Subsequently Govan *et al.* (1981) isolated alginate-producing strains of *Pseudomonas fluorescens*, *Pseudomonas mendocina* and *Pseudomonas putida* after growth of the bacteria on media containing the antibiotic carbenicillin. More recently it has been reported that the plant pathogen *Pseudomonas syringae* pathovar *glycinea* also produces alginate and that the polysaccharide is an essential part of the infection process of this organism (Fett *et al.*, 1986).

STRUCTURE AND PROPERTIES OF ALGINATE

Algal alginates are unbranched (1-4)-linked glycuronans comprised of residues of β-D-mannosyluronic acid and the C5 epimer α-L-gulosyluronic acid. Although these two uronic acids have only minor differences in structure they will adopt different chair conformations (D-mannuronate, $^4C_1$; L-guluronate, $^1C_4$) such that the bulky carboxyl group is in the energetically favoured equatorial position (Fig. 1). Consequently, the resultant glycosidic bonds at positions 1 and 4 will be equatorial in β-D-mannuronate but axial in α-L-guluronate.

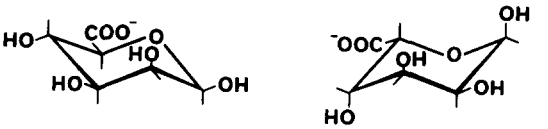

Fig. 1. The component monosaccharides of alginate; D-mannuronate (left) and L-guluronate (right).

The monomers are arranged within the alginate molecule as a series of block structures. These may be homopolymeric [poly(β-D-mannosyluronate) and poly(α-L-gulosyluronate)] or heteropolymeric approximating to an alternating sequence (Haug *et al.*, 1967*a*). Because of the orientation of the glycosidic bonds it has been predicted (Rees,

1972) that regions in which β-D-mannuronate predominate will form an extended ribbon structure, analogous to cellulose, whereas those rich in α-L-guluronate will form a buckled chain (Fig. 2). This has been confirmed experimentally by X-ray diffraction analysis of the partial hydrolysis products of alginate (Atkins *et al.*, 1973*a*; 1973*b*). Furthermore, solution studies using both ^{1}H-NMR (Penman & Sanderson, 1972) and ^{13}C-NMR (Grasdalen *et al.*, 1977) also provide evidence that the uronic acids adopt these different chair forms within the alginate chains.

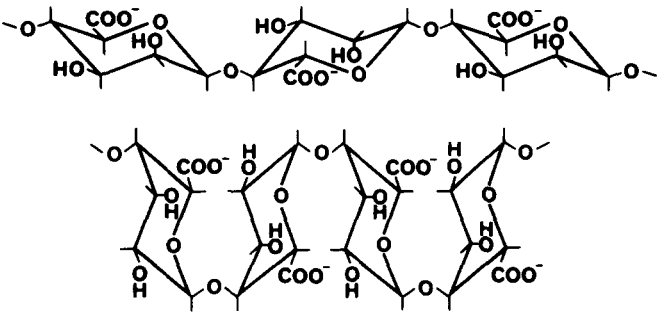

Fig. 2. Chain conformations of poly D-mannuronate (top) and poly L-guluronate (bottom).

A major property of alginate is the ability to form gels in the presence of certain divalent (or multivalent) cations, particularly Ca^{2+}. The physical properties of the gel will depend on the ratio of uronic acids within the polysaccharide chains; alginates rich in L-guluronate form strong but brittle gels whereas those rich in D-mannuronate are weaker but more flexible (Penman & Sanderson, 1972; Rees, 1972). The ratio of D-mannuronate to L-guluronate (M:G ratio) can be used as an index of the nature of the gel that will be formed in the presence of divalent cations (Haug *et al.*, 1967*b*).

The variation in gel strength may be rationalized in terms of the modes of binding of cations by the various block structures that occur within the alginate molecule. All of the block structures are polyanionic and will form intermolecular ionic bonds with di- or multivalent cations. However, regions of polyguluronate are also able to chelate the metal ions because of the spatial arrangement of the ring and hydroxyl oxygen atoms and thus form a much stronger type of interaction. These polyguluronate junction zones have been likened to the cross-section of an 'egg-box' where the Ca^{2+} ions are the 'eggs' within the 'egg-box'-like

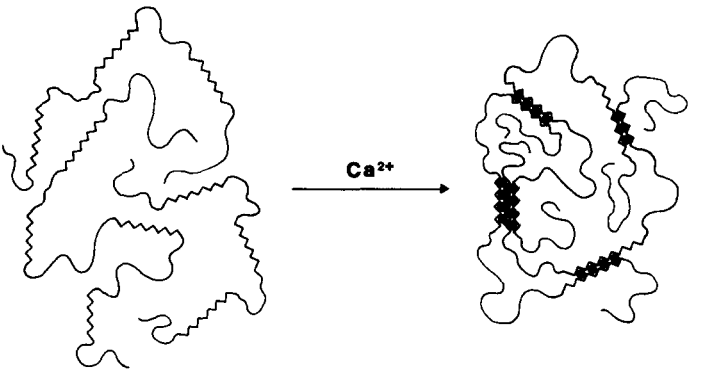

Fig. 3. Gelation of poly L-guluronate blocks with calcium ions; (^^^) represents polyguluronate blocks and (●) represents Ca^{2+}.

cross-section of the polysaccharide chains (Grant *et al.*, 1973) (Fig. 3). The higher affinity of the polyguluronate blocks for Ca^{2+} ions and the fact that most algal alginates contain all three types of block structure will allow the formation of a classical gel structure. The polyguluronate will form the junction zones which will be terminated by regions of non-interaction, i.e. polymannuronate and to a lesser extent the random blocks.

Unlike carrageenan or agar/agarose, alginate generally forms thermostable gels over the range 0–100°C. However, there is almost certainly a transition temperature above which an alginate gel will melt, although this is well above the boiling point of water. For example, the modulus of rigidity of an alginate gel decreases with an increase in temperature. (Andresen & Smidsrod, 1977) and certain mixed gels of pectin and alginate show thermoreversibility (Toft, 1982) indicating that the properties of alginate gels are temperature-dependent.

The structure of bacterial alginates is essentially the same as the algal material although there are some minor but important differences. The bacterial alginate is invariably O-acetylated and this substituent appears to be associated exclusively with the D-mannuronate residues (Davidson *et al.*, 1977*b*; Skjak-Braek *et al.*, 1985). However, results from the chemical analyses of similar preparations of bacterial alginate (Sherbrock-Cox *et al.*, 1984) have shown that the molar ratio of O-acetyl to D-mannuronate is greater than unity in some cases. This indicates that some of the D-mannuronate residues must be di-O-acetylated. Analysis of data from high field ^{1}H-NMR has confirmed this observation and has shown that D-mannuronate may be substituted in either the O-2 or O-3

position with occasional 0-2,3-di-substitution (Skjak-Braek *et al.*, 1986*a*).

The distribution of the various block structures within bacterial alginates may be atypical in some cases. *Azotobacter vinelandii* produces alginates with a range of block structures that are very similar to those found in some species of seaweeds (Table 1). However, detailed analysis of alginates derived from various species of *Pseudomonas* show that the polysaccharide is devoid of polyguluronate blocks (Table 1) and therefore very different from the algal material. There has been some circumstantial evidence that *P. mendocina* may produce alginate with an M:G ratio of less than one, and therefore must contain some polyguluronate block (Hacking *et al.*, 1983). However, this result is derived from infrared analysis of the alginate which at best is only semiquantitative for the estimation of the relative proportions of the two uronic acids (Fillipov & Kohn, 1974). Fett *et al.* (1986) have reported that *Pseudomonas syringae* is also capable of producing alginate and subsequent work (Osman *et al.*, 1986) implies that the polysaccharide may contain trace amounts of the polyguluronate sequence although no evidence has been provided to support this suggestion.

From the commercial point of view it is obviously important to produce alginate that contains a significant proportion of polyguluronate blocks. Clearly, the *Azotobacter* alginate may well be a suitable substitute for the algal material although at present the various *Pseudomonas* species appear to be less useful. The presence of O-acetyl groups in the bacterial alginate is not a problem as they may be removed readily in alkaline conditions (0·1 M-NaOH, 25°C, 20 min). Furthermore, should *Azotobacter* alginate prove to be of commercial significance then it would be relatively straightforward to produce mutants unable to transfer O-acetyl groups to the polysaccharide or precursors although this may have implications for the epimerization of the D-mannuronate residues (see later).

STRUCTURAL ANALYSIS OF ALGINATES

The chemical and physical analysis of the detailed structure of alginates is not easy and although many methods have been devised they all have limitations. Initially it is worth considering some of the methods used to identify and quantitate alginate as some of these have been adapted and extended to provide a more detailed analysis of the structure of the polysaccharide. One of the earliest methods of quantitating the alginate

TABLE 1
Fractional Composition of Alginates as Determined by ^{1}H-NMR

Organism	*Fractional composition*								*Reference*
	F_G	F_M	F_{GG}	F_{MM}	F_{MG}	F_{GM}	F_{GGG}	F_{MMM}	
Seaweeds:									
Laminaria digitata	0·42	0·68	0·27	0·43	0·15	0·15	0·22	0·10	Grasdalen (1983)
Laminaria hyperborea (stipe)	0·70	0·30	0·60	0·20	0·10	0·10	0·56	0·06	
Macrocystis pyrifera	0·39	0·61	0·21	0·43	0·18	0·18	0·20	0·20	
Ascophyllum nodosum	0·43	0·57	0·18	0·32	0·25	0·25	0·13	0·17	
Bacteria:									
Azotobacter vinelandii TL	0·45	0·55	0·43	0·52	0·02	0·02	0·41	0·02	Skjak-Braek *et al.* (1986*a*)
Azotobacter vinelandii IV	0·94	0·06	0·93	0·04	0·01	0·01	—	—	
Pseudomonas fluorescens (10255)	0·40	0·60	0	0·20	0·40	0·40	—	0·40	
Pseudomonas mendocina (10541)	0·26	0·74	0	0·48	0·26	0·26	—	0·26	
Pseudomonas putida (1007)	0·37	0·63	0	0·26	0·37	0·37	—	0·40	
Pseudomonas aeruginosa CF492a	0·26	0·74	0	0·48	0·26	0·26	—	—	Sherbrock-Cox *et al.* (1984)
Pseudomonas aeruginosa (3763)	0·44	0·56	0	0·12	0·44	0·44	—	—	

present in seaweed samples was to decarboxylate the polyuronide with mineral acid (Anderson, 1959). Once the appropriate apparatus has been constructed this method is simple to use and accurate but has the disadvantage that it may only be used in the absence of other compounds that might liberate CO_2.

Alginates have been quantitated colorimetrically using various modifications of the Dische carbazole assay (Bitter & Muir, 1962; Blumenkrantz & Absoe-Hansen, 1973). In one adaptation of this method the differential reactivities of the uronic acids with carbazole, in the presence or absence of borate, have been exploited to allow estimation of M:G ratios (Knutson & Jeanes, 1968). However, all versions of the carbazole assay are compromised to some extent by the susceptability of the methods to interference by a wide range of compounds. The use of other colorimetric methods has also been explored but these are relatively non-specific assays and are not suitable for distinguishing between different polysaccharides (Haug & Larsen, 1962). The degree of substitution of bacterial alginates with O-acetyl groups may also be measured colorimetrically (Buscher *et al.*, 1974). The use of colorimetric assays in conjunction with other methods for the analysis of food hydrocolloids, including alginate, has been reviewed by Southgate (1984).

As the solution properties of alginates are dependent on the chemical composition it is clearly desirable to be able to analyse the M:G ratio and ideally the block structure of the polymer. Most techniques for the determination of the M:G ratio by chemical analysis are dependent on the acid hydrolysis of the alginate to its constituent uronic acids. The protective effect of the carboxyl groups on the glycosidic bonds means that relatively harsh conditions are required for hydrolysis and consequently some decarboxylation occurs particularly of the L-guluronate residues. Therefore, appropriate corrections must be made for the partial loss of L-guluronate when estimating the M:G ratios (Haug & Larsen, 1962). Furthermore, the D-mannuronate and the L-guluronate must be separated both from each other and any side products to allow quantitation. A variety of methods have been developed to quantify the constituent uronic acids including anion-exchange chromatography with colorimetric detection (Haug & Larsen, 1962), GLC (Clamp & Scott, 1969), HPTLC (Wingender *et al.*, 1985) and HPLC with UV detection (Gacesa *et al.*, 1983).

In an attempt to circumvent the problems of the acid hydrolysis of polyuronides other methods of depolymerization have been investigated. Reduction of the carbodiimide-activated carboxyl groups with borohydride proceeds stoichiometrically and the resultant neutral polysaccharide is readily acid hydrolysed to yield the corresponding hexoses. The

monosaccharides can be reduced with $NaBH_4$ to yield the hexitols which are analysed by GC as the *n*-butane boronic acid esters (Vadas *et al.*, 1981). An analogous procedure has also been reported in which the carboxyl groups are methylated and then reduced prior to hydrolysis of the polysaccharide and subsequent analysis by capillary GC (Fazio *et al.*, 1982).

The M:G ratio of alginates may also be determined non-destructively by IR spectroscopy (Fillipov & Kohn, 1974). There is some doubt as to whether this method can be regarded as more than semi-quantitative (Mackie, 1971) and it is certainly not suitable as a definitive test for the presence of alginate although it has been used as such in the past. An advantage of IR spectroscopy is that the presence of O-acetyl substitution may be readily detected by the presence of characteristic absorbance bands at 1250 and 1730 cm^{-1}.

Although the M:G ratio is a useful parameter the properties of a sample of alginate can be predicted more accurately from a knowledge of the block structure. The contribution of individual blocks to the overall structure of alginate may be quantitated by chemical or physical methods. The chemical method is straightforward and has the advantage of not requiring sophisticated equipment. The three types of block structure may be isolated from alginate by partial acid hydrolysis and fractional acid precipitation and quantified using the phenol sulphuric total carbohydrate assay method (Haug *et al.*, 1967*a*). This method is reliable and has been used widely. However, the various blocks should not be considered as the ideal structures as, for example, the poly L-guluronate fraction will routinely contain 10–15% D-mannuronate.

The application of NMR and circular dichroism (c.d.) techniques has led to the development of several non-destructive methods for the analysis of block structure. The c.d. spectrum of alginate is dependent on the M:G ratio and on the arrangement of block structures within the molecule (Morris *et al.*, 1975). However, the distinguishing features of the spectrum are very sensitive to minor variations in Ca^{2+} ion concentration, particularly for samples containing poly G blocks (Morris *et al.*, 1978). Nonetheless, an analytical method has been described (Morris *et al.*, 1980) and this has been applied successfully to the analysis of various alginate samples (Stockton *et al.*, 1980*a*, 1980*b*). However, this method has not found widespread use, largely because few laboratories have the sophisticated instrumentation that is required.

The use of ^{1}H-NMR for the analysis of block structures in alginate has been developed into a routine technique. Originally it was essential to partially degrade and fractionate the alginate into its block structures before analysing each of these separately (Penman & Sanderson, 1972).

However, the method has been extended by the use of higher temperatures (90°C) and solvent suppression techniques so that intact or only minimally depolymerized samples of alginate may be analysed (Grasdalen *et al.*, 1979). Strictly speaking ^{1}H-NMR provides information on nearest neighbour frequency or diad frequency rather than analysis of total block structure. However, data from ^{1}H-NMR and from chemical analysis of alginates correlate well although there are some discrepancies in samples with a high proportion of mixed doublets (Grasdalen *et al.*, 1979). If natural abundance ^{13}C-NMR is used instead then an increase in the resolving power of the technique is obtained although the method is less sensitive than ^{1}H-NMR. For instance analysis of ^{13}C-NMR spectra enables the triad frequencies of the monomers within the alginate chains to be estimated (Grasdalen *et al.*, 1977). However, ^{13}C-NMR is less suited to routine use because of the extended accumulation time that is required to obtain spectra with good resolution. On balance, of all the techniques described, ^{1}H-NMR is probably the most suitable for routine analysis of alginate structure and a 100 MHz Fourier transform machine is perfectly adequate although clearly more information can be obtained from higher power machines.

All of the analytical methods that have been described are only suited for use *in vitro* once a relatively pure sample of alginate has been obtained. Clearly the ability to analyse alginates either in complex mixtures or *in situ* in the original producing organism would be of advantage. One possibility would be to raise antibodies to defined alginate structures and to use standard immunological methodology. Both polyclonal (Vreeland & Laetsch, 1981) and monoclonal antibodies (Vreeland *et al.*, 1984) have been raised against alginate and used to locate the presence of the polysaccharide in sections of algal tissue. However, the ionic interactions between alginate and the antibody are such that the concentrations of various ions can have significant effects on the results that are obtained (Larsen *et al.*, 1985). Although this technique is in its early stages and the antibodies need to be further characterized with respect to the structures to which they bind there can be no doubt that the potential of this technique is great. The use of molecular probes, including antibodies, for the analysis and localization of alginates has been recently reviewed (Vreeland *et al.*, 1987).

BIOSYNTHESIS OF ALGINATE

A putative pathway for alginate biosynthesis was first described for the seaweed *Fucus gardneri* based on measurable enzyme activities (Lin &

Hassid, 1966*a*) and the isolation and identification of several nucleotide sugars (Lin & Hassid, 1966*b*). Subsequently, similar pathways have been described for *A. vinelandii* (Pindar & Bucke, 1975) and for *P. aeruginosa* (Banerjee *et al.*, 1983). Although the basic pathway is now well established (Fig. 4) there is still controversy concerning both the routing of intermediates into the pathway and the final stages of modification, particularly epimerization of the D-mannuronate residues.

In bacteria there is little doubt that the epimerization of D-mannuronate to L-guluronate occurs as a post-polymerization modification. In other words the initial polymeric product is poly D-mannuronate and this is subsequently modified to produce the 'mature' alginate. A mannuronan C5-epimerase from *A. vinelandii* has been purified to homogeneity (Skjak-Braek & Larsen, 1985) and the corresponding enzyme has also been detected in a strain of *P. aeruginosa* (Piggott *et al.*, 1981). The precise step at which epimerization occurs in the seaweeds is still open to debate. As GDP-L-guluronate was tentatively identified in cell-free extracts of *Fucus gardneri* it was originally proposed that epimerization occurred at the nucleotide level and that both uronic acids were incorporated into the alginate during polymerization (Lin &

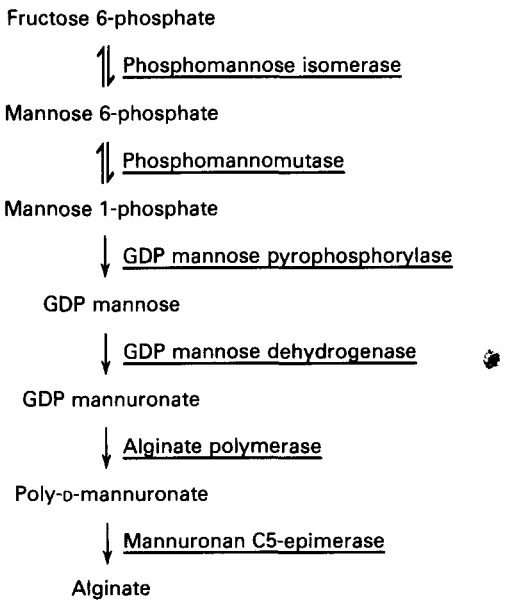

Fig. 4. Pathway for the biosynthesis of alginates. The O-acetylation of selected mannuronate residues in bacteria may possibly occur at the nucleotide or at the polymer level (adapted from Pindar & Bucke, 1975).

Hassid, 1966*b*). However, subsequently other laboratories have demonstrated the presence of a polymer-level epimerase in several different species of seaweed (Madgwick *et al.*, 1973*a*; Ishikawa & Nisizawa, 1981) thus indicating a pathway analogous to that in bacteria. The most recent results may go some way towards reconciling these two views. Growing tips of *Sargassum muticum* initially incorporate ^{14}C-labelled D-sorbitol 6-phosphate into the L-guluronate residues of alginate and it is only subsequently that radioactivity is associated with D-mannuronate (Quillet & de Lestang-Bremond, 1985). They propose that GDP-L-guluronate is synthesized directly from D-sorbitol 6-phosphate (i.e. the same compound as L-gulitol 1-phosphate) via GDP-L-gulose; the alginate is then synthesized from GDP-D-mannuronate and GDP-L-guluronate and subsequently a polymer-level epimerase is able to 'fine-tune' the structure of the polysaccharide. Whether this 'compromise' pathway is generally applicable to the brown algae remains to be seen. However, it is already clear that alginate is not synthesized in this way in bacteria as there is no inversion of the carbon skeleton, *viz*, [6-^{14}C]-labelled glucose produces alginate labelled at C6 (Carlsson & Mathews, 1966; Lynn & Sokatch, 1984; Anderson *et al.*, 1987). Furthermore, the polymer-level epimerase from *A. vinelandii* is unable to convert L-guluronate into D-mannuronate residues (Larsen & Haug, 1971).

Information about the routes by which precursors are channelled into the alginate pathway is of importance for evaluating potential yields of the polysaccharide and for the development of metabolic inhibitors with possible clinical use. The pathway originally proposed for *A. vinelandii*, on the basis of measured enzyme activities (Pindar & Bucke, 1975) assumes the conversion of the carbon sources (D-glucose or D-fructose) directly into fructose 6-phosphate without the involvement of three-carbon intermediates. However, this does not fit well with radiotracer data in which the specific activity of the alginates produced by *A. vinelandii* or the *Pseudomonads* is greater than that of the precursor (Lynn & Sokatch, 1984; Wingender *et al.*, 1985; Anderson *et al.*, 1987). It has been estimated that approximately 80–90% of the glucose is channelled into alginate via the Entner Doudoroff pathway (Fig. 5) whereas the remainder is routed via the pathway described by Pindar & Bucke (1975). The main consequence of metabolizing glucose through the Entner Doudoroff pathway, because of the action of the 2-keto-3-deoxy-6-phosphogluconate aldolase, is that only carbons 4, 5 and 6 of the precursor are available for alginate biosynthesis and the rest of the molecule is 'lost' to pyruvate. Consequently, if glucose is used as a feedstock then no more than a 50% conversion into alginate may be expected. However, D-fructose is phosphorylated directly and is not

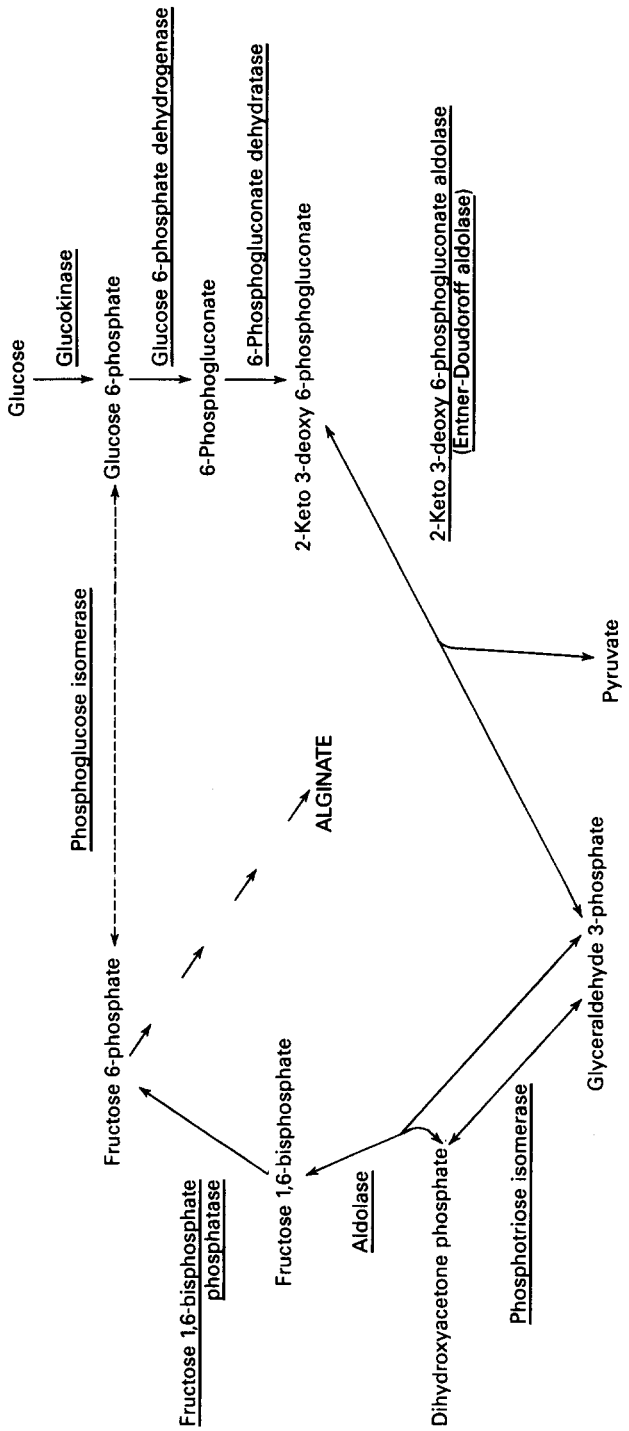

Fig. 5. Metabolism of glucose via the Entner Doudoroff pathway (solid lines). The dotted line indicates the route of direct conversion of glucose into alginate.

metabolized via the Entner Doudoroff pathway (Anderson *et al.*, 1987) and therefore it is reasonable to expect a theoretical conversion of approximately 90% of the monosaccharide into alginate.

Although the measurement of the enzymes involved in bacterial alginate biosynthesis has proved to be relatively straightforward in *A. vinelandii* (Pindar & Bucke, 1975) this has not been the case with *Pseudomonads*. For example, one group has readily measured phosphomannose isomerase activity in strains of *P. aeruginosa* (Piggott *et al.*, 1981) whereas others have not been able to detect the enzyme at all (Banerjee *et al.*, 1983; Narbad *et al.*, 1987). This problem has been partly resolved by the use of gene cloning techniques and complementation studies which have demonstrated that a gene encoding phosphomannose isomerase is present in *P. aeruginosa* and is essential for the biosynthesis of alginate (Darzins *et al.*, 1985*a*; Gill *et al.*, 1986). Recent work has indicated that several of the gene products are expressed transiently and at low levels during the growth cycle of *P. aeruginosa* and therefore the time of harvesting will be critical if enzyme activities are to be detected (Sa-Correia *et al.*, 1987).

Despite the problems associated with the direct measurement of enzyme activities in *P. aeruginosa* great advances have recently been made in the genetics of alginate biosynthesis by this organism. Two structural genes, *algA* and *algD* encoding phosphomannose isomerase and GDP mannose dehydrogenase, respectively, have been cloned and expressed in *E. coli* (Gill, *et al.*, 1986; Deretic *et al.*, 1987*a*) and two regulatory genes have also been identified (Deretic *et al.*, 1986; Goldberg & Ohman, 1987). The genetics and enzymology of alginate biosynthesis in *P. aeruginosa* has recently been extensively reviewed (Deretic *et al.*, 1987*b*; Russell & Gacesa, 1988). Genetic analysis of alginate biosynthesis in *A. vinelandii* and in the brown algae is far less advanced and is likely to remain so in the near future. However, conventional mutagenesis has been used successfully to increase the production of alginate by strains of *A. vinelandii* (Chen *et al.*, 1985).

Whether bacteria will be used for the commercial production of alginate is still open to question although it has been considered to be economically feasible providing that sufficiently high yields of polysaccharide can be obtained (Bucke *et al.*, 1975). At present it appears that the *Pseudomonads* are not suitable because of the inability to produce poly L-guluronate blocks. In contrast *A. vinelandii* will produce alginates that are analogous to those from the brown algae but the organism is not ideal for use in large-scale fermenters largely because of its high oxidation rate. Alginate from *A. vinelandii* behaves in exactly the same way as the algal material although it does have the advantage of greater viscosity

(Chen *et al.*, 1985). Although the use of fermentation techniques for the production of alginate is attractive the feasibility of this approach will depend ultimately on the economics of the process and the stance taken by the appropriate regulatory authorities.

ENZYMIC MODIFICATION OF ALGINATES

An attractive alternative to producing alginates by bacterial fermentation is the use of modifying enzymes to upgrade poor-quality algal material. In principle, mannuronan C5-epimerase, which converts D-mannuronate to L-guluronate at the polymer level, could be utilized for this purpose (Fig. 6).

Studies on the epimerase from *A. vinelandii* have revealed that the type of product that is obtained is dependent on the Ca^{2+} ion concentration (Ofstad & Larsen, 1981; Skjak-Braek *et al.*, 1986*b*). At low Ca^{2+} ion concentration the formation of L-guluronate blocks is favoured whereas at higher concentrations mixed sequences are obtained. The sequence of uronic acids in the product is also dependent on the nature of the starting material. For instance, D-mannuronate residues that are O-acetylated in bacterial alginates are not epimerized by the enzyme (Skjak-Braek *et al.*, 1986*b*). Also regions of alternating sequence cannot be modified as the enzyme is unable to epimerize an isolated D-mannuronate residue in the triad sequence GMG. Therefore alginates from *Macrocystis* and certain bacterial strains will not be amenable to this treatment as they contain large proportions of the GMG-type triad (Grasdalen, 1983). It will be interesting to see whether the corresponding enzymes from other reported sources such as *Pelvetia canaliculata* (Madgwick *et al.*, 1983*a*) and *Ishige okamurai* (Ishikawa & Nisizawa, 1981) are different in their substrate specificities and the type of products that they produce. However, at present the enzymes from these algal sources are too difficult to isolate and too unstable to be of use without modification.

Although the epimerase from *A. vinelandii* has been immobilized successfully with a resultant improvement in stability (Skjak-Braek *et al.*, 1986*b*) there are still several problems associated with its use. Crude preparations of the enzyme are usually contaminated with alginate lyases and sophisticated purification procedures are necessary to remove this enzyme (Skjak-Braek & Larsen, 1985). Another difficulty is that only small quantities of epimerase can be isolated from the culture broth of *A. vinelandii* and that the organism does not produce the enzyme reproducibly. Clearly, if the enzyme is to be used commercially then the gene

for the epimerase will have to be cloned. An alternative possibility is that some of the alginate lyases could be converted into epimerases (Gacesa, 1987) although this idea is unproven at present. However, the prospect of improving the gelling properties of alginates either enzymically or chemically is attractive.

DEGRADATION OF ALGINATES

The enzymes that degrade alginates can be both useful and a problem. Alginate-degrading enzymes of known specificity are potentially useful for elucidating the fine structure of the polysaccharide (see below) and they also hold promise as therapeutic agents in the treatment of mucoid *P. aeruginosa* lung infections in patients with cystic fibrosis (Russell & Gacesa, 1988). However, alginases are also involved in the disease processes of certain phytopathogenic micro-organisms and may also be involved in the spoilage of alginates or alginate-containing foodstuffs (Zeng, 1984).

So far all of the alginases that have been reported are β-eliminases producing 4-deoxy-L-*erythro*-hex-4-ene pyranosyluronate containing oligosaccharides (Fig. 6) via a resonance-stabilized enolate ion intermediate (Gacesa, 1987). This is analogous to the other polyuronate lyases, e.g. polygalacturonate lyase. However, polyuronides such as pectin/pectate are also degraded by hydrolytic enzymes to produce saturated products yet this does not appear to be the case with alginate. The reason for this is unclear although it may be that β-elimination mechanisms are used if the products are to be metabolized further, e.g.

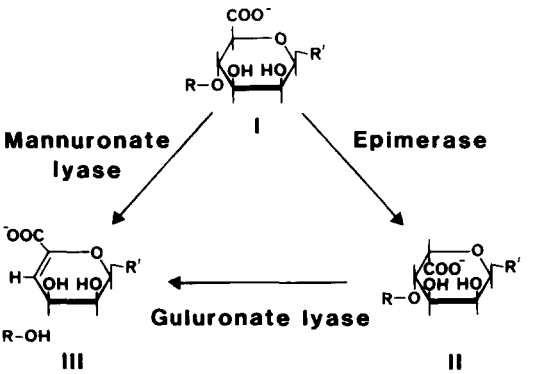

Fig. 6. Reactions catalysed by alginate-modifying enzymes. The compounds are: I, D-mannuronate; II, L-guluronate; and III, 4-deoxy-L-*erythro*-hex-4-ene pyranosyluronate (from Gacesa, 1987).

via the TCA cycle, whereas the hydrolysis products would be suitable for recycling into new polysaccharides. If this is the case then alginate hydrolases are most likely to be found in the tissues of brown algae rather than in bacteria.

Alginate lyases have been isolated from a wide range of organisms including bacteriophage (Davidson *et al.*, 1977*a*), bacteria (e.g. Kashiwabara *et al.*, 1969; Sutherland & Keen, 1981; Hansen & Nakamura, 1985), marine fungi (Wainwright & Sherbrock-Cox, 1981), molluscs (e.g. Nisizawa *et al.*, 1968; Favorov *et al.*, 1979; Muramatsu & Katayose, 1979) and brown seaweeds (Madgwick *et al.*, 1973*b*; Shiraiwa *et al.*, 1975). In most cases only one enzyme has been isolated from a particular source although it is evident that, in bacteria at least, there are usually multiple activities present (Caswell *et al.*, 1986). The alginate lyases are invariably specific for either the β-D-mannuronate or the α-L-guluronate linkage and are typically endo in their mode of action. Very few examples of exo-acting enzymes have been reported (Preiss & Ashwell, 1962; Doubet & Quatrano, 1984) although they should be fairly widespread as many bacteria can use alginate as a sole carbon source.

The use of alginate lyases for the determination of the fine structure of alginates has met with only limited success so far. Principally this is because many of the enzymes are incompletely characterized with regards to sequence specificity and also because of the lack of suitable exo-acting enzymes. There is a vicious circle in that it is difficult to characterize the enzyme unless the sequence of the substrate is clearly defined and the fine structure of the substrate will only be elucidated by the analytical use of appropriate lyases. Nonetheless, some attempts have been made to analyse alginates enzymically and to estimate block length (Boyd & Turvey, 1978; Currie, 1983). The properties of some of the endo-acting alginate lyases that have been well characterized are summarized in Table 2.

Alginate lyases have also been used in conjunction with other enzymes for the removal of the cell walls of brown seaweeds to facilitate the production of protoplasts (Kloareg & Quatrano, 1987). This is an important application as genetic improvement of the alginate-producing seaweeds, either by gene cloning or cell-fusion techniques, will require a reliable method for the generation of viable protoplasts.

APPLICATIONS OF ALGINATES

Alginates have been used widely for a broad range of applications (Table 3) and many facets of the properties of this polysaccharide have been

TABLE 2
Properties of Some Alginate Lyases

Organism	*Sequence specificity*[a]	*Major end product*	*Mechanism*	*Km* (mM)	*Reference*
Klebsiella aerogenes	$G_{\blacktriangle}X$	Trimer	Endo	0·11	Boyd & Turvey (1977, 1978)
Photobacterium?	$MM_{\blacktriangle}M$	Trimer	Endo	1·6	Romeo & Preston III (1986)
Littorina sp.	$M_{\blacktriangle}M$	Trimer?	Endo	0·19	Favorov *et al.* (1979)
Azotobacter vinelandii phage	$M_{\blacktriangle}XM$	Trimer?	Endo	1·0	Davidson *et al.* (1977*a*)
Pseudomonas?	$G_{\blacktriangle}XG$	Dimer/Trimer?	Endo	6·0	Davidson *et al.* (1976)

[a]The arrow indicates the site of cleavage. *G* represents L-guluronate, *M* represents D-mannuronate and *X* represents either D-mannuronate or L-guluronate.

TABLE 3
Some Major Applications of Alginates

Area of application	*Function*	*Specific examples*
Food and drinks industry	Stabilizer	Foam stabilizer (beer).
		Phase-separation retardant (ice cream).
	Viscosifier	Suspension of fruit pulp.
		Thickener for sauces, milk shakes etc.
	Gelling agent	Reconstitution of foods (stoneless fruit, onion rings).
	Film	Coating of fish.
Pharmaceutical industry	Stabilizer	Emulsions in cosmetic preparations.
		Binder for tablets and lozenges.
	Gelling agent	Moulds for dental impressions.
	Film/fibres	Gastroenteric coatings for tablets.
		Haemostatic bandages.
	Therapeutic agents	Anti-acid and anti-ulcer compound.
Other uses	Viscosifier	Printing inks.
	Gelling agent	Enzyme/cell immobilization.

exploited. Although the gelling of alginates in the presence of Ca^{2+} ions is a major property of the polysaccharide it may also be used as a stabilizer/suspending agent, a thickening agent, and the calcium salt may be formed into thread and spun using traditional weaving technology (Kennedy *et al.*, 1984).

One of the major users of alginates is the food and drinks industry. Alginate salts, the free acid and the propylene glycol ester have all been approved for use in foods and drinks by both the FAO/WHO and the EEC although some countries do have specific restrictions on applications (Overeem, 1984). Considerable ingenuity has been used in the formulation of foods containing alginates so that gelation, for instance, may be encouraged, retarded or made to be temperature dependent. Thus the basic properties of alginates can be extended considerably by adjusting the relative proportions of other additives (King, 1983).

The same range of properties of alginates that are important to the food industry are also of relevance in the production of pharmaceuticals. Also, in addition to the use of alginate as a stabilizer or gelling agent for pharmaceutical preparations there are some specific applications. For instance alginate is a useful anti-ulcer agent as the polysaccharide is

insoluble in the presence of acid and therefore forms a protective film on the stomach lining. Also, the buffering capacity of alginate enables it to be used as an anti-acid compound for the treatment of dyspepsia.

There are numerous other uses for alginates, many of which are protected by patents, and these applications have been comprehensively reviewed by Chapman and Chapman (1980).

CONCLUSIONS

The literature on alginates is vast and it is clearly impossible in a review of this size to cover every aspect of the subject. Although alginates have been widely used there is still much to be understood about the biosynthesis, detailed structure, properties and degradation of this polysaccharide. Stanfords original description of alginate (Stanford, 1883) showed a considerable insight into some of the properties of this polysaccharide and this has been the sound basis from which much of the subsequent work has developed. However, more than one hundred years later there are still some very basic questions that need to be answered.

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