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Studies on the Sequence of Uronic Acid Residues in Alginic Acid

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In an electrophoretic medium containing sodium and calcium ions in a suitable proportion, the mobility of alginate fragments depends on their uronic acid composition. By free boundary electrophoresis it has thus been shown that both heterogeneous and homogeneous acid hydrolysis leads to a splitting of the alginic acid molecule into chemically different fragments. These fragments were isolated by fractional precipitation and characterized by the number average degree of polymerization (P_n) and the uronic acid composition. It was concluded that the alginic acid molecule is a block polymer containing long sequences of both guluronic and mannuronic acid residues. At the same time, alginic acid must also contain long sequences of a predominantly alternating structure, as shown by the isolation of a fraction ($P_n = 15$) which, upon acid hydrolysis, gave a diuronide fraction consisting mainly of diuronides containing both monomers.

In a recent publication[1] we have described the heterogeneous hydrolysis of alginic acid and the subsequent isolation of alginate fragments of a number average degree of polymerization of 20, containing a very high percentage of either guluronic or mannuronic acid residues.

The present paper describes a further study of the partial hydrolysis of alginate leading to the formation of components with different uronic acid compositions. Free boundary electrophoresis was used to examine the hydrolysis products, and the components observed by electrophoresis were isolated by repeated fractionations and analysed. The bearing of these results on the structure of the alginate molecule and the course of the heterogeneous hydrolysis of alginic acid is discussed.

EXPERIMENTAL

Materials. Sodium alginate was prepared from *Laminaria digitata*, collected at Tarva, 29/8. The method of preparation is described elsewhere.[2] The alginate contained 61 % mannuronic acid.

Preparation of alginate fractions. A commercial alginate, prepared from *Laminaria digitata*, was hydrolysed with 20 parts of 0.3 N hydrochloric acid at 100°C. After 20 min the solution was removed from the insoluble material by filtration, neutralized and concentrated by evaporation. The solution was thoroughly dialysed against water and the polysaccharide isolated by freeze-drying (Preparation A). The remaining insoluble material was suspended in fresh 0.3 N hydrochloric acid and the hydrolysis continued for 20 h. The solution was removed by filtration and discarded. The insoluble material was suspended in water and dissolved by addition of dilute alkali. The solution was dialysed against water, the volume adjusted to 0.5 % alginate, sodium chloride added to 0.1 N and the solution mixed with approximately equal volumes of 0.025 N hydrochloric acid until a pH of 2.85 was obtained. The resulting suspension was centrifuged, the insoluble material suspended in water and both fractions neutralized and dialysed. The two fractions were isolated by precipitaton with ethanol, washed with ethanol and ether and dried (Preparation B; soluble at pH 2.85 and preparation C; insoluble at pH 2.85). Some properties of the three preparations are given in Table 1.

Table 1.

	P_n	% Mannuronic acid
Preparation A	20.0	65
» B	20.8	92
» C	19.8	13

Hydrolysis of alginate. One part of sodium alginate was washed with an exçess of 0.3 N hydrochloric acid, suspended in 20 parts of 0.3 N hydrochloric acid and hydrolyzed on a boiling water bath. A stream of nitrogen was passed through the suspension. For determining the rate of hydrolysis, samples of 2—3 ml of the suspension were removed at intervals. The samples were centrifuged and the soluble and insoluble phases analysed separately. For examination of the electrophoretic patterns obtained after different degrees of hydrolysis, the following procedure was employed: After a suitable time of hydrolysis the alginic acid suspension was centrifuged, and the soluble phase neutralized, concentrated and dialysed against water. A small part of the insoluble phase was dissolved by suspension in water and addition of alkali, and dialysed against water. The two fractions were analysed for carbohydrate content and reducing power. The main part of the insoluble fraction was resuspended in 0.3 N hydrochloric acid and further hydrolysed. The non-dialysable material of the soluble and insoluble fractions was then dialysed against the electrophoretic medium and examined by electrophoresis. In addition to experiments of the type described above, two samples were hydrolysed for 10 and 20 h, respectively, at 100°C, and the insoluble fractions examined electrophoretically. A third sample was hydrolysed for 10 h, centrifuged, the insoluble material dissolved by suspension in water and addition of dilute alkali, and further hydrolysed for 10 h after addition of hydrochloric acid to 0.3 N.

Homogeneous hydrolysis of alginate was carried out at pH 3.6 by mixing equal volumes of alginate solution (1 %) and citrate buffer, and boiling the solution under reflux.

Methods of analysis. The amount of carbohydrate was estimated by the phenol-sulfuric acid metod [4] and reducing power by the Nelson method.[5] The uronic acid composition of alginates was determined according to Haug and Larsen,[6] and the figures are corrected for the different rates of destruction of the two uronic acids.

Electrophoresis. A sample (4 ml) of the solution (1 %) to be examined was dialysed against 500 ml salt solution over night. For most experiments 0.05 M sodium chloride containing 0.00075 M calcium chloride has been used. Free boundary electrophoresis was carried out in a Perkin Elmer Model 238 instrument equipped with a standard analytical cell. All patterns and mobilities refer to the ascending boundary. In all experiments, the current (10 mA) was applied for 30 min.

Fractionation of degraded alginate

Two examples of typical fractionation experiments are given: 1) 10 g of sodium alginate (8.8 g alginic acid) was hydrolysed as described above for 2 h and the soluble and insoluble phases separated by centrifugation. The insoluble material (6.1 g) was suspended in water, dissolved by neutralization and dialysed against water. The volume was adjusted to 1 % carbohydrate (550 ml), sodium chloride added to 0.1 N and 0.025 N hydrochloric acid added until a pH of 2.85 was obtained (580 ml). The mixture was centrifuged, the centrifugate neutralized, concentrated by evaporation (200 ml) and dialysed against water. A sample was dialysed against the electrophoretic medium and examined by free boundary electrophoresis. One main peak and traces of another was observed (Fig. 6). The material was isolated by precipitation with ethanol (yield 3 g). The fraction precipitated at pH 2.85 was dissolved and dialysed against water. A sample was examined electrophoretically and found to give two peaks and traces of a third. The volume was adjusted to 0.5 % alginate, (450 ml), magnesium chloride added to 0.2 M and the solution mixed with equal volumes of 0.01 M calcium chloride. After separation by centrifugation, ethylendiaminetetraacetic acid (EDTA) was added to both fractions in twice the equivalent amounts of divalent metals present, and the solution dialysed against water. Samples of the two fractions were examined electrophoretically. In both cases two peaks were present. The fraction which was precipitated by calcium (1.2 g) was reprecipitated from a solution (0.5 %), containing 0.2 M magnesium chloride, by addition of one half volume of 0.01 M calcium chloride. The insoluble material was removed by centrifugation, dissolved by suspension in a solution of EDTA and dialysed against water. Electrophoresis revealed only one peak. The fraction was isolated by precipitation with ethanol, washing with ethanol and ether and drying (yield 0.7 g). The soluble fraction from the first precipitation with calcium (0.7 g) was precipitated from a solution (0.5 %), containing 0.1 N sodium chloride, by the addition of 0.025 N hydrochloric acid until a pH of 2.75 was obtained (approximately equal volumes). The precipitate was removed by centrifugation, suspended in water, dissolved by neutralization, and dialysed against water. The electrophoretic pattern showed only one peak, and the material was isolated by precipitation with ethanol, washing with ethanol and ether and drying (yield 0.4 g).

2) Sodium alginate (10 g) was hydrolysed as described above for 20 min and the soluble and insoluble phases separated by centrifugation. The insoluble phase was fractionated as described in example 1). The soluble phase (1.1 g) was neutralized, concentrated to 100 ml by evaporation *in vacuo* and dialysed against water. The non-dialysable fraction (0.5 g) was diluted to 0.5 % carbohydrate, magnesium chloride added to 0.2 M, and a half volume of 0.1 M calcium chloride added to the mixture. The mixture was centrifuged, EDTA added to both fractions and the resultant solutions dialysed against water. Both fractions were examined by free boundary electrophoresis. The soluble fraction (0.7 g) gave only one peak, while the insoluble fraction (0.1 g) gave two peaks (Fig. 9). Both fractions were isolated by precipitation with ethanol.

Preparation and investigation of diuronides

Preparation A was hydrolysed by mixing 10 ml of a solution containing 200 mg with 10 ml of 0.06 N sulfuric acid and hydrolysing for 16 h at 100°C. The number average degree of polymerization was 2.1 and no significant loss took place during the hydrolysis. Preparation B and C were hydrolysed for 18 h at 100°C in citrate buffers at pH 3.6 and 3.8, respectively (homogeneous). The pH was then adjusted to 3.1 and 3.5, respectively, and the hydrolysis continued for another 18 h. The degree of polymerization of preparation B was then reduced to 3.3 with a recovery of 86 %. The pH of the solution of preparation C was adjusted to pH 3.1 and hydrolysed again for 18 h. The degree of polymerization was 2.6 and the recovery 86 %. All the three hydrolysates were fractionated on a column of Sephadex G 25 (25 × 1200 mm) in 0.1 M sodium sulfate. The concentrated hydrolysate (2 ml) was added to the column, and the rate of elution was 7 ml/h. The eluate was examined by the phenol-sulfuric acid method (Fig. 1). The diuronide fractions were examined by paper electrophoresis [3] and chromatography in pyridine:ethyl acetate:acetic acid:water (5:5:1:3).

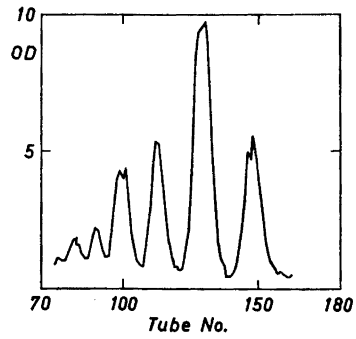


Fig. 1. Elution curve of hydrolysate of preparation A from Sephadex G 25 column.

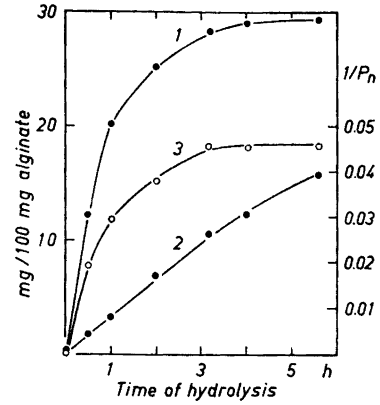


Fig. 2. Hydrolysis of alginic acid (*L. digitata*, Tarva 29/8) in 0.3 N hydrochloric acid. 1 = Soluble material, 2 = Reducing power of the hydrolysate, 3 = 1/P*n* of insoluble fraction.

RESULTS

a) *Hydrolysis in 0.3 N hydrochloric acid.* In the experiments described in the previous paper [1] the hydrolysis was always carried out in 1 M oxalic acid. In order to establish whether hydrolysis follows the same course in mineral acid, hydrolysis was carried out in 0.3 N hydrochloric acid (giving approximately the same pH as 1 M oxalic acid). The results, given in Fig. 2, exhibit the same general features as observed earlier, with a limit of hydrolysis of approximately 30 % and a rapidly decreasing rate of depolymerization of the insoluble phase.

In the following experiments, 0.3 N hydrochloric acid was used for the hydrolysis.

b) *Choice of electrophoretic medium.* The electrophoretic mobility u of a macromolecule in an electric field of field strength E is determined by the ratio between the effective charge of the molecule q and its frictional coefficient f, thus

$$u = Eq/f$$

The substances we want to separate contain equivalent amounts of carboxyl groups and differ only with respect to uronic acid composition. In order to obtain a separation by electrophoresis, a medium must be chosen where the effective charge of the molecule depends on its composition. It is earlier established [7,2] that the selectivity for calcium in a calcium-sodium ion exchange process depends on the uronic acid composition of the molecule. It has also been demonstrated [8] that calcium forms ion pairs with the carboxyl groups of alginate to a larger extent than sodium ions. When the proportion of calcium to sodium ions increases above a certain level, the alginate solution forms a

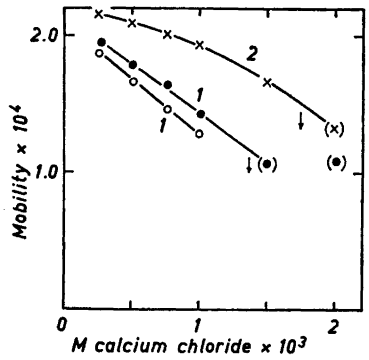


Fig. 3. Ascending mobility of alginate fractions in 0.05 N NaCl containing varying amounts of $CaCl_2$. 1 = Preparation C; 2 = Preparation B. The arrows indicate precipitation or gel formation.

gel. In order to keep the tendency for gel formation as low as possible, a low ionic strength was used.[9] Experiments were carried out with alginate fractions with very different uronic acid compositions; preparation B contained 92 % mannuronic acid while preparation C contained 13 % mannuronic acid (*cf.* Experimental). Sodium chloride (0.05 M) containing various amounts of calcium chloride was used as the electrophoretic media. In all cases the descending boundaries rapidly spread and became too diffuse to allow a correct determination of mobility. Accordingly, as the ascending boundaries were very sharp, they were used to calculate an apparent mobility. The results are given in Fig. 3. Preparation B gave rise to one sharp ascending peak only, while preparation C gave two peaks, both with a mobility lower than that of preparation B.

Based on the results given in Fig. 3, a medium consisting of 0.05 M sodium chloride and 0.00075 M calcium chloride was chosen as a standard medium for the electrophoretic experiments.

c) *Electrophoretic examination of the hydrolysis products.* The products obtained by heterogeneous hydrolysis of alginate in 0.3 N hydrochloric acid

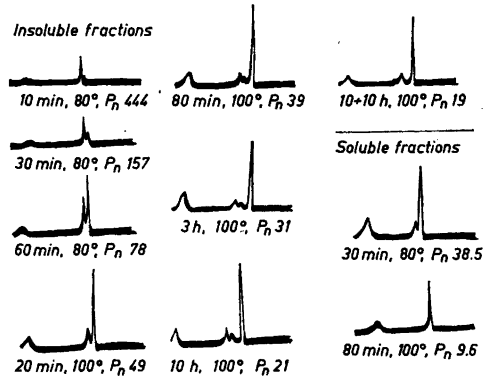


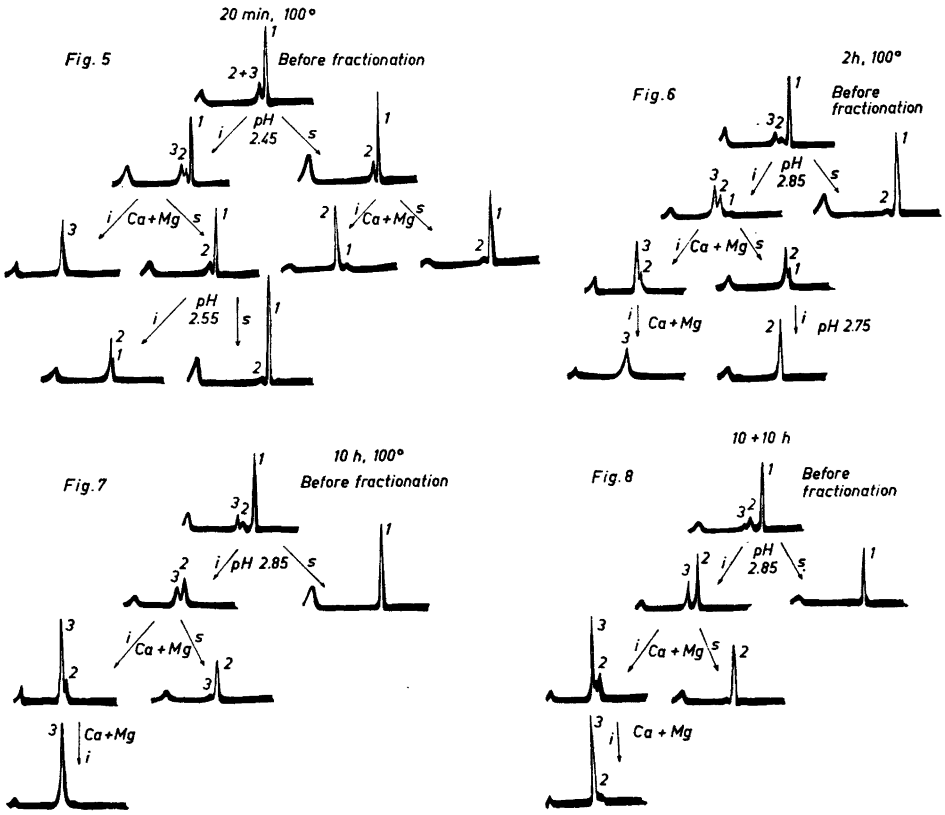
Fig. 4. Ascending pattern of alginate subjected to different degrees of hydrolysis.

Table 2. Electrophoretic examination of alginate hydrolysed in 0.3 N HCl. The degree of polymerization given (P_n) corresponds to the non-dialysable part of the fraction. Amounts given as percentage of total alginate sample.

Hydrolysis	Insoluble fraction							Soluble fraction					
	Amount %			Ascending peaks				Amount %			Ascending peaks		
	Total	Non-dialysable	P_n	No.	Mobilities, $u \times 10^4$			Total	Non-dialysable	P_n	No.	Mobilities, $u \times 10^4$	
80° 10 min	99.6	99.6	444	1			1.46						
20 »	98.7	98.7	200	2	1.53		1.45						
30 »	97.3	97.3	157	2	1.56		1.45	1.9	1.6	38.5	2	1.47	1.34
60 »	96.2	96.2	78	2	1.65		1.49	1.9	1.9	28.5	2	1.67	1.45
120 »	93.0	93.0	59	2	1.75		1.58	3.2	2.5	15.0	2	1.63	1.45
100° 20 »	90.0	84.0	53	2	1.70		1.54	9.6	8.9	16.0	2	1.60	1.42
40 »	83.0	70.0	52	2	1.79		1.55	7.4	4.4	15.5	2	1.80	1.42
80 »	77.0	60.0	39	3	1.73	1.54	1.44	6.0	3.0	9.6	1	1.80	
120 »	74.0	57.0	38	3	1.78	1.53	1.45	3.3	–				
100° 2 h	70.0	61.0	37	3	1.73	1.52	1.37	29.2	0				
3 »	66.0	62.0	31	3	1.80	1.60	1.40	3.8					
4 »	64.0	57.7	32	3	1.82	1.60	1.43	2.2					
6 »	61.6	53.5	27	3	1.87	1.60	1.43	2.2					
100° 10 »	60.9	44.8	21	3	1.77	1.48	1.35	32.4	0				
100° 20 »	53.4	44.2	22	3	1.85	1.53	1.40	31.9	0				
100° 10+ 10 h	43.6	37.2	19	3	1.82	1.53	1.37	33.8+ 15.1	0				

were examined by free boundary electrophoresis. The products in the soluble and the insoluble phases were examined separately. In order to obtain maximum yield of non-dialysable products, the entire soluble phase was removed at intervals, and fresh 0.3 N hydrochloric acid added to the insoluble phase after a small sample of the latter had been removed for examination. The results are given in Table 2, and some of the ascending electrophoretic patterns are shown in Fig. 4.

d) *Fractionation of degraded alginate.* The results given above demonstrate that the alginate molecule is split into components with different electrophoretic mobility by heterogeneous acid hydrolysis. Attempts were made to isolate these components by fractionation and to determine the uronic acid composition and the average degree of polymerization of electrophoretically pure fractions. Samples of alginate, degraded at 100°C in 0.3 N hydrochloric acid for 20 min, 2 h, 10 h, and 10 + 10 h, were fractionated. The



Figs. 5—8. Ascending electrophoretic patterns of fractions prepared from the insoluble part of alginate hydrolysed at 100° in 0.3 N HCl for 20 min, 2 h, 10 h, and 10 + 10 h. s and i refers to soluble and insoluble fractions, respectively, and the peaks are numbered from 1 to 3 with decreasing mobility.

Table 3. Yield and composition of fractions obtained from alginate (containing 61 % mannuronic acid) after varying degrees of hydrolysis.

		20 min, 100°	2 h, 100°	10 h, 100°	10 + 10 h, 100°
Soluble material	Yield, % of total	9.6	27.2	35	43.2
	% mannuronic acid	59	67	66	68.6
	P_n	12	4	1.8	
Insoluble, non-dialysable					
Peak I	Yield, % of total	47.2	34.4	26	13.7
	% mannuronic acid	80.0	86.5	89	92
	P_n	45	35	30	15.1
Peak II	Yield, % of total	14.2	7.4	10.4	12.5
	% mannuronic acid	30.6	21.8	13.8	6.3
	P_n	35	20.0	19	17.5
Peak III	Yield, % of total	16	11.6	6	2
	% mannuronic acid	22	12.3	7.8	9.1
	P_n	90	70	57	32

samples were first fractionated by precipitation with acid; in most cases a pH of about 2.8 was found convenient. The soluble fraction at this pH contained mainly, or exclusively, the component responsible for the fastest moving peak. The insoluble fraction gave two peaks by electrophoresis, and the components responsible for the two peaks were separated by fractional precipitation with calcium ions in the presence of magnesium ions. The component corresponding to the slowest moving peak was precipitated by this method, while the component giving the peak with intermediate mobility was soluble. A detailed example of a fractionation experiment is given in the Experimental section. The general trend of the fractionation of each of the four degraded alginates is illustrated by the ascending patterns of the fractions in Figs. 5—8.

The uronic acid composition and the number average degree of polymerization were determined for the electrophoretically pure fractions, and the results are given in Table 3. The yields of the fractions were estimated and are also given in the table.

As shown in Fig. 3, the non-dialysable part of the fraction which passes into solution during hydrolysis, also gave two peaks on electrophoresis. A sample of this material, isolated after 20 min at 100°C, was fractionated with calcium and magnesium. The ascending patterns of the fractions are shown in Fig. 9, and the composition and number average degree of polymerization are given in Table 4.

Table 4. Fractionation by precipitation with calcium and magnesium of the non-dialysable material soluble by hydrolysis for 20 min at 100°.

	Before fractionation	Soluble	Insoluble
Yield, % of total	8.9	7.9	1.0
% Mannuronic acid	59	60.6	58.5
P_n	16	15	40

e) *Homogeneous acid hydrolysis.* The results described above were all obtained by heterogeneous hydrolysis. It is possible that in this case all bonds are not equally accessible, *i.e.* some bonds are more or less protected against hydrolysis. The results previously published as well as curve 3 in Fig. 2 show that this really is the case; the insoluble fragments formed during the first part of the hydrolysis were further hydrolyzed only at a very low rate.

In order to determine whether a partial protection of the molecule is essential for the hydrolytical splitting of alginate into components with different uronic acid compositions, alginate was degraded by homogeneous hydrolysis, and samples were investigated by free boundary electrophoresis. The degradation was carried out at pH 3.7, where alginate is completely soluble,[10] and where the rate of hydrolysis still is considerable.[11] The ascending patterns together with the number average degree of polymerization is given

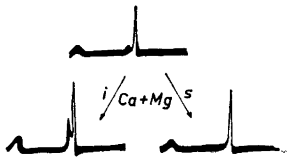


Fig. 9. Fractionation of the non-dialysable part of the material present in the hydrolysate after 20 min, 100° in 0.3 N HCl.

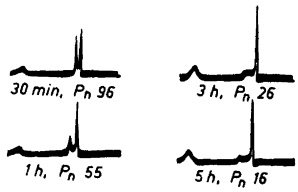


Fig. 10. Homogeneous hydrolysis at pH 3.7, 100°. Ascending boundaries.

in Fig. 10. The results show that the alginate molecule is split into fragments with different mobilities, also by homogeneous hydrolysis.

f) *Investigation of the diuronides.* The fraction of the alginate sample which is dissolved in the hydrolysate after 20 min has approximately the same uronic acid composition as that of the total alginate (Table 4), and the same applies to the main component of this fraction isolated by fractionation with calcium and magnesium, and shown to be electrophoretically pure (Fig. 9). This result shows that fragments are formed during the hydrolysis of alginates which contain both uronic acids in the same molecule in approximately equal amounts. However, the results do not imply anything about the sequence of the two uronic acid residues in these fragments. Attempts were made to obtain some information on this point by examination of the diuronides obtained by partial hydrolysis.

Preparation A, B, and C were hydrolysed as described in the Experimental section, and the diuronide fractions isolated by column chromatography on Sephadex G 25 in 0.1 M sodium sulfate. The diuronides from each of the three preparations were subjected to paper electrophoresis and chromatography, and the results are given in Table 5, where the mobilities are given relative

Table 5. Diuronides prepared from alginate fractions.

	P_n of hydrolysate	Yield of diuronides	Composition of diuronide fractions: Chromatography R_g: 0.26	0.32	Paper-electrophoresis M_m: 1.18	1.38
Prep. A	2.1	30	×××	×	×	×××
» B	3.3	8.5	(×)	×××	×××	(×)
» C	2.6	14	×××		×××	

to that of glucose and mannuronic acid by chromatography and paper electrophoresis, respectively.

DISCUSSION

Alginic acid is a linear polysaccharide composed of two different monomers, which to some extent, are linked together in the same molecule.[12] For most linear polysaccharides containing two different monomers, the distribution of the monomers along the polymer chain is described as alternating or random. Previous work has shown that the distribution of monomers in the alginate molecule must be of a different kind, being characterized by long sequences of the same type of uronic acid residue. This unusual monomer distribution has been further studied by free boundary electrophoresis.

The medium used for the free boundary electrophoresis is chosen to give a different effective charge, and consequently a different mobility for molecules with different chemical composition. A splitting of the boundary into separate peaks should, therefore, indicate that the sample contains fractions with different chemical compositions, or, possibly, with different degrees of polymerization. That this really is the case has been demonstrated by fractional precipitation and examination of the fractions by electrophoresis and chemical analysis. Even if the appearance of more than one peak in the electrophoretic pattern may be taken as evidence for chemical heterogeneity, the presence of only one peak is not a proof of chemical homogeneity. This may be seen by inspecting the electrophoretic pattern of the insoluble fraction of the alginate after 20 min at 100°C in Fig. 5, where only two peaks were visible before fractionation, while the presence of a third peak was revealed by electrophoretic examination of the fractions. Even when no further splitting of a peak is observed when the material is subjected to fractionation, a certain degree of chemical heterogeneity may be expected for material giving rise to only one peak. It should, therefore, not be assumed that the composition of the electrophoretically pure fractions, given in Table 2, represents the composition of chemically homogeneous entities.

It should also be emphasized that the severe lack of enantiography makes an interpretation of changes in mobilities doubtful, and an attempt to estimate the amounts of the different fractions by inspection of the peaks in the complex pattern of the unfractionated material may be quite misleading. Another limitation of the use of the electrophoretic method is due to gel formation. Undegraded alginate is impossible to examine by this method.

Even when these limitations of the method are taken into account, the electrophoretic examination of the hydrolytic products of the alginate can give information about the course of the acid hydrolysis of alginate and of the constitution of the alginate molecule. Fig. 4 and Table 2 demonstrated that only very slight degradation was sufficient to lead to a splitting of the molecule into chemically different components. A clear separation into two components was observed after hydrolysis at 80°C for 20 min. The number average degree of polymerization of this material was 200, and less than 2 % of the total alginate was dissolved in the hydrolysate.

Electrophoretic examination of the products formed by homogeneous hydrolysis demonstrated that splitting of the molecule into chemically different components also occurred in this case. It is reasonable to assume that no part of the alginate molecule is protected against hydrolysis in homogeneous

solution. Therefore, this observation indicates that random degradation of alginate also leads to a splitting of the alginate molecule into chemically different fractions.

After 20 min of heterogeneous hydrolysis at 100°C, the degree of polymerization of the insoluble fraction had been reduced to 53, and 10 % of the material was dissolved in the hydrolysate. The major part of this soluble material was an electrophoretically homogeneous material with an average degree of polymerization of 16 and an uronic acid composition approximately equal to that of the whole alginate. The electrophoretic homogeneity of this material shows that the uronic acid residues must occur in the same molecules. According to the investigation of the diuronide composition, this material consisted to a large extent of alternating mannuronic and guluronic acid residues. The very high yield of diuronides obtained by hydrolysis of Preparation A (Table 5) may also be an indication of alternating structure, assuming that the two types of glycosidic bonds are broken at different rates.

The rest of the alginate molecule (90 %) was insoluble in acid and was split into three components, one containing 80 % mannuronic acid residues and the other two containing 30 and 22 % mannuronic acid residues, respectively. As the hydrolysis proceeded further, the three components in the insoluble fraction remained well defined. The degree of polymerization of each component decreased (Table 3), and the yield decreased, except for the guluronic rich fraction with the lowest degree of polymerization (Peak 2). This observation indicates that material was transferred from the guluronic rich component with a high degree of polymerization (Peak 3) to the guluronic rich component with low degree of polymerization (Peak 2). Apart from this, the main feature of the hydrolysis was a transfer of material from the insoluble fraction to the hydrolysate. The relatively small decrease in the degree of polymerization of each of the components of the insoluble phase compared with the considerable amount of material which was removed, indicates that the material was mainly removed from the chain ends of the fragments. The steady change of the uronic acid composition of the three fractions in the direction of homopolymers, shows that the material removed from the insoluble fragments during hydrolysis must have a more intermediate uronic acid composition than the rest of the fragments.

If we assume that all the guluronic acid residues in the mannuronic acid rich component (Peak 1) are placed at the chain ends as alternating mannuronic-guluronic acid residues, and vice versa for the two guluronic acid rich components (Peaks 2 and 3) the lengths of unbroken homopolymer sequences may be calculated (Table 3). The amounts of such sequences may also be estimated from the yields given in Table 3. The results are given in Table 6.

The block sizes obtained in this way were remarkably independent of the time of hydrolysis, as long as the insoluble fragments were not dissolved and reprecipitated (10 + 10 h). The transference of material between the two guluronic rich components is clearly shown in the table, and the sum of guluronic rich homopolymer blocks was almost constant throughout the hydrolysis. Apart from demonstrating the marked protection of the homopolymer fragments, the table also gives an indication of the average minimum lengths

Table 6. Hypothetical lengths and amounts of homopolymer sequences.

Time of hydrolysis	Peak 1		Peak 2		Peak 3	
	P_n	Amount	P_n	Amount	P_n	Amount
20 min	28	28.4	13.6	5.5	50.4	9.0
2 h	25.7	25.5	11.3	4.2	52.8	8.9
10 h	23.2	20.3	13.7	7.5	48.1	5.1
10 + 10 h	12.7	11.5	15.3	10.9	23.0	1.7

of the blocks in the alginate molecules, provided our assumptions are valid.

The significance of the two guluronic acid rich components is not yet understood. It is possible that the component giving Peak 3 contains some mannuronic acid residues inside the chains, and that the transference of material from this component to the component with a lower degree of polymerization is due to a splitting of the molecule at these, presumably less protected, points in the chain. In that case, the correct average length of guluronic acid blocks should be given by the component with the lowest degree of polymerization.

The results of this study thus indicate that the alginate molecule contains blocks of mannuronic acid residues, blocks of guluronic acid residues, and at the same time a fraction with a predominantly alternating structure. It is possible, however, that no sharp distinction exists between the fractions with mainly alternating structure and the homopolymer blocks: that the former rather should be regarded as composed of short "blocks" and that "blocks" of intermediate sizes also occur in the molecule. More information is needed about the structure of the material which is dissolved in the hydrolysate.

In our previous paper we presented some evidence for the guluronic and mannuronic blocks being in the same molecule, and this is further confirmed by the results in this work, where the splitting of the molecule into chemically different components has been observed electrophoretically. Whether the fragments containing long sequences of predominantly alternating mannuronic-guluronic acid residues are also in the same molecules, is less clear, but some evidence will be presented in the subsequent paper which indicates that this is the case.

The characterization and description of the structure of a linear heteropolysaccharide like alginate, where the distribution of monomers is neither random nor alternating, is a problem which is not yet adequately resolved. A description of such a structure must be given by means of a probability function. Related problems are encountered in the field of synthetic copolymers. Recently, Painter [13] introduced a statistical method for a general description of monomer distribution in linear polysaccharides containing two monomers. Attempts will be made to apply Painter's model to the structure of alginate.

It is well known for synthetic copolymers that the distribution of two different monomers along the polymer chain is important for the properties of the polymers. Block polymers have properties significantly different from polymers with the same overall composition, but with a predominantly alternating structure. It is possible that for polysaccharides the properties also depend on the distribution of monomers. Attempts to correlate monomer sequence and properties of alginates will be reported in a subsequent paper.

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