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THE RELATIVE EXTENSION OF ALGINATES HAVING DIFFERENT CHEMICAL COMPOSITION*

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ABSTRACT

From light-scattering and viscosity experiments, it was found that the relative extension of the three types of "blocks" in alginate increased in the order "MG-blocks"<"MM-blocks"<"GG-blocks", both in 0.1M aqueous sodium chloride and in the unperturbed state. From a comparison with calculated free-rotation dimensions, it was concluded that this was due to a difference in the hindrance to rotation around the glycosidic linkage in the different blocks. Calculations by statistical mechanics, using Kitaygorodsky potential functions for the non-bonded interaction between atomic groups in adjacent dimers, yielded unperturbed dimensions which were in agreement with the above order, only when the L-guluronic acid residue was assumed to adopt the *1C* ($^1C_4$) conformation.

INTRODUCTION

Previous experimental work on the solution properties of sodium alginate[1,2] has mostly dealt with one alginate sample, prepared from *Laminaria digitata*, with a ratio for D-mannuronic and L-guluronic acid residues of 1.6. This sample has been shown to behave in aqueous sodium chloride solution as a very extended coil at all ionic strengths. Extrapolation of its molecular dimensions to infinite ionic strength yielded a high value of the Kuhn-segment length ($A_m = 155$ Å). By using a modification of the Burchard–Stockmayer–Fixman extrapolation procedure[3,4], the unperturbed dimensions were also estimated to be high [$(A_m)_\theta = 135$ Å]. This result suggested that the high extension of the uncharged alginate molecule was mainly due to a high degree of mechanical inflexibility in the polymer chain. A further understanding of the solution properties of alginate requires a knowledge of the flexibility of the different types of monomer sequence in alginate. It has been shown[5–8] that alginate contains long, homopolymeric blocks of each monomer, together with

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blocks of the alternating sequence. We have now investigated alginate samples containing different amounts of the three types of structure, and have attempted to estimate their relative extension in the unperturbed state. The results are discussed in terms of the geometry of, and the hindered rotation in, the alginate chain. It seems quite certain, from X-ray data[9,10] and from conformational energy estimations by Angyal[11] and Vijayalakshmi and by Rao[12] on β-D-mannose, that the most-probable ring-conformation of the D-mannuronic acid residues is the *C1* form ($^4C_1$). The L-guluronic acid residues in alginate have been shown to exist in the *1C* conformation in the crystalline acid-form[9,10]. This conformation is also suggested to predominate in crystalline potassium and ammonium polyguluronate[13]. N.m.r. results[14] and conformational energy estimations[11,12] indicate that the *1C* conformation is the most stable form of α-L-gulose, but the conformation of L-guluronic acid residues in the alginate chain will depend on the relative desirability of having a carboxyl group or the glycosidic linkages equatorial. The conformation in aqueous sodium chloride must therefore be regarded as uncertain, and we are here trying to explain the observed difference in flexibility among the different types of structure by letting the L-guluronic acid residues adopt either the *1C* or the *C1* conformation.

MATERIALS AND METHODS

Experimental methods. — The alginates were prepared as previously described from *Laminaria digitata*[15], *Laminaria hyperborea*[15], and from the receptacles of *Ascophyllum nodosum*[16]. Some alginates were degraded[17] at pH 4 to give samples of different molecular weights. Some acid-soluble alginate fractions were prepared by fractional precipitation at pH 1.4 of some slightly degraded alginate samples[18] from *Ascophyllum nodosum*. The alginate samples were characterized with regard to chemical composition[15] and sequence by fractional precipitation, after heterogeneous hydrolysis, as described by Haug *et al.*[16].

Viscosity measurements were made at 20.0° in a Zimm–Crothers Model A, low-shear, rotating-cylinder viscometer, as described previously[2,19]. Sodium chloride was, in all cases, used for adjusting the ionic strength.

Light-scattering experiments were carried out as described previously[1].

Theoretical methods. — The co-ordinates of the monomers were derived from the idealized xylose co-ordinates given by Settineri and Marchessault[20], by suitable epimerisations and by addition of a carboxyl group at C-5. Bridge parameters used were those obtained by Chu and Jeffrey[21] for cellobiose, except that, in some cases, the bridge angle was varied in the range 115–117°.

For a given set of dihedral angles about C-1–O, O–C-4′ (see Fig. 1 in the work of Whittington[22]), and, in some cases, the C-5–C-6 bonds, the co-ordinates of the dimer in this conformation were derived from the monomer co-ordinates by suitable rotations and translations.

A given conformation was classified as allowed when all inter-atomic distances between atoms in the two monomers were greater than the prescribed values and forbidden when at least one such distance was less than the prescribed value. The parameters given by Rees and Skerrett[23] were used, except that the carboxyl group was treated as a single, bulky atom as described elsewhere[22]. The parameters are given in Table I of ref. 22.

The energy of each conformation was calculated by using the Kitaygorodsky potential[24] for interactions between atoms in the two monomers. Rotation of the carboxyl group about the C-5–C-6 bond was included so that each oxygen atom in the carboxyl group was considered explicitly. The Kitaygorodsky parameters used are given in Table I of ref. 22.

From an examination of molecular models, it appears that, to a good approximation, the energy of a short oligomer can be written as the sum of the energy of the dimers. That is, rotation about one pair of glycosidic bonds is almost independent of rotation about the neighbouring pair of glycosidic bonds. If, in a given polymer, the monomer conformation is fixed, the distance between adjacent pairs of bridge oxygen atoms is fixed. Under these assumptions, the treatment of the conformational statistics is staightforward and we followed an approach described elsewhere[25], taking explicit account of the rotation of the carboxyl group about the C-5–C-6 bond. The free-rotation dimensions of the alternating structure were calculated by the same[25] technique, by letting all the possible conformations of the dimer have the same energy.

RESULTS AND DISCUSSION

Estimation of the relative extension of different alginates by light-scattering measurements

The weight-average molecular weight, M_w, and the z-average radius of gyration $(R_G)_z$ were measured at ionic strength 0.1 for two alginate samples containing 70 and 10% of L-guluronic acid residues (G.A.). The results are given in Table I, together with results obtained earlier on a sample containing 38.5% of G.A. The main result in Table I is that the three samples have a high and very similar degree of extension. It is clear, however, that the sample rich in G.A. residues is somewhat more-extended than the sample of intermediate composition. The difference between the sample rich in D-mannuronic acid residues and that of intermediate composition is doubtful. Knowing that the sample of intermediate composition contains homopolymeric blocks of L-guluronic acid and D-mannuronic acid residues having, respectively, a higher and the same degree of extension as the total sample, the presence of the alternating sequence seems to reduce the total extension of the alginate molecule. The above results therefore suggest that the relative extension (at ionic strength 0.1) of the three types of sequence increases in the following series: "MG-blocks"<"MM-blocks"<"GG-blocks".

Very recently, Brucher *et al.*[26] measured the molecular weight, the radius of gyration, and the intrinsic viscosity, at ionic strength 0.1, of two alginate fractions having different chemical composition. Their finding, that the fraction having the highest content of L-guluronic acid had the most-extended conformation, agrees with the above results. They could, however, not decide if this was due to the "MG-blocks" or the "MM-blocks", or both, being less-extended than the "GG-blocks".

TABLE I

WEIGHT-AVERAGE MOLECULAR WEIGHT, M_w, AND z-AVERAGE RADIUS OF GYRATION $(R_G)_z$ FOR ALGINATE SAMPLES OF DIFFERENT CHEMICAL COMPOSITION[a]

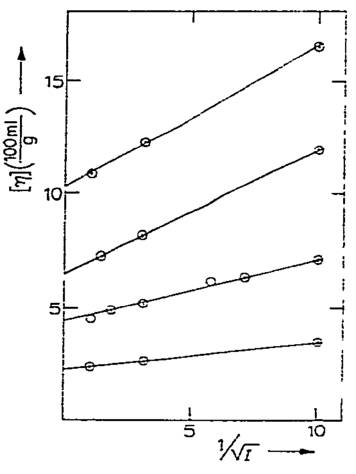
Alginate	L-*Guluronic acid residues* (%)	M_w	$(R_G)_z$ (Å)
Laminaria hyperborea, stipes, Hustad 4/5	70	500,000	1330
Laminaria digitata, Tarva 29/8	38.5	500,000	1050[b]
Ascophyllum nodosum, receptacles	10	180,000	730
Laminaria digitata, Tarva 29/8	38.5	180,000	650[b]

[a]Solvent: 90mM sodium chloride and 10mM sodium fluoride in water. [b]Average values taken from Fig. 7 of ref. 1.

Determination of B-*values*

It is not possible from the above results to deduce if the difference in the relative extension of the three types of blocks is due to a difference in their unperturbed dimensions or to a difference in their expansion caused by the solvent or by electrostatic interaction in the polyelectrolyte chain. The use[27] of the second virial coefficient, as determined by light scattering, as a measure of the expansion due to solvent or electrostatic interaction, and calculation of the unperturbed dimensions, is very hazardous in the present case. This is due to the presence in alginate of cell particles which limit the accuracy of the determination of the second virial coefficient[1]. Recently, however, a method was developed[19] that allowed the estimation of the relative, unperturbed dimensions of the molecular chain in polyelectrolytes from measurements of the intrinsic viscosity at different ionic strengths. A parameter B, which is the value of the slope, S, of the straight line relating the intrinsic viscosity to the inverse square root of the ionic strength for the case when the intrinsic viscosity is 1.0 (100 ml/g) at ionic strength $I = 0.1$, was shown to be inversely related to accepted parameters for chain extension.

Samples of different molecular weights of the two alginates used in the preceding paragraph and of an acid-soluble alginate (known to contain a high proportion of the alternating sequence[18]) were used for the determination of B-values. The intrinsic viscosity, $[\eta]$, was determined for a series of ionic strengths between 0.01 and 1.0. The plotting of $[\eta]$ against $1/\sqrt{I}$ resulted, in all cases, in straight lines, as shown for the sample rich in guluronic acid residues in Fig. 1. Corresponding values of S and the intrinsic viscosity at $I = 0.1$, $[\eta]_{0.1}$, are given for all the samples in Fig. 2. Curves 1–4 in the figure are results presented earlier for polyphosphate, polyacrylate, alginate from *Laminaria digitata*, and native DNA, respectively. It is seen that the difference between the different alginate samples is relatively small, but it is significant that the sample rich in guluronic acid has lower, and the sample rich in the alternating sequence has higher, *S*-values than the sample from *Laminaria digitata*. The sample rich in mannuronic acid is not significantly different from the *L. digitata* sample.



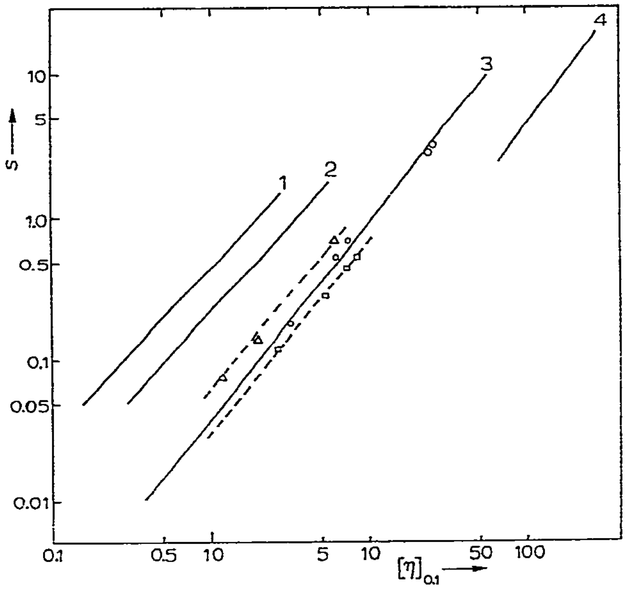


Fig. 1. The intrinsic viscosity as a function of the reciprocal square root of the ionic strength for four alginate samples containing 72.5% of L-guluronic acid residues.

Fig. 2. The ionic-strength dependence of $[\eta]$ plotted against the intrinsic viscosity in 0.1M sodium chloride solution. △, Acid-soluble alginate fraction, 35.5% of L-guluronic acid residues (G.A.); ○, alginate from *Ascophyllum nodosum* receptacles, 9% of G.A; □, alginate from *Laminaria hyperborea*, 72.5% of G.A. Curves 1–4 are results given previously[19] for polyphosphate, polyacrylate, alginate from *Laminaria digitata*, and double-stranded DNA, respectively.

The *B*-values for the four alginates are given in Table II, together with fractionation results[16] after heterogenous hydrolysis in 0.3M hydrochloric acid. The solubilized material (S_{2h}) after degradation for 2 h is very rich in the alternating sequence. The part of the insoluble material (I_{2h}) soluble at pH 2.85 ($S_{2.85}$) contains ~90% D-mannuronic acid residues, whereas the corresponding insoluble material ($I_{2.85}$) contains ~90% of the L-guluronic acid residues. The amounts of these fractions may, therefore, be regarded as a relative measure of the three types of blocks in alginates. In Table II, values are also given for K_θ, $(A_m)_\theta$, and C_∞. The values of K_θ are obtained from the empirical correlation curve between B and K_θ

(Fig. 4 of ref. 19). The Kuhn statistical segment-lengths, A_m, are calculated from the equation:

$$A_m = \left[\frac{K_\theta}{\Phi}\right]^{2/3} \cdot \frac{M_0}{b_0}, \tag{1}$$

where Φ is the Flory viscosity constant (assumed to be 1.75×10^{21} (ref. 2), M_0 is the monomer weight, and b_0 is the projection along the fibre axis of the monomer length, as obtained from X-ray diffraction studies. The characteristic ratio, C_∞, is defined by the equation

$$C_\infty = \lim_{n\to\infty} \frac{(\overline{r^2})\theta}{n \cdot l^2}, \tag{2}$$

where n is the degree of polymerization, l is the virtual bond-length (the distance between adjacent bridge oxygens[22]), and $\overline{r^2}$ is the mean-square end-to-end distance. The C_∞-values were calculated by the equation[22]

$$C_\infty = (A_m/l) - 1\,. \tag{3}$$

The magnitudes of A_m and C_∞ are dependent on b_0 and l, which are different in the two conformations, and they were consequently calculated under the assumptions of both *C1* and *1C* conformations of the L-guluronic acid residues.

By comparing the fractionation results in Table II with the parameters for chain extension, it is seen that the relative, unperturbed dimensions increase in the order: "MG-blocks"<"MM-blocks"<"GG-blocks". This order is the same as that found from the light-scattering results at ionic strength 0.1.

Before attempts are made to explain this order of flexibility on the molecular level, the extent to which the unperturbed dimensions depend on any specific solvation of the monomer residues should be questioned. Attempts have therefore been made to compare the intrinsic viscosity at infinite ionic strength in different solvents. Because of the low solubility of alginate in most solvents, the comparison has been limited to water, 8M aqueous urea, and 20% aqueous ethanol. The intrinsic viscosities of an alginate sample from *L. digitata* at different ionic strengths are given in Fig. 3. It is seen that the intrinsic viscosity at infinite ionic strength is the same in each solvent. Although water is a major part of each solvent, large proportions of urea or ethanol should affect the intrinsic viscosity at infinite ionic strength if the unperturbed dimensions were critically dependent on any specific interaction between water and alginate. The lack of such an effect suggests that the explanation for the difference in flexibility among the different "blocks" in alginate should be looked for within the polymer chain. This conclusion is further supported by recent near-infrared and n.m.r. results[26] on alginate solutions where no specific interaction between alignate and water was observed.

The unperturbed dimensions of polysaccharides are, in general, dependent on the geometry of the monomer units and the freedom of rotation around single bonds in the chain[25].

TABLE II

CHARACTERIZATION OF MONOMER COMPOSITION AND SEQUENCE, AND OF THE EXTENSION, OF DIFFERENT ALGINATE SAMPLES

Sample	L-*Guluronic acid* (%)	S_{2h} "*MG-blocks*"	I_{2h}		B	K_0 ($\times 10^3$)	A^a		B^a	
			$S_{2.85}$ "*MM-blocks*"	$I_{2.85}$ "*GG-blocks*"			$(A_m)_0$ (Å)	C_∞ (Å)	$(A_m)_0$ (Å)	C_∞ (Å)
Ascophyllum nodosum, receptacles	9	9.1	79	8	0.040	11	130	23	132	24
Laminaria hyperborea, stipe, Hustad 4/5	72.5	25	0	75	0.031	15	155	27	175	37
Laminaria digitata, Tarva 29/8	38.5	30	41	29	0.040	11	130	23	138	27
Acid-soluble alginate fraction	35.5	65	25	10	0.065	5.8	95	16	99	19

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[a] *A*, Calculated from K_0 by using equations 1 and 3 of the text, assuming that $b_0 = 5.15$ Å (Ref. 9) and $l = 5.5$ Å (Ref. 22), *i.e.* that all the uronic acid residues are in the *C1* conformation. *B*, Calculated from K_0 by assuming that $b_0 = 5.15$ Å and $l = 5.5$ Å for the mannuronic acid residues, and $b_0 = 4.35$ Å (Ref. 10) and $l = 4.35$ Å (Ref. 22) for the guluronic acid residues (*1C* conformation).

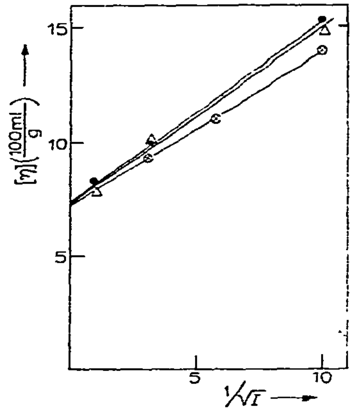

Fig. 3. The intrinsic viscosity as a function of the reciprocal square root of the ionic strength. ●, 8M Aqueous urea; △, water; ⊗, 20% aqueous ethanol.

Effect of monomer geometry on the dimensions of freely rotating chains

Yathindra and Rao[28] have calculated the characteristic ratio, C_f, for the case of free rotation around the two single bonds of different types of glycosidic linkages. They assumed complete rigidity of the sugar rings and constancy of the bond angle at the oxygen of the glycosidic linkage. According to Macki[29], this angle is between 116° and 117° in crystalline polymannuronic and polyguluronic acids. No angles have been reported for the alternating sequence, but Arnott and Scott[30], who have done a literature survey of X-ray diffraction data on (1→4)-linked polysaccharides, state that the average value of this angle is 116.5°, with a range from 115.7° to 117.6° and a standard deviation of 0.7°. In Table III are given calculated C_f-values with two bond

TABLE III

THE CHARACTERISTIC RATIO, C_f, FOR POLYSACCHARIDES CALCULATED WITH THE ASSUMPTION OF FREE ROTATION AROUND THE SINGLE BONDS OF THE GLYCOSIDIC LINKAGE; DIFFERENT VALUES FOR THE BOND ANGLE, θ, AT THE GLYCOSIDIC OXYGEN ATOM

Type of linkage[a]	(1e, 4′e)	(1a, 4′a)	*Alternating* [(1e, 4′a) (1a, 4′e)]
Corresponding dimer residue in alginate[b]	M(*C1*)−M(*C1*) G(*C1*)−G(*C1*) *etc.*	G(*1C*)−G(*1C*)	[M(*C1*)−G(*1C*) G(*1C*)−M(*C1*)]
C_f ($\theta = 116°$)[c]	2.44	1.91	
C_f ($\theta = 117°$)[d]			2.14
C_f ($\theta = 117.5°$)[c]	2.58	2.00	

[a] The nomenclature used for characterisation of the different types of linkage is the same as that used by Yathindra and Rao[28]. [b] The abbreviation is such that M(*C1*)−G(*1C*) means D-mannuronic acid in the *C1* conformation, and L-guluronic acid in the *1C* conformation, with L-guluronic acid at the reducing end. [c] Data taken from ref. 28. [d] Own calculation.

angles inside this range for the possible types of linkage in alginate with D-mannuronic acid in the *C1* conformation and L-guluronic acid in the *1C* conformation. All the linkages would be diequatorial if the L-guluronic acid residues adopt the *C1* conformation, and the free rotation would obviously then be identical for the different type of monomer sequence, as indicated in the first column of Table III.

Table III shows that the different types of linkage give free-rotation dimensions that differ only slightly. The steric factor, σ, defined by the equation[28] $\sigma = (C_\infty/C_f)^{1/2}$, is seen, by comparison with the experimental results in Table II, to be high (2.7–4.3) and to increase in the order poly(MG) < poly(M) < poly(G). It is therefore a difference in the hindrance to rotation around the glycosidic linkages that causes the differences in unperturbed dimensions.

Estimation of the hindered rotation around the glycosidic linkages

Ramachandran *et al.*[31] introduced the "hard sphere" calculation for polysaccharides as a very simple, but very approximate, way of estimating the hindered rotation around the glycosidic linkages. In this type of calculation, complete rigidity of the rings and constancy of the bond angle at the glycosidic oxygen are assumed. The rotational freedom about the two single bonds of the glycosidic linkage is investigated by stepwise rotation in a computer. The number of rotational isomers which are free from steric interaction (overlapping of van der Waal's radii of atoms from the two adjacent rings) is listed and compared with the total number of isomers (with steps of 10°, the total number is $36^2 = 1296$). We have carried out hard-sphere calculations for the different possible dimer residues in alginate. The results are given in Table IV. In addition to the details of the assumption given in Table IV, it should be mentioned that the carboxyl group is treated as a compact sphere having a diameter which is the mean between the maximum and minimum "diameter" of the group as a function of rotation[22].

TABLE IV

ESTIMATION OF THE NUMBER OF "FULLY ALLOWED" CONFORMATIONS[a] IN DIMERS

Dimer residue	*MM*	*MG*	*GM*	*GG*
Both M and G in *C1* conformation	14	42	14	42
M in *C1* and G in *1C* conformation	14	22	2	7

[a] This term is taken from the work of Rees and Skerrett[23] and refers to the choice of distances between pairs of atoms which are "allowed", *i.e.* which do not lead to interaction; a discussion of the coordinates used in the calculation is found in the work of Whittington[22]. Total number of rotational isomers is $36^2 = 1296$. The bond angle at the linking oxygen was assumed to be 117°.

Two main points emerge from the results in Table IV. First, the number of "fully allowed" conformations is extremely low, compared to the total possible number. This is in agreement with the large σ-values obtained above. Second, the observed higher stiffness of "GG-blocks" than "MM-blocks" can be rationalized by these calculations only when it is assumed that the L-guluronic acid residues exist in the *1C* conformation. Since the alternating block contains both the MG and the GM residue, and since Table IV suggests that the "local flexibility" in these two dimers is widely different, it is not possible by these calculations to predict the overall flexibility of the alternating block.

Calculation of unperturbed dimensions, assuming separability of conformational energies

In this theory, which is described in detail by Flory[32], knowledge of chain geometry, as obtained by X-ray diffraction, is utilized together with knowledge of the potential energy of interaction between pairs of atoms, as obtained by the study of small molecules in the vapour phase. Rotations around single bonds are performed by using a computer, with calculation of the energy of interaction between adjacent monomers as a function of the torsion angles. The dimensions corresponding to the statistical mechanical average over all the dihedral angles are then calculated. Since only near-neighbour interactions are considered, unperturbed dimensions are obtained in this way. A large number of assumptions are involved in this theory, but despite this, it has been very successful in reproducing data for relatively non-polar vinyl polymers in non-polar solvents (θ-solvents)[32].

The theory is currently being used on polysaccharides. Although its application here seems more doubtful because of the polar nature of both polymer and solvent, the results obtained so far on amylose[33,34] and cellulose[35] have been very valuable in understanding their solution properties.

We have calculated C_∞-values for the three types of monomer sequence in alginate by assuming the *C1* conformation for the D-mannuronic acid residues and both *C1* and *1C* conformations for the L-guluronic acid residues. The results are given in Table V. Kitaygorodsky potential functions[24] were used in these calculations. The carboxyl group was treated by including rotation about C-5–C-6, and the bridge angle was varied from 115° to 117°. The details of the calculations are given in "Methods".

The results in Table V give a quantitative picture of the same trends as observed from the "hard-sphere" calculations. It is only when the L-guluronic acid exists in the *1C* conformation that the sequence dependence of C_∞ is the same as that observed

TABLE V

CALCULATED VALUES FOR THE CHARACTERISTIC RATIO, C_∞, ALLOWING FOR ROTATION AROUND THE C-5–C-6 BOND, AND USING THE KITAIGORODSKY POTENTIONAL FUNCTIONS

Bridge angle (degrees)	*Poly-man* (C1)	*Poly-gul* (C1)	*Poly-gul* (1C)	*Poly-[man*(C1) – *gul*(C1)]	*Poly-[man*(C1) – *gul*(1C)]
115	66	31	253	27	13
116	65	31	251	25	13
117	62	30	249	24	13

experimentally. The variation with bridge angle over the range given by Arnott and Scott[30] is small.

With the L-guluronic acid residues in the *1C* conformation, the calculations suggest a rather pronounced, higher stiffness of the homopolymeric blocks, and especially of the guluronic acid blocks, than of the alternating structure. At first sight, this seems to be in disagreement with the relatively small differences between the alginate samples as shown in Table II. However, Whittington[36], using a Monte Carlo method[37] for generating a monomer sequence resembling the sequences in alginate isolated from *Laminaria digitata*, showed that the blocks with the lowest C_∞-value markedly reduced the average dimensions as compared to pure polymannuronic or polyguluronic acid. Since all the alginate samples used probably contain the alternating sequence, its existence will, to a large extent, mask large differences in flexibility among the homopolymeric blocks if they were there. A more-explicit knowledge of the monomer sequence in alginates is, therefore, needed before a quantitative comparison of experimental and calculated dimensions can be made.

CONCLUSIONS

Both light-scattering and viscosity measurements indicate that the relative dimensions of the three types of building blocks in alginate increase in the order: "MG-blocks"<"MM-blocks"<"GG-blocks". This sequence may be explained by theoretical considerations about the hindered rotation around the glycosidic linkage if the L-guluronic acid residues are assumed to adopt the *1C* conformation in solution.

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