Relation between Primary Structure and Chain Flexibility of Random Coil Polysaccharides: Calculation and Experiment for a Range of Model Carrageenans

By **Edwin R. Morris,* David A. Rees,*** and **E. Jane Welsh,** Unilever Research, Colworth Laboratory, Sharnbrook, Bedford MK44 1LQ
Lawrence G. Dunfield and **Stuart G. Whittington,*** Department of Chemistry, University of Toronto, Toronto M5S 1A1, Canada

In an attempt to relate conformational features to overall chain flexibilities of polysaccharides as determined by hydrodynamic volume and (for polyelectrolytes) response to ionic strength, a series of carrageenans was derived from the same alternating copolymeric backbone (2) by controlled chemical introduction of 3,6-anhydro-D-galactose 2-sulphate residues in place of D-galactose 2,6-disulphate. This change is equivalent to replacement of residues in the $^4C_1$ chair conformation with ' stiff ' linkages to their neighbours, by those in the $^1C_4$ conformation joined by ' flexible ' linkages. Chain flexibilities are characterised in terms of the parameter B introduced by Smidsrød and Haug, which is determined from the response of intrinsic viscosity to ionic strength. Semi-empirical values are calculated for comparison by assuming a chain with fixed, standard bond lengths, angles, and ring conformations, within which motions are constrained only by van der Waals attractions and repulsions. Standard methods of configurational statistics lead to values for the Kuhn persistence length A_m, which were then converted by use of an empirical relationship into B values. Experimental evidence is given that residue replacement does indeed occur at random, as supposed in the theoretical calculations. Good agreement is found between calculation and experiment and we therefore propose a simple method for semi-quantitative prediction of statistical segment length and response of polysaccharide polyelectrolyte to salt, taking into account (i) stereochemical features which determine local freedom of rotation about glycosidic and aglycone bonds and (ii) geometry of individual sugar residues in the chain.

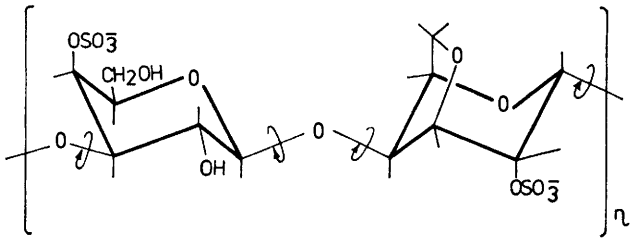
POLYSACCHARIDES as a class differ from most naturally occurring proteins and nucleic acids, not only in the chemical nature of their constituent residues, but also in the patterns in which those residues are arranged. It is common to find long regular sequences of many identical ' repeating units,' which may themselves contain any number from one to five or even more monosaccharide residues, in obvious contrast with the variable

pattern of residues in globular proteins and in nucleic acids. For many of the more interesting polysaccharides, such regular sequences are interrupted occasionally by insertion of atypical residues. When the conditions are appropriate for regions of ordered secondary structure to exist, the interruptions can terminate such ordered regions and this may represent part of their biological function. However, the influence of interruptions on the properties of polysaccharides in the random coil form is less well understood, and it is the purpose of this paper to begin to explore this problem experimentally as well as by configurational statistics. The question is important not only for polysaccharides with built-in (covalent) interruptions but also [1] for ostensibly regular polysaccharides because thermodynamic relationships between alternative ring conformations for monosaccharides are such that a minor proportion of these residues must, at any instant, exist in higher energy ring forms.

A particularly favourable polysaccharide system with which to explore the influence of covalent interruptions on random coil properties, is the carrageenans. These constitute an important group of matrix copolysaccharides which occur naturally in marine red algae. The gel-forming ability [2] of certain members has been studied in some detail and shown to involve ordered conformational states (double helices) of the regular repeating parts of the covalent structure. The relation between covalent structure and solution properties in the random coil form is less well understood, but is also likely to relate to biological function of these polysaccharides.

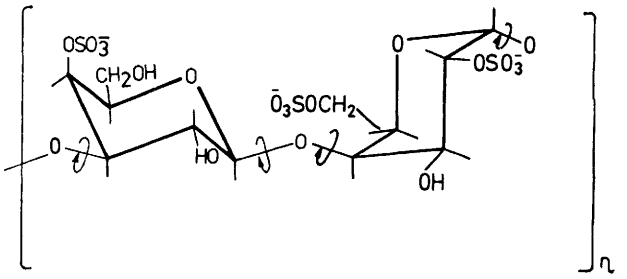
The idealised covalent structure [3,4] of gel-forming carrageenans is an $(A\text{–}B)_n$ repeating arrangement of 4-linked 3,6-anhydro-α-D-galactose residues sulphated to varying extents and 3-linked β-D-galactose 4-sulphate (1), interrupted by a proportion of the A residue being present as galactose 2,6-disulphate or 6-sulphate with concomitant ring inversion from $^1C_4$ to $^4C_1$ conformation (2). ι- and λ-carrageenans are of particular interest because they represent the two extremes *i.e.* in ι-carrageenan the A residue is almost exclusively in the $^1C_4$ form whereas in the λ-form it is in the $^4C_1$ conformation.[5]

The conformational properties of carrageenans have been explored previously [6] by model-building calculations using hard sphere potentials to represent the steric effects of the atoms directly bonded to the rings. It appears that both ι- and λ-carrageenan are fairly extended molecules which are able to form helices with a small number of disaccharide residues per turn. The fibre conformation of ι-carrageenan has been characterised as a double helix with three disaccharide residues per turn,[7] whereas λ-carrageenan, although less well characterised, would appear to adopt a distorted ribbon-like form in the solid state.[6] Calculations [6] confirm the result that would be expected from qualitative considerations, that freedom of rotation about bonds between adjacent sugar residues in the polysaccharide chain, is greatly restricted by an increase in the steric bulk of equatorial substituents adjacent to the glycosidic oxygen on each ring, and it is also relatively restricted when bonds to the glycosidic oxygen are axial rather than equatorial. On these grounds, ι-carrageenan is expected to be considerably stiffer than λ-carrageenan [compare (1) and (2)].



(1)

Curved arrows represent bond rotations



(2)

Curved arrows represent bond rotations

The backbone geometry of λ-carrageenan can be converted into that of ι- in a controlled way by treatment with alkaline borohydride.[8] Consequently it is possible to prepare a series of polysaccharides with differing amounts of the A residue in the 3,6-anhydride form. This is equivalent to the controlled introduction of the alternate chair form of the residue A, with controlled increase in flexibility of the glycosidic bonds. In alkali-modified λ-carrageenan, sulphation of the B residue in the 2-position prevents double helix formation, and the polymer can therefore be kept in the random coil form. Characterisation of the solution properties should then show trends which can be compared with calculations of the coil dimensions as a function of the amount of

[1] D. A. Brant and W. L. Dimpfl, *Macromolecules*, 1970, **3**, 655; D. A. Brant, *Quart. Rev. Biophys.*, 1976, **9**, 4, 527.

[2] D. A. Rees, *Adv. Carbohydrate Chem. Biochem.*, 1969, **24**, 267; D. A. Rees in 'MTP International Review of Science, Biochemistry Series One,' Butterworths, London, 1975, vol. 5, p. 1; D. A. Rees and E. J. Welsh, *Angew. Chem. Internat. Edn.*, 1977, **16**, 214.

[3] N. S. Anderson, T. C. S. Dolan, A. Penman, D. A. Rees, G. P. Mueller, D. J. Stancioff, and N. F. Stanley, *J. Chem. Soc.* (*C*), 1968, 602.

[4] N. S. Anderson, T. C. S. Dolan, and D. A. Rees, *J. Chem. Soc.* (*C*), 1968, 596.

[5] N. S. Anderson, T. C. S. Dolan, C. J. Lawson, A. Penman, and D. A. Rees, *Carbohydrate Res.*, 1968, **7**, 468.

[6] D. A. Rees, *J. Chem. Soc.* (*B*), 1969, 217; D. A. Rees in 'MTP International Review of Science, Organic Chemistry Series One,' Butterworths, London, 1973, vol. 7, p. 251.

[7] N. S. Anderson, J. W. Campbell, M. M. Harding, D. A. Rees, and J. W. B. Samuel, *J. Mol. Biol.*, 1969, **45**, 85; S. Arnott, W. E. Scott, D. A. Rees, and C. G. A. McNab, *J. Mol. Biol.*, 1974, **90**, 253.

[8] D. A. Rees, *J. Chem. Soc.*, 1961, 5168; 1963, 1812.

anhydride present, and provide a good test of our ideas about the influence of ring conformation and substitution pattern around the glycosidic bonds.

To characterise the unperturbed dimensions of the polymers experimentally we have made use of a technique devised by Smidsrød and Haug [9] which relies on the ability of a polyelectrolyte to respond to changes in salt concentration. At high ionic strength the charges are screened so that coulombic repulsion terms have little influence on the conformation of the polymer. At lower ionic strength, these interactions increase in importance and the polymer conformation will adapt. If the polymer is initially very extended, then little conformation change is expected since the charges are already as far apart as possible. Similarly, if the polymer is stiff in the sense of having little freedom of rotation about backbone bonds, it can respond little to the change in ionic strength. In either of these cases, the intrinsic viscosity of the polymer is therefore insensitive to changes in salt concentration. Less stiff or less extended polymers should show greater response to changes in ionic strength.

EXPERIMENTAL

Polysaccharide Samples.—λ-Carrageenan was specially prepared for us from *Chondus crispus* by Marine Colloids, Inc. (sample number REX 5400). Analysis showed a carbohydrate content of 57%. All subsequent residue contents are expressed in terms of this carbohydrate content. On this basis the content of 3,6-anhydrogalactose was 10.2% and that of galactose was 89.8%. Analyses of carrageenan samples are summarised in Table 1.

TABLE 1

Analysis of carrageenan samples

Sample number	% 4-linked residues in anhydride form	% Consecutive carrabiose content	*B* Value
1	20.3	5.6	0.053
2	40.3	16.3	0.065
3	54.0	27.1	0.067
4	69.2	46.6	0.075
5	81.5	68.7	0.083

Chemical Modification of λ-Carrageenan.[8]—The controlled conversion of α-D-galactose 2,6-disulphate into 3,6-anhydro-D-galactose 2-sulphate residues was achieved by the alkaline borohydride elimination reaction. To prepare fully modified material, λ-carrageenan (250 mg) was dissolved in water (50 ml) and sodium borohydride (50 mg) was added. The solution was allowed to stand at room temperature overnight. This reduction of terminal sugar units prevents large molecular weight loss during exposure to alkali. The solution was made N with respect to sodium hydroxide and further sodium borohydride (250 mg) was added. The solution was placed in a water-bath at 80 °C for 5 h to ensure complete chemical modification. The solution was cooled and neutralised with the same volume of N-hydrochloric acid solution. Part of the solution (10 ml) was retained for chemical analysis and the remainder (90 ml) was divided into 5 parts for intrinsic viscosity measurements.

Since the reaction in N-sodium hydroxide reaches completion quickly, a lower alkali concentration and/or shorter were used for preparation of partially modified samples. 0.1N-Sodium hydroxide solution was used to prepare samples 2 and 3, and 0.5N-alkali for sample 4.

Analytical Methods for Sugar Composition.—Total carbohydrate content was estimated [10] by pipetting the polysaccharide solution (1.0 ml) into a test tube with aqueous phenol (5% w/v; 1 ml). AnalaR concentrated sulphuric acid (5 ml) was added quickly, directly onto the surface of the solution. After 30 min, when the solution had cooled, the optical density was measured in a Unicam SP 800 spectrophotometer (maximum absorption 485 nm), calibrated against known amounts of D-galactose and methyl 3,6-anhydro-α-D-galactoside.

The content of 3,6-anhydro-D-galactose was estimated colorimetrically by the resorcinol–hydrochloric acid assay.[11] Solution (0.5 ml, containing 10—100 μg of 3,6-anhydro-D-galactose) was mixed in a test tube with resorcinol–hydrochloric acid solution (10 ml). The latter reagent was made up immediately before use by mixing hydrochloric acid (*d* 1.18; 200 ml), resorcinol (0.13% in ethanol; 20 ml), and deionised water (40 ml). The tube was placed in a water-bath at 80 °C (10 min) and then in cold water (5 min). The resulting crimson solution was measured in a Unicam SP 800 spectrophotometer (maximum absorbance 485 nm). The amount of anhydro-sugar present was calculated by reference to a standard curve, obtained by calibrating the reagents with methyl 3,6-anhydro-α-D-galactoside.

The D-galactose content was given by the phenol–sulphuric acid assay [11] having first substracted the contribution that was calculated to be due to the anhydride sugar.

'*Consecutive Carrabiose Content.*'[12]—This expresses the fraction of total 3,6-anhydride which can be split out as carrabiose derivatives (B–A) where B is galactose and A is anhydrogalactose. Thus *n* consecutive carrabiose residues give *n* — 1 carrabiose disaccharide units in this way. Mild methanolysis splits not only all 3,6-anhydrogalactosyl bonds but also a small proportion of galactosyl bonds. Through control experiments using carrabiose dimethyl acetal it is possible to calculate the yield of carrabiose derivatives which would have been formed in the absence of galactosyl cleavage. Aqueous solutions of the polysaccharides were made up with an estimated 3,6-anhydride concentration between 0.1 and 0.3 mg ml^{-1}. Known volumes of each solution (containing *ca.* 5 mg polysaccharide) were withdrawn and freeze-dried in triplicate, and the residue boiled under reflux for 30 min with methanolic hydrogen chloride (0.2%; containing 2,2-dimethoxypropane; 20 ml), then neutralised with silver carbonate. The suspension containing excess of silver carbonate was allowed to stand at room temperature for at least 20 min to ensure complete neutralisation before addition of an accurately measured volume (0.25 ml) of a standard methanolic solution of sucrose octa-acetate (*ca.* 2% concentration) and thorough mixing, filtration, and evaporation to dryness. The residue was thoroughly dried *in vacuo*, then dissolved in pyridine–acetic anhydride mixture (1 : 1 v/v; 5 ml) and left to stand at room temperature overnight, before evaporation to dryness once more. The mixture of sugar acetates was dissolved in diglyme (1.0 ml) for analysis by g.l.c. Analysis was by injection (1 μl) onto

[9] O. Smidsrød and A. Haug, *Biopolymers*, 1971, **10**, 1227.
[10] M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, *Analyt. Chem.*, 1956, **28**, 350.
[11] W. Yaphe, *Analyt. Chem.*, 1960, **32**, 1327; D. A. Rees and E. Conway, *Biochem. J.*, 1962, **84**, 411.
[12] C. J. Lawson, D. A. Rees, D. J. Stancioff, and N. F. Stanley, *J.C.S. Perkin I*, 1973, 2177.

an OV-1 column (3% on Gas Chrom P) in a Pye 104 chromatograph at 258 °C; retention times were 16 min for carrabiose dimethyl acetal and 18 min for sucrose octa-acetate. The peak areas were estimated by multiplication of peak heights and widths at half height giving an accuracy of ±2—3%.

For the control experiment, known weights of carrabiose dimethyl acetal hexa-acetate (*ca.* 5 mg each in triplicate) were dissolved in redistilled methanol (5 ml) containing 2,2-dimethoxypropane (0.25 ml). A solution of sodium methoxide was prepared by adding 2,2-dimethoxypropane (3 ml) to redistilled methanol (100 ml), followed after 30 min by bright sodium (50 mg). A sample of this solution (0.25 ml) was added to the hexa-acetate solution at room temperature and left for 16 h. After neutralisation with a piece of solid carbon dioxide and evaporation to dryness, deacetylation was shown to be complete by the ^{1}H n.m.r. spectrum in deuterium oxide solution. This residue was treated quantitatively (in parallel with the polysaccharide sample) with methanolic hydrogen chloride followed by the same sequence of stages as described above. G.l.c. analysis was then used to determine the recovery of carrabiose derivative (typically this was *ca.* 75%). This figure was then used to adjust the amounts of carrabiose derivatives recovered from the polysaccharides, to give the consecutive carrabiose content.

Viscosity Measurements and Derivation of B *Values.*—Intrinsic viscosity measurements were carried out on a low shear concentric cylinder Couette viscometer based on the design by Ogston and Stanier.[13] Zero shear relative viscosities were obtained from the slope of the linear low shear portion of the stress–strain curve. Extrapolation to zero concentration was by combined Huggins [$(\eta_{rel} - 1)/c$ *versus* c] [14] and Kraemer ($\ln\eta_{rel}/c$ *versus* c) treatment.[15]

The intrinsic viscosities of native, fully alkali modified, and three intermediate samples of λ-carrageenan were measured at five different ionic strengths in order to derive their B values (see Table 1). Polysaccharide solutions (0.25% w/v; 18 ml) were extensively dialysed against 0.010, 0.015, 0.025, 0.060, and 0.25M-sodium chloride solutions. Measurements were on volumes of 10 ml and each solution was diluted with buffer to give four concentrations having relative viscosities within the range 1.2—3.0. The derivation of B values was in two stages. (i) Intrinsic viscosity $[\eta]$ was plotted against the reciprocal of the square root of ionic strength to determine the slope of the straight line plot S. (ii) To overcome the problem that the absolute magnitude of S depends on molecular weight as well as on flexibility, S is expressed in terms of the intrinsic viscosity at a fixed ionic strength. This is necessary because the molecular weight decreases during alkali modification as a result of side reactions. To compensate for molecular weight effects Smidsrød and Haug [9] normalised as follows to give the flexibility parameter B: $B = S/([\eta]_{0.1MNaCl})^{1.3}$.

Calculation of the Unperturbed Dimensions.—The methods [1,16,17] used for calculating the unperturbed dimensions of polysaccharides have been described in detail elsewhere and therefore we give only a rather brief account of our procedure. The copolymer is represented by the sequence of vectors ($\vec{l}_i$; $i = 1,2 \ldots n$) joining adjacent oxygen atoms. The end-to-end vector of the polymer in a particular conformation is simply the sum of these n vectors and the mean-square length of the polymer is the square of this vector averaged over all conformations of the polymer [equation (1)].

$$\langle R_n^2 \rangle = \sum_{i=1}^{n} l_i^2 + 2 \sum_{i=2}^{n} \sum_{j=1}^{i-1} \langle \vec{l}_i \cdot \vec{l}_j \rangle \qquad (1)$$

The co-ordinate system on each monomer is defined such that the x-axis of the local co-ordinate system is directed along the vector joining the glycosidic oxygen atoms of this monomer residue. If $\boldsymbol{T}_i$ is the rotation matrix which transforms from the co-ordinate system relative to the $(i + 1)$th monomer to the co-ordinate system relative to the ith monomer, then the mean-square length of the n-mer can be written as (2) where l is the root-mean-square monomer residue length.

$$\langle R_n^2 \rangle = nl^2 + 2(l\ 0\ 0) \sum_{i=2}^{n} \sum_{=1}^{i-1} \langle \boldsymbol{T}_i \ldots \boldsymbol{T}_{j-1} \rangle \begin{pmatrix} l \\ 0 \\ 0 \end{pmatrix} \qquad (2)$$

We introduce the approximation that, under theta conditions the total energy of the polymer can be written as the sum of the energies of the constituent dimers and from this equation (3) follows.

$$\langle \boldsymbol{T}_i \boldsymbol{T}_{i+1} \ldots \boldsymbol{T}_{j-1} \rangle = \langle \boldsymbol{T}_i \rangle \langle \boldsymbol{T}_{i+1} \rangle \ldots \langle \boldsymbol{T}_{j-1} \rangle \qquad (3)$$

The angular brackets denote Boltzmann averages over all rotatable side groups and over the glycosidic and aglycone bonds of the dimer. For a strictly alternating copolymer the summations can be carried out analytically but for a stochastic copolymer it is convenient to couple Monte Carlo techniques for the generation of a sample of monomer sequences with the $\boldsymbol{G}$ matrix technique of Flory and Jernigan which allows one to calculate the dimensions of a polymer with any given sequence of monomers. If we define the matrix as (4) we arrive at expression (5).

$$\boldsymbol{G}_i = \begin{pmatrix} 1 & \boldsymbol{l}_{i'}\langle \boldsymbol{T}_i \rangle & 0 \\ \boldsymbol{O} & \langle \boldsymbol{T}_i \rangle & \boldsymbol{l}_i \\ 0 & \boldsymbol{O} & 1 \end{pmatrix} \qquad (4)$$

$$C_n \equiv \langle R_n^2 \rangle / nl^2 = 1 + (2/nl^2)\ (1\ 0\ 0\ 0\ 0) \prod_{i=1}^{n-1} \boldsymbol{G}_i \begin{pmatrix} 0 \\ l \\ 0 \\ 0 \\ 1 \end{pmatrix} \qquad (5)$$

We have calculated the averaged $\boldsymbol{T}$ matrices for all possible dimers ignoring the sulphate substituents, using a Kitaigorodsky potential to represent the interaction between non-bonded atoms, and making use of the idealised co-ordinates utilised in earlier hard sphere calculations. We generated a sample of monomer sequences in which each 4-linked residue was independently taken to be the $^4C_1$ or $^1C_4$ form with suitable probability to reproduce the desired overall composition. We then used the $\boldsymbol{G}$ matrix approach to calculate the dimensions of each polymer in the sample and hence to estimate the characteristic ratio for various overall chemical compositions.

RESULTS AND DISCUSSION

By controlled alkaline borohydride treatment of λ-carrageenan, it was possible to prepare samples of in-

[13] A. G. Ogston and J. E. Stanier, *Biochem. J.*, 1968, **109**, 43.
[14] M. L. Huggins, *J. Amer. Chem. Soc.*, 1942, **64**, 2716.
[15] A. Haug and O. Smidsrød, *Acta Chem. Scand.*, 1962, **16**, 1569.
[16] S. G. Whittington, *Biopolymers*, 1971, **10**, 1617; S. G. Whittington in ' Structure of Fibrous Biopolymers,' eds. E. D. T. Atkins and A. Keller, Butterworths, London, 1975, p. 307.
[17] O. Smidsrød, R. M. Glover, and S. G. Whittington, *Carbohydrate Res.*, 1973, **27**, 107.

creasing anhydride content. This reaction followed first-order kinetics (Figure 1), so providing partial justification for comparing the properties of the products with predictions made from a theoretical model in which the conversion was considered to occur at random. However, there are further influences on the overall distribution of anhydride residues which must be considered

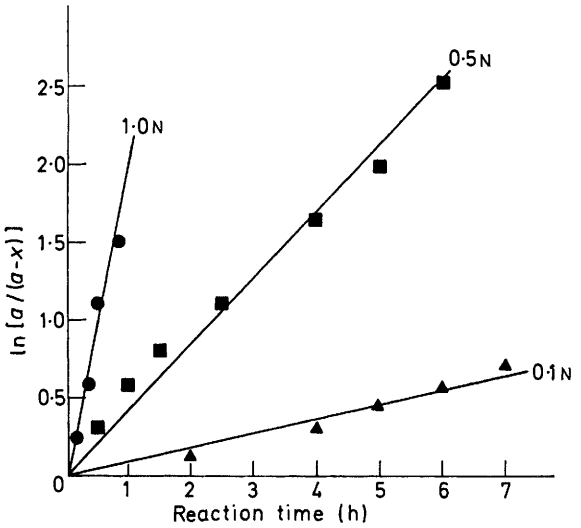


FIGURE 1 Alkaline borohydride treatment of λ-carrageenan at different levels of alkali, showing first-order kinetics in the formation of 3,6-anhydride residues

too. The starting material already contained 20% of its 4-linked residues in the anhydride form; having beeen introduced biologically, these need not have been inserted at random. Secondly, the final limit of conversion was at 80% of the 4-linked residues. If this were caused by sulphate hydrolysis in competition with sulphate elimination, the unconverted residues would be expected to be distributed at random; on the other hand, a random pattern need not be expected if the limit were caused by the presence of alkali-resistant 4-linked galactose residues, such as those in ξ-carrageenan which lack 6-sulphate.[18] To evaluate the effect of these secondary influences on the distribution of anhydride residues, we used the criterion of ' consecutive carrabiose contents,' which is essentially a method of next-neighbour analysis (see Experimental section for details); it measures the proportion of 3,6-anhydrogalactose residues which is adjacent to another disaccharide unit which also contains 3,6-anhydrogalactose rather than galactose 2,6-disulphate. Assuming a purely random distribution of anhydride residues at each stage, we calculated the ' consecutive carrabiose content ' that would be expected for a series of degrees of conversion of 2,6-disulphate into 3,6-anhydride. It was found that the experimental points for all five carrageenan samples fell closely on the curve which was calculated in this way (Figure 2). This justifies the assumption made in the calculation of chain dimensions, that the two types of 4-linked residues replace each other at random in each sample.

Native λ-carrageenan having a B value of 0.053 (Table 1) is a ' stiff ' random coil polymer comparable to sodium alginate (0.04) [17] and sodium hyaluronate (0.065).[9] With modification to introduce anhydride residues, there is a progressive increase in B value (Table 1) but even at the limit of conversion, the chain remains fairly stiff (B 0.083) compared [9] with, say, carboxymethylamylose (0.20) and dextran sulphate (0.23). The increase in flexibility parameter with anhydride content over the range accessible by chemical modification cannot be explained by loss of sulphate groups and hence by the lowering of charge density, because this would decrease the response of coil dimensions to ionic strength and hence if this did contribute to the measured value of B (which is unlikely [9]) it would be in the opposite sense to our experimental observations. Another secondary effect which occurs during alkali modification is a degree of chain cleavage as shown by the intercepts of the intrinsic viscosity plots in Figure 3. The influence, if any, of this side reaction on the flexibility parameter would also be in the opposite sense to our observations. We therefore conclude that, at least qualitatively, the change in B values (Table 1) reflects a genuine increase in flexibility as a result of the controlled conversion of $^4C_1$ residues into $^1C_4$.

We next examine whether it is possible to explain the trend in the experimental results (Table 1) quantitatively. Our first attempts using configurational statistics with very simple energy functions were remarkably successful. Each sugar residue was regarded as having a fixed ring shape, so that any flexibility in the chain arose from rotations about the bonds to glycosidic oxygens [see (1) and (2)]. Constraints on these rotations were assumed to be

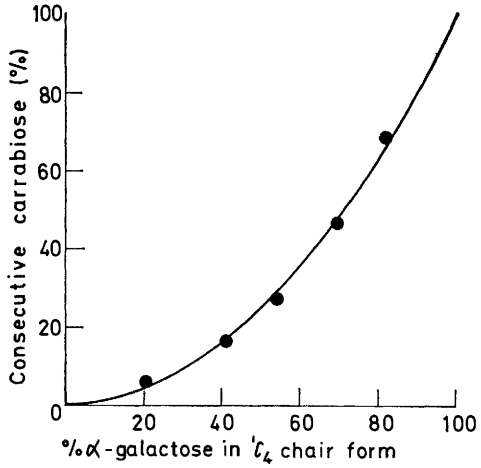


FIGURE 2 Comparison of the curve calculated for the variation of consecutive carrabiose assuming reaction occurs randomly along the chain, as a function of consecutive carrabiose, with the experimental points

caused only by van der Waals repulsion, the energy of which is supposed to increase exponentially within the contact distance, and van der Waals attraction, the energy of which is supposed to decrease with the inverse sixth power of distance. Note that many factors are

[18] A. Penman and D. A. Rees, *J.C.S. Perkin I*, 1973, 2182.

excluded from this model which must surely be relevant to the behaviour of real molecules, such as deformation of bond angles, occurrence of a proportion of residues in

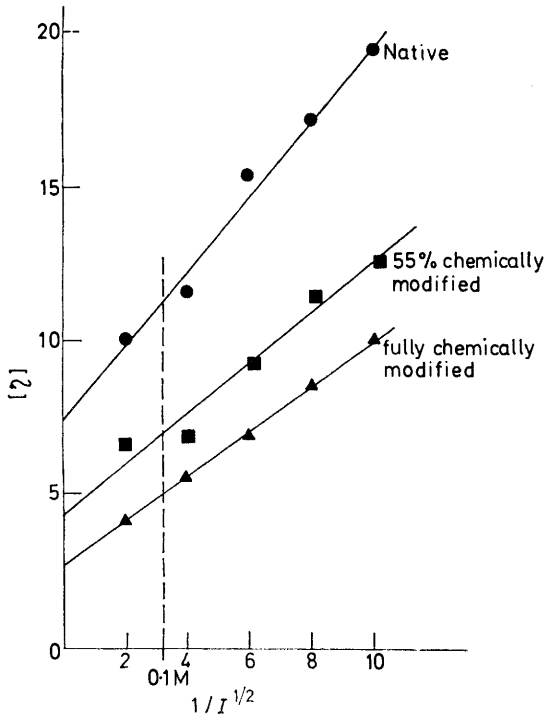


FIGURE 3 Relationship between intrinsic viscosity and reciprocal of the square root of ionic strength for native and chemically modified λ-carrageenan

higher energy ring conformations, and the influence of other energy terms on the internal motions such as hydrogen bonding, electrostatic and dipolar interactions, and influence of solvent. The results of the calculations are shown in Figure 4 for both carrageenan and agarose at different degrees of conformational interconversion of the 4-linked residue, expressing the overall chain dimensions in terms of the characteristic ratio, C_∞,[19] which is a measure of the mean-square end-to-end length relative to the length at maximum extension. Note that the dimensions of carrageenan decrease with the conversion, in qualitative agreement with the experimental evidence (Table 1). The curve calculated for agarose is strikingly similar, despite the fact that the 4-linked residue has the opposite optical configuration (it is L-galactose 6-sulphate or 3,6-anhydro-L-galactose). The fact that the two diastereoisomeric polysaccharide backbones gave results that were so similar, reinforces the notion that will be discussed further below, that the overall chain dimensions are determined to a good first approximation by two simple local effects, freedom of rotation at each glycosidic bridge and the fixed shape of each sugar residue, and longer range effects are less important. The calculations predict that the decrease in the unperturbed dimensions with the amount of anhydride would be sharper at first. This is not unexpected since similar results have been observed theoretically and experimentally in other copolysaccharides. For example, the introduction of a few flexible links into alginate by periodate oxidation is observed to increase the B value sharply.[20] Unfortunately, however, we had no carrageenan samples with sufficiently low content of anhydride residues to allow this comparison to be made directly.

To develop further the quantitative comparison between theory and experiment, we looked for an empirical correlation between the B value and some parameter that could be derived from the calculations. We noticed that a good linear relationship can be demonstrated using the data collected by Smidsrød and Haug,[9] between B and $1/A_m$ where A_m is the Kuhn persistence length which is in turn readily calculated from the characteristic ratio by equation (6) in which l is the monomer residue length.

$$A_m = (C_\infty + 1)l \qquad (6)$$

The calculated C_∞ values for carrageenan were therefore converted to segment lengths using a mean residue length of 4.74 Å for 10% 4-linked galactose in the $^4C_1$ form, reducing to 4.38 Å for 90% conversion into $^1C_4$. These results were then converted to B values using an empirical relationship (Figure 5). When the experimental points are plotted on this theoretical curve (Figure 6), the agreement is found to be remarkably good. This suggests that the simplified model for polysaccharide structures on which our predictive calculations were

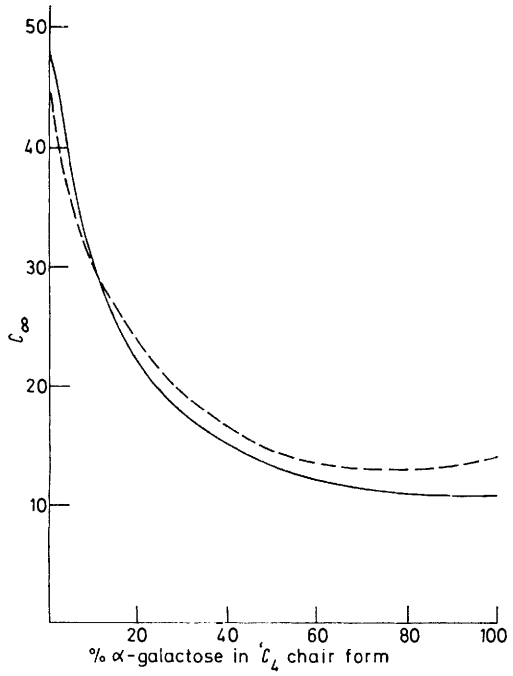


FIGURE 4 Dependence of characteristic ratio on the percentage of 4-linked residues in the $^1C_4$ chair form for carrageenan (solid line) and agarose (dotted line)

based, does in fact include the main features that dominate the form and flexibility of the chains in solution.

[19] P. J. Flory, 'Principles of Polymer Chemistry,' Cornell University Press, Ithaca, 1953.

[20] O. Smidsrød and T. J. Painter, *Carbohydrate Res.*, 1973, **26**, 125.

This invites analogy with the conclusion [21] that emerged from a recent theoretical study of the molecular dynamics

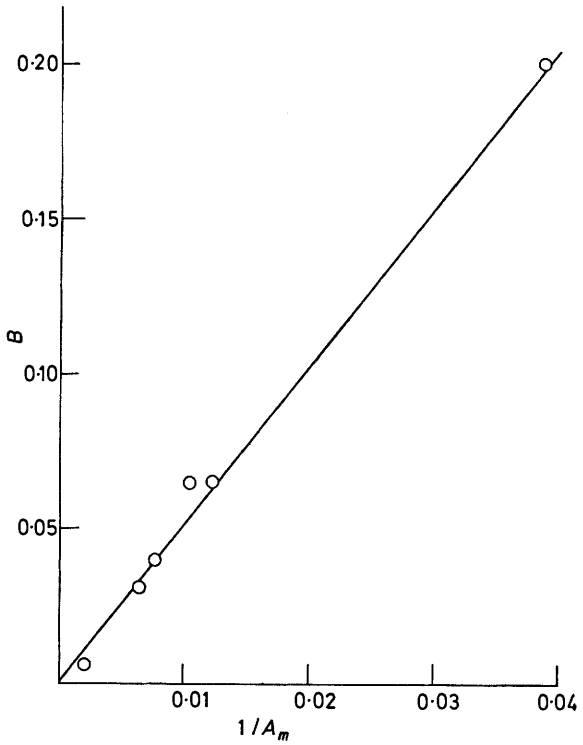


FIGURE 5 Relationship between the empirical parameter of flexibility (B) and the reciprocal of the statistical segment length (A_m) for a series of polyelectrolytes. In order of increasing B values these were deoxyribonucleic acid, alginate relatively rich in polyguluronate, alginate relatively rich in polymannuronate, alginate relatively rich in alternating blocks, carboxymethylcellulose, and carboxymethylamylose (data taken from refs. 9 and 17)

of a globular protein, that motions within the structure are largely determined by constraints of the covalent

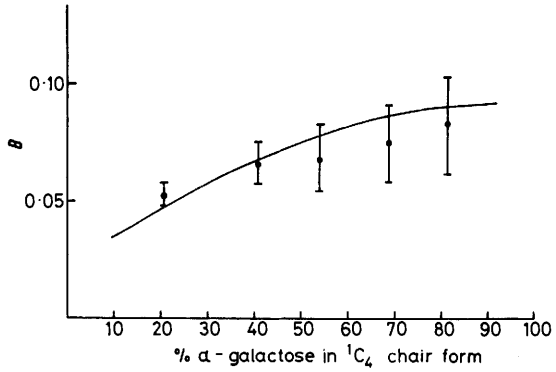


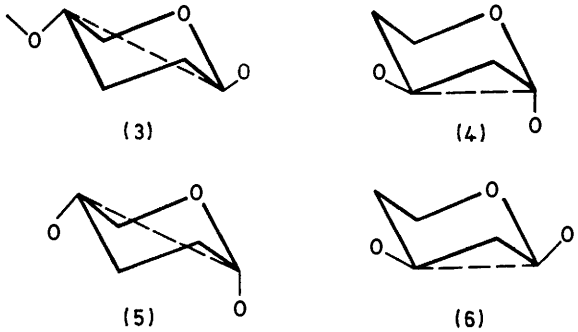
FIGURE 6 Comparison of the curve calculated for the variation of the parameter of flexibility (B) for carrageenans of increasing anhydride content with the experimental points. Error bars are based on standard deviations for the ionic strength dependence of intrinsic viscosity plotted as indicated in Figure 3.

framework and by hard sphere repulsions. We must point out, however, that limitations do show up in this type of model when it is submitted to more searching comparison with experiment, for example, in attempting to predict the temperature coefficient of the characteristic ratio.[1] Our aim here is the more pragmatic one of predicting the major change in random coil behaviour with the composition of sugar residues, to understand the differences in biological and technological properties. Our approach seems entirely adequate for this.

If the chain flexibilities are indeed dominated by such simple features, we may next ask whether they can be understood (and hence, in future, predicted) by qualitative conformational analysis. From earlier work the important considerations are as follows.

(i) *Local Interactions.*—We have previously shown [6,22] that bond rotations at a glycosidic bridge become more restricted with the number of axial bonds to the glycosidic oxygen and with the number of bulky equatorial substituents surrounding the glycosidic oxygen. In comparing the relative local flexibilities of different polysaccharide chains (below), we shall add these numbers together to rank the chains very roughly in order of the number of ' steric crowding factors.'

(ii) *Local Geometry.*—This principle was first defined [22] in relation to ordered rather than disordered shapes of polysaccharide chains, when it emerged from model-building calculations that fundamentally different types of overall chain contour were generated for homopoly-



saccharides depending on the geometrical relationship between glycosidic and aglycone bonds *across* a sugar residue. When these bonds subtend a large dihedral angle [120—180° *e.g.* (3) and (4) where the projection is down the vector shown by a dotted line], the chain is designated ' Type A' and any ordered forms are extended and ribbon-like.[22] When the dihedral angle is close to 0° [*e.g.* (5) and (6)] the chain is designated ' Type B ' and ordered forms are hollow helices.[22] Subsequent calculations [23] revealed that Type A and B chains also showed family differences in their predicted random coil dimensions. For Type A chains, C_∞ is usually predicted to be of the order of 100, whereas for Type B chains the values are 10 or less. Of the residues which do not fit into the categories of Type A and B, only the 1,6-linked type is common enough to be important; chains of 1,6-linked residues have an extra source of flexibility because

[21] J. A. McCammon, B. R. Gelin, and M. Karplus, *Nature*, 1977, **267**, 585.

[22] D. A. Rees and W. E. Scott, *J. Chem. Soc.* (*B*), 1971, 469.

[23] S. G. Whittington and R. M. Glover, *Macromolecules*, 1972, **5**, 55.

TABLE 2

Conformational analysis and flexibility ranking of polysaccharides

Polysaccharide [a]	A_m/Å	B	Residue type(s)	Steric crowding factors [b]	Refs.
Alginate, relatively rich in poly-guluronate	155	0.031	A	<4	17
Alginate, relatively rich in poly-mannuronate	130	0.040	A	>2	17
λ-Carrageenan		0.053	A + B	3.5	This work
Carboxymethyl-cellulose	82	0.065	A	3	9
Alginate, relatively rich in alternating blocks	95	0.065	A + A	~3	17
Hyaluronate		0.065	A + B	3	9
Chemically modified λ-Carrageenan		0.083	A + B	1	This work
Carboxymethyl-amylose	25.6	0.20	B	4	9
Amylose xanthate		0.22	B	4	9
Dextran sulphate		0.23	1,6-linked	3	9

[a] These are listed in order of increasing chain flexibility, as indicated by measured values of A_m and/or B. [b] Average value per glycosidic oxygen; for explanation of this term, see text.

the glycosidic system involves three bonds rather than only two.

In an attempt to explore the extent to which these simple insights can be useful in ranking the flexibilities of polysaccharides, we have listed (Table 2) the experimental evidence for a series of polysaccharides, with a conformational classification in terms of the content of Type A and B residues and the number of steric crowding factors. Some caution is clearly necessary in extrapolating from homo- to hetero-polysaccharides because the comparison of experimental results for alginate rich in alternating blocks with those for alginate rich in each of the two homopolymeric blocks, shows that some sequence dependence does exist. Nevertheless we can observe from Table 2 that for each polysaccharide type (A, B, or A + B), the measured stiffness does indeed correlate with the number of steric crowding factors; that Type B and also 1,6-linked polymers tend to be more flexible than other types; and on the limited evidence available so far, it would seem that alternating copolymers of Type A and B residues are comparable in stiffness with Type A polymers having the same degree of steric crowding at the linkages. We conclude that conformational analysis in terms of residue type and steric crowding is likely to be reliable for rough prediction of the relative chain flexibilities of polysaccharides for which experimental data are not yet available.

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