Carbohydrate Research, 35 (1974) 259–263

Note

Molecular conformation in gels of cellulose sulfate*

STRUTHER ARNOTT, D. W. L. HUKINS†,
Department of Biological Sciences,
ROY L. WHISTLER, AND C. W. BAKER
Department of Biochemistry, Purdue University, West Lafayette, Indiana 47907 (U.S.A.)
(Received December 21st, 1973; accepted for publication, February 18th, 1974)

Cellulose sulfates and carrageenans both form highly viscous solutions, but only κ- and ι-carrageenans have been exploited as industrially useful gels[1,2]. X-Ray diffraction work has shown that base-modified ι-carrageenan exists in oriented gels as coaxial, double helices[3]. Double helices are believed to be responsible for junction-zone formation in both carrageenan and agarose (agaran) gels[4], but junction-zones in other polysaccharides do not necessarily involve double helices. We now provide direct evidence that, in cellulose sulfate gels, the molecules are not double helical.

Samples of cellulose sulfate were prepared by direct sulfation of cotton-linter cellulose with the Me_2SO–SO_3 complex[5] to produce sulfates of d.s. 0.94–1.88. The majority of the sulfate ester groups are at primary positions[5,6]. The best diffraction pattern was in fact obtained from Sample 1 (Table II), which approximates to cellulose 6-sulfate.

Gels of the sodium salt of cellulose sulfate (2% concentration) were oriented by drying (at 66% relative humidity) between the rounded ends of glass rods[7]. X-Ray diffraction patterns were obtained in the same way as in previous work on keratan sulfate[8], except that shorter exposure times (~2 h) were required. Fig. 1 shows a typical diffraction pattern of cellulose sulfate, which is characteristic of equally spaced, helical molecules packed ~15 Å apart with their axes parallel, but with no further regular interactions between molecules[9]. The spacing of layer lines (Fig. 2) corresponds to an axial repeat (*c*) of 10.3 ±0.1 Å. Therefore, the β-D-(1→4)-linked polysaccharide chain is nearly fully extended and cannot accommodate a second coaxial chain. Because *c* is very similar to the values observed for cellulose[10] and chitin[11], we assume that the cellulose sulfate backbone has the same two-fold, helical conformation. The intensity distribution of the diffraction pattern is compatible with this.

Computer methods were used to build a molecular model and to investigate

*Dedicated to Dr. Horace S. Isbell, in honor of his 75th birthday.
†Present address: Laboratory of Molecular Biophysics, Department of Zoology, South Parks Road, Oxford, England.

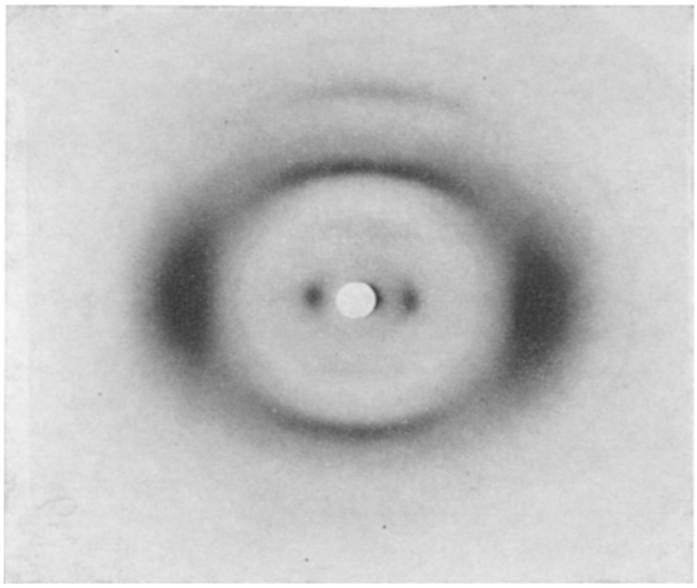

Fig. 1. X-Ray diffraction pattern of an oriented gel of cellulose sulfate. The direction corresponding to the molecular axes is vertical. For this particular pattern, the fiber of dried gel made an angle of 82° with the X-ray beam.

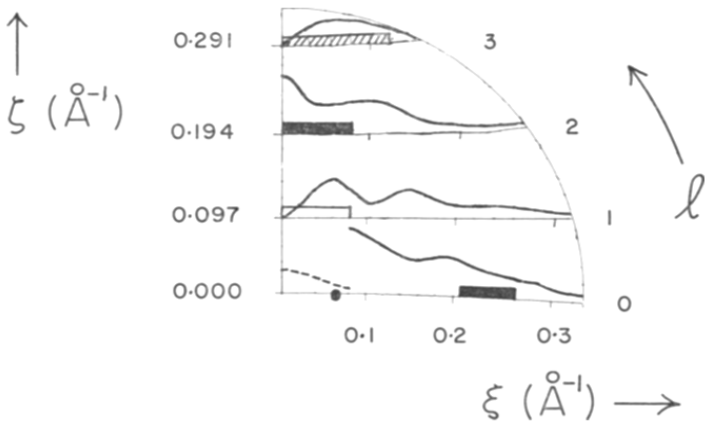

Fig. 2. Comparison of observed and calculated diffraction patterns of cellulose sulfate. The figure represents the upper-right quadrant of the pattern with ξ and ζ the lateral and axial reciprocal spacings. On each layer line (with index l), the observed features of the pattern are marked filled, shaded, or unfilled for strong, medium, or weak intensities, respectively. The filled circle on layer line $l = 0$ is a Bragg reflexion[9]. Curves on each layer line represent the intensity distribution calculated from the coordinates of Table I, as described in Ref. 8, except that the marked difference in axial and lateral disorder was accounted for by replacing the term $\exp(-B \sin^2\theta/\lambda^2)$ with $\exp(-B'\xi^2/4 - B''\zeta^2/4)$, where $B' = 20$ and $B'' = 4$. The dashed curve is reduced by a factor of 10^{-1}.

possible sulfate conformations[8]. The best average conformation for the pyranose ring derived by Arnott and Scott[12] was changed slightly to make a 2.7 Å hydrogen bond between O-3 and O-5 of successive residues. Two fully staggered sulfate conformations are allowed; for both, the conformation-angle θ[C-5–C-6–O-6–S] is exactly 180°, while θ[O-5–C-5–C-6–O-6] is either 60° or −60°. The coordinates of our model, which is in reasonable agreement with the intensity distribution of the diffraction pattern (Fig. 2), are given in Table I. Fig. 3 shows a computer-generated drawing of the cellulose sulfate model, which resembles the two-fold, helical models of the mucopolysaccharides dermatan sulfate[13] and keratan sulfate[8], although the β linkages are (1→3) and the (1→4) linkages α in these mucopolysaccharides. Molecules of keratan sulfate are packed in stretched films 14.8 Å apart in a similar manner to the packing of cellulose sulfate molecules in oriented gels[8].

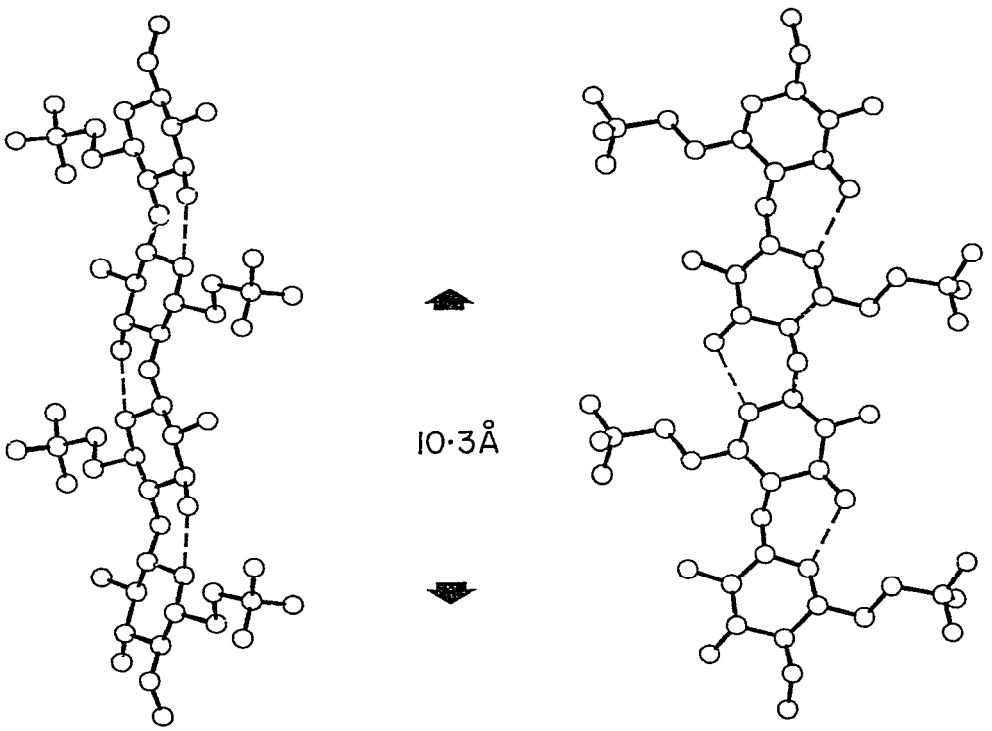

Fig. 3. Two different views of the cellulose sulfate model. The dashed lines are the hydrogen bonds between O-3 and O-5 of successive residues.

Our results show that the formation of multi-stranded, coaxial helices is not always necessary for polysaccharide gel-formation. The similarity between molecular models proposed for the chain conformations in cellulose, cellulose sulfate, and chitin indicates that sulfation at C-6 does not greatly influence the preferred conformation about the β-D-(1→4) linkages in polysaccharides.

TABLE I

CYLINDRICAL POLAR COORDINATES[a] OF THE ATOMS IN ONE RESIDUE OF THE CELLULOSE SULFATE MODEL

	r (Å)	θ (degrees)	z (Å)
C-1	0.38	−8.7	−2.39
C-2	1.52	−23.3	−3.39
C-3	1.22	3.3	−4.74
C-4	0.24	180.0	−5.15
C-5	1.31	162.6	−4.08
C-6	2.68	179.2	−4.45
O-1	0.90	−55.6	−1.19
O-2	2.77	−9.2	−2.91
O-3	2.12	−16.5	−5.70
O-4	0.90	124.4	−6.34
O-6	3.64	164.2	−3.43
O-7	5.49	−175.0	−3.84
O-8	5.63	163.3	−4.94
O-9	5.99	165.3	−2.61
S	5.16	169.9	−3.71

[a]The z axis coincides with the two-fold screw axis of the helix. Atoms O-7, O-8, and O-9 are sulfate oxygens. Coordinates of the atoms in successive residues may be generated by adding 180° to θ and 5.15 Å to z. Sulfate-group coordinates correspond to the conformation angle C-4–C-5–C-6–O-6 having a value of 60°. To convert into Cartesian coordinates see Ref. 8.

EXPERIMENTAL

General methods. — Total sulfur was determined[14] by flask combustion. The d.s. values determined from the percentage of sulfur present in the sulfated samples are recorded in Table II. Intrinsic viscosities (dl/g) were determined[15] for 2% (w/v) solutions in aqueous sodium and/or potassium chloride. These values are recorded in Table II. All non-freeze-dried products were dried (24 h, 55°) under diminished pressure over anhydrous potassium hydroxide.

TABLE II

D.S. AND INTRINSIC VISCOSITY OF THE CELLULOSE SULFATES

Sample	*S* (%)	*D.s.*	*dl/g*
1	11.68	0.94	2.49[a]
2	15.80	1.60	2.54[a]
3	17.04	1.88	2.68[a]

[a]Determined in 2% aqueous potassium chloride.

Cellulose sulfates (**1**, **2**, *and* **3**). — Three samples of dry, cotton-linter cellulose (12.2 g, 0.075 basic mole) were separately sulfated[17] with freshly prepared Me_2SO–SO_3 complex (35.7 g, 0.215 mole; 53.4 g, 0.337 mole; 71.4 g, 0.430 mole). The products were neutralized (10% KOH) and purified by dialysis[16]. The final products were isolated by freeze-drying.

ACKNOWLEDGMENTS

The X-ray work was supported by a grant from NSF (to S.A.) and a research fellowship from the Jane Coffin Childs Memorial Fund (to D.W.L.H.).

REFERENCES

1 R. L. WHISTLER, U. S. Pat. 3,507,855 (1970).
2 R. G. SCHWEIGER, *Carbohyd. Res.*, 21 (1972) 219.
3 N. S. ANDERSON, J. W. CAMPBELL, M. M. HARDING, D. A. REES, AND J. W. B. SAMUEL, *J. Mol. Biol.*, 45 (1969) 85.
4 I. C. M. DEA, A. A. MCKINNON, AND D. A. REES, *J. Mol. Biol.*, 68 (1972) 153.
5 R. L. WHISTLER, A. H. KING, G. RUFFINI, AND F. A. LUCAS, *Arch. Biochem. Biophys.*, 121 (1967) 358.
6 R. L. WHISTLER AND W. W. SPENCER, *Arch. Biochem. Biophys.*, 95 (1961) 36.
7 W. FULLER, F. HUTCHINSON, M. SPENCER, AND M. H. F. WILKINS, *J. Mol. Biol.*, 27 (1967) 507.
8 S. ARNOTT, J. M. GUSS, D. W. L. HUKINS, I. C. M. DEA, AND D. A. REES, *J. Mol. Biol.*, in the press.
9 S. ARNOTT, *Trans. Amer. Crystallogr. Ass.*, 9 (1973) 31.
10 R. R. SUNDARARAJAN AND R. H. MARCHESSAULT, *Can. J. Chem.*, 50 (1972) 792.
11 D. CARLSTRÖM, *Biochim. Biophys. Acta*, 59 (1962) 361.
12 S. ARNOTT AND W. E. SCOTT, *J. Chem. Soc. Perkin II*, (1972) 324.
13 S. ARNOTT, J. M. GUSS, D. W. L. HUKINS, AND M. B. MATHEWS, *Biochem. Biophys. Res. Commun.*, 54 (1973) 1377.
14 T. F. ALCINIO, A. I. COHEN, AND M. E. EVERHARD, *Treatise Anal. Chem., Part II*, 12 (1965) 57.
15 H. A. SWENSON, *Methods Carbohyd. Chem.*, 3 (1963) 84.
16 J. W. GREEN, *Methods Carbohyd. Chem.*, 3 (1963) 95.
17 R. L. WHISTLER, *Methods Carbohyd. Chem.*, 6 (1972) 426.