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A comparison of the conformation of sucrose octasulfate, free and bound to acidic fibroblast growth factor

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Abstract

The conformation of sucrose octasulfate free in solution has been determined based on high-resolution NMR spectroscopy. Three-bond $^{1}H-^{1}H$ scalar coupling constants, laboratory and rotating frame NOEs, long-range $^{1}H-^{13}C$ scalar coupling constants, and chemical-shift temperature and ionic-strength dependence were used, aided by molecular mechanics calculations. By modification of a pulse sequence designed for measuring long-range $^{1}H-^{13}C$ coupling constants, it was possible to obtain an accurate value for the $^{3}J_{C2f-H1g}$ despite the confounding presence of $^{3}J_{HH}$ of similar value. Free sucrose octasulfate appears to assume a conformation significantly different from any of the eight conformations observed bound to acidic fibroblast growth factor, as determined in a previous X-ray crystallographic study [X. Zhu, B.T. Hsu, and D.C. Rees, *Structure*, 1 (1993) 27–34]. Strong electrostatic interactions between guest and host may be the dominant factor in deformation of sucrose octasulfate. The implications of this study for protein–carbohydrate interactions and the effects of the presence of sulfate groups on the flexibility of sucrose are also discussed.

Keywords: Sucrose octasulfate; Conformation; Acidic fibroblast growth factor

Abbreviations: aFGF, acidic fibroblast growth factor; DQCOSY, double quantum filtered homonuclear shift correlation spectroscopy; HMQC, heteronuclear multiple quantum correlation; NOE, nuclear Overhauser effect; ROE, rotating-frame Overhauser effect; ROESY, rotating frame Overhauser effect spectroscopy; SOS, sucrose octasulfate; TOCSY, total correlation spectroscopy; TSP, sodium 3-(trimethylsilyl)propanesulfonate.

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1. Introduction

Sucrose octasulfate (SOS, Fig. 1) used in the treatment of gastric ulcers, has been shown to bind acidic fibroblast growth factor [1] (aFGF) with a higher affinity than does heparin [2], and it also inhibits angiogenesis. Its antiulcer properties have been proposed to arise from its stabilization of FGFs [2]. Zhu et al. [1] found that SOS bound to aFGF in a 1:1 complex in eight crystallographically independent conformations, while aFGF, the host, showed much less conformational variability. This prompted us to study the conformation of free K_8SOS in solution with high-resolution NMR spectroscopy and molecular mechanics calculations and compare the conformations of this molecule in its free and bound states.

2. Results and discussion

Analysis of crystal structures of SOS.—Atomic coordinates obtained from the Cambridge Structural Data Base [3] and the Brookhaven Protein Data Bank were used to calculate torsion angles and ring puckering parameters for the crystal structures of K_8SOS heptahydrate [4] and the SOS–aFGF complex [1], respectively. The results of the calculations based on these data are shown in Table 1 and in Figs 2–4. The conformation of the crystal K_8SOS was labeled as S_0, and the eight conformations of SOS bound to aFGF were labeled in accordance with the original Protein Data Bank file.

The glucose ring in crystalline K_8SOS is very close to a perfect $^4C_1$ chair, just slightly distorted towards $B_{2,5}$ as indicated by a very small θ value and a ϕ_2 value close to 300° (see Fig. 2). In contrast, when bound to aFGF, the glucose ring in all eight conformations deviates significantly from a $^4C_1$ chair conformation. Actually, the pyranose ring in S_2, S_5, and S_6 should be described as $^2H_1$, an E_5 distorted towards $^0H_5$, and an E_1 distorted towards $^0H_1$, respectively. It is interesting to note that most of the conformations (except S_7) fall in the range of $\phi_2 = -60°$ to $+90°$.

The furanose ring in crystalline K_8SOS is in the $^5T_4$ conformation. This five-membered ring also exhibits great conformational variability when bound to the protein, although most of the observed conformations (except S_7) fall in one quadrant of the

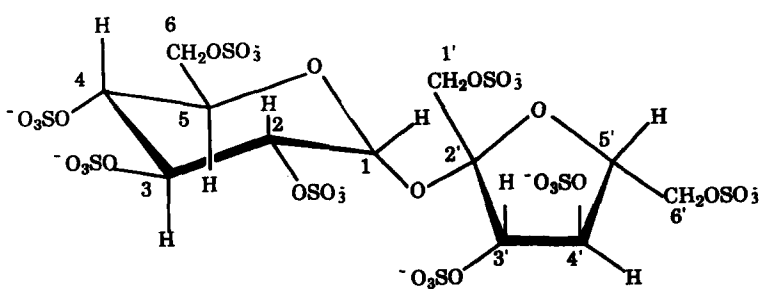

Fig. 1. Sucrose octasulfate with carbons labeled. According to convention, the atoms in the fructose moiety are primed, and those in the glucopyranose moiety are unprimed.

Table 1
Conformational parameters, in degrees, derived from X-ray crystallographic studies of SOS

Source	K_8SOS [a]			SOS–aFGF complex [b]					
Conformer parameter [c]	S_0	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8
ϕ	107	84.4	100.0	135.8	98.5	142.2	108.9	12.4	131.9
ψ	-42	-22.6	1.3	-64.5	-5.3	-100.3	-11.9	1.2	-24.7
Q	0.60	0.64	0.46	0.64	0.60	0.65	0.51	0.60	0.58
θ	1.7	18.3	45.1	33.2	18.0	43.1	40.2	11.9	26.7
ϕ_2	293.6	80.4	90.6	340.0	33.1	308.4	52.7	180.3	348.6
P	222.9	278.3	326.6	307.2	266.8	283.6	334.2	87.7	290.3
θ_m	30.1	18.6	31.4	21.3	18.6	20.6	23.4	40.8	30.3

[a] Derived from data available from Cambridge Data Base, deposited by Nawata et al. [4].
[b] Derived from data available from the Protein Data Bank, deposited by Zhu et al. [1].
[c] These parameters are defined as follows: ϕ, ψ are the interglycosidic torsion angles; Q is the total puckering amplitude of a pyranose ring; ϕ_2 and θ are the phase angle and azimuthal angle, respectively, of the conformational sphere for a pyranose ring; θ_m is the puckering amplitude of a furanose ring; P is the phase angle of the pseudorotation for a furanose ring.

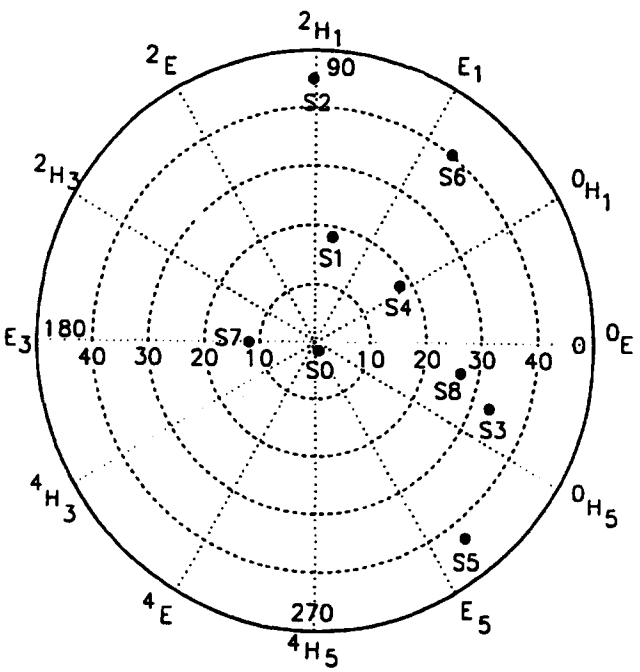

Fig. 2. Stereogram of the puckering parameters for the glucose ring of crystal SOS in different conformations. Labels are explained in text. Note that the half-chair, H, conformations are at $\theta = 50.8°$ and the half-boats, E, at $\theta = 54.7°$.

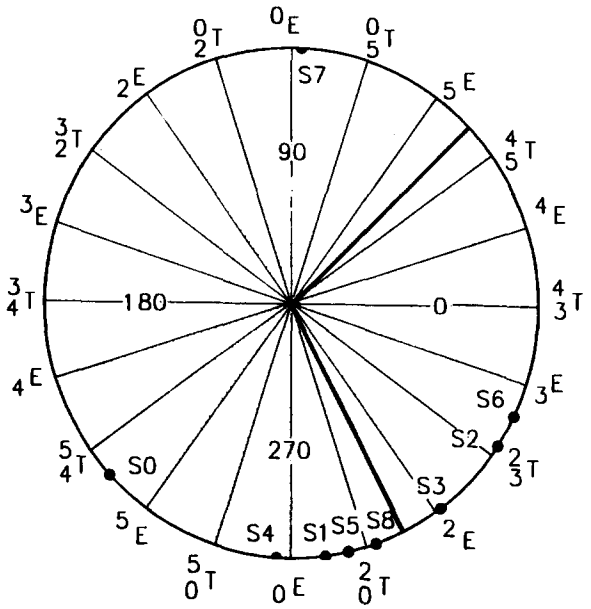

Fig. 3. The conformational wheel for the furanose ring of SOS in different conformations. The region between the solid radial lines ($P = -63$ to $+45°$) is found from molecular mechanics calculations.

conformational wheel as shown in Fig. 3. All conformations of the furanose ring in the bound state are significantly different from the conformation found in crystalline K_8SOS heptahydrate.

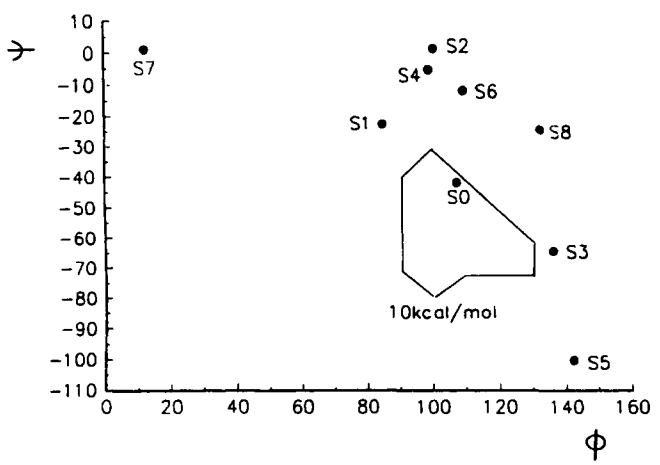

Fig. 4. The glycosidic torsion angles of SOS in different conformations. The solid line represents the 10 kcal/mol contour level from molecular mechanics calculations.

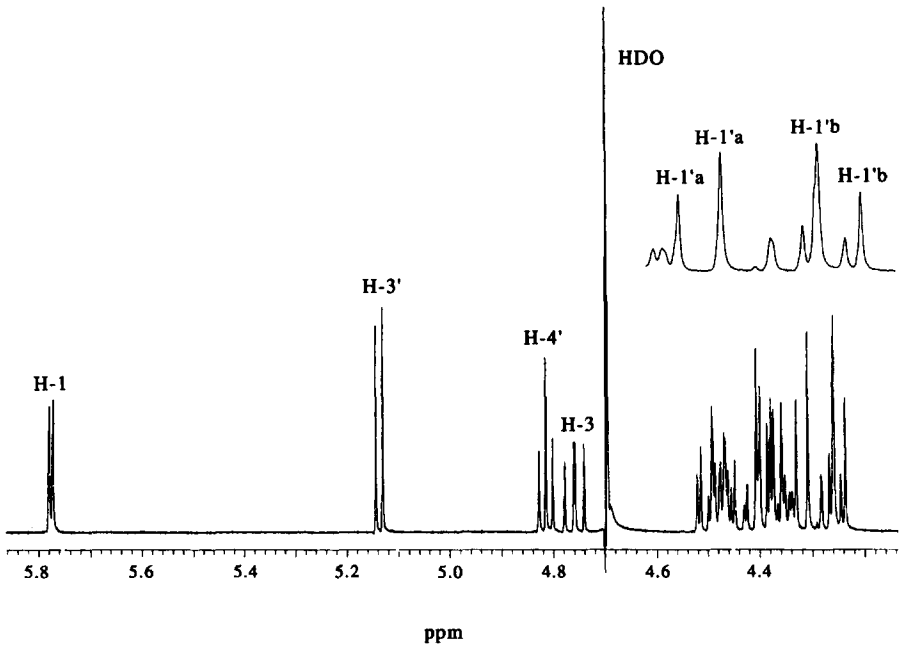

Fig. 5. The proton spectrum of K_8SOS of 85 mM at 500 MHz, 25°C. The insertion is the H-1'a and H-1'b region.

As can be seen from inspection of Table 1, all the glycosidic torsion angles of SOS bound to aFGF also deviate significantly from the values of S_0, the conformation found in crystalline K_8SOS heptahydrate.

NMR data.—^1H and ^{13}C NMR chemical shift assignments were based on two-dimensional DQCOSY, TOCSY, and HMQC experiments and confirmed by ROESY experiment (spectra available on request). Our assignments (see Fig. 5) agree with those reported by Silvey [5], except for H-1′ and H-6′ and, therefore C-1′ and C-6′. In a well-resolved proton spectrum, the two-spin system of H-1′ is clearly identifiable (see insertion of Fig. 5). Stereospecific assignment of methylene protons generally requires a concerted use of both $^3J_{HH}$ and $^3J_{CH}$ [6]. This strategy could not be applied to SOS due to the lack of a proton on C-2′.

Initially, pulse sequence A of Adams and Lerner [7] was used to measure the long-range scalar coupling between C-2′ and H-1. However, because of the near equality of this coupling constant with the three-bond H-1–H-2 coupling, the center two lines of what should be a doublet of doublet cancel each other, rendering accurate measurement impossible. Purely in-phase peaks were obtained by adding a refocusing delay (Fig. 6). From the resultant spectrum, shown in Fig. 7, J_{COCH} for C-2′ and H-1 is found to be 3.8 Hz. This is very close to the value of 3.9 Hz reported by Duker and Serianni [8] for labeled sucrose. The H-1′a–C-3′ coupling of 2.4 Hz was also measured using the unmodified pulse sequence A of Adams and Lerner [7]. The H-1′b–C-3′ coupling

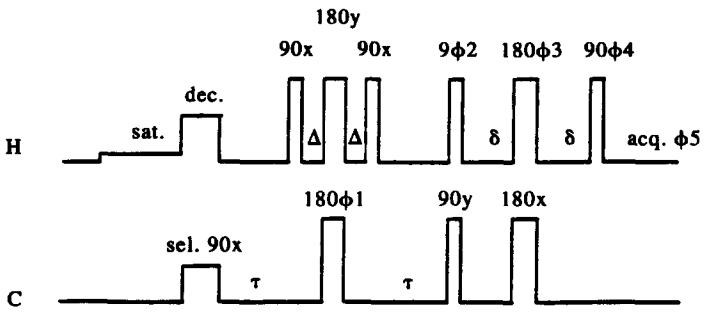

Fig. 6. A modified version of pulse sequence A of Adams and Lerner (ref. [7]). τ and Δ have the same meanings as in the original sequence, and 2δ is the added refocusing delay. The pair of 180° pulses in the middle of this delay is to refocus chemical shift evolutions. The phase cycles are: $\phi_1 = x, y, -x, -y$; $\phi_2 = \phi_3 = 4(x), 4(y), 4(-x), 4(-y)$; $\phi_4 = 4(-x), 4(-y), 4(x), 4(y)$; $\phi_5 = -x, x, -x, x, -y, y, -y, y, x, -x, x, -x, y, -y, y, -y$.

constant could not be extracted due to its overlap with H-6′b–C-5′ and H-6′b–C-4′ couplings.

Chemical shifts and $^3J_{HH}$ values of easily distinguishable peaks were measured as a function of temperature (6–50°C) and concentration (10–85 mM). To assess the effect of ionic strength, a sample of 10 mM K_8SOS with 1 M added KCl was also studied at 50°C. The results are shown in Tables 2 and 3.

Only $n-3$ torsion angles [therefore $(n-3)$ ($^3J_{HH}$s)] are needed to specify the conformation of an n-membered ring. In the case of SOS, we have just enough

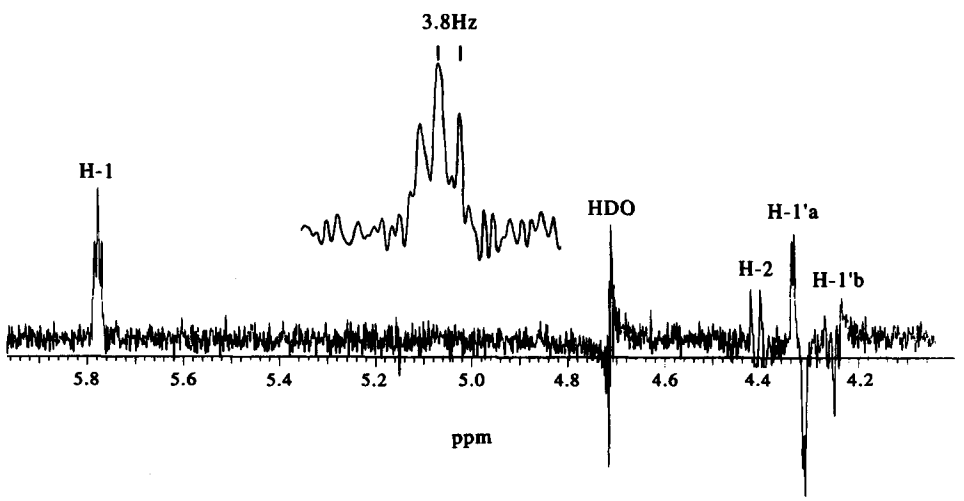

Fig. 7. The spectrum obtained with the modified pulse sequence A (see Fig. 6) with a 60 ms refocusing delay. The insertion is the H-1 peak. Other peaks are from residual HDO at 4.7 ppm, transferred antiphase coherence by application of a purging pulse to H-2 at 4.41 ppm, and the two-bond heteronuclear coupling to the two H-1s at 4.32 and 4.25 ppm, respectively.

Table 2
Temperature dependence of chemical shift and couplings [a]

Temperature (°C)	Chemical shifts (ppm)						$^3J_{HH}$ coupling constants [d] (Hz)					
	H-1	H-3	H-1′a	H-1′b	H-3′	H-4′	H-1–H-2	H-2–H-3	H-3–H-4	H-1′a–H-1′b	H-3′–H-4′	H-4′–H-5′
6	5.790	4.783	4.332	4.258	5.158	4.863	3.53	9.99	9.02	11.04	6.22	6.23
18	5.784	4.770	4.327	4.256	5.149	c	3.54	9.99	8.91	11.00	6.46	c
30	5.776	4.763	4.322	4.254	5.141	4.818	3.58	9.94	8.88	11.01	6.64	6.65
50	5.773	4.760	4.317	4.250	5.133	4.796	3.59	9.99	8.76	11.01	6.84	6.99
50 [b]	5.767	4.767	4.315	4.258	5.144	4.829	3.53	9.89	8.78	10.88	6.46	6.47

[a] Concentration of the sample was 10 mM. All chemical shifts were measured referencing to internal TSP with an error less than ±0.001ppm. $^3J_{HH}$ values were measured with an error of ±0.05 Hz.
[b] 10 mM sample with 1 M KCl.
[c] Signals obscurred by HDO.
[d] Values calculated for the glucose ring of S^{00} were: H-1–H-2, 3.56 Hz; H-2–H-3, 9.99 Hz; H-3–H-4, 9.69 Hz; The values calculated for the fructose ring from the global minimum along the pseudorotation path were H-3′–H-4′, 7.63 Hz; H-4′–H-5′, 7.62 Hz.

well-resolved couplings to specify the conformations of the two rings. It is clear from Tables 2 and 3 that most proton chemical shifts and $^3J_{HH}$ values are virtually independent of temperature, concentration and ionic strength, over the ranges studied. Only the chemical shift of H-4′ and vicinal proton–proton couplings $^3J_{H\text{-}3',H\text{-}4'}$ and $^3J_{H\text{-}4',H\text{-}5'}$ change with changes in temperature, concentration, or ionic strength. $^3J_{H\text{-}3,\,H\text{-}4}$ also changes slightly with temperature. The results reported here are consistent with position-4 of the glucose ring being more accessible to solvent. Further support for this explanation is a report from Silvey [5] that the carbon at position-4 of the glucose ring is the most easily desulfated.

The two H-1′ protons have a mismatch of 12° in the rotating frame, so the relay of NOE via TOCSY transfer from H-1′b to H-1′a would be about 17% [9]. Therefore the ROE difference method cannot be used to extract accurate interglycosidic distances. Instead, one-dimensional NOE difference experiments were performed by selectively exciting the H-1 resonance over a temperature range of 6–50°C on a 10 mM sample. NOEs of a 10 mM sample with 1 M KCl were also measured at 50°C. At 6°C all NOEs were found to approach zero. The results are listed in Table 4. The largest deviation of

Table 3
Concentration dependence of chemical shifts and couplings in K_8SOS at 30°C

Concn (mM)	Chemical shifts (ppm)						$^3J_{HH}$ coupling constants (Hz)					
	H-1	H-3	H-1′a	H-1′b	H-3′	H-4′	H-1–H-2	H-2–H-3	H-3–H-4	H-1′a–H-1′b	H-3′–H-4′	H-4′–H-5′
10	5.776	4.763	4.322	4.254	5.141	4.818	3.58	9.94	8.88	11.01	6.64	6.65
45	5.780	4.770	4.324	4.259	5.147	4.832	3.54	9.98	8.88	11.00	6.51	6.59
85	5.779	4.773	4.325	4.260	5.152	4.840	3.53	9.96	8.86	10.96	6.45	6.41

Table 4
Apparent distance ratios (interglycosidic:intraglycosidic) from NOE measurements on K_8SOS [a]

Temperature (°C)	18	30	50	50 [b]
H-1–H-1′a:H-1–H-2	1.18	1.21	1.17	1.25
H-1–H-1′b:H-1–H-2	1.02	1.05	1.06	1.06

[a] Concentration of K_8SOS was 10 mM. The estimated error in measurements was 5%. Interproton distances were calculated by taking the distance between H-1 and H-2 as 2.45 Å (r_o), based on Nawata et al. [4], and using the equation $r/r_o = (k_o/k)^{1/6}$ where k_o, k are the NOE buildup rates, respectively, for the standard proton pair (H-1, H-2) and the interglycosidic proton pair (H-1, H-1′a or H-1, H-1′b). By this calculation, we obtained interproton distances of 2.55 Å for H-1–H-1′a and 2.92 Å for H-1–H-1′b, averaged over the three temperatures measured.
[b] Sample was 10 mM K_8SOS with 1 M KCl added.

the distance ratio was less than 4%. Thus, within experimental error, temperature and ionic strength have no effect on the interglycosidic distances under the conditions investigated.

Molecular mechanics calculations.—The crystal structure of K_8SOS heptahydrate [4] was used as the starting point in our calculations after deletion of potassium ions and water molecules. First the molecule was allowed to relax without any constraints. The resultant structure was used in a relaxed Ramachandran mapping in steps of 20° for both ϕ and ψ. It was found that the glucose ring had to be constrained to a $^4C_1$ chair to avoid irreversible deformation of the glucose ring into a boat form during a full 0° to 360° mapping. A single low-energy region $\phi = 50$–$140°$, $\psi = -80$–$0°$ was found in this way. All areas outside this region were found to have prohibitively high energies (30 kcal/mol higher than the energy of the global minimum). This low-energy region found from the above search was further refined in the steps of 10° for both ϕ and ψ with no contraints. The glucose ring was found to retain the $^4C_1$ conformation. According to this refinement, an energy contour of 10 kcal/mol above the global minimum (denoted as S^{00}) was drawn in Fig. 4.

The global minimum (S^{00}) found in the above search was used to drive the Altona–Sundaralingam phase angle P of the fructose ring for a full cycle in steps of 9°. The puckering amplitude of the fructose ring found in crystalline K_8SOS heptahydrate was used. No other torsion angles were contrained, but the glycosidic torsion angles found in the low-energy paths in the pseudorotation cycle did not deviate from those in S^{00}. Two regions were found along the pseudorotation pathway within 10 kcal/mol relative to the minimum are $P = -63$ to $+45°$ (containing the global minimum for pseudorotation, marked in Fig. 3) and $P = 189$–$252°$.

Rotation of side groups was found to have negligible effects on the minimal values of ϕ, ψ and P. The local minima of O-1′–C-1′–C-2′–C-3′ were also found by rotation around O-1′–C-1′–C-2′–C-3′, producing three minimum regions with energy differences of less than 3 kcal/mol. These three regions corresponds to *tg*, *gg*, and *gt*.

Comparison of calculated and NMR-derived conformations.—The conformations of uncharged carbohydrates can be reliably calculated using the MM2 method [10]. The force-field parameters of the sulfate group successfully explained the conformational flexibility of sufate-containing iduronate ring [11,12]. In the case of SOS, however, error

in molecular mechanics calculations could arise from the presence of the eight sulfate groups. Therefore we based our conformational analysis mainly on NMR measurements using the Boltzmann distribution of low-energy conformers as an additional constraint to fit both conformations and their energies to experimental data at different temperatures. Conformations which do not satisfy Boltzmann distributions were discarded.

The Karplus equation modified for hexopyranose rings [13] was used to calculate the $^3J_{HH}$ values for the glucose and fructose rings in the conformation S^{00}. The results are given in the footnote to Table 2. The calculated $^3J_{H\text{-}1,H\text{-}2}$ and $^3J_{H\text{-}2,H\text{-}3}$ values are in excellent agreement with experimental values. These two couplings also show the least changes with variations in temperature, concentration, and ionic strength, indicating rigid O-5–C-1–C-2–C-3 and C-1–C-2–C-3–C-4 dihedral angles. The calculated $^3J_{H\text{-}3,H\text{-}4}$ is slightly smaller than the experimental values. This coupling constant also decreases with increase in temperature. Since the other two dihedral angles are fixed, the only available degree of freedom of the pyranose ring conformation is vibration along the $\phi_2 = 120°$ path on the Cremer–Pople sphere; i.e. along the path connecting $^4C_1$ to $^{2,5}B$. For any deviation from $^4C_1$, which corresponds to a torsion angle of 180° for H-3–C-3–C-4–H-4, a smaller value of J would be expected according to the Karplus relation. Therefore relatively minor flexing of the glucose ring could lead to smaller experimental $^3J_{H\text{-}3,\ H\text{-}4}$ values.

The three-bond proton–proton coupling constants (therefore, the corresponding ring torsion angles) of the fructose ring showed significant changes on change in temperature, concentration and ionic strength, indicating that conformational averaging exists in the fructose ring. The conformations obtained from molecular mechanics calculations were used to fit the experimentally determined three-bond coupling constants, using the Boltzmann distribution as an additional constraint. Of the two low-energy regions found by driving the Altona–Sundaralingam phase parameter P, the one with $P = 189–252°$ corresponds to $^3J_{H\text{-}3',H\text{-}4'}$, $^3J_{H\text{-}4',H\text{-}5'}$ values of < 1 Hz, while the other region corresponds to values between 4.5 and 7.8 Hz. The $P = 189–252°$ region could not be the major conformer due to its vanishingly small J. Nor could it be the minor conformer, because in that case a decrease in J would have resulted as temperature increased according to the Boltzmann distribution. So the region where $P = 180–252°$ can be safely ruled out. Conformational averaging within the $P = -63–45°$ region is the only interpretation consistent with the experimental results. To satisfy the Boltzmann distribution, the major conformer must be from the upper end of the marked arc on the conformational wheel (around ^4E, see Fig. 3), which would yield J values below the observed average. The minor conformer(s) must be from around the center of the marked arc or the lower end (around ^2_3T), as both would yield J values above the observed average. All these are significantly different from the conformation found in K_8SOS heptahydrate (S_0).

Using the equation formulated by Tvaroska et al. [14]

$$^3J_{CH} = 5.7\cos^2\theta - 0.6\cos\theta + 0.5 \quad (1)$$

the dihedral angle H-1–C-1–O-1–C-2′ is calculated to be $\pm 35.5°$ or $\pm 135.5°$. Only the value of $-35.5°$ agrees reasonably well with molecular mechanics calculations and NOE contraints. This fixes ϕ to be approximately 85°, which is in reasonable agreement with the calculated value (see Fig. 4).

In the conformer S^{00}, the distance between H-1 and H-2 is 2.45 Å. Since the H-1–C-1–C-2–H-2 dihedral angle has been found to be rigid, this distance provides a good reference for calculations of distances based on NOE data. The interglycosidic distances are therefore calculated based on this internal standard and shown in Table 4. These values are consistent with motional averaging resulted from free rotation of the O-1′–C-1′–C-2′–C-3′ torsion angle with $\phi = 85°$ derived from long-range $^{1}H–^{13}C$ coupling constants (see above). If the conformations found bound to aFGF were easily accessible in solution by free SOS, a temperature-dependence of NOE values would have been detected since the population distribution of those conformations would change with temperature. Since the interglycosidic distances were found to be unaffected by temperature change, and only one low-energy conformation was found in the molecular mechanics calculations, the interglycosidic linkage appears to be relatively rigid.

Some insights about the effect of the sulfate group on the flexibility of sucrose can be gained by comparing the conformation of SOS to that of sucrose in solution. Like SOS, the glucose ring of sucrose maintains a rigid $^{4}C_{1}$ chair form, while the fructose ring undergoes conformational averaging [15,16]. The conformational conversion in sucrose is along a ${}^{2}_{3}T–{}^{4}_{5}T$ path [17], almost identical to the arc found for SOS (see Fig. 3). These observations lead to the conclusion that the presence of sulfate groups has little impact on the flexibilities of the glucose and fructose rings. However, since the interglycosidic NOE of sucrose does depend on temperature [18], the interglycosidic linkage in sucrose is not rigid [17]. The mechanism reponsible for the quenching of internal motions around the interglycosidic linkage of SOS is still unclear.

3. Conclusions

Some biological background needs to be mentioned here when extrapolating these results to the physiological milieu. The co-crystal obtained by Zhu et al. [1] was a 1:1 complex of SOS–aFGF. Arakawa et al. [19] reported a stoichiometry of two molecules of SOS bound per molecule of aFGF in solution, based on equilibrium dialysis. The form of SOS used clinically, sucralfate, is an amorphous complex of aluminum hydroxide and SOS^{8-} [5]. Recently, Zheng et al. [20] reported that K_8SOS stimulated cell growth but was not cytoprotective of rat gastric cells in vitro, whereas aluminum hydroxide administered by itself was cytoprotective. Their observations suggest that the antiulcer activity of sucralfate has two components, protection and stimulation of gastric epithelial cells, and that SOS itself contributes mainly to the latter.

On the basis of the NMR study and molecular mechanics calculations reported here, our conclusion is that in its free state SOS takes a significantly different conformation from those bound to aFGF under the conditions used by Zhu et al. [1]. Although the bound conformations have only been determined to 2.47 Å resolution, including possible deviation resulted from the use of molecular mechanics type parameters in the refinement program, it is clear that they are high-energy conformations significantly distorted from the low-energy free conformation in both solution and crystalline K_8SOS heptahydrate. There are numerous other examples of oligosaccharides where a negatively

charged group, typically sialic acid, is an absolute requirement for recognition by a protein. It is interesting to note that SO_4^{2-} can substitute functionally for sialic acid [21]. The strong electrostatic attractions between the negatively charged sulfate groups with the largest region rich in positively charged amino acid residues on aFGF [1] could play the dominant role in this deformation of the rings of SOS. The diverse conformational changes induced by these strong interactions between host and guest are not accessible to free ligands.

4. Materials and methods

Materials and sample preparation.—Potassium sucrose octasulfate (K_8SOS) was obtained from Toronto Research, Inc. and used without further purification. No peaks from impurities were visible in a one-dimensional ^{1}H NMR spectrum. Solutions of this compound were prepared by repeated lyophilization from D_2O. The final samples, 10, 45 and 85 mM, were prepared using nominally 99.996% D_2O (Cambridge Isotope Laboratory). Samples for NOE measurements were degassed at aspirator pressure in an ultrasonic bath, followed by saturation with nitrogen gas.

Nomenclature.—The atomic labeling of sucrose octasulfate (fructose moiety, primed numbers; glucose moiety, unprimed numbers) is given in Fig. 1. The conformation about glycosidic linkage bonds is described by the following torsion angles (similar to those of sucrose given by du Penhoat et al. [22]):

$$\begin{aligned} \phi &= \text{O-5} - \text{C-1} - \text{O-1} - \text{C-2}' \\ \psi &= \text{C-1} - \text{O-1} - \text{C-2}' - \text{O-5}' \end{aligned} \tag{2}$$

The orientation of the sulfate group on O-1′ is referred to as gauche–gauche (*gg*), gauche–trans (*gt*) and trans–gauche (*tg*) based on the torsion angles O-5′–C-2′–C-1′–O-1′ and C-3′–C-2′–C-1′–O-1′, respectively.

The Cremer–Pople parameters (Q, θ, ϕ_2) [23] as defined by Jeffrey and Yates [24], were used to describe the conformation of the glucose ring. The ring oxygen atom is designated atom 1, the anomeric carbon atom is atom 2, and thence the numbering is clockwise around the ring. The conformations, however, are described using standard carbohydrate notation (see Fig. 1). In this notation, a perfect $^4C_1$ conformation lies exactly on the north pole of the Cremer–Pople sphere.

Although the Cremer–Pople parameters could also be used on five-membered rings, we chose instead the equivalent, more widely used Altona–Sundaralingam parameters (P,θ_m) [25]. In our notation the original numbering of the fructose ring is preserved; thus $P = 0°$ corresponds to a ^4_3T conformation.

NMR spectroscopy.—The NMR spectra were recorded on a Varian UNITY 500 MHz NMR spectrometer. Data were processed off-line on SUN Sparcstations using standard Varian software. All experiments were performed at 25°C on an 85 mM sample dissolved in D_2O, except where noted.

Two-dimensional phase-sensitive DQCOSY, TOCSY, ROESY and HMQC spectra were recorded with standard pulse sequences to assign the ^{1}H and ^{13}C NMR spectra. A

mixing time of 40 ms was used in the TOCSY experiment. Spin-locking in the ROESY experiment was achieved using the sequence proposed by Kessler et al. [26]. The effective spin-lock field was 1.4 kHz. The carrier frequency was placed to the low-field side of the H-1 resonance at 6.17 ppm to minimize TOCSY transfer [27]. The mixing time in the ROESY experiment was 300 ms.

One-dimensional TOCSY experiments with a *z*-filter [28] using an I-BURP-2 excitation pulse [29] were performed to obtain $^{3}J_{HH}$ assignments for the glucose ring. The mixing time was 50 ms. The *z*-filter delays used were 0.0, 0.73, 2.76, and 3.49 ms.

One-dimensional transient NOE difference spectroscopy using an I-BURP-2 excitation pulse was used to measure NOEs across the glycosidic linkage. A preacquisition delay equal to at least 5–10 times the longest T_1 was used in all cases. The NOE buildup delays were randomized. A 2-Hz line broadening was used to facilitate cancellation of unwanted signals.

Long-range $^{1}H-^{13}C$ coupling constants were measured at 25°C with a modified version of pulse sequence A described by Adams and Lerner [7]. A refocusing delay with a final purging pulse was added to this pulse sequence to get in-phase splittings as shown in Fig. 6. The refocusing delay used was 60 ms.

Conformational analysis.—Molecular mechanics calculations were performed with the MM2* (1987) force field as implemented in MacroModel V3.5 ×. This force field differs from the original MM2 force field in that it uses partial charge treatment of electrostatics instead of the MM2 standard dipole–dipole electrostatics. Parameterization of sulfate groups by Ragazzi et al. [11] was incorporated into the MM2* force field used in MacroModel. A total net charge of 0.3e [12] was assigned to each sulfate group to account for the partial screening effect of counterions. An effective value of 3.0 was taken for the dielectric constant [11].

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