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Interpretation of the Reduction Potential of 6,6′-Dithiosucrose Cyclic Disulfide by Comparison of the Conformations of 6,6′-Dithiosucrose Cyclic Disulfide, 6,6′-Dithiosucrose, and Sucrose in Aqueous Solution[1]

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Abstract: 6,6′-Dithiosucrose (sucrose dithiol, $Suc(SH)_2$) is a weakly reducing species. The equilibrium constant for reduction of mercaptoethanol disulfide, ME^{ox}, by sucrose dithiol in aqueous solution is K_{eq} = 0.3 M = $\{[SucS_2][Me^{red}]^2\}/\{[Suc(SH)_2][ME^{ox}]\}$, where $SucS_2$ (6,6′-dithiosucrose cyclic disulfide, sucrose disulfide) is the cyclic disulfide formed from the oxidation of sucrose dithiol. Measurements of values of T_1 and J using ^1H NMR spectroscopy indicate that sucrose and sucrose dithiol adopt indistinguishable conformations in water. The conformations of sucrose dithiol and sucrose disulfide are similar but distinguishable. Molecular mechanics calculations indicate that two of the possible structures of sucrose disulfide have relatively low energies. Comparison of coupling constants calculated for these structures with experimental coupling constants from ^1H NMR spectra indicated that one of the two structures was more probable; this more probable structure had the conformation more similar to that of sucrose. The reduction potential of the $Suc(SH)_2/SucS_2$ (an 11-membered ring) couple is similar to that of an *n*-alkane-1,*n*-dithiol/cyclic disulfide (n = 5 or 6) couple; the value of EC (effective concentration = K_{eq} = 0.3 M) characterizing oxidation of the two thiols of $Suc(SH)_2$ to a disulfide is similar to that for oxidation of the two thiols of hexane-1,6-dithiol (K_{eq} = 0.2 M) and pentane-1,5-dithiol (K_{eq} = 3.6 M). The similarity of these values of EC suggests that $SucS_2$ is a relatively strain-free structure and reflects the proximity of the two thiol groups in this carbohydrate.

Introduction

This paper explores the usefulness of the redox reaction linking thiols and disulfides (eq 1) in defining the conformation of the representative disaccharide sucrose (αGlc(1↔2)βFru*f*). The

$$\text{HS-6-Glc-}\alpha\text{(1,2)-}\beta\text{Fru-6′-SH} \underset{+2e^-,\,+2H^+}{\overset{2e^-,\,-2H^+}{\rightleftharpoons}} \text{S-6-Glc-}\alpha\text{(1,2)-}\beta\text{Fru-6′-S} \quad (1)$$

thiol–disulfide interchange reaction has been developed by Creighton and by Kim as a probe of conformations in biochemical systems, particularly in studies of oligopeptides containing two cysteine moieties.[3-6] The assumption underlying these studies is that the redox potential of a dithiol reflects the geometry of the SH groups; that is, the tendency for the dithiol to be oxidized to the corresponding disulfide is higher if the favored conformation of the thiol reactant resembles that of the disulfide product.

Measurement of the change in free energy for oxidation of a dithiol to a disulfide is normally accomplished using thiol–disulfide interchange. Direct electrochemical measurements of redox potentials for thiol–disulfide couples are not reliable because the surfaces of most electrodes are reactive to organosulfur compounds[7] and the redox reactions are typically not thermodynamically reversible. The most straightforward experimental measurements of equilibrium constants for strongly reducing thiols (typically, dithiols capable of forming stable cyclic disulfides, eqs 2 and 3) are based on equilibration of these species with a stable

$$\text{HSRSH} + \overbrace{\text{SR′S}} \overset{K}{\rightleftharpoons} \overbrace{\text{SRS}} + \text{HSR′SH} \quad (2)$$

$$K = \frac{[\overbrace{\text{SRS}}][\text{HSR′SH}]}{[\text{HSRSH}][\overbrace{\text{SR′S}}]} \quad (3)$$

cyclic disulfide of known structure and redox potential (e.g., reduced (DTT) and oxidized (DTT^{ox}) dithiothreitol, lipoic acid, and similar materials (eq 4)). This method is, however, difficult

$$\epsilon = \epsilon^{\circ}_{DTT} - 0.029\,58 \log \frac{[\text{DTT}][\text{cyclic disulfide}]}{[DTT^{ox}][\text{dithiol}]} \quad (4)$$

to apply to weakly reducing dithiols, and the equilibrium for these species is often measured with respect to a more readily reduced noncyclic disulfide such as oxidized glutathione (GSSG), oxidized mercaptoethanol (ME^{ox}), or cystine (eqs 5 and 6). These systems

$$\text{HSRSH} + (\text{HOCH}_2\text{CH}_2\text{S})_2 \overset{K_{ME}}{\rightleftharpoons} \underset{ME^{ox}}{\overbrace{\text{SRS}}} + \text{HOCH}_2\text{CH}_2\text{SH} + \text{mixed disulfides} \quad (5)$$

$$K_{ME} = \frac{[\overbrace{\text{SRS}}][ME^{red}]^2}{[\text{HSRSH}][ME^{ox}]} = \text{EC} \quad (6)$$

based on noncyclic disulfides can be applied to weakly reducing dithiols, since the concentrations of the equilibrating species can be adjusted to values convenient for the measurements of the relevant concentrations.[8,9] By convention, this equilibrium

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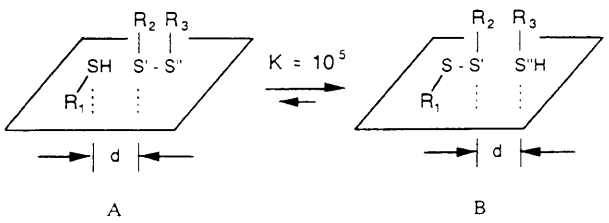


Figure 1. Equilibrium constant for thiol–disulfide interchange depends strongly on the C–S–S–C dihedral angle of the participating groups. Assuming that $R_1 = R_2 = R_3$, that steric interactions between the R groups have no influence on K, and that the change in the CSSC angle is 90° (as shown), the equilibrium constant K is 10^5.

constant (relative to a noncyclic disulfide) is often given the name "effective concentration" (EC, eq 6) because it has units of concentration.[3] The EC is often interpreted as the concentration of monothiol, RSH, required in an intermolecular reaction (eq 7)

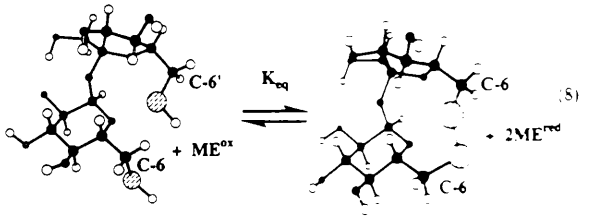
$$2RSH + R'SSR' \overset{K}{\rightleftharpoons} RSSR + 2R'SH \qquad (7)$$

to give the same conversion to product as is achieved by HSRSH in an analogous intramolecular reaction (eq 5, $R'SSR' = ME^{ox}$) when the two reactions are conducted under similar conditions. The energy of a CSSC unit depends strongly on the CSSC dihedral angle, and the EC may vary by 10^5 M for the same SS distance, depending upon the conformation of the two SH groups (Figure 1).[10] Since the energy of the disulfide depends strongly on the CSSC dihedral angle and constraints on this angle may be very different in an intramolecular reaction and an intermolecular "model" for it, interpretation of EC (especially low values of EC) in terms of the proximity of the thiol groups must be guarded. In a hypothetical situation in which the distance of S′ from S in Figure 1A is the same as that of S″ from S′ in B (that is, the "concentration" is the same) but the CSSC dihedral angle is 0° in A and 90° in B, the equilibrium will still lie strongly in favor of B.

In principle, thiol–disulfide interchange is a particularly attractive reaction with which to measure values of EC (or equilibrium constant) and to draw approximate inferences concerning proximity and conformation. The equilibration of thiols with disulfides can be accomplished at room temperature in water at neutral or alkaline values of pH or in polar organic solvents.[8] The reaction occurs in very high yield, and its mechanism is simple and well understood. Kim correctly emphasized, when using this reaction to study conformations in polypeptides, that it is sufficient (and is experimentally a great simplification) to measure only the concentrations of the reacting species used to define the value of EC; other species (polymers, mixed disulfides) need not be considered.[3,4] The system is thus analytically tractable.

The physical organic chemistry of thiol–disulfide interchange is well established.[8] Interchange between thiols and disulfides involves initial deprotonation of the thiol to thiolate, nucleophilic attack of the thiolate anion on the disulfide group along the S–S axis, and protonation of the new thiolate anion generated in this process. For monothiols, the rates and equilibrium constants for many thiol–disulfide interchange reactions follow Brønsted relationships. Reference values of EC (either in that form or as values of equilibrium constants or of ϵ_0') are available for many compounds, especially for α,ω-dithiols that form cyclic monomeric disulfides upon oxidation.[8] A comparison of values of EC from model compounds with the value of EC for a new cyclic monomeric disulfide provides a measure of the stability of the latter. The further interpretation of this number in terms of conformation is one subject of this study.

This paper reports the first test of the value of measurements of EC based on thiol–disulfide interchange to examine the conformations of disaccharides in solution. As a test case, we determined the equilibrium constant for sucrose having hydroxyl groups on the 6 and 6′ carbon atoms replaced by thiols (eq 8).



We selected this system for three reasons. First, sucrose disulfide (as its peracetylated derivative) was a known compound, although it had not been established that peracetylated sucrose disulfide was accessible from peracetylated sucrose dithiol under equilibrium conditions. Second, previous conformational analyses of sucrose had defined the system as one with preferred conformations, placing C-6 and C-6′ close to one another.[11-13] Sucrose thus represents a system that might be expected to give a value of EC for disulfide bond formation between thiols at C-6 and C-6′ that is high relative to disulfide bond formation in dithiol analogs of other oligosaccharides having more open conformations. Third, there are independent reasons in organoleptic chemistry and metabolism for interest in these compounds, since sucrose is a centrally important compound in nutrition and metabolism.[14]

Previous work on conformational analysis of carbohydrates has relied heavily on measurements of nuclear Overhauser effects (NOE) and on semiempirical calculations to define conformation. Values of NOE vary with the inverse sixth power of the distance between the nuclei involved and therefore provide information concerning weighted time-averaged conformation of the sugars in solution.[22] Values of NOE can be influenced significantly by conformers that do not dominate the equilibrium. The most commonly used semiempirical method of calculating the conformation of sugars is the HSEA (hard-sphere exo anomeric) method.[23,24] This method assumes pyranose and/or furanose rings to be rigid and calculates low-energy conformations using nonbonded steric interactions and torsional interactions about the glycosidic linkage.

Verifying interferences about carbohydrate structure obtained by NOESY and ROESY techniques or derived from computation is not straightforward.[25] Oligosaccharides are notoriously difficult to crystallize and are thus difficult subjects for X-ray analysis. Furthermore, the relevance of the solid-state conformation of oligosaccharides to the solution conformation of oligosaccharides is uncertain.

Examination of equilibrium constants for dithiol analogs of sugars as a method of inferring the conformations of the parent sugars is obviously indirect and difficult. Alternative methods for examining conformations of sugars are, however, themselves imperfect. A new method offering complementary information would be useful, provided that the strengths and weaknesses of the method were clearly understood. The potential advantages of comparing dithiols and cyclic monomeric carbohydrate disulfides at equilibrium are (1) a high value of equilibrium constant

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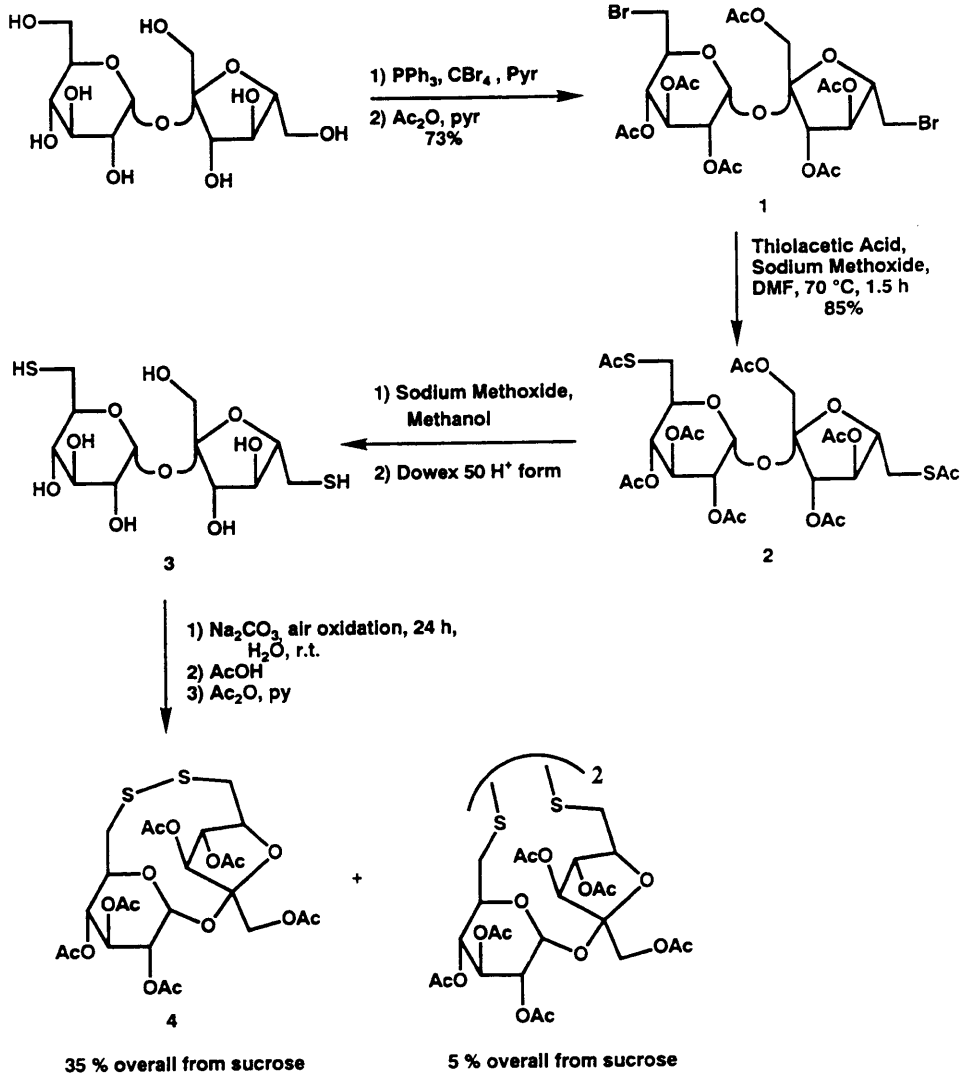
Scheme I. Synthesis of Sucrose Disulfide 4



can indicate (qualitatively) that a conformation with the two thiol groups proximate is accessible; (2) comparison of values of NOE and T_1 for dithiol and disulfide can show whether the conformation of the dithiol is similar to the more conformationally restricted disulfide; and (3) examination of product mixtures can establish whether multiple forms of the disulfide having similar energies exist; the determination of the concentration dependence of these forms might be useful in separating enthalpic and entropic contributions to their stability.

The crystal structure of sucrose (Figure 2) has been determined by neutron diffraction.[26] The structure contains two intramolecular hydrogen bonds: one between OH-1′ and O-2 and one between OH-6′ and O-5 (Figure 2). Several studies have shown that the latter hydrogen bond is not present in solution.[12-20] Lemieux and Bock used HSEA calculations and values of T_1 (D_2O and DMSO-d_6) to show that sucrose has a conformationally rigid structure in solution.[12] The intramolecular hydrogen bonding of sucrose in DMSO-d_6 has been determined using SIMPLE (secondary isotope multiplet NMR spectroscopy of partially labeled entities).[13-16] These studies indicated that the favored conformation of sucrose has the C-1′ hydroxyl hydrogen bonded to the C-2 oxygen as in the crystalline state but no hydrogen bond between OH-6′ and O-5. An additional conformation containing a hydrogen bond between OH-3′ and O-2 was, however, shown to be significant. Later studies using ^1H NMR spectroscopy and calculations concluded that sucrose may be more flexible than

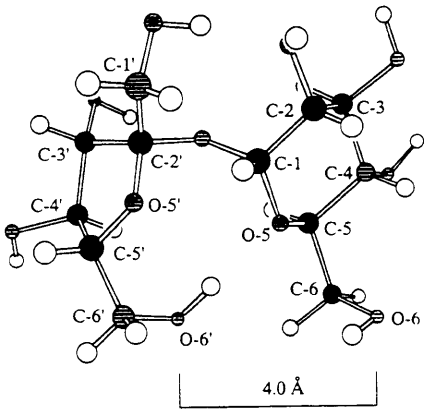


Figure 2. Neutron diffraction structure of sucrose:[27] O-6 and O-6′ are 4.0 Å apart. Oxygens and hydrogens are labeled according to the nearest carbon.

previously thought[18,19] and suggested that the hydrogen bond between the hydroxyl group at C-2 and the C-1′ hydroxyl is not important in determining the conformation[18] and that this hydrogen bond is not persistent in solution.[20]

Results and Discussion

Synthesis. Scheme I outlines an adaptation of the method of Hough[11] for the synthesis of 6,6′-dithiosucrose. We oxidized the dithiol to the disulfide by stirring the dithiol at low concentration in a solution saturated with air. We quenched the oxidation

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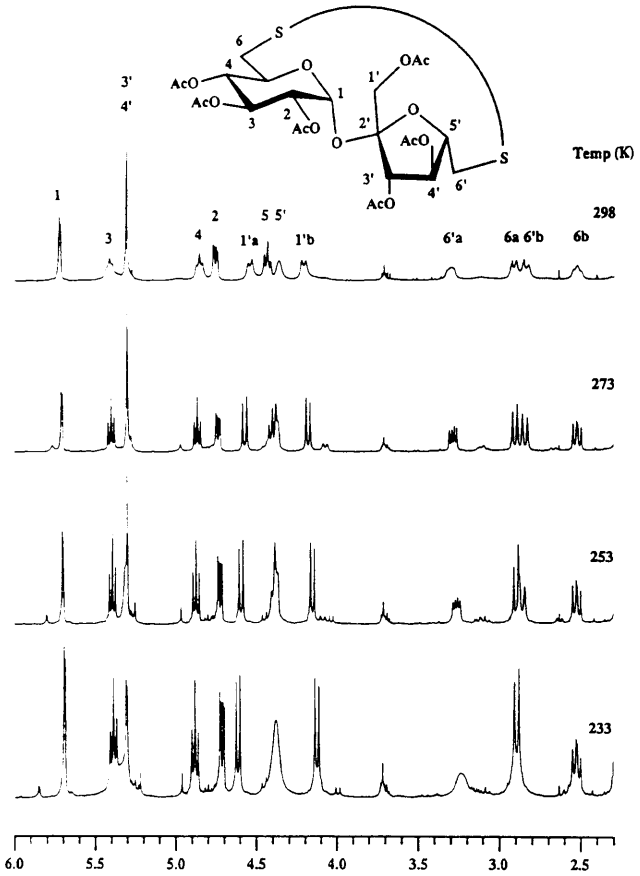

Figure 3. Variable temperature ^1H NMR (500 MHz) spectra of sucrose disulfide hexaacetate in $CDCl_3$.

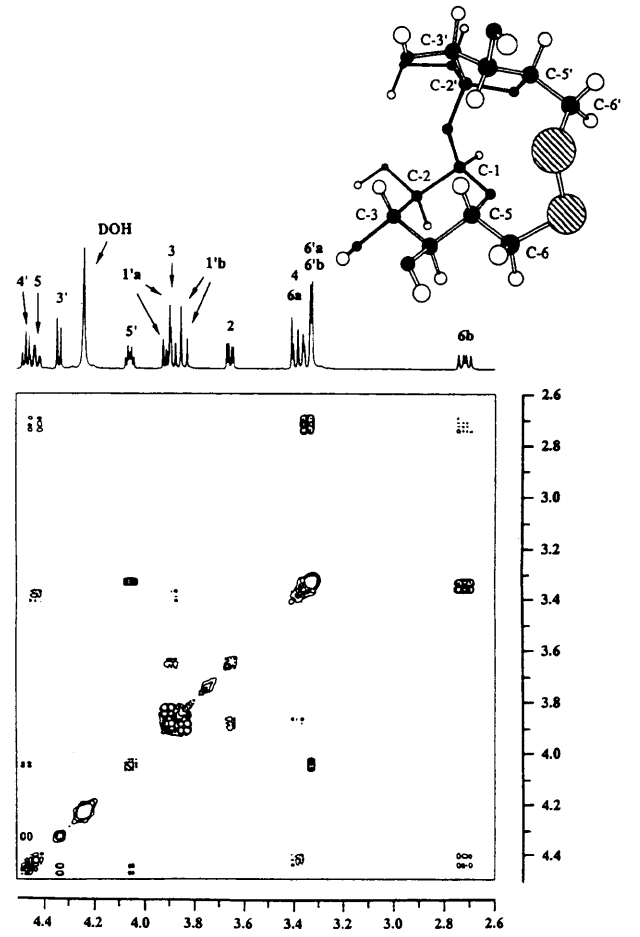

Figure 4. 2D HOMOCOSY (^1H) of sucrose disulfide in D_2O at 353 K.

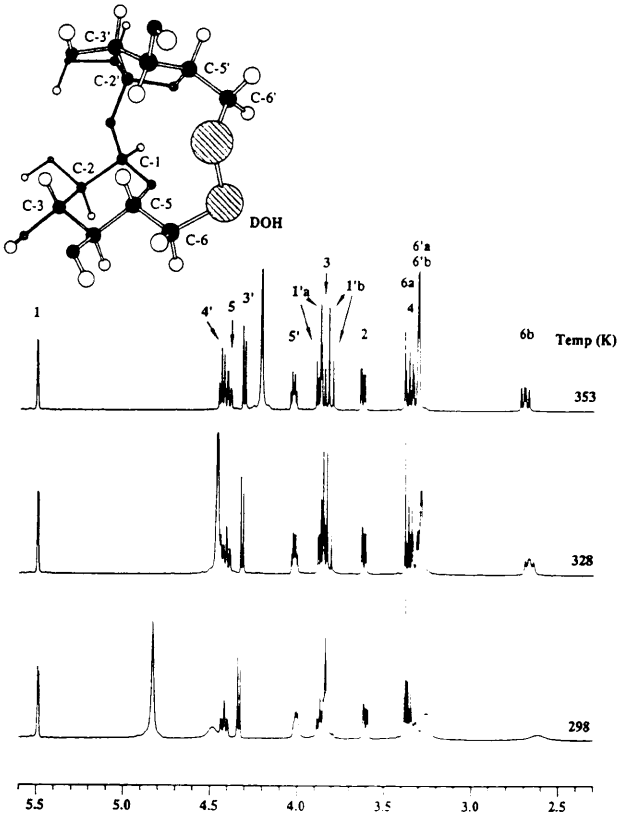

Figure 5. Variable temperature ^1H NMR (500 MHz) spectra of sucrose disulfide in D_2O.

reaction with acetic acid, acetylated the disulfide with acetic anhydride, and purified it by column chromatography on silica gel. Deacetylation in base afforded the free sucrose disulfide. In addition, two dimeric cyclic disulfides (head-to-head and head-to-tail) were isolated as minor products of this reaction.

Characterization. Monomer. We assigned the resonances in the ^1H NMR spectrum of the peracetylated cyclic monomer of sucrose disulfide (acetylated sucrose disulfide) using a 2D HOMOCOSY experiment. At room temperature in $CDCl_3$ the ^1H NMR spectrum of the acetylated sucrose disulfide showed broad lines due to conformational interchange (Figure 3). At 273 K the ^1H NMR spectrum of this molecule indicated the presence of two conformations in a ratio of ca. 10:1. The ^1H NMR peaks due to the two conformations coalesced above 273 K. Below 273 K, the peaks in the ^1H NMR spectrum of the two conformers broadened further due to lower energy conformational exchange, possibly "ring flipping". The peaks in the ^{13}C NMR spectrum were partially assigned using a $^1H/^{13}C$ 2D HETEROCOSY experiment.

The resonances in the ^1H NMR spectrum of sucrose disulfide (formed by deacetylating the cyclic monomer of sucrose disulfide hexaacetate) were also assigned using a 2D HOMOCOSY experiment (Figure 4). At room temperature in D_2O, the ^1H NMR spectrum of sucrose disulfide showed broad lines due to conformational interchange. At 353 K, the peaks of the sucrose disulfide were sharp and represented a single time-averaged conformation (Figure 5). The freezing point of water prevented spectral observation of the free cyclic monomer at temperatures below 273 K.

Dimer. Several observations support the assignment of a dimeric structure (head-to-head; head-to-tail) to two of the minor products of the oxidation: (1) FAB mass spectrometric analysis of this peracetylated material showed an M + 1 peak consistent with the molecular weight of the dimer; (2) vapor-phase osmometry measurements of the peracetylated material gave an approximate molecular weight (1390 g/mol) consistent with that of a dimer (1248 g/mol); (3) retention times using HPLC and TLC were longer for the free sucrose disulfide dimer than for the free cyclic

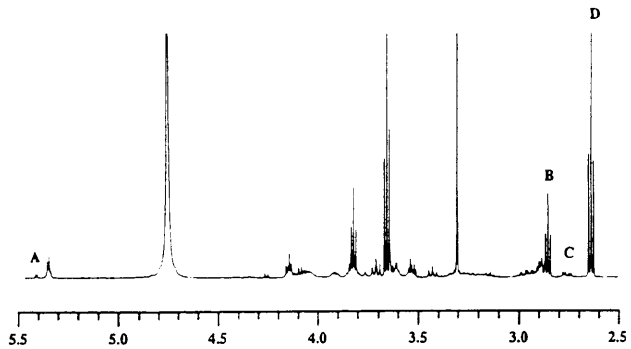

Figure 6. ^{1}H NMR of the equilibrium between sucrose dithiol and mercaptoethanol. Peak A is the H-1 proton of sucrose disulfide. Peak B is the $(HOCH_2CH_2S)_2$ hydrogen. Peak C is the H-6b hydrogen of sucrose dithiol. Peak D is the $HOCH_2CH_2SH$ hydrogen.

monomer of sucrose disulfide; (4) Ellman's test[27] of the peracetylated and free dimers indicated that there were no free thiols; and (5) reduction of the free dimers with DTT regenerated sucrose dithiol and indicated that formation of dimer was reversible under mild conditions. We did not determine which species was the head-to-head dimer and which was the head-to-tail dimer. The ^{1}H and ^{13}C NMR spectra of the two dimers showed no conformational exchange on the NMR time scales at 298 K.

Measurement of Equilibria for Thiol–Disulfide Interchange of Sucrose Dithiol. Preliminary experiments established that sucrose disulfide could be observed by equilibration of sucrose dithiol and the disulfide of β-mercaptoethanol.[28] We determined the equilibrium constant K_{eq} (eq 6, R = sucrose) of sucrose dithiol and mercaptoethanol disulfide at several concentrations using ^{1}H NMR spectroscopy. By integrating the peaks characteristic of the four compounds—the H-1 peak of the cyclic monomeric sucrose disulfide, the H-6b peak of the sucrose dithiol, and the peaks due to the methylene protons adjacent to the sulfur atoms in mercaptoethanol and mercaptoethanol disulfide—we determined the mole fraction of each component. Mixed disulfides and oligomers (typically HS-sucrose-(S-S-sucrose)$_n$-SH) were also present; their concentrations depended on the initial concentrations of sucrose dithiol and mercaptoethanol disulfide. Figure 6 shows a representative spectrum of an equilibrium mixture at 25 °C. The measured value of EC was constant over a range of concentrations of sucrose dithiol and mercaptoethanol disulfide from 4 to 40 mM. At concentrations higher than 40 mM, the formation of dimers, polymers, and mixed disulfides involving sucrose dithiol made ^{1}H NMR spectroscopic analysis difficult. Table I gives the average value of EC for sucrose disulfide and values of EC for other cyclic disulfides.[8] To ensure that the measured values of EC represent true equilibrium values, we measured the equilibrium constant starting from sucrose disulfide and mercaptoethanol and obtained the same value of K_{eq} (0.3 M).

The value of EC (or K_{eq} = 0.3 M) for sucrose dithiol, which forms an 11-membered cyclic disulfide upon oxidation, is similar to that of hexane-1,6-dithiol (K_{eq} = 0.2 M), which forms an 8-membered cyclic disulfide, and pentane-1,5-dithiol (K_{eq} = 3.6 M), which forms a 7-membered cyclic disulfide. This similarity means that the two thiol groups of sucrose dithiol can reach a conformation that places them in close proximity and in a geometry similar to that in an unstrained disulfide group; this conformation must be the ground state of sucrose dithiol or energetically close to it. This result implies that the two thiol groups of sucrose dithiol are in close proximity to each other in the ground state.

Measurement of the Rate of Reduction of Sucrose Disulfide. DTT reduces sucrose disulfide approximately 1.5 times faster than it does mercaptoethanol disulfide. This small difference in rate implies that the energy difference between the ground state of sucrose disulfide and its transition state for thiol disulfide interchange with DTT is almost the same as that for mercaptoethanol disulfide. The similar rates of reduction for sucrose disulfide and for a strain-free disulfide (mercaptoethanol disulfide) imply that the disulfide group of sucrose disulfide is also strain-free.

Table I. EC of Various Cyclic Disulfides

structure	EC (M)
6,6′-sucrose disulfide	0.3
	0.2[a]
	3.6[a]
	180.0[a]

[a] Values not determined in this paper were taken from ref 8 after correction for a systematic error in that paper.

The CSSC Dihedral Angle of Sucrose Disulfide. In an effort to understand the formation of sucrose disulfide in greater detail, we wished to determine the CSSC dihedral angle of sucrose disulfide experimentally. Unfortunately, no presently available technique accurately measures the CSSC dihedral angle in solution.[29] UV spectroscopic data are, however, compatible with the hypothesis that the dihedral angle in sucrose disulfide is strainless: the UV spectra of peracetylated sucrose disulfide and of sucrose disulfide show a peak at 250 nm. Absorption at this wavelength is characteristic of unstrained noncyclic disulfides. In the absence of evidence to the contrary (vide infra), we conclude that the CSSC dihedral angle is ca. 90°.[30]

Conformational Analysis Using Molecular Mechanics. We investigated the lowest energy conformations of sucrose dithiol and sucrose disulfide using the crystal structure of sucrose, molecular mechanics, and ^{1}H NMR spectroscopy. As our initial model we used the crystal structure of sucrose. The crystal structure of sucrose places the C-6 and C-6′ oxygens 4.0 Å apart. Rotation of the C-6 hydroxymethyl group by 120° places the two oxygens 2.3 Å apart. This rotamer is the favored conformation for simple glucopyranosides in solution.[12] The replacement of the CH_2OH group at C-6 and C-6′ in this new rotamer with CH_2SH groups places the two sulfur atoms 1.8 Å apart with a CSSC dihedral angle of 130°. Since a typical sulfur–sulfur bond length is 2.05 Å, this conformation of the dithiol or disulfide is unfavorable; thus the sucrose disulfide or sucrose dithiol must deform from this conformation.

Due to the inadequacies in the first model, we investigated the possible conformations of sucrose disulfide by energy minimization of the various C-6 and C-6′ rotamers of sucrose disulfide using force-field calculations.[31] These calculations produced the two low-energy conformations shown in Figure 7. We hypothesize that interchange between these two conformers could cause the

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(28) Equilibrations using DTT or other small-ring cyclic disulfides are cleaner because they do not produce mixed disulfides, but they are not always practical due to the high reduction potentials of small dithiols.

(29) Raman Spectroscopy: At present there is some controversy over whether the CSSC dihedral angle varies with frequency (Raman). See: Zhao, W.; Bandekar, J.; Krimm, S. *J. Am. Chem. Soc.* **1988**, *110*, 6891–6892, and references therein. Our preliminary experiments with Raman spectroscopy indicated that large quantities of sample and numerous scans were required to achieve reasonable signal to noise. Circular Dichroism (CD): Disulfides obey a quadrant rule with null points at 0, 90, and 180° CSSC dihedral angles. Disulfides with CSSC dihedral angles of close to 90° sometimes give ambiguous results (cystine). Also, an M-helical disulfide with <90° CSSC dihedral angle gives a negative CD, as does a P-helical disulfide with a CSSC dihedral angle of >90°. See Gottarelli, G.; Samori, B. In *Chem. Ethers, Crown Ethers, Hydroxy Groups Their Sulphur Analogues*; Patai, S., Ed.; Wiley: Chichester, UK, 1980; Vol. 1, pp 279–298.

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(31) Still, W. C.; Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caulfield, C.; Chang, G.; Hendrickson, T. *MacroModel V2.0*; Department of Chemistry, Columbia University, New York, NY.

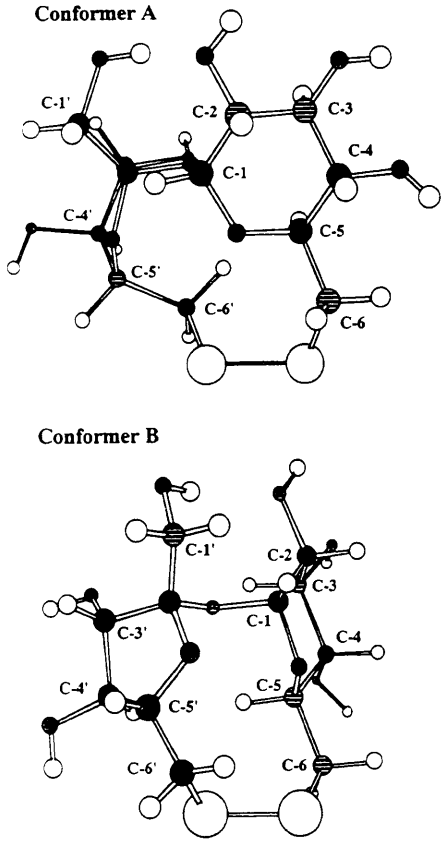

Figure 7. Structures of sucrose disulfide minimized by force field calculations. The CSSC angle in conformer A is 118°; that in conformer B is 96°.

conformational exchange observed by ^1H and ^{13}C NMR spectroscopy. The rotameric conformation of the C-6 hydroxymethyl group is similar in both conformations (the O-5–C–C–S-6 dihedral angle is 45° for conformer A and 81° for conformer B), but the rotameric conformation of the C-6′ hydroxymethyl group changes (the O-5′–C–C–S-6′ dihedral angle is −98° for conformer A and 63° for conformer B). The CSSC dihedral angle is 118° for conformer A and 96° for conformer B. The conformations of the glucopyranose rings (excluding the rotameric conformation at C-6) are similar in sucrose and in the structures A and B.

In conformer A, the fructofuranose ring adopts a conformation similar to that in sucrose. In conformer B, the fructofuranose ring has been deformed considerably from that in sucrose. For example, the dihedral angles between H-3′–C–C–H-4′ and H-4′–C–C–H-5′ are approximately 90° in conformer B and 160° in sucrose and conformer A. Other studies have indicated that the fructofuranose ring is flexible in solution.[18,19]

Conformational Analysis Using Experimentally Derived Values of T_1. We also used values of T_1 from NMR spectroscopy to probe the conformation of sucrose dithiol and sucrose disulfide. Values of T_1 measure the exponential increase with time of the intensity of the nuclear magnetization vector in the z direction for a particular nucleus (^1H or ^{13}C). Relative values of T_1 within an organic molecule are influenced primarily by the distances between the observed atom (^1H or ^{13}C) and other hydrogen atoms in the molecule (eq 9).[24] Provided that certain constraints are met, eq

$$\frac{1}{(T_1)_i} = \sum_{j \neq i}^{j=n} \frac{c}{r_{ij}^6} \qquad (9)$$

9 describes the value of T_1 for atom i (^1H or ^{13}C), where c is a constant (dependent on the correlation time and empirically determined for carbon; the value of c for protons is calculated from the value of c for carbon),[32] r_{ij} is the distance between the observed atom i and a proton j in the molecule, and n is the number of protons in the molecule. An important assumption in this treatment is that the molecule is rigid and tumbles isotropically. For sucrose, the assumption of rigid rotation may be incorrect. In particular, if a methylene group rotates internally as the molecule tumbles, then the value of T_1 for that methylene group will be larger than the calculated value.

(32) $c_{proton} = (^3/_2)[(\gamma_H)^2/(\gamma_C)^2]c_{carbon}$. Harris, R. K. *Nuclear Magnetic Resonance Spectroscopy*; Pitman: London, 1983; p 89.

Table II. Observed Values of T_1 for Sucrose (Suc), Sucrose Dithiol ($Suc(SH)_2$), and Sucrose Disulfide ($SucS_2$)[a]

	T_1 (s)		
atom	Suc	$Suc(SH)_2$	$SucS_2$
H-1	1.02	1.05	0.79
H-2	1.28	1.29	1.37
H-3	1.96	2.00	1.75
H-4	1.53	1.50	1.19
H-5	*b*	0.97	0.92
H-6	0.49	0.45	*c*
H-1′	0.43	0.43	0.41
H-3′	1.86	1.82	1.66
H-4′	1.40	1.53	1.55
H-5′	1.12	*b*	1.04
H-6′	0.49	0.45	0.42
C-1	0.56	*d*	0.58
C-6	0.30	*d*	0.28
C-1′	0.31	*d*	0.32
C-6′	0.41	*d*	0.41

[a] The values of T_1 were determined at 293 K using 27 delays and fitting the resulting curve to an exponential. [b] These values were not determined due to overlapping peaks in the ^1H NMR spectrum. [c] The peaks in the ^1H NMR spectrum were too broad to determine a T_1 value. [d] These values were not determined.

Comparison between Observed Values of T_1 for ^{13}C Nuclei in Sucrose and Sucrose Disulfide. The values of T_1 for methine carbons of sucrose vary from 0.53 to 0.60 s, while those of sucrose disulfide vary from 0.54 to 0.62 s. Since the experimental error in a single measurement is 5% (0.05–0.06 for comparison of two measurements), the results for sucrose and sucrose disulfide are indistinguishable. The reason that the values of T_1 for ^{13}C are so similar for these two molecules is that the sum of terms in $1/r_{ij}^6$ (eq 9) is dominated by the directly attached proton.

The values of T_1 for the three corresponding methylene carbons of sucrose and of sucrose disulfide are also similar (Table II), and therefore, by analogy with the known conformational motions of sucrose,[12] we conclude that the rotations of the C-1′ CH_2OH and C-6 CH_2S- groups of sucrose disulfide are hindered but that the C-6′ CH_2S- group of sucrose disulfide is still relatively mobile. The mobility of the C-6′ CH_2S- group in sucrose disulfide might result from conformational exchange involving rotation of this group.

Comparison between Observed Values of T_1 for the Hydrogens in Sucrose and Sucrose Dithiol. The values of T_1 for the corresponding methine hydrogens in sucrose and sucrose dithiol differ by less than 3%, except for those at C-4′. This exception may be due to differences in the populations of the three predominant rotameric conformations of the C-6′ CH_2OH group in sucrose relative to those of the C-6′ CH_2SH group in sucrose dithiol. This difference in rotameric population will cause differences in the $1/r^6$ time-averaged distance between H-4′ and H-6′ and thus in the value of T_1 for H-4′. Overall, the data in Table II suggest that the replacement of OH groups at C-6 and C-6′ with SH groups causes few (if any) changes in conformation.

Comparison between the Observed Values of T_1 for the Hydrogens of Sucrose and Sucrose Disulfide. Table II shows only two relatively large differences between the values of T_1 of sucrose and sucrose disulfide: in sucrose disulfide, the values of T_1 for H-1 and H-4 are 20% lower than those in sucrose or sucrose dithiol. The lower value of T_1 for H-4 is due to the different C-6 rotamer predominating in sucrose disulfide (molecular mechanics calculations indicate that the O-5–C–C–S-6 dihedral angle is approximately 60° rather than the −60° in sucrose).[12,26] The lower value of T_1 for H-1 in sucrose disulfide relative to that for H-1 in sucrose results from the closer proximity of the H-1′ and H-1

Table III. Observed and Calculated Coupling Constants of Sucrose (Suc), Sucrose Dithiol ($Suc(SH)_2$), Sucrose Disulfide ($SucS_2$), Sucrose Octaacetate (Asuc), Sucrose Dithioacetate Hexaacetate ($Asuc(SH)_2$), and Sucrose Disulfide Hexaacetate ($AsucS_2$) at Various Temperatures[a]

		J_{ij} (Hz)										
		Suc			$SucS_2$						$AsucS_2$	
					obsd		calcd					
atom H-i	atom H-j	obsd (298 K)	calcd	$Suc(SH)_2$ obsd (298 K)	(298 K)	(353 K)	A	B	Asuc obsd (298 K)	$Asuc(SH)_2$ obsd (298 K)	obsd[d] (273 K)	obsd[e] (273 K)
1	2	3.6	3.6	3.9	3.7	3.7	3.6	3.6	3.7	3.6	4.0	4.1
2	3	9.8	9.8	10.0	9.8	9.7	9.8	9.8	10.4	10.4	10.2	10.1
3	4	9.7	9.3	9.4	9.6	9.3	9.3	9.3	9.9	9.7	9.8	9.8
4	5	9.3	9.7	9.4	9.7	9.3	9.3	9.3	9.8	9.7	9.9	9.9
5	6	*b*		**5.8, 3.0**	**9.8, 2.4**	**9.7, 2.4**			*b*	**5.6, 3.2**	**11.0, 1.0**	**10.4, sm**
6	6	*b*		14.5	*c*	14.4			*b*	14.4	14.3	14.2
1′	1′	*b*		*b*	*c*	12.5			5.8	12.2	12.6	12.7
3′	4′	8.8	8.2	**8.8**	**7.8**	**7.2**	**7.6**	**0.6**	**5.9**	**6.0**	*b*	**2.5**
4′	5′	8.6	8.0	**8.3**	*c*	**7.2**	**8.8**	**0.9**	**6.1**	**5.9**	*b*	**2.6**
5′	6′	*b*		**7.7, 5.3**	*c*	**4.4, 4.4**			*b*	**7.0, 5.8**	**7.7, sm**	**8.5, sm**
6′	6′	*b*		*b*	*c*	*b*			*b*	14.0	14.9	14.6

[a] The coupling constants in boldface are mentioned in the text. The coupling constants were calculated according to Haasnoot, C. A. G.; De Leeuw, F. A. A. M.; Altona, C. *Tetrahedron* **1980**, *36*, 2783–2792, and Herve du Penhoat, C.; Imberty, A.; Roques, N.; Michon, V.; Mentech, J.; Descotes, G.; Perez, S. *J. Am. Chem. Soc.* **1991**, *113*, 3720–3727. [b] These values were not determined due to overlapping peaks in the ^1H NMR spectrum. [c] These values were not determined due to conformational interchange which broadened the peaks. [d] Taken in $CDCl_3$. [e] Taken in a mixture of $CDCl_3$ (0.5 mL) and C_6D_6 (0.2 mL).

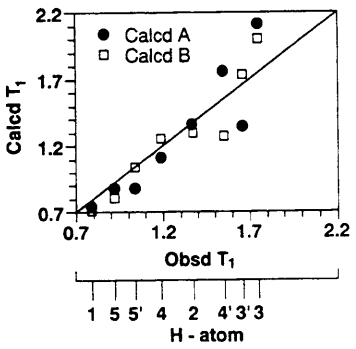

Figure 8. Comparison of the experimentally derived values of T_1 (s) with the calculated values of T_1 (s) for conformers A and B (Figure 7). The straight line is T_1(calcd) = T_1(obsd). Deviations from this line indicate disagreement between observed and calculated values.

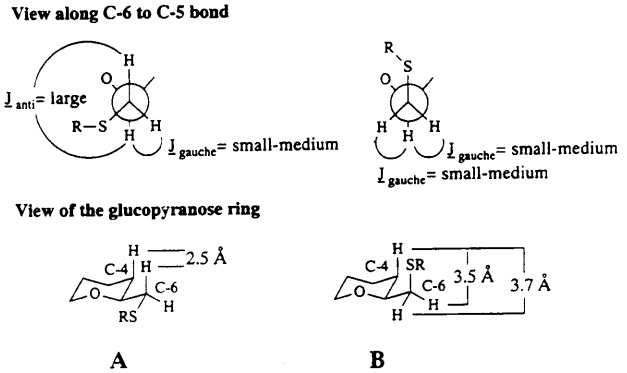

Figure 9. Two possible rotamers about the C-6–C-5 bond of sucrose dithiol (R = H) or sucrose disulfide (R = SR′) and the resulting coupling constants. The distance between H-4 and H-6 in each rotamer is taken from the crystal structure of sucrose.

hydrogens in sucrose disulfide (compared to the analogous hydrogens in sucrose). This argument is supported by NOE measurements: equilibrium NOEs between H-1 and H-1′ were larger in sucrose disulfide (9% NOE for irradiation of H-1 and 13% NOE for irradiation of H-1′) than in sucrose (5% NOE for irradiation of H-1 and 8% NOE for irradiation of H-1′). The equilibrium NOEs between H-1 and H-2 were indistinguishable (13% for both).

Conformational Analysis Using Calculated Values of T_1 for Hydrogens. Additional structural analysis of these compounds involved comparison of the values of T_1 determined experimentally for sucrose and sucrose disulfide with the values of T_1 calculated, using eq 9, from the crystal structure of sucrose[33] or from the low-energy structures of sucrose disulfide determined by molecular mechanics (conformers A and B, Figure 7). For sucrose, the calculated values of T_1 were on average 13% different from the experimentally determined values. For sucrose disulfide, the calculated values of T_1 were also on average quite different from the experimentally determined values (12% for conformer A and 10% for conformer B, Figure 8). In short, none of the calculated values of T_1 compared well with the experimental values of T_1. These differences from calculated values of T_1 may indicate that the molecule is not rigid, especially within the furanose ring. The flexibility of the furanose ring in sucrose disulfide is supported by our result that conformational exchange occurs on the NMR time scale and is also supported by a recent report for sucrose.[19]

Conformational Analysis Using Observed ^1H NMR Coupling Constants of Sucrose, Sucrose Dithiol, and Sucrose Disulfide. The ^1H NMR coupling constants of sucrose and sucrose dithiol differ by less than 0.3 Hz (Table III); this close correspondence suggests that the conformations of these two molecules are similar.

The ^1H NMR coupling constants of sucrose dithiol and sucrose disulfide differed substantially for $J_{5,6}$ and $J_{5',6'}$ (Table III). These differences indicate that the mix of rotamers around the C-5–C-6 bond is unique for each of these molecules. In sucrose dithiol, coupling constants between H-5 and both H-6 hydrogens are relatively small; these hydrogens are therefore probably gauche to each other (Figure 9). In sucrose disulfide, one of the coupling constants between H-5 and H-6 is large (9.7 Hz); therefore, one of the protons on C-6 is probably anti to H-5 (Figure 9). These observations are consistent with the values of T_1, which show a large difference between H-4 in sucrose dithiol (1.50 s) and H-4 in sucrose disulfide (1.19 s).

In rotamer A (Figure 9), one of the H-6 hydrogens is anti to H-5 and close to H-4 (2.5 Å). This proximity will lower the value of T_1 (which is $1/r^6$ dependent) for H-4. In rotamer B (Figure 9), both H-6 hydrogens are gauche to H-5 and far away from H-4 (3.4 Å). Taken together, the observations in this and the preceding paragraph indicate that the C-5–C-6 bond in sucrose dithiol adopts

(33) The value of c in eq 9 was calculated from the values of T_1 (^{13}C) for sucrose. This equation was then used along with distance (r_{ij}) information estimated from the postulated structure to determine values of T_1 for ^1H. We calculated values of T_1 (^1H) for sucrose by averaging the calculated values of T_1 for the three rotamers at C-6′ (O-5′–C–C–S-6′ dihedral angles of 60, 180 and −60°). We assumed that the C-6 and C-1′ hydroxymethyl groups were rigid based on their low values of T_1 for ^{13}C. The discrepancy between calculated and experimental values of T_1 for C-3′ and C-1 hydrogens is greater than 20°, probably because of inaccuracies in modeling the rotameric configuration at C-1′; assumptions concerning the rotameric configuration of C-1′ (the O-5′–C–C–O-1′ angle) influence values of T_1 for H-1 and H-3′ significantly. The calculated values of T_1 for the two conformers (A and B, Figure 2) of sucrose disulfide were compared with experimental values.

a conformation similar to that of rotamer B and the C-5–C-6 bond in sucrose disulfide adopts a conformation similar to that of rotamer A.

The coupling constants between H-5′ and H-6′ are also different for sucrose dithiol and sucrose disulfide. The C-6′ CH_2S- is probably freely rotating in sucrose dithiol but more constrained in sucrose disulfide. The two small coupling constants between H-6′ and H-5′ in sucrose disulfide indicate that the two H-6′ protons are gauche to H-5′.

Comparison between Observed Coupling Constants and Coupling Constants Calculated from the Two Theoretical Conformations (A and B, Figure 7) of Sucrose Disulfide and Sucrose Disulfide Hexaacetate. The data in Table III show that the fructofuranose ring hydrogens of sucrose disulfide have large coupling constants ($J_{3'4'}$ = 7.2 Hz, $J_{4'5'}$ = 7.2 Hz) consistent with dihedral angles (H-3′–C–C–H-4′ and H-4′–C–C–H-5′) closer to 180 than to 90°. The fructofuranose ring hydrogens of conformer A have dihedral angles close to 180° (calculated $J_{3'4'}$ = 7.6 Hz, $J_{4'5'}$ = 8.8 Hz),[18] while those of conformer B have dihedral angles close to 90° (calculated $J_{3'4'}$ = 0.6 Hz, $J_{4'5'}$ = 0.9 Hz).[18] These results suggest that the structure of the fructofuranose ring of sucrose disulfide is more similar to that of conformer A than to that of conformer B. The glucopyranose ring of conformer A, conformer B, and sucrose disulfide have similar coupling constants and are all in the chair conformation ($^4C_1$). From these observations we conclude that sucrose disulfide appears to have a conformation similar to that of conformer A in Figure 7.

In sucrose disulfide hexaacetate, the coupling constants between H-5 and H-6 have one large value and one very small value (Table III). This observation is consistent with one H-6 being anti to H-5. The coupling constants between H-5′ and H-6′ have one large value and one very small value which is consistent with one H-6′ being anti to H-5′. The coupling constants within the fructofuranose ring of sucrose disulfide hexaacetate are small ($J_{3'4'}$ = 2.6 Hz, $J_{4'5'}$ = 2.5 Hz). Taken together, these factors suggest that sucrose disulfide hexaacetate exists in a conformatioin similar to that of conformer B in Figure 7. Thus we conclude that sucrose disulfide hexaacetate appears to adopt a conformation (conformer B in Figure 7) that is different from sucrose disulfide (conformer A in Figure 7).

Conclusions

(1) *Sucrose dithiol is more strongly reducing than might have been expected for a compound forming an 11-membered disulfide ring.* The K_{eq} (or the effective concentration, EC) for the reaction between sucrose dithiol and mercaptoethanol disulfide is 0.3 M. This value is similar to the values for cyclic disulfides with smaller ring sizes (Table I). The relatively strong reducing character of $Suc(SH)_2$ reflects a conformation that allows the two thiol groups to reach a geometry similar to that of the disulfide. We infer that the disulfide is essentially strain-free.

(2) *Sucrose dithiol and sucrose adopt similar conformations in solution.* Coupling constants for sucrose dithiol and sucrose differ by less than 0.3 Hz. The values of T_1 (^{1}H) for sucrose dithiol and sucrose are within 3% of each other except at H-4′. This exception is probably due to a differential population of the various rotameric configurations (O-5′–C–C–(O-6′ or S-6′) dihedral angle) of the C-6′ CH_2OH or CH_2SH group.

(3) *Sucrose disulfide adopts a conformation similar to that of structure A, one of two low-energy structures calculated using molecular mechanics (Figure 7); this conformation is similar to that of sucrose dithiol in solution, although it differs in the OCCS dihedral angles and in the proximity of H-1 to H-1′.* The difference in the O-5–C–C–S-6 dihedral angle of sucrose disulfide and sucrose dithiol lowers the value of T_1 for H-4 (1.19 and 1.50 s, respectively, Table II) and changes the H-5–H-6 coupling constants (9.7, 2.4 and 5.8, 3.0 Hz, respectively, Table III). The shorter H-1′–H-1 distance in sucrose disulfide (vs sucrose dithiol) lowers the value of T_1 for H-1 (0.79 and 1.05 s, respectively, Table II) and increases the NOE between H-1 and H-1′ (8.9% in sucrose disulfide and 5.4% in sucrose). Coupling constants (Table III) indicate that the fructofuranose and glucopyranose rings of sucrose disulfide, sucrose dithiol, and sucrose adopt similar conformations in solution. Sucrose disulfide and structure A (Figure 7) have similar fructofuranose and glucopyranose rings and OCCS dihedral angles.

(4) *Molecular mechanics calculations suggest an origin for the proximity of H-1–H-1′ in sucrose disulfide.* When the C-6 and C-6′ CH_2OH groups in the crystal structure of sucrose (Figure 1) are replaced by CH_2SH groups (sucrose dithiol) and rotated to the OCCS dihedral angle found in one of the two structures obtained by molecular mechanics (structure A, Figure 7, similar in structure to sucrose disulfide), the two C-6 and C-6′ sulfurs are 2.21 Å apart (rotamer 1 of sucrose dithiol) and the CSSC dihedral angle is 108°. Since a typical S–S bond length is 2.05 Å, the two sulfur atoms in rotamer 1 of sucrose dithiol must be moved about 0.15 Å to form the disulfide. We propose that this movement causes pivoting about the glycosidic linkage, which in turn causes a closer interaction between H-1 and H-1′ and between O-5 and O-5′. The shorter distance between H-1 and H-1′ in sucrose disulfide relative to sucrose dithiol and sucrose is observed experimentally (NOE and T_1, see above).

(5) *The use of thiol–disulfide interchange equilibria to determine the conformations of sucrose works well.* Using values of T_1, NOEs, and coupling constants, we have shown that sucrose disulfide adopts a conformation similar to that of sucrose. All of the available techniques thus point to the same conclusions concerning conformation. The close proximity of the thiol groups in sucrose dithiol and the value of EC (0.3 M) for this compound indicates that a value of EC can be interpreted in terms of simple proximity and to a conformation of the CSSC group that is essentially strain-free.

Experimental Section

General. Measurements of equilibrium constants were carried out under an atmosphere of argon. Deuterated solvents were obtained from MSD. Nondeuterated solvents and sucrose were obtained from Fisher Chemicals. Other chemicals were obtained from Aldrich Chemical Company. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM 500 spectrometer at ambient temperature unless specified. The lowest energy conformatioins of sucrose disulfide were calculated with Macromodel V2.0 using the MM2(85) parameter set.[31]

2,3,4-Tri-*O*-acetyl-6-bromo-6-deoxy-α-D-glucopyranosyl 1,3,4-Tri-*O*-acetyl-6-bromo-6-deoxy-β-D-fructofuranoside (1). Sucrose (1.96 g, 5.73 mmol) and pyridine (110 mL) were heated to 100 °C to dissolve the sucrose and cooled to 0 °C. Triphenylphosphine (10.0 g, 38 mmol) was added. At 0 °C, carbon tetrabromide (6.5 g, 20 mmol) was added in three batches over 15 min. The reaction mixture was heated at 70 °C for 1.5 h, quenched with methanol (20 mL), and cooled to room temperature. Acetic anhydride (75 mL) and DMAP (101 mg) were added. After 2 h, the reaction mixture was added to a biphasic mixture of methylene chloride (250 mL), 1 N HCl (100 mL), and water (100 mL). Concentrated HCl (approximately 60 mL) was added slowly until the pH of the water layer was less than 2. The layers were separated, and the aqueous layer was extracted with methylene chloride (2 × 100 mL). The combined organic layers were then washed with saturated aqueous sodium bicarbonate (250 mL). The combined organic phases were dried ($MgSO_4$), filtered, and concentrated at aspirator pressure to give a brown solid. This solid was partially recrystallized from ethyl acetate/heptane. The precipitate (Ph_3PO) was discarded, and the supernate was concentrated at aspirator pressure. Purification by chromatography on silica gel (eluant 2:1 to 1:2 hexane/ethyl acetate) gave 6,6′-dibromo-6,6′-dideoxysucrose hexaacetate (3.0 g, 73% yield):[34] ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.65 (d, J = 3.6 Hz, C-1), 5.41 (dd, J = 10.2, 9.5 Hz, C-3), 5.39 (d, J = 5.1 Hz, C-3′), 5.37 (t, J = 5.0 Hz, C-4′), 5.03 (t, J = 9.7 Hz, C-4), 4.84 (dd, J = 10.4, 3.7 Hz, C-2), 4.29 (ddd, J = 10.0, 5.3, 2.7 Hz, C-5), 4.25 (td, J = 6.7, 4.8 Hz, C-5′), 4.22 (d, J = 12.5 Hz, C-1′a), 4.19 (d, J = 12.4 Hz, C-1′b), 3.62 (d, J = 6.6 Hz, C-6′a and C-6′b), 3.49 (dd, J = 11.5, 2.7 Hz, C-6a), 3.37 (dd, J = 11.6, 5.4 Hz, C-6b), 2.14 (s, 3 H), 2.08 (s, 3 H), 2.08 (s, 3 H), 2.07 (s, 3 H), 2.03 (s, 3 H), 1.99 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.09, 170.03, 169.48, 169.35, 104.43, 90.36, 81.09, 77.06, 76.14, 70.61, 70.18, 69.28, 69.19, 62.38, 31.61, 31.14, 20.75, 20.63, 20.59, 20.54, 20.52, 20.48. Anal. Calcd for $C_{24}H_{32}O_{15}Br_2$: C, 40.02; H, 4.48. Found: C, 39.81; H, 4.41.

2,3,4-Tri-*O*-acetyl-6-*S*-acetyl-6-thio-α-D-glucopyranosyl 1,3,4-Tri-*O*-acetyl-6-*S*-acetyl-6-thio-β-D-fructofuranoside (2). The acetylated sugar

(34) Anisuzzaman, A. K. M.; Whistler, R. L. *Carbohydr. Res.* **1978**, *61*, 511–518.

1 (625 mg, 0.87 mmol), dimethylformamide (10 mL), thioacetic acid (550 μL, 7.71 mmol), and sodium methoxide (205 mg, 3.80 mmol) were mixed and heated at 70 °C for 1.5 h. The solution was concentrated at aspirator pressure and purified on silica gel (2:1 to 1:2 hexane/ethyl acetate) to provide 6,6′-dithioacetate sucrose hexaacetate (524 mg, 85% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.51 (d, J = 3.6 Hz, C-1), 5.37 (t, J = 9.7 Hz, C-3), 5.35 (d, J = 6.0 Hz, C-3′), 5.29 (t, J = 5.9 Hz, C-4′), 4.93 (t, J = 9.7 Hz, C-4), 4.80 (dd, J = 10.4, 3.6 Hz, C-2), 4.22 (ddd, J = 10.0, 5.6, 3.2 Hz, C-5), 4.10 (d, J = 12.2 Hz, C-1′a), 4.06 (d, J = 12.1 Hz, C-1′b), 4.08–4.04 (m, C-5′), 3.31 (dd, J = 14.0, 5.8 Hz, C-6′a), 3.18 (dd, J = 14.0, 7.0 Hz, C-6′b), 3.18 (dd, J = 14.4, 3.2 Hz, C-6a), 3.12 (dd, J = 14.5, 5.6 Hz, C-6b), 2.32 (s, 3 H), 2.31 (s, 3 H), 2.11 (s, 3 H), 2.06 (s, 3 H), 2.05 (s, 3 H), 2.05 (s, 3 H), 2.04 (s, 3 H), 1.95 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 194.58, 194.52, 170.09, 170.02, 169.96, 169.78, 169.70, 169.65, 103.68, 89.94, 79.94, 76.75, 75.77, 70.16 (2 C), 69.46, 69.33, 62.89, 31.82, 30.41, 30.36, 29.62, 20.72, 20.63, 20.58, 20.55, 20.51, 20.44.

6-Thio-α-D-glucopyranosyl 6-Thio-β-D-fructofuranoside (3). The dithioacetate **2** (327 mg, 0.46 mmol) was dissolved in methanol (20 mL, argon-purged, 2.5 mg NaOMe/mL methanol). After 1 h the reaction was acidified with ion-exchange resin (Dowex 50-X-8, H^+ form), filtered, and concentrated at 1 Torr to provide 6,6′-dithiosucrose: ^{1}H NMR (500 MHz, D_2O) δ 5.23 (d, J = 3.9 Hz, 1 H, C-1), 4.03 (d, J = 8.8 Hz, 1 H, C-3′), 3.96 (t, J = 8.3 Hz, 1 H, C-4′), 3.79 (ddd, J = 9.7, 5.8, 3.0 Hz, 1 H, C-5), 3.69 (td, J = 7.7, 5.3 Hz, 1 H, C-5′), 3.59 (t, J = 9.4 Hz, 1 H, C-3), 3.48 (s, 2 H, C-1′), 3.40 (dd, J = 10.0, 3.9 Hz, 1 H, C-2), 3.30 (t, J = 9.4 Hz, 1 H, C-4), 2.85 (dd, J = 14.5, 2.9 Hz, 1 H, C-6a), 2.76 (d, J = 5.0 Hz, 1 H, C-6′a), 2.75 (d, J = 7.7 Hz, 1 H, C-6′b), 2.63 (dd, J = 14.5, 5.8 Hz, 1 H, C-6b); ^{13}C NMR (125 MHz, D_2O) δ 103.7, 92.3, 82.2, 77.3, 76.7, 72.4, 72.2, 71.4, 71.2, 61.4, 27.4, 25.2.

6,6′-Dithiosucrose Cyclic Disulfide Hexaacetate (4). The dithioacetate **2** (435 mg, 0.61 mmol) and sodium methoxide (100 mg, 1.85 mmol) were dissolved in methanol (10 mL). After 3 h the solution was diluted with water (1000 mL) and adjusted to pH 9.0 with sodium carbonate. After 36 h of being stirred in air, the reaction was quenched with glacial acetic acid (60 mL) and concentrated at 1 Torr. The residue was acetylated with acetic anhydride (10 mL) in pyridine (40 mL) using DMAP as a catalyst. After 6 h, the solution was diluted with methylene chloride (100 mL) and passed through a plug of silica gel (50 mL, using ethyl acetate as the eluant). Further purification on silica gel (1:2 hexane/ethyl acetate going to ethyl acetate) yielded 215 mg (56%) of pure **4** and 32 mg (8%) of a mixture of sucrose disulfide dimers (dimers A and B, head-to-head and head-to-tail, which were partially separated by this purification). The NMR data for the major conformer of sucrose disulfide hexaacetate[11] are listed below: ^{1}H NMR (500 MHz, $CDCl_3$, 273 K) δ 5.71 (d, J = 4.0 Hz, C-1), 5.40 (t, J = 9.8 Hz, C-3), 5.30 (s, C-4′ and C-3′), 4.87 (t, J = 9.9 Hz, C-4), 4.74 (dd, J = 10.2, 4.0 Hz, C-2), 4.58 (d, J = 12.6 Hz, C-1′a), 4.43–4.36 (m, C-5 and C-5′), 4.19 (d, J = 12.5 Hz, C-1′b), 3.29 (dd, J = 14.9, 7.7 Hz, C-6′a), 2.91 (d, J = 14.1 Hz, C-6a), 2.85 (d, J = 14.7 Hz, C-6′b), 2.52 (dd, J = 14.3, 11.0 Hz, C-6b), 2.22 (s, 3 H), 2.11 (s, 3 H), 2.08 (s, 3 H), 2.08 (s, 3 H), 2.03 (s, 3 H), 2.01 (s, 3 H); ^{1}H NMR (500 MHz, C_6D_6 (0.2 mL) $CDCl_3$ (0.5 mL), 273 K) δ 5.74 (d, J = 4.1 Hz, C-1), 5.43 (t, J = 9.8 Hz, C-3), 5.27 (d, J = 2.5 Hz, C-3′), 5.10 (t, J = 2.6 Hz, C-4′), 4.85 (t, J = 9.9 Hz, C-4), 4.72 (dd, J = 10.1, 4.0 Hz, C-2), 4.61 (d, J = 12.7 Hz, C-1′a), 4.38 (t, J = 10.4 Hz, C-5), 4.30 (br d, J = 8.4 Hz, C-5′), 4.18 (d, J = 12.7 Hz, C-1′b), 3.20 (dd, J = 14.7, 8.5 Hz, C-6′a), 2.88 (d, J = 14.1 Hz, C-6a), 2.54 (d, J = 14.5 Hz, C-6′b), 2.45 (dd, J = 14.2, 10.9 Hz, C-6b), 2.13 (s, 3 H), 1.96 (s, 3 H), 1.93 (s, 3 H), 1.91 (s, 3 H), 1.89 (s, 3 H), 1.87 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$, 273 K) δ 170.32, 170.12, 170.07, 169.97, 169.88, 169.60, 104.86 (C-2′), 90.30 (C-1), 83.27 (C-5′), 78.25 (C-3′ or C-4′), 76.92 (C-3′ or C-4′), 70.89 (C-4), 70.78 (C-2), 69.88 (C-3), 66.23 (C-5), 61.46 (C-1′), 39.55 (C-6′ or C-6), 38.33 (C-6′ or C-6), 20.80, 20.71, 20.51, 20.37. The C-2′ carbon was assigned due to its chemical shift. The other secondary carbons were assined using a 2D ^{1}H/^{13}C HETEROCOSY spectrum. Anal. Calcd for $C_{24}H_{32}O_{15}S_2$: C, 46.15; H, 5.16. Found: C, 45.99; H, 5.07.

The NMR data for sucrose disulfide dimer A are as follow: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.58 (d, J = 3.4 Hz, C-1), 5.44 (t, J = 10.3 Hz, C-3), 5.43 (d, J = 6.0 Hz, C-3′), 5.35 (t, J = 5.7 Hz, C-4′), 4.91 (t, J = 9.7 Hz, C-4), 4.86 (dd, J = 10.5, 3.4 Hz, C-2), 4.25 (dt, J = 8.2, 5.4 Hz, C-5′), 4.21 (ddd, J = 9.9, 7.8, 2.7 Hz, C-5), 4.16 (d, J = 12.2 Hz, C-1′a), 4.05 (d, J = 12.2 Hz, C-1′b), 3.28 (dd, J = 14.4, 8.2 Hz, C-6′a), 3.20 (dd, J = 14.4, 5.2 Hz, C-6′b), 2.93 (dd, J = 13.6, 2.7 Hz, C-6a), 2.79 (dd, J = 13.6, 8.3 Hz, C-6b), 2.15 (s, 3 H), 2.10 (s, 3 H), 2.09 (s, 3 H), 2.09 (s, 3 H), 2.06 (s, 3 H), 1.99 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.16, 170.11, 169.95, 169.83, 169.79, 104.12, 90.20, 81.34, 77.64, 76.10, 71.48, 70.18, 69.22, 69.00, 63.08, 42.53, 40.80, 20.80, 20.71, 20.66, 20.62, 20.60, 20.47.

The NMR data for sucrose disulfide dimer B are as follow: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.64 (d, J = 3.4 Hz, C-1), 5.42 (d, J = 4.9 Hz, C-3′), 5.34 (t, J = 10.0 Hz, C-3), 5.31 (t, J = 4.8 Hz, C-4′), 5.08 (t, J = 9.7 Hz, C-4), 4.94 (dd, J = 10.4, 3.4 Hz, C-2), 4.37 (dt, J = 7.6, 5.1 Hz, C-5′), 4.32 (d, J = 11.9 Hz, C-1′a), 4.15 (dt, J = 9.8, 4.4 Hz, C-5), 3.98 (d, J = 11.9 Hz, C-1′b), 3.24 (dd, J = 14.0, 5.6 Hz, C-6′a), 3.12 (dd, J = 14.1, 5.0 Hz, C-6a), 3.00 (dd, J = 14.0, 7.8 Hz, C-6′b), 2.90 (dd, J = 14.1, 3.6 Hz, C-6b), 2.19 (s, 3 H), 2.12 (s, 3 H), 2.12 (s, 3 H), 2.07 (s, 3 H), 2.02 (s, 3 H), 1.98 (s, 3 H). The following ^{13}C NMR data are for a 1:1 mixture of dimer A and B: ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.26, 170.15, 170.10, 170.06, 170.02, 169.94, 169.82, 169.77, 169.69, 169.64, 104.22 (B), 104.12 (A), 90.29 (B), 90.20 (A), 81.51 (B), 81.33 (A), 78.16 (B), 77.63 (A), 76.68 (B), 76.10 (A), 71.48 (A), 70.72 (B), 70.18 (A), 69.60 (B), 69.49 (B), 69.21 (A), 69.00 (A), 68.31 (B), 63.08 (A), 62.95 (B) 42.52 (A), 42.39 (B), 41.26 (B), 40.79 (A), 21.03, 20.90, 20.79, 20.71, 20.65, 20.61, 20.58, 20.56, 20.45. Assignments were made using spectral subtraction.

6,6′-Dithiosucrose Cyclic Disulfide (5). Sucrose disulfide hexaacetate **4** (62 mg, 0.10 mmol) was added to methanol (10 mL, containing 1 mg/mL of sodium methoxide). After 1.5 h the reaction was quenched with ion-exchange resin (Dowex-50-X-8 H^+ form), filtered, concentrated at 1 Torr, and dissolved in D_2O: ^{1}H NMR (500 MHz, D_2O, 297 K) δ 5.30 (d, J = 3.7 Hz, C-1, 1 H), 4.34–4.24 (br s, C-4′, 1 H), 4.23 (td, J = 9.8, 2.4 Hz, C-5, 1 H), 4.15 (d, J = 7.8 Hz, C-3′, 1 H), 3.83–3.80 (m, C-5′, 1 H), 3.68 (t, J = 9.6 Hz, C-3, 1 H), 3.65 (s, C-1′, 2 H), 3.42 (dd, J = 9.8, 3.8 Hz, C-2, 1 H), 3.17 (t, J = 9.7 Hz, C-4, 1 H), 3.15–3.10 (br s, C-6a, 1 H), 3.10–3.04 (br s, C-6′, 2 H), 2.50–2.36 (br s, C-6b, 1 H); ^{13}C NMR (125 MHz, D_2O, 297 K) gave many broad peaks; ^{1}H NMR (500 MHz, D_2O, 357 K) δ 5.92 (d, J = 3.7 Hz, C-1, 1 H), 4.87 (t, J = 7.2 Hz, C-4′, 1 H), 4.84 (td, J = 9.7, 2.4 Hz, C-5, 1 H), 4.74 (d, J = 7.2 Hz, C-3′, 1 H), 4.45 (dt, J = 7.2, 4.4 Hz, C-5′, 1 H), 4.31 (d, J = 12.5 Hz, C-1′a, 1 H), 4.29 (t, J = 9.3 Hz, C-3, 1 H), 4.24 (d, J = 12.5 Hz, C-1′b, 1 H), 4.06 (dd, J = 9.7, 3.8 Hz, C-2, 1 H), 3.79 (t, J = 9.3 Hz, C-4, 1 H), 3.77–3.70 (m, C-6a (1 H) and C-6′ (2 H), 3 H), 3.12 (dd, J = 14.4, 9.5 Hz, C-6b, 1 H); ^{13}C NMR (125 MHz, D_2O, 357 K) δ 102.68, 90.91, 78.18, 76.18, 74.29, 71.54, 71.49, 69.83, 66.43, 59.06, 39.55, 36.38.

Deacetylation of Dimer A. The acetylated dimer A was deprotected as described above for sucrose disulfide hexaacetate using 2 mL of basic methanol: ^{1}H NMR (500 MHz, D_2O) δ 5.24 (d, J = 3.7 Hz, C-1), 4.03 (d, J = 8.5 Hz, C-3′), 3.98 (dd, J = 8.4, 7.8 Hz, C-4′), 3.95–3.90 (m, C-5), 3.82 (td, J = 7.9, 4.7 Hz, C-5′), 3.62 (dd, J = 9.7, 9.4 Hz, C-3), 3.52 (d, J = 12.6 Hz, C-1′a), 3.47 (d, J = 12.6 Hz, C-1′b), 3.41 (dd, J = 10.0, 3.7 Hz, C-2), 3.23–3.13 (m, C-4, C-6′a, C-6a), 3.15 (dd, J = 13.7, 8.3 Hz, C-6′b), 2.77 (dd, J = 14.2, 8.2 Hz, C-6b); ^{13}C NMR (125 MHz, D_2O) δ 104.0, 92.37, 80.60, 77.80, 76.84, 72.91, 72.25, 71.18, 70.61, 61.60, 43.75, 40.15.

Equilibrations. All thiol–disulfide equilibrations were performed in D_2O (argon purged, 50 mM pD 7.0 phosphate buffer). Sucrose dithiol was prepared by deacetylating sucrose dithioacetate hexaacetate as described above and redissolving it in the buffer solution. Mercaptoethanol and mercaptoethanol disulfide were also dissolved in this buffer to make up stock solutions. NMR tubes containing a mixture of these solutions were then allowed to equilibrate (until a constant value for K_{eq} was obtained, approximately 1 day), and the K_{eq} was calculated by NMR integration (K_{eq} = [sucrose disulfide][mercaptoethanol]^2/[sucrose dithiol][mercaptoethanol disulfide] = EC). Initial concentrations: tube A sucrose dithiol (40 mM), mercaptoethanol (50 mM), mercaptoethanol disulfide (50 mM) [K_{eq} = 0.32 M]; tube B sucrose dithiol (9.7 mM), mercaptoethanol disulfide (5.0 mM) [K_{eq} = 0.23 M]; tube C sucrose dithiol (7.4 mM), mercaptoethanol disulfide (5.7 mM) [K_{eq} = 0.31 M]. From these data, we conclude that K_{eq} = EC = 0.3 M.

Determination of Molecular Weight by Vapor-Phase Osmometry (VPO). Determinations of molecular weights were made with a Wescan Model 233 vapor pressure osmometer operated at 55 °C. The molecular weights were measured in toluene at concentrations of 1–15 mg/mL. At each concentration, 3–6 measurements were taken. A calibration curve was generated using sucrose octaacetate (MW 678.6). Sucrose disulfide hexaacetate (found MW 598, calcd MW 624) and sucrose disulfide hexaacetate dimer (found MW 1390, calcd MW 1248) were analyzed by VPO.

Determination of the Rate of Reduction of Sucrose Disulfide. Four stock solutions were made up: (1) SDS solution (13 mg (0.03 mmol) of sucrose disulfide in 2.0 mL of deoxygenated D_2O), (2) 10 mM DTT solution (6.2 mg of DTT in 4.0 mL of buffered deoxygenated D_2O (100 mM PO_4, pD 7.0)), (3) 10 mM ME^{ox} solution (6.2 mg in 4.0 mL of deoxygenated D_2O), (4) DCl solution (9% wt soln of DCl in D_2O).

In each of four NMR tubes (sealed and deoxygenated) was placed 250 μL of the 10 mM ME^{ox} solution. To each tube was added 250 μL of the 10 mM DTT solution. After 1, 6, 11, and 21 min, the reactions were quenched with 25 μL of the DCl solution. Proton NMR spectra were

then acquired of each tube. The procedure was repeated with the 10 mM ME^{ox} solution being replaced by the 10 mM SDS solution.

The peaks in the NMR spectrum corresponding to oxidized and reduced mercaptoethanol were integrated. The changes in these integrals over time were used to calculate the rate of reduction $[(1/c_{final} - 1/c_{initial}) = kt]$ (k = 0.0044 mM^{-1} min^{-1}). A similar procedure was used for sucrose disulfide (k = 0.0067 mM^{-1} min^{-1}).

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