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Carbohydrate Research 289 (1996) 25–37

CARBOHYDRATE RESEARCH

Conformation of cyclic and linear (1 → 2)-β-D-glucans in aqueous solution

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Received 22 January 1996; accepted 15 April 1996

Abstract

The conformations of cyclic (1 → 2)-β-D-glucan chains having degree of polymerization (dp) 17 to 24 were characterized by means of small-angle X-ray scattering and Monte Carlo simulation. The results indicate that cyclic (1 → 2)-β-D-glucan chains adopt the shape of a doughnut-like ring with a thickness of about 10 Å for all the samples. The diameter of the annulus for the cyclic glucan having dp 21 is estimated to be only about 4–5 Å. Two linear (1 → 2)-β-D-glucans possessing dp 19 and 21 prepared by acid hydrolysis of a cyclic glucan and subsequent fractionation showed different scattering profiles from those obtained for cyclic glucans having the corresponding dp. Although the Monte Carlo simulation does not completely reproduce the scattering profiles observed by small-angle X-ray scattering, linear (1 → 2)-β-D-glucans seem to possess a characteristic cylindrical shape with cross-sectional diameters of 11.8 and 13.2 Å for linear glucans of dp 19 and 21, respectively. © 1996 Elsevier Science Ltd.

Keywords: (1 → 2)-β-D-Glucan; Cyclic structure; Small-angle X-ray scattering; Monte Carlo simulation

1. Introduction

Most microbial polysaccharides fulfill their physiological functions under conditions which correspond to the ordering of polysaccharides chains. That is, the conformation in the ordered state is considered to be one of the most significant factors in determining the functions of polysaccharide chains.

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PII S0008-6215(96)00134-6

Cyclic (1 → 2)-β-D-glucans, often referred to as cyclosophorans (designated CS in this paper), are produced by gram-negative bacteria such as *Agrobacterium* and *Rhizobium* [1,2]. The degree of polymerization (dp) of cyclosophorans isolated from the culture broth is dependent on the bacterial strain [3], and the typical dp ranges from 17 to 24. The largest molecular weight reported corresponds to dp 40 [4].

Although the exact physiological role of cyclic (1 → 2)-β-D-glucans is not known, this polysaccharide is thought to play an important role in adapting the bacteria to changes in environmental osmolality by regulating the osmotic balance between the cytoplasm and the periplasmic space [5,6]. An alternative function was suggested in mediating bacterium–plant host interactions during infection of the host [6–8]. The physiological function of cyclic (1 → 2)-β-D-glucan is assumed to be closely related to its conformation [9].

Several conformational studies of cyclic (1 → 2)-β-D-glucan have been performed by computer modelling and NMR methods [10–15]. The ^{13}C and ^{1}H NMR spectra of cyclosophorans having dp 17–24 show only six and seven signals, respectively, suggesting that the glucose residues of CS are equivalent on the NMR time scale. The homopolymeric nature of the glucans and conformational identity of the glucose residues make these molecules difficult subjects for structure determination by NMR techniques [9].

In this report, the conformation of cyclic and linear (1 → 2)-β-D-glucans in aqueous solution was investigated by small-angle X-ray scattering (SAXS). The observed SAXS profile was compared with those calculated directly from the atomic coordinates of these glucans generated by the Monte Carlo method.

2. Materials and methods

Sample preparation.—Cyclosophoran mixtures [16] produced by *Agrobacterium radiobactor* TFO12607 and *Rhizobium phaseoli* AHU1133 were provided through the courtesy of Daikin Industries Ltd, Japan. The former mixture (A-23-9) was first fractionated into seven cyclosophoran peaks by HPLC on two sequentially connected ODS columns (120AQ, 2.5 cm i.d. × 25 cm, YMC, Japan) as shown in Fig. 1a. Here the number attached to CS denotes the degree of polymerization. The HPLC was performed on a Jasco HPLC equipped with a refractive-index detector. The eluent was aqueous 4.5% (v/v) MeOH with a flow rate 5 mL/min, and the temperature of the column was maintained at 35 °C. Three mL of the sample solution at 66 mg/mL was injected for each fractionation. Since CS19 and CS20 were not separated with the ODS columns, the fraction containing both CS19 and CS20 was further fractionated by HPLC with a Polyamine II column (2.5 cm i.d. × 25 cm, YMC, Japan, Fig. 1b). The eluent was 53:47 MeCN–water with a flow rate of 5.0 mL/min, and the temperature of the column was maintained at 35 °C. The second mixture contains more than 75% of cyclosophoran having dp 17 (CS17), and CS17 was easily isolated from the mixture by HPLC separation with the ODS column system mentioned above.

Linear (1 → 2)-β-D-glucan (designated as LS) was prepared by acid hydrolysis (0.08 M CF_3CO_2H, 100 °C, 30 min) of CS21 and subsequent fractionation by HPLC with the

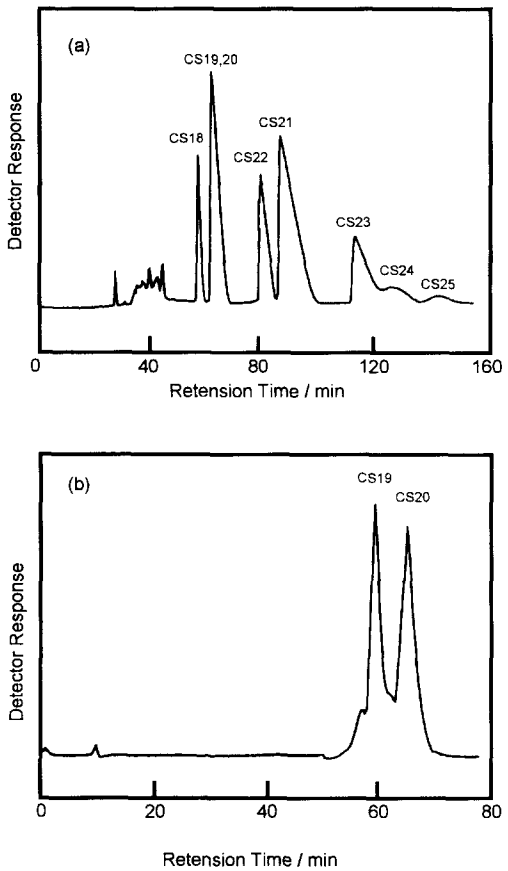

Fig. 1. Two-step HLPC separation of cyclic (1 → 2)-β-D-glucans. (a) Preparative HPLC of a mixture of cyclosophorans (A-23-9) on ODS column (YMC, 120 AQ ODS). (b) HPLC separation of CS19 and CS20 on an NH_2-bonded column (YMC, Polyamine II).

ODS column (data not shown). The equipment and conditions for HPLC used were the same as those used for cyclosophorans. All the samples obtained by HPLC were further purified by gel-filtration chromatography on a Bio-Gel P-4 column (extra fine, 2.5 × 100 cm) with water as the eluent to remove minor compounds probably eroded from the ODS and Polyamine II columns. The purity of all the samples was found to be better than 95% using the Polyamine II column (4.6 mm i.d. × 25 cm. YMC, Japan, data not shown).

The molecular weights of each fraction were confirmed by fast-atom bombardment mass spectroscopy (JOEL-JMS-SX102A mass spectrometer). This technique has been used previously [2]. The accelerating voltage was 5 kV for all the samples. Xenon was used as the bombarding gas, and the atom gun was operated at 6 keV. A 1-thioglycerol and glycerol mixture (1:1) was used as the matrix. Gas–liquid chromatography (GLC) of the alditol acetate from fully methylated CS17 and CS21 each showed a single peak.

which is identified as the alditol acetate from 3,4,6-tri-*O*-methyl-D-glucose, confirming a cyclic structure composed of (1 → 2)-β-D-glucose residues for these samples.

Small-angle X-ray scattering.—Small-angle X-ray scattering (SAXS) was observed from aqueous solutions of cyclic glucans (CS17, CS18, CS19, CS20, CS21, CS22, CS23, and CS24) and linear glucans (LS19 and LS21) at 25 °C. The concentrations of solutions measured were 40, 20, and 10 mg/mL for the cyclic glucans, and 25 and 12.5 mg/mL for the linear glucans. The SAXS experiments were performed with the SAXES optics (small-angle X-ray scattering equipment for solutions) installed at BL10C in the Photon, Japan [17]. Incident X-rays from synchrotron radiation were monochromatized to $\lambda = 1.488$ Å with a double-crystal monochrometer, and then focused to the focal point at the cell position with a bent focusing mirror. The scattered X-rays were detected by the one-dimensional position sensitive proportional counter (PSPC) with effective length 160 mm positioned at a distance of 1000 mm from the sample holder. The SAXS intensities were accumulated for a total measuring time of 1800 s in order to attain a sufficient S/N ratio. The observed range of the magnitude of the scattering vector was from $q = 2.50 \times 10^{-2}$ Å^{-1} to $q = 0.375$ Å^{-1}, which is equivalent to Bragg spacings from 251 to 16.8 Å. The exact camera distance was calibrated by using the diffraction peaks of collagen fiber (long period = 6.7×10^2 Å) at the 6th, 9th, and 11th orders. Data were collected on a CAMAC system controlled by a NEC PC9801RX.

A flat cell ($1 \times 0.5 \times 0.2$ cm^3) fitted with a pair of quartz windows (20 μm thick) was centered in the incident X-ray beam. The cell was thermostated by circulating water of a constant temperature though the cell holder.

The scattered intensities were corrected for variation of the incident X-ray flux by monitoring the beam with an ionizing chamber placed in front of the thermostated sample holder. The excess scattering intensities were evaluated by subtracting the scattering intensities of water from those of the solutions. The particle scattering function, $P(q)$, is defined by $P(q) = I(q)/I(0)$, where $I(q)$ is the scattered intensity at scattering vector q and $I(0)$ is the corresponding intensity extrapolated to $q = 0$.

Monte Carlo simulation.—(1 → 2)-β-D-Glucan chains were generated in conformations consistent with the disaccharide conformational energy map by the Monte Carlo method [18–21]. Here the glucose residue is assumed to be rigid and replaced with a virtual bond connecting the neighboring oxygen atoms of the glycosidic linkages. The energy map for β-sophorobiose (Fig. 2) was calculated as a function of the dihedral angles ϕ and ψ by using molecular mechanics [19]. Here the dihedral angles ϕ and ψ are defined as $\phi = \theta$[H-1, C-1, O, C-2′] and $\psi = \theta$[C-1, O, C-2′, H-2′]. In the calculation, nonbonded van der Waals contributions and electrostatic contributions due to partial charges were taken into consideration [19]. The atomic coordinates for the glucose units were adopted from the crystallographic data of β-sophorobiose [21], and the bond angle τ at the glycosidic oxygen was usually fixed to 113.6°. The occurrence probability for a given ϕ, ψ pair was obtained by normalizing the Boltzmann factor associated with each ϕ, ψ pair by the sum of all such Boltzmann factors [18–21]

Among the conformations of chains in the Monte Carlo sample for a given dp, those with end-to-end distances less than 1.5 Å were considered to be examples of cyclic (1 → 2)-β-D-glucans. The ensemble of linear (1 → 2)-β-D-glucan chains used for computing averages was composed of 500 chains. Calculation of scattering factors was

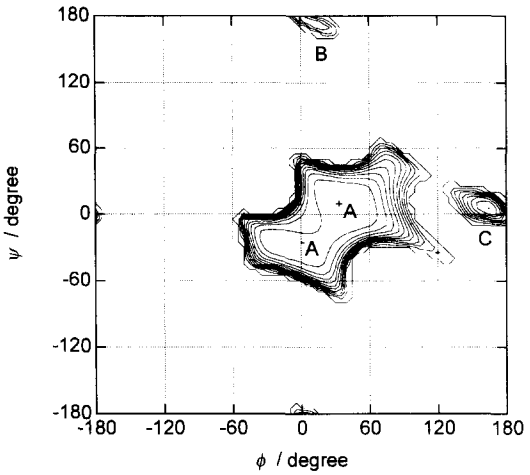

Fig. 2. Conformational energy map for the β-sophorobiose unit. The geometry used for the calculation was taken from the literature [22], with $\tau = 113.6°$. Contours are shown at intervals of 1.0 kcal mol^{-1} only for energies less than 10.0 kcal mol^{-1} The region of the energy well wherein $\psi > -20°$ was defined as conformation $^{+}$A and the region wherein $\psi < -20°$ was defined as conformation $^{-}$A according to York [15].

performed by regarding all carbon and oxygen atoms as spherical scatterers with corresponding van der Waals radii, as described previously [20,21,23]. The radii of carbon and oxygen atoms are taken to be 1.67 and 1.50 Å, respectively. All the O-6 atoms of the glucose chain sequence were affixed to the pyranose ring at a gauche–trans (gt) conformation with respect to the torsion angle θ[O-5, C-5, C-6, O-6] and the torsion angle θ[C-4, C-5, C-6, O-6], respectively [24].

The scattering profile $I(q)$ was calculated as a function of the magnitude of the scattering vector q from the atomic coordinates of the (1 → 2)-β-D-glucan chains in the Monte Carlo sample according to the Debye formula [23]

$$I(q) = \sum_{i=1}^{n} g_i^2/\phi_i^2(q) + 2\sum_{i=1}^{n-1}\sum_{j=i+1}^{n} g_i g_j \phi_i(q)\phi_j(q)\frac{\sin(d_{ij}q)}{d_{ij}q}, \quad (1)$$

$$q = (4\pi/\lambda)\sin(\theta/2), \quad (2)$$

where g_i and $\phi_i(q)$ denote the atomic scattering factor and the particle scattering factor of the ith atom, respectively. Here d_{ij} is the distance between the ith and jth atoms, and the particle scattering factor for each atom is assumed to be approximately given by that of a rigid sphere possessing a radius equivalent to the van der Waals radius of the atom as

$$\phi_i(q) = 3\left[\sin(R_i q) - (R_i q)\cos(R_i q)\right]/(R_i q)^3. \quad (3)$$

The simulated scattering profiles are directly compared with those observed by SAXS by normalizing with respect to the scattered intensity at the zero angle.

3. Results and discussion

The small-angle X-ray scattering profiles reveal the difference in the structure of cyclic and linear (1 → 2)-β-D-glucan chains in aqueous solution (Fig. 3) in the region of $q > 0.15$ Å^{-1}. Scattering data for the cyclic species were found to be concentration dependent and were extrapolated to infinite dilution. The radius of gyration R_G was evaluated from the initial slope of the Guinier plots ln $I(q)$ versus q^2 according to the Guinier approximation [25], which reads

$$I(q) \propto \exp(-R_G^2 q^2/3). \quad (4)$$

The radius of gyration R_G is smaller for a cyclic (1 → 2)-β-D-glucan than for its linear homologue as summarized in Table 1, indicating that a cyclic glucan chain assumes a compact conformation which may be represented roughly by a flat disk with a hollow in

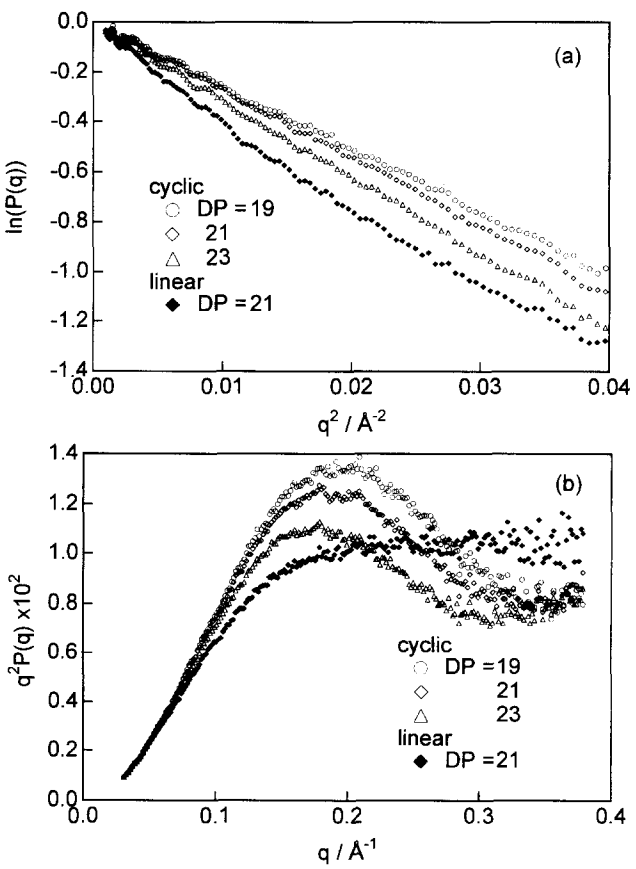

Fig. 3. SAXS profiles of cyclic and linear (1 → 2)-β-D-glucans in terms of (a) Guinier plots [ln $P(q)$ versus q^2] and (b) Kratky plots [$q^2P(q)$ versus q]; CS19 (○), CS21 (◇), CS23 (△) and LS21 (◆).

Table 1
Summary of parameters estimated from SAXS data for cyclic and linear (1 → 2)-β-D-glucans

Sample	R_G (Å)	T [a] (Å)	R_C [b] (Å)
CS17	7.8	10.0	
CS18	8.1	10.0	
CS19	8.5	10.0	
CS20	8.3	10.0	
CS21	8.6	10.5	
CS22	8.4	10.7	
CS23	8.9	10.8	
CS24	10.6	9.8	
LS19	11.1		5.9
LS21	12.0		6.6

[a] The measured thickness of the cyclic glucan assuming it to have the shape of a flat disk.
[b] The measured cross-sectional radius of a cylinder for the linear glucan assuming it to have the shape of a rigid cylinder.

the middle. The thickness T of cyclic (1 → 2)-β-D-glucans was evaluated according to the Guinier plots for thickness [25]

$$q^2 I_{\text{flatdisk}}(q) \propto \exp(-q^2 T^2/12)/A, \tag{5}$$

where A denotes the cross-sectional area. The thickness was found to be around 10 Å and to be independent of the molecular weight from CS17 to CS24 as summarized in Table 1. As shown in Fig. 3, the scattering from linear (1 → 2)-β-D-glucan chains is characterized by a plateau in the Kratky plots, whereas that from cyclic glucans possesses a peak.

Fig. 4 shows the dp-dependence of the probability that the terminal units of a simulated (1 → 2)-β-D-glucan chain approach one another within a distance of 6 Å. The

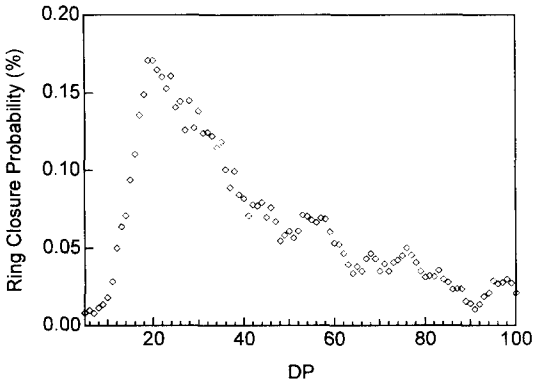

Fig. 4. The dp-dependence of the probability that the terminal glucose units of Monte Carlo (1 → 2)-β-D-glucan chains approach within a distance of 6 Å. A total of 10^5 chains was generated and examined for their end-to-end distance at each dp.

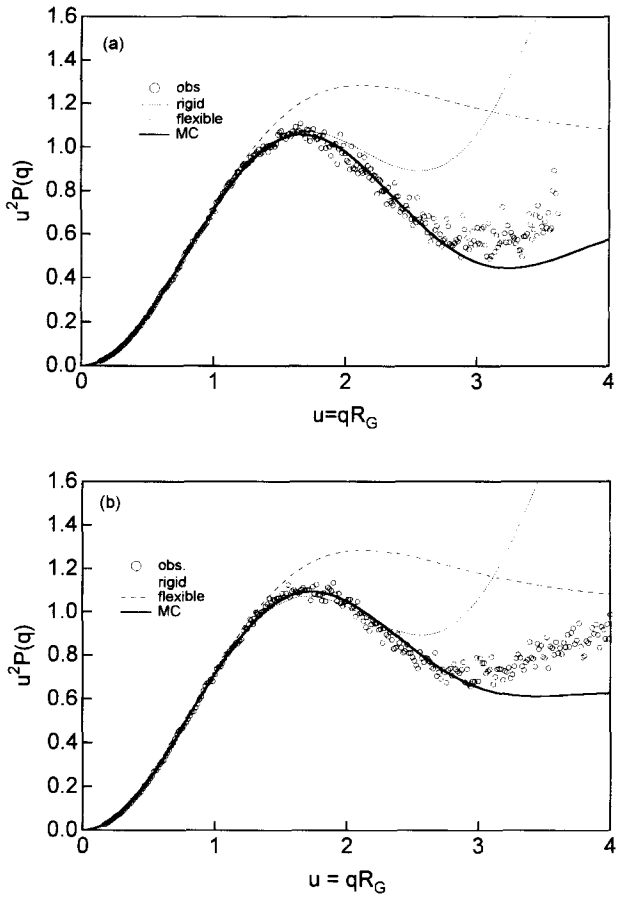

Fig. 5. A normalized Kratky plot of SAXS for (a) CS17 and (b) CS21 along with curves calculated for the Monte Carlo chains (————), the rigid ring (· · ·) and the flexible ring (---), respectively. Open circles are the observed data.

highest probability is seen in the dp range from 15 to 40. This result might reflect the occurrence in nature of cyclic (1 → 2)-β-D-glucans containing from 17 to 40 glucose residues [4]. We have collected 'pseudo-cyclic glucans' from Monte Carlo-generated linear (1 → 2)-β-D-glucan chains whose ends are separated by less than 1.5 Å, and a representative model chain among them was selected for examination based on agreement of the calculated and observed R_G.

Here R_G was calculated directly from the atomic coordinates of the Monte Carlo sample chains. Although the model chain is not closed covalently to form a ring, using it to simulate for cyclic (1 → 2)-β-D-glucan chains yields a scattering profile which agrees reasonably with that observed by SAXS as shown in Fig. 5 for CS17 and CS21. The deviation from the observed profile at $u\ (\equiv q\ R_G) > 3$ can be reduced by introducing a smaller apparent scattering unit in eq (3), but its physical significance cannot be

explained explicitly at this stage. It should be noted that similar deviations at large q have been observed for the simulation of other linear glucans [20,21].

The observed SAXS profile is also compared with those calculated from two elementary models: (1) a rigid ring [26,27] and (2) a flexible (Gaussian) ring [28,29]. Here the particle scattering factors for the two models are given as

$$P_{\text{rigid}}(q) = N^{-1} \sum_{n=1}^{N} \frac{\sin[(2u)\sin(\pi n/N)]}{(2u)\sin(\pi n/N)} \tag{6}$$

and

$$P_{\text{flexible}}(q) = (2/u)\exp(-u^2/4)D(u^{1/2}/2), \tag{7}$$

respectively, where u and $D(x)$ denote the reduced scattering vector and the Dawson integral, defined as

$$u \equiv qR_G \tag{8}$$

$$D(x) = \int_0^x \exp(t^2)\,dt. \tag{9}$$

Comparison of the observed scattering profile with those of these two models confirms the rather stiff nature of a cyclic (1 → 2)-β-D-glucan chain where the conformational freedom is suppressed by linking end-to-end. The deviations in the higher q range are due partly to failure of the models to take into account the effect of the molecular thickness.

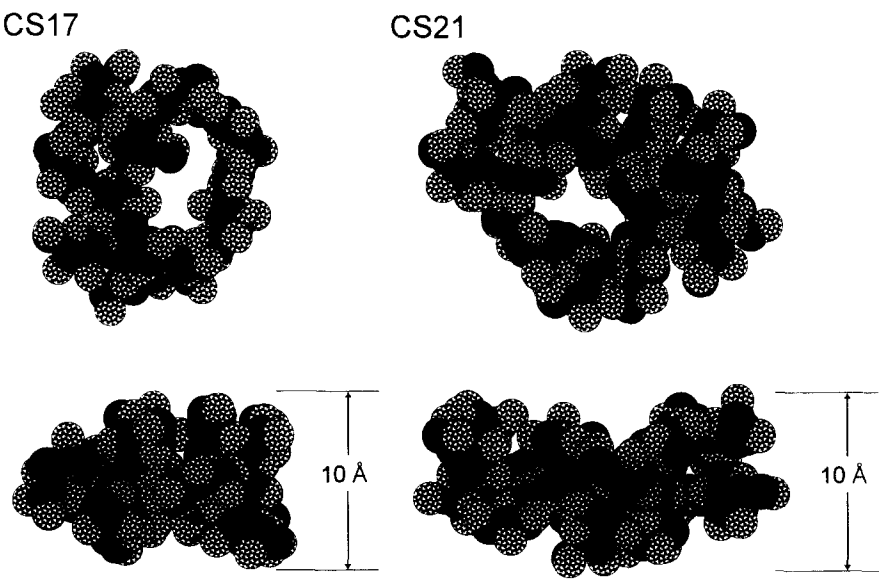

Fig. 6. Space filling models of 'cyclic' (1 → 2)-β-D-glucans based on Monte Carlo simulation: CS17 (left) and CS21 (right). The model chains have end-to-end distance of less than 1.5 Å. Hydrogen atoms are not shown.

Typical conformations of the simulated cyclic CS17 and CS21 chains are shown in Fig. 6. The shape is an irregular doughnut-like ring for both molecules. Since the (1 → 2)-β linkage produces a rather thick cylindrical conformation (see the conformation of linear (1 → 2)-β-D-glucans in Fig. 8), the cavity in a cyclic (1 → 2)-β-D-glucan chain may be too small to form an inclusion complex with relatively large molecules. Actually, the diameter of the ring annulus for CS 21 is only about 4–5 Å. All the glycosidic linkage torsion angles are found within region A of the conformational energy map of Fig. 2. Thirteen of 20 linkages are in region $^{+}$A, seven are in region $^{-}$A. No special mode of arranging $^{+}$A and $^{-}$A is observed.

Previous conformational analysis of the cyclic (1 → 2)-β-D-glucans [10–15] by molecular modelling and NMR has been somewhat inconclusive [9]. The structural model for CS17 proposed by Serrano et al. [11] involves alternation of low-energy conformation A with an energetically unfavorable conformation. This model yields a

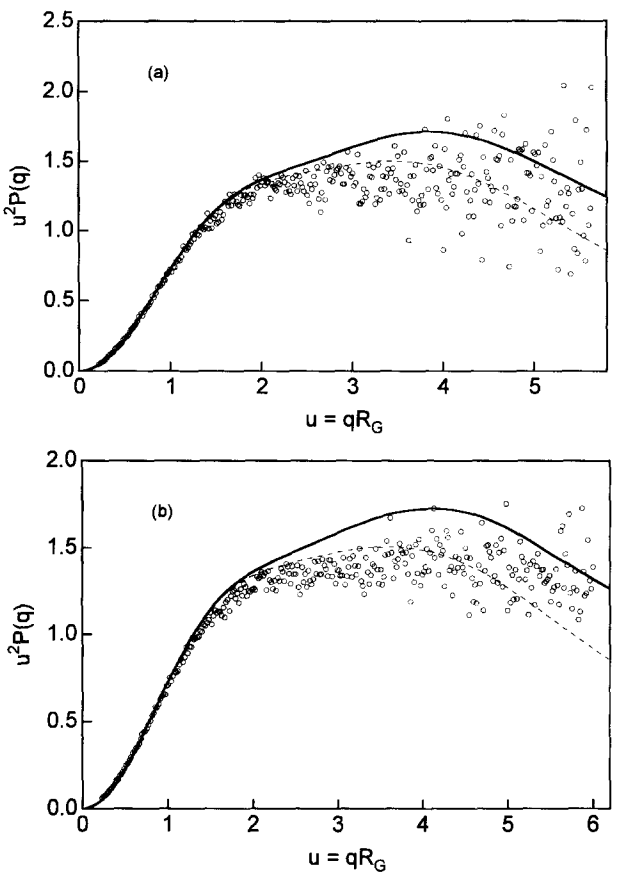

Fig. 7. A normalized Kratky plot of SAXS for (a) LS19 and (b) LS21. Calculated scattering profiles (————) were obtained from the atomic coordinates of simulated linear (1 → 2)-β-D-glucan chains (500 chains). Doubling of the radius of scatterers (see also eq 3) improves the fit (---). Open circles are the observed SAXS intensities.

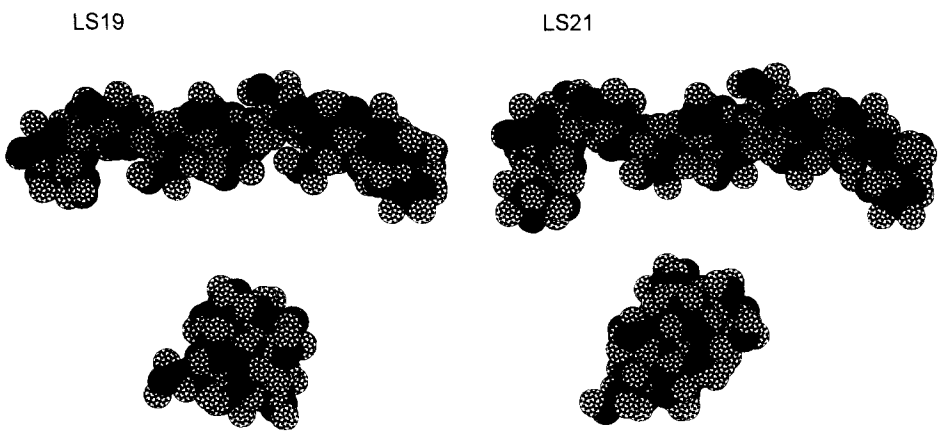

Fig. 8. Typical space filling models of linear (1 → 2)-β-D-glucan: LS19 (left) and LS21 (right). Each of these chains was chosen to have an end-to-end distance close to the mean value for the corresponding 500 chains. Hydrogen atoms are not shown.

rather symmetric macrocycle with an internal diameter of about 15 Å. Metropolis Monte Carlo investigations [13] suggest a relatively symmetric structure with a small polar cavity for CS18 to CS24. In contrast to these symmetric geometry models, Andre et al. [14] reported that MM3 molecular mechanics calculations predict a nonsymmetric conformer with a small cavity of 3.7 Å in diameter as the lowest energy form. Fair agreement was found between NMR experimental data and corresponding average values predicted by molecular modelling [14]. York [15] has suggested that ring closure in these cyclic glucans may be achieved by interrupting a perfectly alternating +A–A pattern of linkage conformations with two 'frame shifts' at which the alternating pattern is broken. The present model is very similar to Andre's model in terms of the asymmetrical nature of the molecules, a small cavity size, and the distribution of dihedral angles of glucosidic linkage. It should be noted that R_G calculated from the atomic coordinates of the Serrano model for CS17 is 9.4 Å, while the measured R_G for this cyclosophoran is just 7.8 Å. We should note that the evaluation of various models proposed previously in light of the SAXS data may be an appropriate topic for further research.

The Monte Carlo simulation for linear (1 → 2)-β-D-glucans yields less satisfactory results with respect to the scattering profile (Fig. 7). Although the radii of gyration evaluated from the generated LS19 and LS21 chains are in good agreement with observed values by SAXS, deviations in scattering profile become apparent at larger q where $u \equiv qR_G > 1.3$. The tendency of this deviation suggests an apparent thickness of the experimental scattering units as reflected that is much larger than that of the simulated chains. Here chain thickness is associated with the cross-sectional radius R_c evaluated from SAXS according to the cross-sectional Guinier approximation for a cylinder-like molecule [25]

$$qI_{\text{cylinder}} \propto \exp\left(-q^2R_c^2/4\right)/H, \tag{10}$$

where H denotes the height of a cylinder. As summarized in Table 1, the measured cross-sectional radius tends to increase with molecular weight from 5.9 Å (LS19) to 6.6 Å (LS21). Although the Guinier plots for cross-section exhibit a good linearity, suggesting a rod-like character, the evaluated cross-sectional diameter (11.8 and 13.2 Å, respectively, for LS19 and LS21) is considerably larger than the thickness evaluated for cyclic (1 → 2)-β-D-glucan chains (10 Å) and has an apparent molecular-weight-dependence. The experimental values of the cross-sectional radius are also larger than the corresponding values, 5.30 and 5.64 Å, respectively, estimated from the Monte Carlo simulations of linear (1 → 2)-β-D-glucan chains with $\tau = 113.6°$. Phenomenologically, the thickening effect may be taken into account either by introducing a larger radius in eq (3) or by choosing a larger bond angle τ. For example, the calculated cross-sectional radius increases approximately 12% by doubling the radius of the scattering units as demonstrated in Fig. 7. The calculated cross-sectional radius also increases with increasing bond angle τ from 5.15 Å ($\tau = 110.0°$) to 5.30 Å ($\tau = 113.6°$) and 5.53 Å ($\tau = 120.0°$) for the linear glucan chains having dp 19, and from 5.39 Å ($\tau = 110.0°$) to 5.64 Å ($\tau = 113.6°$) and 5.83 Å ($\tau = 120.0°$) for the glucans having dp 21. Theoretical fitting the experimental scattering profile is improved by increasing the size of the scattering units or increasing the bond angle.

In this study we have used a simple rigid residue energy map for generating the glucan chains. We note, however, the possibility that flexible-residue modelling [30] might improve agreement with SAXS experimental data. In addition, a more realistic model of the interactions between solvent and sugar residues might also be included in the simulation. Refinement of the simulation is a matter for further investigation.

Acknowledgements

We would like to thank Dr. T. Higashiura of Daikin Industries, Ltd. for the gift of cyclosophoran mixtures and Mr. H. Isuda for technical assistance with the Monte Carlo simulation. S.K. is grateful to Professor D.A. Brant for helpful discussions and suggestions during the preparation of this manuscript. This work was performed under the approval of the Photon Factory Advisory Committee (proposal No. 91-217).

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