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21. Virus isolates were collected from various regions of the world by three organizations: WHO, the Henry M. Jackson Foundation for the Advancement of Military Medicine (HMJFAMM), and NIAID. Isolates from the WHO Network for HIV-1 Isolation and Characterization were obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH. Isolates from HMJFAMM were provided by J. Mascola, Walter Reed Army Institute of Research, Rockville, MD, and F. McCutchan, Henry M. Jackson Research Laboratory, Rockville, MD. Isolates from NIAID were provided by J. Bradac, Division of AIDS, NIAID, NIH.
22. A. J. Conley *et al.* [*Proc. Natl. Acad. Sci. U.S.A.* **91**, 3348 (1994)] have further recently reported that an antibody to gp41 neutralizes five of six primary isolates tested. A detailed comparison of IgG1 b12 and a number of human anti-envelope monoclonal antibodies in the neutralization of a panel of primary isolates under equivalent conditions will be presented elsewhere (J. P. Moore *et al.*, in preparation).
23. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; E, Glu; I, Ile; K, Lys; L, Leu; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; and V, Val.
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25. HIV-1 IIIB and HIV-1 MN are viruses with an extensive history of passage in transformed T cell lines [M. Robert-Guroff *et al.*, *Nature* **316**, 72 (1985)]. Stocks of these strains grown in H9 cells were passaged in mitogen-stimulated PBMCs to prepare viruses that had been grown in the same cells as the primary viruses, to eliminate the influence of any host cell–dependent epigenetic factors on virus neutralization (*5*). The stock of PBMC-grown MN was a gift from A. N. Conley (Merck Research Labs).
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27. Infectious culture supernatants containing virus and free gp120 were treated with 1% Nonidet-P40 nonionic detergent to provide a source of gp120 [J. P. Moore *et al.*, *AIDS* **3**, 155 (1989)]. An appropriate volume of inactivated supernatant was diluted with a buffer containing tris-buffered saline (TBS), 1% NP-40, 10% fetal bovine serum, and a 100-μl sample was added for 2 hours at room temperature to microplate wells (Immulon II, Dynatech) coated with sheep polyclonal antibody D7324 (Aalto Bio Reagents, Dublin, Ireland). This antibody was raised to peptide APTKAKRRVVQREKR (*23*), derived from the COOH-terminal 15 amino acids of the clade B IIIB isolate. Unbound gp120 was removed by washing with TBS, and bound gp120 was detected with CD4-IgG (1 μg/ml), or with monoclonal antibody, diluted in TMTSS buffer essentially as described [J. P. Moore *et al.*, *AIDS* **4**, 307 (1990); J. P. Moore *et al.*, *J. Virol.* **68**, 469 (1994)]. Bound ligand was then detected with an appropriate alkaline phosphatase–conjugated antibody to IgG, followed by AMPAK (Dako Diagnostics). Absorbance was read at 492 nm (A_{492}). Each virus was tested against CD4-IgG in triplicate and against IgG1 b12 in duplicate. All A_{492} values were corrected for nonspecific antibody binding in the absence of added gp120 (buffer blank). The mean, blank-corrected A_{492} values for CD4-IgG and IgG1 b12 were then calculated and the A_{492} ratios of IgG1 b12:CD4-IgG determined. This normalization procedure permits allowance to be made for the different amounts of gp120 captured onto the solid phase by D7324 when comparing antibody reactivity with a panel of viruses. Binding ratios of 0.50 or greater were deemed to represent strong antibody reactivity; ratios from 0.25 to 0.50 were considered indicative of moderate reactivity; values <0.25 were designated as representative of essentially negative monoclonal antibody reactivity.
28. Supported by NIH grants AI33292, AI27742, AI35168, and AI25541 and by Johnson and Johnson. We are grateful to the original library donor for his cooperation and to H. C. Lane and R. Walker for clinical samples. We thank R. Bastidas, R. Rozenshteyn, R. Austin, R. Graff, and P. Morden for technical support and W. P. Yang for surface plasmon resonance measurements. We thank R. Chanock for critical review of the manuscript. C.F.B. is a Scholar of the American Foundation for AIDS Research and the recipient of an Investigator Award from the Cancer Research Institute.

7 June 1994; accepted 7 September 1994

Crystal Structure of β-D-Cellotetraose Hemihydrate with Implications for the Structure of Cellulose II

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The crystal structure of β-D-cellotetraose shows the same molecular packing as cellulose II, with two antiparallel molecules in the unit cell. For cellulose II, the orientation of the C6–O6 bonds has been described as gauche-trans and trans-gauche, respectively, for the two antiparallel molecules, which otherwise have identical conformations. In contrast, in β-D-cellotetraose all C6–O6 bonds are gauche-trans, but the conformations of the two antiparallel molecules are different. Energy minimization and molecular dynamics studies suggest that the structure of cellulose II should be reinvestigated in light of these findings.

Although cellulose is the most abundant biological macromolecule and has been studied for 73 years with x-ray diffraction methods (*1*), there are still open questions concerning details of its three-dimensional structure. This holds for naturally occurring cellulose I and for cellulose II, which is obtained from cellulose I by treatment with alkali (mercerization).

In view of the fact that x-ray fiber diffraction techniques yield insufficient data for the complete description of a molecular structure at the atomic level, these techniques are usually complemented by model building. Using these methods, investigators have shown for cellulose I and II that the chainlike molecules are formed by (1→4)-linked β-D-glucopyranoses in the $^4C_1$ chair conformation, which are alternately rotated 180° along the chain axis. In cellulose I, all molecules are arranged parallel (*2*). In cellulose II, they are antiparallel and orientated along the unique *c* axis in the monoclinic unit cell with $a = 8.01$ Å, $b = 9.04$ Å, $c = 10.36$ Å, and $\gamma = 117.1°$, space group $P2_1$, with two molecules A and B in the asymmetric unit (*3–5*). Molecule A is located in the corner of the *a*, *b* plane of the unit cell and the antiparallel molecule B is in the center (Fig. 1) and shifted 2.24 Å (*3*) or 3.0 Å (*4*) along *c*. The torsion angles φ and Ψ, which describe rotation about the interglucose link, are identical in molecules A and B, but the orientation of the exocyclic C6–O6 bond, χ, has been described as *tg* (trans-gauche) for A and *gt* (gauche- trans) for B (*3*) [see (*6*)]. The conformation of cellulose II is stabilized by intramolecular hydrogen bonds O3···O5′ (A and B) and O2···O6′ (only A) (Table 1), and a number of intermolecular hydrogen bonds connect the molecules into sheets parallel to the (010) and (120) planes (Fig. 1). Because these interactions are not identical as reported in (*3*) and (*4*), the structure of cellulose II is still under debate and other models have been discussed, with major emphasis on the orientation of the C6–O6 bonds (*7*). One of the best methods to resolve the structural ambiguities would be x-ray diffraction on single crystals of oligo-β-D-celluloses. We describe here the crystal structure of β-D-cellotetraose (*8*), which exhibits a molecular

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packing that is comparable to that of cellulose II.

β-D-Cellotetraose crystallizes in the triclinic space group *P*1 with unit cell parameters a = 8.023 Å, b = 8.951 Å, c = 22.445 Å, α = 89.26°, β = 85.07°, and γ = 63.93° (*8*). After transformation to a cell with obtuse angle γ, a = 8.026 Å, b = 9.030 Å, c = 22.445 Å, α = 93.68°, β = 85.10°, and γ = 116.96°, the structure of the cell is comparable to that of cellulose II given above except for the c axis, which accommodates a D-glucopyranose tetramer. In both unit cells, the asymmetric unit contains two molecules in antiparallel arrangement, and in β-D-cellotetraose a water molecule forms hydrogen bonds with the ends of three adjacent molecules. Because hydrogen atoms of O–H groups and of water could not be located in this crystal structure, hydrogen bonds are discussed on the basis of short O···O contacts (*9*).

The eight glucopyranoses in the two β-D-cellotetraose molecules adopt the typical $^4C_1$ chair form, and all C6–O6 bonds are in the *gt* orientation (*6*). Adjacent glucoses are rotated 180° so that systematic intramolecular hydrogen bonds can form (Fig. 2A), with O3···O5′ within 2.78 to 2.91 Å and O3···O6′ within 3.02 to 3.35 Å (omitting O33A···O64A, 3.62 Å, to the terminal glucose) (Table 1). This arrangement can be interpreted as three-center bonds, in which O3–H donates simultaneously to O5′ (major component) and O6′ (minor component) of the adjacent glucose (*7*, *9*). A possible alternative is a conventional two-center bond, O3–H···O5′, as suggested for cellulose I and cellulose II (*2*–*5*).

The two β-D-cellotetraose molecules A and B differ in their overall conformations. The superposition of molecules A and B (Fig. 3) shows that glucoses 1 through 3 differ in their puckering, which affects mainly the orientations of the C3–O3 bonds. According to the Cremer and Pople parameters (*10*), the glucopyranoses in A are "standard" and in B are more strained, probably as a result of packing effects or hydrogen-bonding interactions. These differences are also reflected in the φ, Ψ torsion angles for glucoses 2 and 3 in β-D-cellotetraose; angles φ differ by 11°, whereas angles Ψ are similar in A and B (*6*).

With respect to the direction of the c axis, translation-related β-D-cellotetraose molecules form "infinite" strands (Fig. 2A). Adjacent molecules are hydrogen-bonded O1···O4′ (2.83 Å), O1···O3′ (2.70 Å), and O2···O4′ (3.28 Å), and only slight rearrangement was required to form the covalent β(1→4) linkage as in cellulose II.

As shown in Fig. 1, the β-D-cellotetraose molecules are tilted with respect to the *ac* (010) plane. This permits two types of intermolecular hydrogen bonds to form, first between parallel molecules (A or B) in the *ac* (010) plane and second between antiparallel molecules (A and B) in the (120) plane (see Fig. 1). Both types of hydrogen bonds lead to planar, hydrogen-bonded sheets of β-D-cellotetraose molecules that interpenetrate each other. No hydrogen bonds are observed in the direction of the b axis.

The sheets formed by parallel β-D-cellotetraose molecules in the (010) plane are stabilized by intermolecular O2···O6 hydrogen bonds. Adjacent sheets A, B are antiparallel at a van der Waals distance of 3.5 Å and are shifted relative to each other along the crystallographic c axis by 2.5 Å. This displacement corresponds to half a glucose and was also indicated for cellulose II (*3*–*5*). It permits optimum molecular packing, which is tighter for cellulose II than for cellulose I (*3*, *4*).

In the (120) plane, adjacent β-D-cellotetraose molecules are in an antiparallel orientation and are engaged in a more complex network of hydrogen bonds (Fig. 2B). The interactions are O2A···O2B, O3A···O6B, O6A···O3B, O6A···O5B, and O6A···O6B (Table 1). The hydrogen-bonding patterns in both sheets are periodic in spite of small twists out of the molecular plane observed for the terminal glucoses.

To explore the validity of β-D-cellotetraose as a model for cellulose II, we constructed a cellulose polymer by translation

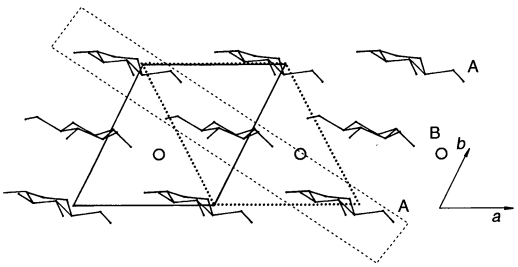

Fig. 1. Packing of β-D-cellotetraose in the *ab* plane. Only one glucopyranose is shown for each molecule. Molecules A and B are oriented parallel in sheets in the *ac* plane; A is antiparallel to B. The circle indicates the water molecule. Solid lines represent the crystal unit cell; dotted lines indicate an unconventional unit cell comparable to that used in fiber diffraction studies (*2*–*5*); dashed lines designate the section (120) of the unit cell described in Fig. 3.

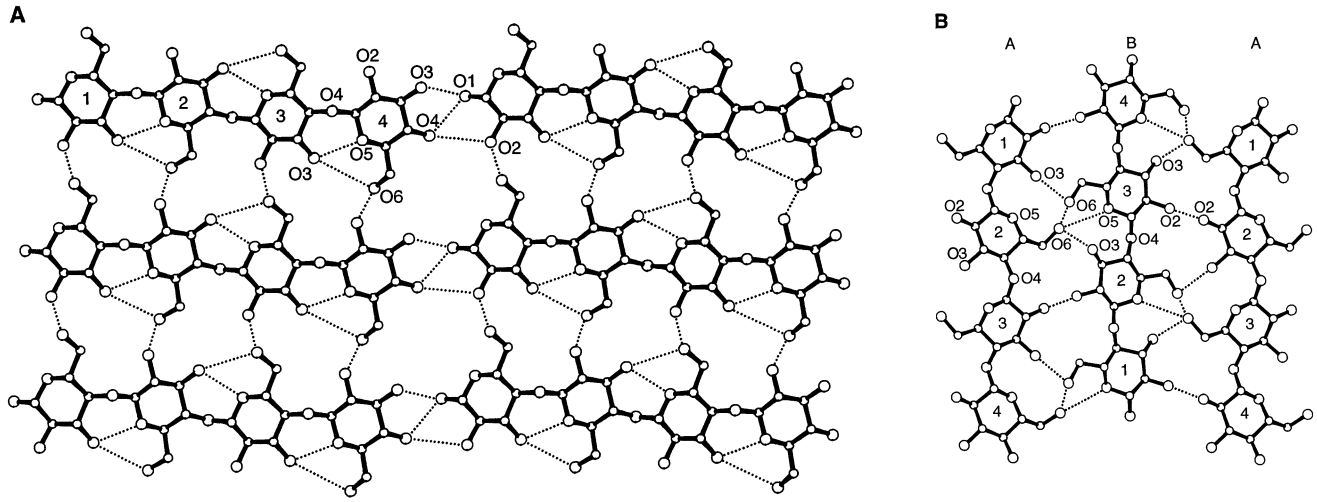

Fig. 2. (**A**) Description of the sheets of parallel molecules (A or B in Fig. 1). Dotted lines indicate short O···O contacts that are probably hydrogen bonds. The intermolecular hydrogen bonds involving O1 connect adjacent molecules in the c direction to form "pseudopolymeric" cellulose II. (**B**) Packing arrangement and hydrogen bonding (dotted lines) of sheets containing antiparallel β-D-cellotetraose molecules; section (120) (see Fig. 1). Intramolecular hydrogen bonds are not drawn for the sake of clarity; they are as shown in (A).

Table 1. Hydrogen-bonding interactions in cellulose II from Kolpak and Blackwell (*3*) and Stipanovic and Sarko (*4*) and in the crystal structure of β-D-cellotetraose (average values). In the model of cellulose II derived on the basis of the crystal structure, the same hydrogen bonds are formed as in the crystal, with differences in bond lengths smaller than 0.06 Å. The labels *gt* and *tg* refer to molecules with the respective orientations of their C6–O6 bonds.

Kolpak and Blackwell			Stipanovic and Sarko			Crystal structure		
Orientation	Bond	Length (Å)	Orientation	Bond	Length (Å)	Orientation	Bond	Length (Å)
				Intramolecular				
gt	O3–O5′	2.69	*gt*	O3–O5′	2.70	*gt*	O3A–O5′A	2.79
							O3A–O6′A	3.27*
tg	O6–O2′	2.73	*tg*	O6–O2′	2.76	*gt*	O3B–O5′B	2.87
	O3–O5′	2.69		O3–O5′	2.70		O3B–O6′B	3.15
				Intermolecular, parallel to (010) (Fig. 2)				
gt	O6–O2	2.76	*gt*	O6–O2	2.97	*gt*	O6A–O2A	2.70
tg	O6–O3	2.67	*tg*	O6–O3	2.65	*gt*	O6B–O2B	2.64
				Intermolecular, parallel to (120) (Fig. 3)				
	O2A–O2B	2.77†		O2A–O2B	2.62†		O2A–O2B	2.72
				O3A–O6B	2.80		O3A–O6B	3.22
				O6A–O3B	2.84		O6A–O3B	3.13
							O6A–O6B	2.65
							O6A–O5B	3.25

*Omitting O33A–O64A (3.62 Å). †Molecule A has *gt* and molecule B has *tg* conformation.

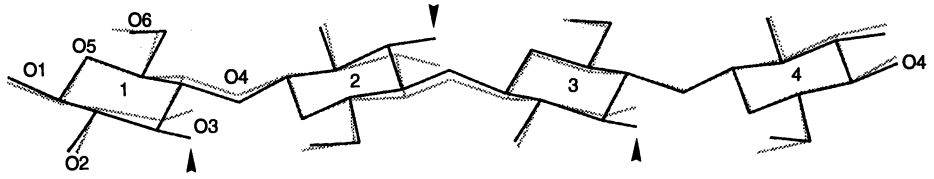

Fig. 3. Superposition of β-D-cellotetraose molecules A (black) and B (gray) by least squares fit (root-mean-square deviation 0.25 Å). Glucoses 1 to 3 show similar differences (affecting the orientation of the C3–O3 bond, see arrowheads) in their conformation; glucose 4 experiences end effects.

of only the central cellobiose fragment of the two molecules A and B to avoid end effects. This model was fitted by least squares to the fiber structure and into the unit cell of cellulose II (*3*) (root-mean-square deviation 0.17 Å, omitting the O6 groups). Energy minimization of both models with periodic boundary conditions on the crystal lattice clearly indicated that our model was superior to that reported in (*3*) by 10 kcal mol^{-1} (*11*). In addition, molecular dynamics simulations were performed. The structures were averaged over the last 40 ps of the simulation and then subjected to energy minimization (*12*); in this case also, our model was more stable than the model based on (*3*) by 4 kcal mol^{-1}.

These results suggest that the fiber structure of cellulose II should be reinvestigated to determine whether the differences in D-glucopyranose conformation and torsion angles in the β-D-cellotetraose crystal structure can be verified and whether the C6–O6 bonds are all in the same *gt* orientation; this was also indicated by a singlet for C6 in the ^{13}C cross polarization–magic angle spinning nuclear magnetic resonance spectrum of cellulose II (*13*, *14*). It has been questioned, however, whether the fiber x-ray data are of sufficient quality to permit a conclusive answer if fine details of a structure are considered [calculated energy values are more sensitive to structural changes (including hydrogen bonds) than crystallographic *R* factors (*7*)].

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5. J. Blackwell *et al.*, *Fiber Diffraction Methods* (American Chemical Society, Washington, DC, 1980), pp. 315–334.
6. Torsion angles φ and Ψ are defined as φ ($O5_i$–$C1_i$–$O4_{i-1}$–$C4_{i-1}$) and Ψ ($C1_i$–$O4_{i-1}$–$C4_{i-1}$–$C3_{i-1}$), and χ is defined as C4–C5–C6–O6 [International Union of Pure and Applied Chemistry–International Union of Biochemistry Joint Commission on Biochemical Nomenclature, *Eur. J. Biochem.* **131**, 5 (1983)]. In β-cellotetraose, average φ, Ψ values are 100°, −97° for A and 89°, −94° for B; the average χ is 187° (A) and 172° (B). In cellulose II (*3*) φ, Ψ are similar for A and B: φ ≈ 94°, Ψ ≈ −97°; χ is 174° (A), −70° (B).
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8. Platelike crystals (0.4 mm by 0.1 mm by 0.01 mm) were obtained by slow diffusion of isopropanol into an aqueous solution of β-D-cellotetraose (Seigaku Corporation, Tokyo, Japan). For x-ray experiments, they were mounted in glass capillaries. Lattice constants were derived from the diffraction angles of 81 strong reflections measured with an Enraf-Nonius FAST area detector mounted on an FR 571 x-ray generator with rotating anode, Ni-filtered CuK_α radiation, wavelength λ = 1.541 Å. X-ray data were collected with an Enraf-Nonius CAD4 diffractometer mounted on the same generator (2165 reflections) and with the synchrotron at Hamburg DESY (2613 reflections). The data sets (diffractometer data, 8 to 1.5 Å, and synchrotron data, 1.9 to 1.0 Å) were merged (R_{merge} = 8.7%). The structure was determined by a combination of Patterson-search and direct methods [SIR92 (*15*)] and refined anisotropically with SHELX76 (*16*) to an *R* factor of 8.5% for 2793 unique reflections $F_o > 1\sigma(F_o)$. Hydrogen atoms could not be located from the electron density maps; positions of C–H hydrogens were computed and used in the refinement. Average standard deviations are 0.02 Å for bond lengths, 1° for bond angles, and 2° for torsion angles.
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11. We simulated "infinite" crystal structures as observed in cellulose II (*3*) by applying periodic boundary conditions with the program DISCOVER (*15*). The energies of the models were minimized until the maximum derivative of the energy function was less than 0.1 kcal mol^{-1} $Å^{-1}$. The final energy was 49 kcal mol^{-1} for the model based on β-D-cellotetraose and 59 kcal mol^{-1} for the model taken from (*3*).
12. After energy minimization (*13*), we calculated a 50-ps molecular dynamics simulation at 300 K, using constant volume and constant temperature (canonical ensemble). After 10 ps of equilibration time, the structures were sampled every 0.2 ps, generating 200 structures over 40 ps of simulation time. Examination of the trajectories showed no structural clustering. The 200 structures were averaged, and the averaged structures were subjected to energy minimization until the maximum derivative was less than 0.001 kcal mol^{-1} $Å^{-1}$. We carried out all simulations with the program DISCOVER 3.1, using the consistent valence force field (CVFF) (*17*) and an energy distance cutoff of 15 Å. The final energies were 42 and 46 kcal mol^{-1} for the models obtained from β-D-cellotetraose and from (*3*), respectively. The conformations of the χ angles (*6*) did not change significantly, but the φ, Ψ angles converged such that the conformation of the fiber model was changed toward our model. The values of φ and Ψ of our initial model were 99° and −97° (A) and 90° and −94° (B); after energy minimization, the values were 93° and −90° (A); and 85° and −89° (B) (the difference in φ remains). The values of φ and Ψ of the initial fiber model (*3*) were 93° and −96° (A) and 95° and −98° (B); after energy minimization the values were 91° and −89° (A) and 83° and −95° (B) (the differences in φ are greater than in the initial model).
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18. This work was supported by Deutsche Forschungsgemeinshaft (Sa 196/12-4), by Bundesministerium für Forschung und Technologie (03-SA3FUB), and by Fonds der Chemischen Industrie.

20 July 1994; accepted 2 September 1994