Pure & Appl. Chem., Vol. 68, No. 11, pp. 2187–2192, 1996.
Printed in Great Britain.

Structural reporter parameters for the characterisation of crystalline cellulose

Andreas P. Heiner and Olle Teleman

Biotechnology and Food Research, VTT Technical Research Centre of Finland
POB 1500, FIN-02044 VTT (Espoo), FINLAND

Abstract

We recently reported molecular dynamics simulations of the two native forms of crystalline cellulose [A. P. Heiner, J. Sugiyama and O. Teleman, Carbohydr. Res., *in press*]. From these a number of molecular properties have been calculated and show that local structure differs in triclinic and monoclinic cellulose, but also between the so-called odd and even subphases of monoclinic cellulose. Although all glucose rings remain close to the $^4C_1$ chair conformation, pucker analysis shows that the odd subphase tends towards the ^2E envelope. Literature data were systematised into a relation between certain torsion angles and solid state ^{13}C NMR chemical shifts. Torsion angles were found to be important for the C-6 chemical shift but not for the C-1 and C-4 carbon atoms.

Natural cellulose consists of long parallel homopolymers of β-(1→4)-linked D-glucose monomers. Despite its importance in a number of industrial applications, relatively little is known about the detailed structure at mesoscopic or microscopic level. The debate about the atomic structure of the crystalline part of cellulose was revived in 1984 when Atalla and vanderHart proposed two phases for the native crystalline structure based on results obtained by solid state ^{13}C CP-MAS NMR experiments [1,2]. Further support was found by Raman spectroscopy [3] and infrared spectroscopy [4,5,6]. The structure of the two phases was solved by Sugiyama et al. using electron diffraction [7]. In both phases the polymers are arranged in a parallel-up fashion. The Iα phase is triclinic with one cellobiose residue per unit cell. The Iβ phase turned out to be monoclinic, and very similar to the model proposed by Sarko and Muggli [8], with two cellobiose moieties per unit cell. That natural cellulose consists of two phases explains almost all earlier experimental data and a linear combination of the two phases can yield reflections in agreement with all published diffraction experiments. Recently, allomorph surfaces were also identified from atomic force micrographs [9].

So far, computer simulation of cellulose under full crystalline periodic boundary conditions [10,11] have used earlier models for the cellulose as starting geometries. We recently reported 1 ns molecular dynamics simulations of both phases [12], starting from the experimental coordinate set of Sugiyama et al. [7]. The computer simulations of the Iβ phase indicated that several structural properties were different for the two chains in the unit cell. The most notable of these were that the ring plane of the glucose moieties in the so-called "odd" planes are placed at an angle to the (1,0,0)-plane of about 10°, whereas the glucose rings in the "even" planes are parallel to that plane. Another property that differed between the odd and even subphases was the distribution of the χ torsion angle, C-4—C-5—C-6—O-6. The existence of two subphases, the odd and the even, in the monoclinic phase seems to explain the presence of further resonances in some ^{13}C CP-MAS NMR spectra [12,13].

In this communication we take the structural description of the crystalline cellulose further. In order to identify suitable structural reporter parameters we have analysed the puckering behaviour for the two native crystal forms following the nomenclature of Cremer and Pople [14] as applied by for instance Dowd et al. [15]. We also report the correlation of published ^{13}C

NMR data with torsion angles, and use that for the prediction of chemical shifts based on the dynamic behaviour of the cellulose during the simulations. Finally, we use results of quantum mechanical calculations of nuclear shielding [16] to predict chemical shift differences for C-1 and C-4 carbons between the cellulose phases.

Methods

The preliminary unrefined structures of crystalline cellulose Iα and Iβ obtained by electron diffraction [7] were used as starting structures. The monoclinic phase (Iβ) was simulated in a periodic box of 3×3×3 unit cells yielding a total of 1512 united atoms and the triclinic phase (Iα) in a box of 4×6×3 (a×b×c) unit cells with a total of 2016 united atoms. The simulations, which lasted 1 ns, were performed as described in [12] using the GROMOS87 force field [17] and a modified version of the GROMOS87 program suit [18].

Pucker parameters were analysed according to Cremer and Pople [14]. Examples of puckered conformations as a function of the Q,Θ,Φ parameters can be found in [15].

^{13}C NMR chemical shift data were taken from [19,20]. Since the correlation between shift and torsion is periodic, the data were refitted with a sinusoidal function. This function was then used to average shifts from the torsion angle distributions obtained from the simulations.

Chemical shift predictions were also made on the basis of quantum chemical calculations. At the 3-21G and the 6-31G* levels, Durran et al. [16] calculated the nuclear shielding effect for a model system representing an α-(1→4)-glucan as a function of the torsion angles φ,ψ of the glycosidic linkage. Although absolute chemical shifts could not be obtained, the chemical shift difference between C-1 and C-4 was reproduced with reasonable accuracy. For computational reasons only a small part of the glucan was included in the calculation, and in the model the only difference between an α-(1→4)-glucan and a β-(1→4)-glucan is the chirality of C-1. For symmetry reasons the φ,ψ map of the nuclear shielding therefore also applies to a β-(1→4)-glucan if φ → -φ, at least to the extent that the limited molecular fragment is a good model for a disaccharide. The φ,ψ values from the MD simulations were used together with the shielding map of Durran et al. to generate predictions for relative chemical shifts.

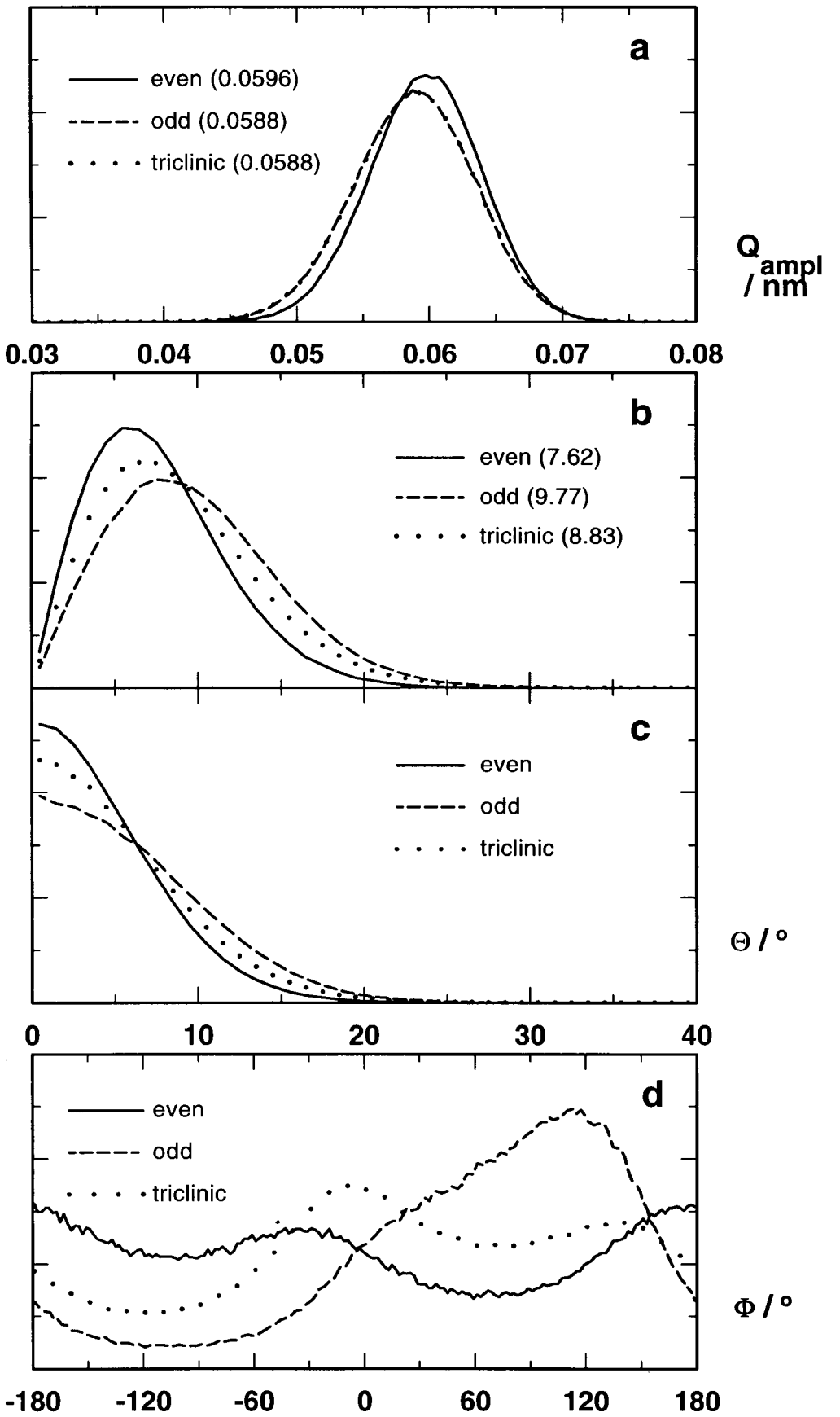
Results and discussion

The preliminary analysis of the MD simulations showed that the local structure differed between triclinic and monoclinic cellulose, but also between the odd and even subphases. We earlier characterised these differences in terms of primary geometric properties [12] but puckering parameters are better for this purpose. Figure 1 depicts distributions for the Q,Θ,Φ pucker parameters as derived from the Iα and Iβ simulations. For the Iβ phase the pucker parameters are evaluated separately for the odd and even subphases. In all cases the conformation character is essentially $^4C_1$. The deviation from this chair is small as seen from the Θ parameters, and the value of Q is typically 0.06 nm, i. e. close to the ideal $^4C_1$ value of $Q = R/\sqrt{6} = 0.408R = 0.0628$ nm for a chair with equal bond lengths and tetrahedral angles ($R_{C\text{-}C}$ = 1.54 Å). The average Θ is between 7° and 10° for the three phases. The volume element on the Φ,Θ unit sphere is $\sin\Theta d\Phi d\Theta$. Correction for this gives the population density (Fig. 1c) which shows that $^4C_1$ is the most densely populated single state in all cases.

The differences between the three phases are clearly characterised by the Φ parameter, which indicates toward which conformation the deformation of the glucose moiety tends (Fig. 1d). In the triclinic cellulose the glucose ring has a preference for Φ=0° and a lesser one for Φ=150°. These values correspond to tendencies towards the ^OE and $^2H_3$ conformations, respectively, which would be reached when $3\cos^2\Theta=1$, i. e. when Θ ≈55°. The monoclinic even subphase has an even Φ distribution without pronounced tendencies, but the monoclinic odd subphase possesses a strong preference for 0°<Φ<150° with a maximum at Φ=120°. This corresponds to a tendency towards the ^2E conformation.

Figure 2 shows typical conformations for the glucose rings in the three phases. Although the deformation from $^4C_1$ is small, the deformations

Figure 1. Distributions of pucker parameters (in arbitrary units) calculated from MD simulations of cellulose. Average puckering amplitude and colatitude are given in brackets. **a)** Puckering amplitude Q (nm). **b)** Puckering colatitude Θ (°). **c)** Puckering colatitude Θ but corrected for the size of the volume element, i. e. the distribution of Θ/sinΘ. **d)** Puckering azimuth Φ (°).


a
even (0.0596)
odd (0.0588)
triclinic (0.0588)
Q ampl / nm
0.03
0.04
0.05
0.06
0.07
0.08
b
even (7.62)
odd (9.77)
triclinic (8.83)
c
even
odd
triclinic
Θ / °
0
10
20
30
40
d
even
odd
triclinic
Φ / °
-180
-120
-60
0
60
120
180

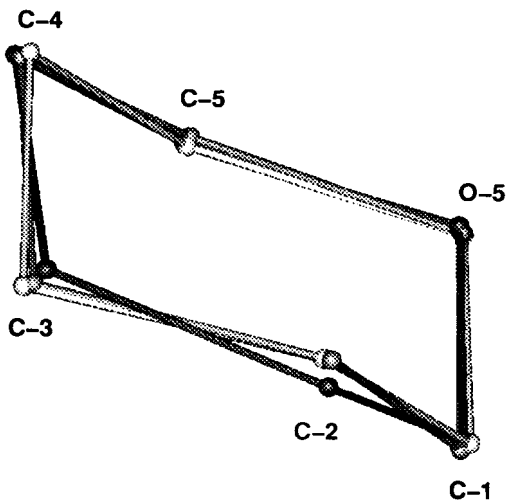


Figure 2. Typical glucose ring conformations the monoclinic even (light grey), monoclinic odd (middle grey) and triclinic (dark grey) cellulose phases.

are statistically significant and can be used to characterise the cellulose in question.

The chemical shift of an atom in NMR spectra is a consequence of its chemical environment. Based on shifts and dihedral angles for various celluloses and related small sugars, Horii and co-workers [19] observed a linear correlation between the ^{13}C chemical shift of C-1' ($\delta_{C\text{-}1'}$) and φ (H-1'—C-1'—O-4—C-4), $\delta_{C\text{-}4}$ and ψ (C-1'—O-4—C-4—H-4), and between $\delta_{C\text{-}6}$ and χ (C-4—C-5—C-6—O-6). Because of the periodic nature of the dihedral angle these correlations are by necessity also periodic. For the torsion angles of the glycosidic linkage, the experimental data is confined to narrow regions ($10° < \varphi < 60°$, $-30° < \psi < -10°$) so that it is not possible to expand the linear correlations to periodic ones. For χ there is experimental data for several rotamers and fitting the shift data to a sine function produces:

$$\delta_{C\text{-}6} = 63.8 + 2.5 \sin(\chi - 71.7°)$$

where $\delta_{C\text{-}6}$ is given in ppm. This may be considered as an analogue to the Karplus equation [21] but in terms of chemical shift rather than spin-spin coupling constants. These correlations were used to predict chemical shifts for C-1, C-4 and C-6 from the torsion angle distributions obtained in the molecular dynamics simulations. Table 1 gives the predicted and experimental shifts for the three cellulose phases.

Figure 3 shows the data underlying the correlation as well as the distributions of the corresponding torsion angles. For φ and ψ it is clear that the uncertainty in the linear fit is considerable and that, in fact, the existence of a correlation between the torsion angle and the chemical shift is questionable. This is further underlined by the fact that use of the correlation to calculate chemical shifts for C-1 and C-4 does not produce agreement with the experimental shifts. For C-6 the situation is considerably better. Not only is the correlation between torsion angle and shift manifestly periodic, but also its use to calculate chemical shifts from the simulations produce close agreement with the experimental shifts. In fact, the calculated shift values are much closer to the experimental ones than suggested by the uncertainty in the fit. We can thus conclude that the χ torsion angle is indeed the main determining factor for the C-6 chemical shift.

A reason that the fit produces agreement for C-6 but not for C-1 or C-4 may be that for C-6 the complete covalent environment is described by the torsion angle. This is not the case for the other two carbon atoms.

Durran et al. [16] approached the chemical shift using *ab initio* methods to calculate the nuclear shielding as a function of φ and ψ for C-1 and C-4 in a model $\alpha(1\rightarrow4)$-glucan. This model glucan only possesses the C-1 and C-2 carbons on the non-reducing side of the glycosidic linkage. In consequence of this and for symmetry reasons the nuclear shielding for the $\beta(1\rightarrow4)$-glucan will be the same as for the $\alpha(1\rightarrow4)$-glucan but with a φ value of the opposite sign. We used these nuclear shielding results again to predict chemical shifts for C-1 and C-4 from the simulated torsion angles, the results are given in Table 1. Since the calculated shieldings and the chemical shift do not have a common reference point, we arbitrarily added a constant to all C-1 shifts such as to produce agreement with the experimental shift for monoclinic even cellulose, and similarly to the C-4 shifts.

The resulting shifts are very similar for the three cellulose phases, both for C-1 and C-4, but

Figure 3. Torsion angle distributions and predictions of chemical shift for the even (full lines), odd (dashed lines) and triclinic (dotted lines) cellulose subphases. Chemical shift data are shown as diamonds. **a)** φ, **b)** ψ; the outlying datapoint enclosed by a circle was excluded from the fit, **c)** χ.

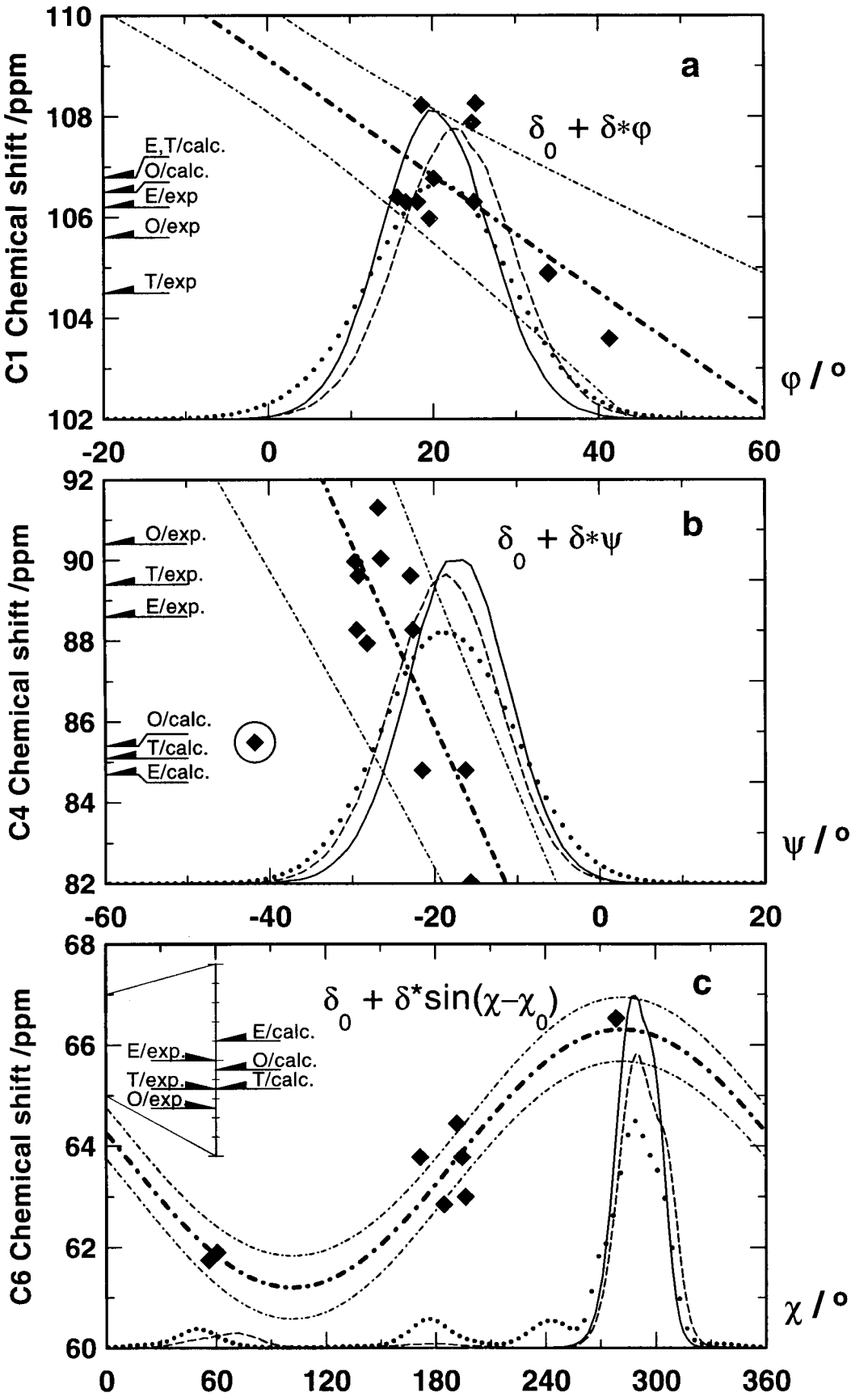

a
$\delta_0 + \delta*\varphi$
C1 Chemical shift /ppm
E,T/calc.
O/calc.
E/exp
O/exp
T/exp
φ / °
b
$\delta_0 + \delta*\psi$
C4 Chemical shift /ppm
O/exp.
T/exp.
E/exp.
O/calc.
T/calc.
E/calc.
ψ / °
c
$\delta_0 + \delta*\sin(\chi-\chi_0)$
C6 Chemical shift /ppm
E/calc.
E/exp.
O/calc.
T/exp.
T/calc.
O/exp.
χ / °

Table 1. Experimental and predicted chemical shifts for C-1, C-4 and C-6 in ppm.

Atom	Cellulose subphase	Experimental	Simulation + Horii correl. [19]	Simulation + *Ab initio* correl. [16][a]
C-1	Even	106.2	106.8	—[a]
	Odd	105.6	106.5	106.5
	Triclinic	104.5	106.8	106.4
C-4	Even	88.6	84.7	—[a]
	Odd	90.4	85.4	89.2
	Triclinic	89.4	85.1	88.8
C-6	Even	66.0	66.2	—
	Odd	65.5	65.9	—
	Triclinic	65.7	65.7	—

[a]) The *ab initio* calculation provides a value for the nuclear shielding, which has no absolute reference point with respect to the chemical shift. In order to compare the shielding data to the shifts a shift was added to make exact agreement for the even phase. The comparison is therefore valid only for the odd and triclinic cases.

do not show very good agreement with the experimental shifts. Contrary to the case of α(1→4)-glucans, but in complete agreement with what we observed from the experimental torsion angle / shift correlation [19] we conclude that the glycosidic linkage torsion angles are not very important for the C-1 and C-4 chemical shift differences between the three cellulose phases.

Conclusion

Although the net structural differences are small between the three cellulose subphases, they are significant and thus valid characterisation tools. We are performing molecular dynamics simulations of cellulose surfaces [22], and will apply the tools presented here to the characterisation of the molecular layers in cellulose surfaces.

Acknowledgements. We are grateful to D. M. Durran for making his numerical nuclear shielding data available and to Drs. J. Sugiyama and A. Teleman for valuable discussion.

References

1. R. H. Atalla and D. L. vanderHart, *Science* **223** (1984), 283-285.
2. D. L. vanderHart and R. H. Atalla, *ACS Symp. Ser.* **340** (1987), 88-118.
3. J. H. Wiley and R. H. Atalla, *ACS Symp. Ser.* **340** (1987), 151-168.
4. A. J. Michell, *Carbohydr. Res.* **173** (1988), 185-195.
5. J. Sugiyama, J. Persson and H. Chanzy, *Macromolecules* **24** (1991) 2461-2466.
6. A. J. Michell, *Carbohydr. Res.* **241** (1993), 47-54.
7. J. Sugiyama, R. Vuong and H. Chanzy, *Macromolecules* **24** (1991), 4168-4175.
8. A. Sarko and R. Muggli, *Macromolecules* **7** (1974) 486-494.
9. L. Kuutti, J. Peltonen, J. Pere and O. Teleman, *J. Microsc.* **178** (1995), Pt. 1, 1-6.
10. L. M. J. Kroon-Batenburg and J. Kroon, The First Electronic Glycoscience Conference, Oxford, 1995, *submitted.*
11. B. J. Hardy and A. Sarko, Polymer (1995), *in press.*
12. A. P. Heiner, J. Sugiyama and O. Teleman, *Carbohydr. Res.* **273** (1995), 207-223.
13. F. Horii, H. Yamamoto, R. Kitamaru, M. Tanahashi and T. Higuchi, *Macromolecules* **20** (1987), 2949-2951.
14. D. Cremer and J. A. Pople, *J. Am. Chem. Soc.* **97** (1975), 1354-1358.
15. M. K. Dowd, A. D. French ad P. J. Reilly, *Carbohydr. Res.* **264** (1994), 1-19.
16. D. M. Durran, B. J. Howlin, G. A. Webb and M. J. Gidley, *Carbohydr. Res.* **271** (1995), C1-5.
17. J. E. H. Koehler, W. Saenger and W. F. van Gunsteren, *Eur. Biophys. J.* **15** (1987), 197-210.
18. W. F. van Gunsteren and H. J. C. Berendsen, GROningen MOlecular Simulation (GROMOS) Library Manual, Biomos, Nijenborgh 4, Groningen, The Netherlands.
19. F. Horii, A. Hirai and R. Kitamaru, *ACS Symp. Ser.* **340** (1987), 119-134.
20. P. S. Belton, S. F. Tanner, N. Cartier and H. Chanzy, *Macromolecules* **22** (1989), 1615-1617.
21. M. Karplus, J. Chem. Phys. **30** (1959), 11, but see also C. A. G. Haasnoot, F. A. A. M. de Leeuw and C. Altona, *Tetrahedron* **36** (1980), 2783-2792.
22. A. P. Heiner and O. Teleman, Poster Abstract, The First Electronic Glycoscience Conference, Oxford, 1995.