Solvent effects on the unperturbed chain conformation of polysaccharides*

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A theoretical account of solvent effects on the unperturbed dimensions is given for two representative polysaccharides, amylose and cellulose. A solvation energy term is calculated for each conformational state by evaluating the contributions of cavity formation and of the interaction between solvent and solute. A significant change in the profiles of conformational surfaces is found in the three solvents considered (water, dioxane and dimethylsulphoxide). As a straightforward consequence, unperturbed chain dimensions are predicted to be solvent-dependent, as already found experimentally for the two polysaccharides considered, as well as for other polar polymers.

(Keywords: polysaccharides; amylose; cellulose; solvent effect; unperturbed dimensions)

INTRODUCTION

The terms 'solvation' or 'solvent effect', etc., are often used not only to refer to, but also to explain, the macroscopic effects that originate from macromolecule–solvent interactions. Some theoretical results have been obtained that are in satisfactory agreement with the experimental data[1], but only for small-sized solutes and non-polar solvents. However, the computational complexity of the intra- and intermolecular interactions existing between a macromolecular solute molecule and the surrounding solvent has hampered widespread use of theoretical formalisms[2], and has often led to either basically geometrical or merely statistical pictures of solute–solvent interaction being used, with a severe loss of reality in the description of the solution.

An account of the interactions of individual solvent molecules with a polymeric solute, although possible *in principle*, is practically unfeasible on a routine basis, because of the excessive degrees of freedom (both orientational and positional) of all the atomic groups. For all the preceding reasons, we have explored an approach to study the solvent dependence of conformational properties that might be able, in addition to the classical conformational calculation of a chain, to include solvent interactions, provided they are limited to a contact surface[3,4].

Much experimental data and plausible arguments have been reported on the insensitivity of the unperturbed dimensions of many polymers to solvent media[5]. However, it was recognized that notable exceptions exist, as in the case of highly polar polymers[6], polydimethylsiloxane in fluorinated solvent mixtures[7] and cellulose derivatives[8]. More recently, some changes in the unperturbed dimensions of amylose have been observed in binary water/dimethylsulphoxide (DMSO) mixtures[9]. These findings suggest that changes in the macroscopic properties observed in different solvents at least partially originate from variations in the short-range polymer–solvent interactions, which influence the population of the conformational isomers.

This paper presents the preliminary theoretical results of an effort to understand the effect of the solvent on the conformational properties of carbohydrate chain molecules. It is therefore an extension of the previous work of Tvaroska[10,11] to calculate chain properties from the whole set of conformational states of maltose and cellobiose dimeric units. The results show that the calculated changes in the population of the conformational energy levels of the polysaccharidic chains support the experimental data giving different unperturbed dimensions.

THEORY

The computation of the conformational energy has already been successfully employed for predicting the most stable crystalline forms of a chain in order to fit fibre X-ray diffraction data, as well as for the understanding of some statistical properties of dilute solutions of most common polysaccharides[12]. The basic assumption in the computation of the conformational energy surface of an uncharged dimeric unit relies upon the fact that, quite often, only the non-bonding interactions between atoms belonging to nearest-neighbour residues are important. Along with the calculation, two hypotheses are made: the absence of 'very' long-range interactions (that is, in the unperturbed conditions) and the explicit presence of the solvent is neglected, although the energy calculation may have been parametrized in order to fit the experimental data in solution. The former approximation can, in fact, be experimentally simulated using Θ conditions.

Limiting our attention to the dimeric residue, the total conformational energy, G_{tot}, is given by the sum of the contribution due to the solvent-free conformational state,

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G_{conf}, and the solvation contribution, G_{solv}:

$$G_{tot} = G_{conf} + G_{solv} \qquad (1)$$

The description of general methods for the computation of the energy associated with the conformational states has been given by many authors. Specifically, the calculation of the conformational energy surface for the dimeric units of maltose and cellobiose have been reported[13–15]. Among these studies, a comparative analysis of potential surfaces using several theoretical approximations has been carried out by Lipkind *et al.*[15], who stressed the importance of taking into account the total potential energy surface of the molecules for the correct reproduction of the experimental data.

The term G_{solv} refers to the energy required to create a cavity within the solvent of a size suitable to accommodate the solute in a given conformation and also the energy of interaction of the solute with the solvent[16]. The calculation of this quantity is the main goal of the present work, and a description of the computational algorithms is given below. The G_{solv} contribution has been evaluated for all accessible conformations of cellobiose and maltose, i.e. for those conformers which do not exceed 5 kcal mol^{-1} with respect to the minimum energy.

Thermodynamics of solvation

Condensed phases are characterized by intense attractive forces, which eventually determine the cohesion, and, in a pure solvent, the result of these forces is a sort of 'internal pressure'[17]. The process of solvation of a solute in a pure solvent can be arbitrarily split into a thermodynamic process in which work is first done against these cohesive forces of the pure liquid and subsequently the solvent molecules surrounding the solute are relaxed to interact with it.

The 'scaled particle theory' (SPT) has provided an idealization of the first step. This theory, developed by Reiss and coworkers[18], has allowed the almost quantitative calculation of some thermodynamic properties of simple non-polar solutions. The real success of the SPT resides in the simplicity of the mathematical relationships, which contain simple variables like density, temperature and the sizes of molecules.

Although the original proposal of the SPT was strictly limited to a system of non-polar 'hard spheres', extensions to many more polar systems have been reported in the more recent literature[10,11,16,18,19]. The approach followed in this paper is described in detail by Beveridge[16] and was previously used by Tvaroska and Kozar[19]. Only a brief description is given here.

For dilute solutions the reversible work required to bring a solute molecule into the solvent is equal to that required for the corresponding process in pure solvent. Therefore, in the absence of solute–solute interactions, the solvation process can be split into two steps (*Figure 1*):

(a) A cavity is created in the solvent. The molar reversible work, G_{cav}, for this step is equal to that required to introduce a mole of hard-core spherical molecules of a given radius into the solvent.

(b) The 'real' molecule is introduced into the cavity and the solvent interacts with the molecule. The molar reversible work, G_{int}, is equal to that required to bring the rigid sphere to a given interaction potential. This is equivalent to saying that the spherical particle is given a polarizability and a charge distribution that simulate the real molecules.

The total solvation energy is given by

$$G_{solv} = G_{cav} + G_{int} \qquad (2)$$

where G_{int} can be split further into the following contributions:

$$G_{int} = G_{el} + G_{disp} + G_{spec} \qquad (3)$$

that is electrostatic (G_{el}), dispersion (G_{disp}) and specific (G_{spec}) contributions.

Throughout this paper the effects of specific interactions have been neglected, in view of the fact that the aim is the calculation of free energies to estimate relative populations. This approximation merely means that the number of nearest-neighbour molecules is assumed to be constant.

Calculation of the solvation energy contribution

The energy of cavity formation for a solution of hard spheres has been derived from the probability that a solvent molecular centre is excluded from a cavity of

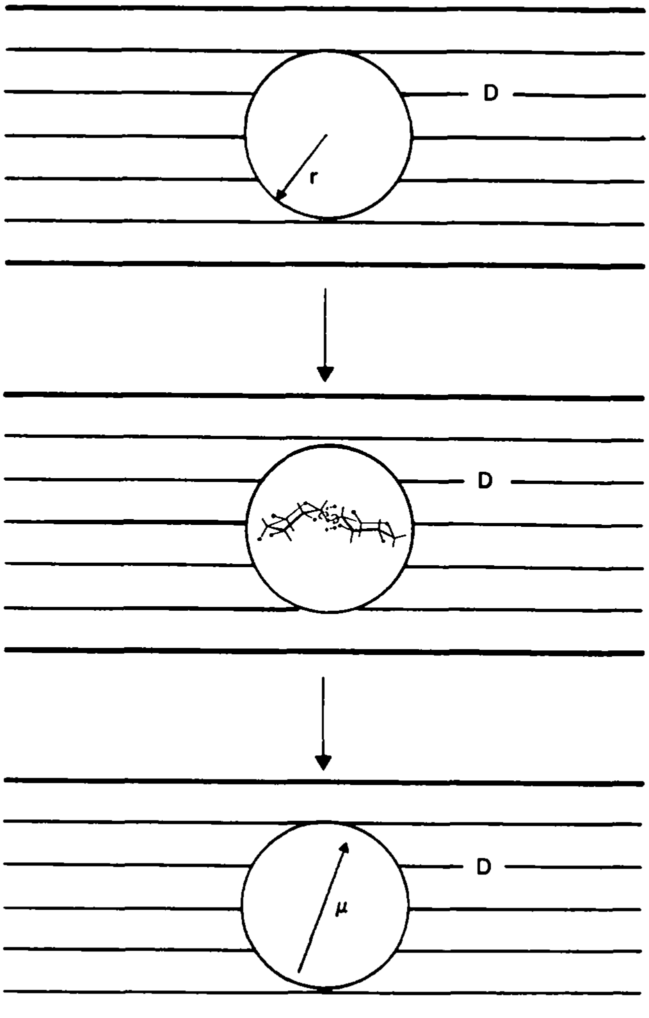

Figure 1 Scheme of the theoretical approach for the calculation of the solvation energies. First, a cavity of proper size is created in the solvent. Secondly, the real molecule, with a given conformational energy, is inserted in the cavity. Thirdly, the surrounding molecular layer of the solvent interacts with the solute molecules with given charge displacement

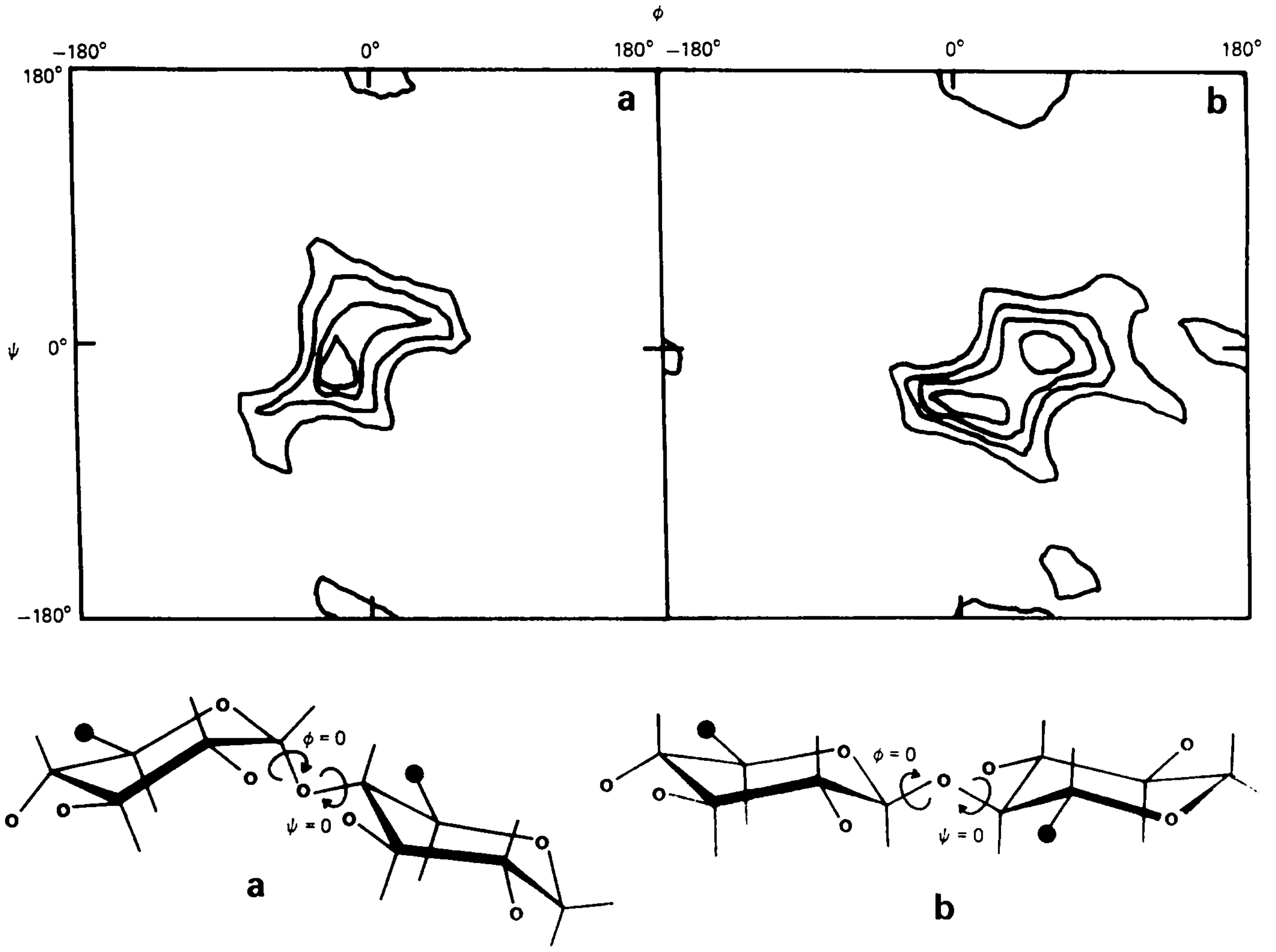

Figure 2 Conformational energy maps calculated for D-maltose (a) and for D-cellobiose (b), according to the procedure of ref. 12. Contours are (1) −1, 0, 5, 25 kcal mol^{-1} and (b) −1, 0, 2, 10 kcal mol^{-1}, respectively

radius r. The radial distribution function $g(r, \rho)$ is defined through the radius r and the solvent numerical density ρ ($\rho = N/V$, where N is the number of solvent molecules of diameter σ_1 in the volume V). The resulting equation of this treatment, as given in detail by Reiss[18], is:

$$G_{\mathrm{cav}}/RT = -\ln(1-y) + \left(\frac{3y}{1-y}\right)\mathscr{R} + \left[\frac{3y}{1-y} + \frac{9}{2}\left(\frac{y}{1-y}\right)^2\right]\mathscr{R}^2 + \frac{yP}{\rho kT}\mathscr{R}^3 \quad (4)$$

where $y = \pi\sigma_1^3\rho/6$ is the reduced density of the solvent at pressure P and $\mathscr{R} = \sigma_2/\sigma_1$ is the ratio of solute diameter to solvent diameter. In the same equation k and R are the Boltzmann constant and the gas constant, respectively.

In this approach, G_{cav} depends solely upon solvent density and solvent and solute dimensions. In other words, it is a measure of the cohesive (i.e. attractive) forces between solvent molecules. The diameter of a rigid sphere equivalent to the solute molecule has been calculated from the atomic van der Waals radii and from the internal molecular coordinates[20]. Geometrical algorithms to calculate molecular volumes and surfaces have been provided, together with other data[10,11], by I. Tvaroska of the Slovak Academy of Sciences, Bratislava, Czechoslovakia.

The electrostatic contribution G_{el} between the solute and solvent has been calculated through a modification of the Onsager reaction-field theory, taking into account, in a solvent medium of constant dielectric constant D, the reaction potential induced by the solute dipole and quadrupole[21] (third step in *Figure 1*):

$$G_{\mathrm{el}} = \frac{KX}{1-lX} + \frac{3HX}{5-X} + bF\left[1 - \exp\left(-\frac{bF}{16RT}\right)\right] \quad (5)$$

with

$$K = \mu^2/r^3$$
$$H = Q^2/r^5$$
$$l = 2(n_2^2 - 1)/(n_2^2 + 2)$$
$$X = (D-1)/(2D+1)$$
$$F = 0 \qquad \text{for } D \leq 2$$
$$F = \left(\frac{(D-2)(D+1)}{D}\right)^{1/2} \qquad \text{for } D > 2$$

and

$$b = 4.35\left(\frac{T}{300}\right)^{1/2}\left(\frac{\sqrt{2}}{r_{12}}\right)^3\left(K + \frac{Hr^2}{r_{12}^2}\right)^{1/2}$$

where r is the radius of the cavity and μ, Q and n_2 are dipole moment, quadrupole moment and refractive index

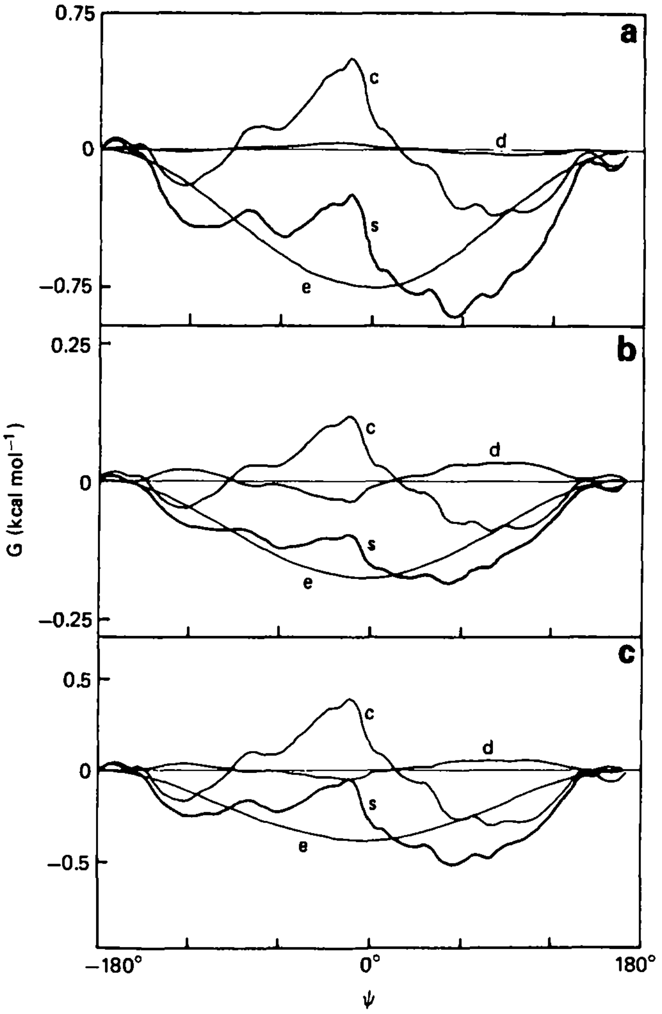

Figure 3 Perturbation of the solvent ((a) water, (b) dioxane, (c) DMSO) on a section of the conformational map of maltose. The relative change of energy is plotted for the contributions (c = cavity, e = electrostatic, d = dispersion) calculated with equations (4)–(6). The total solvation energy (s) is also reported

of solute, respectively; r_{12} is the average distance of separation between the centres of solvent and solute molecules[19].

Dispersion interactions (both attractive and repulsive) have been taken into account by the semiempirical combination of the London attractive and the Born repulsive potential, as provided by Birge *et al.*[22]:

$$G_{\mathrm{disp}} = -(1-\delta)\tfrac{3}{2}K_r N_1\left(\frac{I_1 I_2}{I_1+I_2}\right)\alpha_1\alpha_2 r_{12}^{-6} \qquad (6)$$

where α and I are molecular polarizability and ionization potential, K_r is an empirical constant ($K_r = 0.2$–0.7) and in our calculations the representative value of 0.5 was used. For the proportionality constant $(1-\delta)$ we used the value[23] 0.564. The number N_1 of nearest-neighbour solvent molecules was calculated as reported by Tvaroska and Kozar[19]. Physicochemical data on the solvents have been collected from the literature[24], for the temperature of 298.16 K.

RESULTS AND DISCUSSION

Effect of the solvent on the conformational energy surface

It is not the purpose of this paper to enter into a critical comparison of the previous results on the potential surfaces of maltose and cellobiose. Rather, we wish to focus on the perturbation of such surfaces due to solvent and to make provisions for the solvent dependence of amylose and cellulose chain dimensions in solution, on the basis of the conformational surface calculated for the related dimeric units. *Figures 2a* and *2b* show the surface energies calculated for maltose and cellobiose (as in ref. 13), as a function of the conformational angles ψ and ϕ defined in the figures. The standard structural geometries of Hybl, Rundle and Williams[25] and of Arnott and Scott[26] have been used for α-D-glucose and for β-D-glucose, respectively, and the glycosidic bridge angles were assigned the values of 115° and 117.5° for maltose and cellobiose, respectively. These structural parameters have been chosen to conform with the previous calculation[13], and throughout all the paper the temperature of 298.16 K is used.

Figures 3a, 3b and *3c* show the effects of the solvents water, DMSO and dioxane, respectively, on the maltose conformations generated by rotating the angle ψ, while ϕ is kept constant at −30°. The cavity term in each solvent system is a strict function of the size of the molecule, which is determined by the geometrical orientation of the two glucose residues. It is conceivable that lower energies are required for those conformers which are actually unstable on the basis of the Ramachandran map, while the more expanded conformations present the larger G_{cav} due to the larger cavities to be created in the solvent media.

It has been shown[27] that the dipole moment depends greatly on the configuration of the anomeric carbon C1. An even larger variation of the dipole moment for the maltose dimer as a function of the rotational angle ψ is indicated from the effect of the electrostatic contribution shown in *Figures 3a–c*. In fact, the calculated value of μ for maltose has two large maxima around (ψ, ϕ) values of (−40°, 0°) and (180°, 140°). It turns out that the electrostatic contribution is closely related to the (ψ, ϕ) angles. The stabilizing effect of this contribution is also a function of the dielectric constant of the medium and therefore increases passing from dioxane ($D = 2.21$) to DMSO ($D = 46.68$) to water ($D = 78.30$), at 298.16 K.

The dispersion term is a significant favourable contribution for the absolute value of the solvation energy; however, it shows a very low conformational dependence. Although included in all the calculations, it is difficult to assess its importance for the purpose of the present paper.

The perturbation introduced by the explicit account of the solvent results in the modification of the isoenergetic levels previously calculated[13–15]*. The results are shown in *Figures 4* and *5*, for maltose and cellobiose, respectively, in the three solvents considered here. The overall conformational contours, indeed, seem only little modified by the presence of the solvent, because of the constancy of the external constraints. More important are the changes that occur in the lower energy regions.

It is worth emphasizing that the (ψ, ϕ) pair, corre-

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* All previous calculations have been carried out by using standard methods and potential functions, as described in detail by Brant[12]. Potential functions have been, over the years, refined to fit mainly the experimental data in water. Throughout this paper, these calculations are referred to as 'standard conditions' only because we have used the same set of structural parameters explicitly to take into account the presence of the solvent.

sponding to the energy minimum, change their position from one solvent to another. In the maltose map, they go from the value of (−20°, −30°) in the previous 'standard' calculations to (−10°, −20°) in water and DMSO. In the cellobiose map, the minimum changes from (0°, 50°) in 'standard' conditions to (−30°, −20°) in all the three solvents. The solvation energy calculated in dioxane is in general small and does not significantly affect the previously calculated surface energy maps for either of the dimers (compare *Figures 4a* and *4c*, *Figures 5a* and *5c*). The perturbation due to the solvation becomes more clear from the change of probabilities associated with the conformers, which are reported in the histogram plots for maltose and cellobiose (*Figures 6* and *7*).

The most evident effect of solvent on the probabilities of the various conformers of maltose is the sharpening of the peak probability conformation at $(\psi, \phi) = (-10°, -20°)$. This preferred conformation would favour (if it is considered to be a unique spatial conformation)

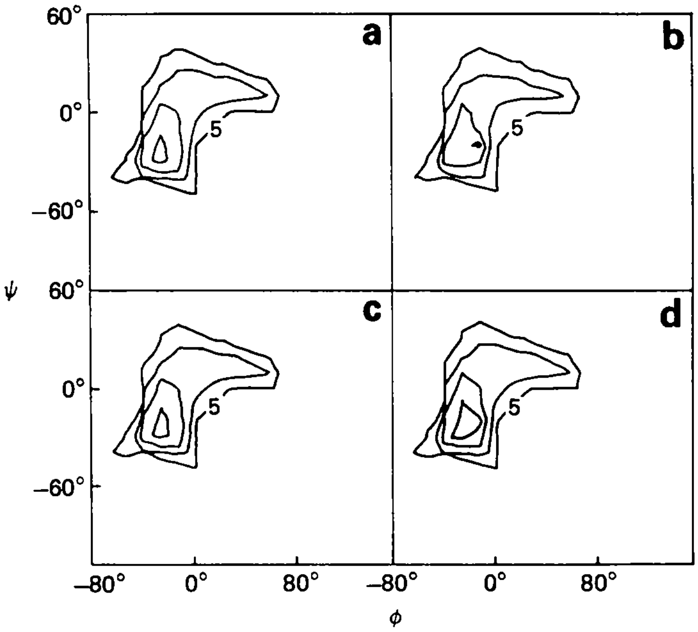

Figure 4 Portions of the conformational energy maps of maltose calculated including solvation and internal (conformational) energies: (a) standard conditions; (b) water; (c) dioxane; (d) DMSO. Contours are 0.1, 1, 2 and 5 kcal mol^{-1} above the minimum

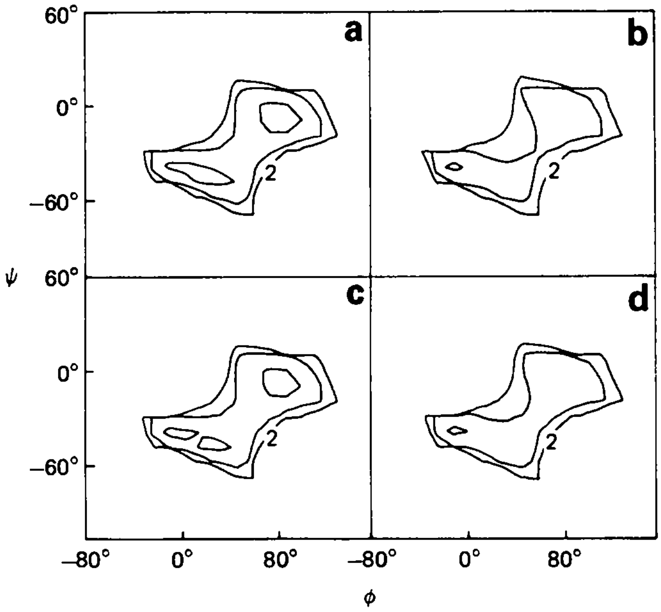

Figure 5 Portions of the conformational energy maps of cellobiose calculated as in *Figure 4*. Contours are 0.2, 1 and 2 kcal mol^{-1} above the minimum

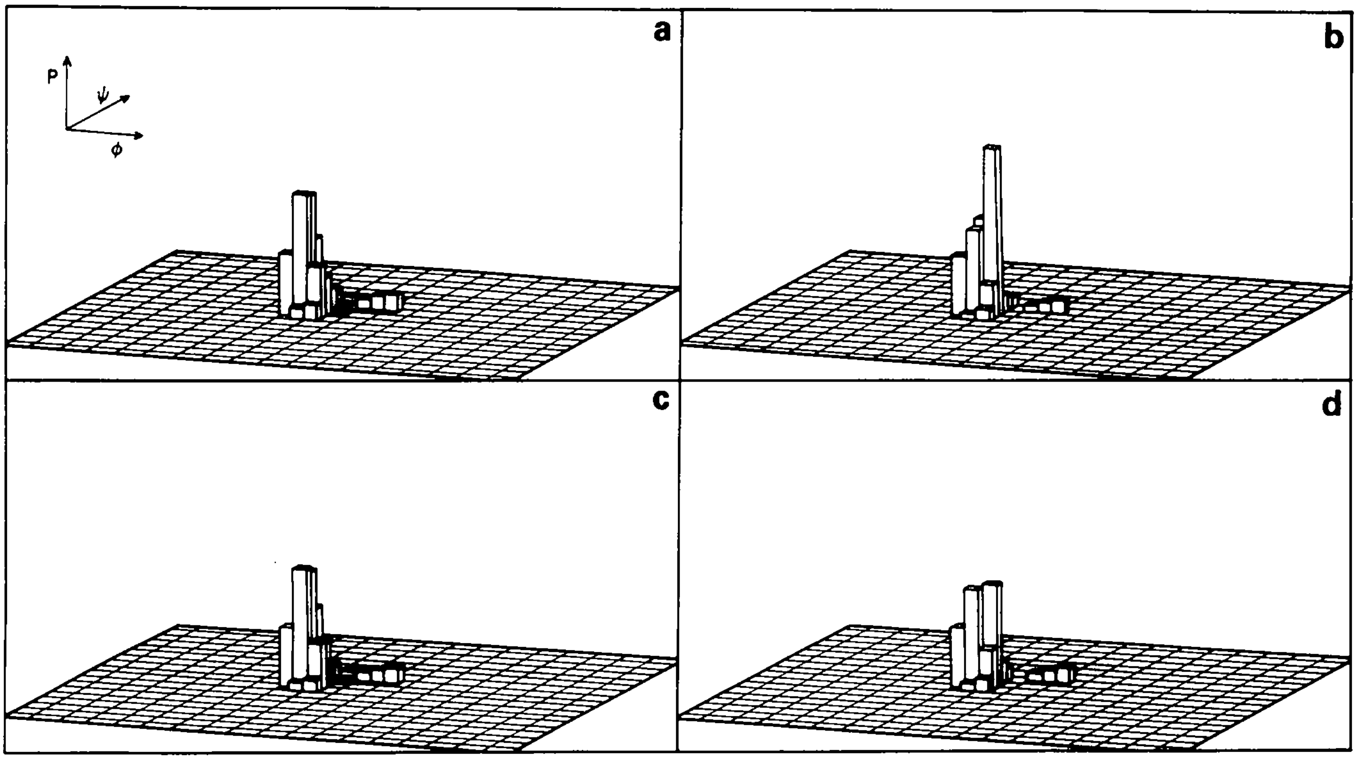

Figure 6 Histograms of probabilities of maltose conformers in (a) standard conditions, (b) water, (c) dioxane and (d) DMSO

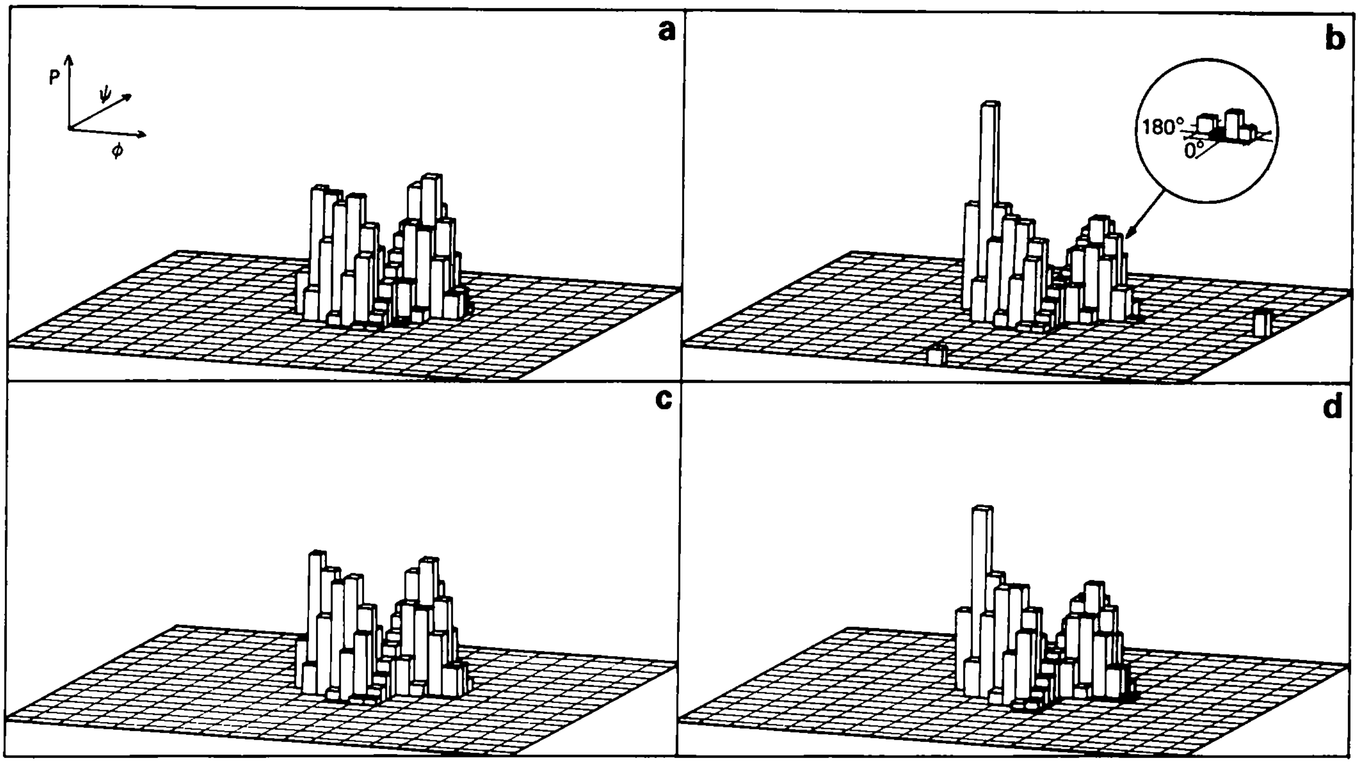

Figure 7 Histograms of probabilities of cellobiose conformers in (a) standard conditions, (b) water, (c) dioxane and (d) DMSO

a left helical twist with six monomers per turn and a pitch of 1.75 Å/monomer. In addition, it is also of interest to note that, in the case of cellobiose in water, a conformation having $(\psi, \phi) \approx (0^\circ, 180^\circ)$ becomes more populous. The conformation seems to be close to that claimed by Kochetkov[28] as contributing significantly to their experimental data from n.m.r. studies.

Statistics of unperturbed chain dimensions

The effects of the solvent on the conformational properties of macromolecular solutes have been taken into account by evaluating the solvation free energy for the dimeric unit of maltose and cellobiose, respectively, and not for a polysaccharide chain as a whole. This approach is consistent with the calculation of the conformational energy surface of the dimer as a starting point for the prediction of average chain properties.

In order to compare the dimensions of the polymeric chains and the effects of different solvents on the relative populations of conformers, the dimensionless characteristic ratio, C_n, and its asymptotic value, C_∞, defined as:

$$C_\infty = \lim_{n\to\infty} C_n = \lim_{n\to\infty} \frac{\langle r^2 \rangle}{nL^2} \qquad (7)$$

are conveniently used[5]. Here $\langle r^2 \rangle$ is the mean-square end-to-end distance for the unperturbed chain, n is the degree of polymerization and L is the so-called 'virtual bond', connecting a pair of adjacent glycosidic bridge oxygens. We chose $L = 4.25$ Å and $L = 5.47$ Å for α- and β-D-glucose, respectively[13]. Furthermore, the rate of convergence to C_∞ with increase in chain length, described in terms of the correlation function f_n, and $n(95\%)$ gives additional information on the topological behaviour of a chain, i.e. on the directional persistence of the chain[5,13]. The quantity $n(95\%)$ is defined as the degree of polymerization required for C_n to reach 95% of its asymptotic limit and the value of f_n is the mean projection of a unit vector along the nth virtual bond of the chain onto a unit vector aligned with the initial virtual bond.

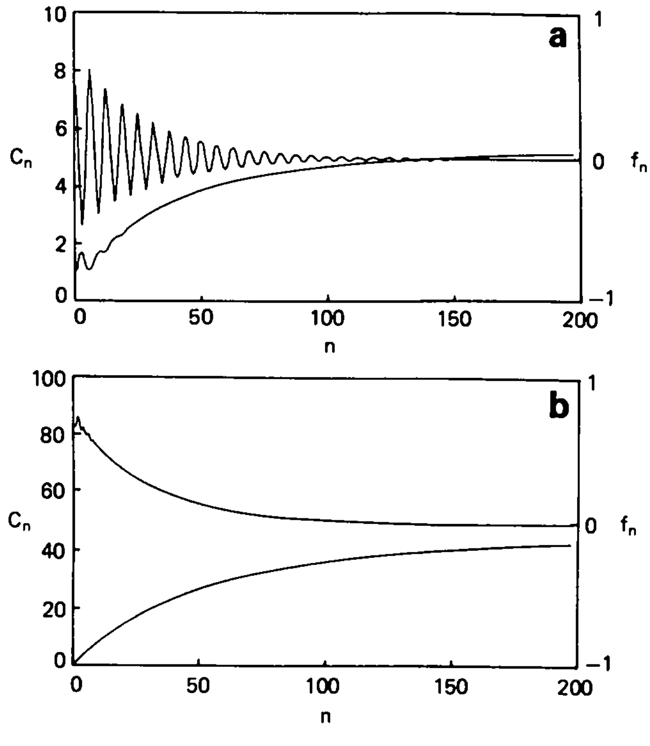

Figure 8 Characteristic ratio, C_n, and correlation function of the virtual bond n with respect to the first bond, f_n, as functions of the degree of polymerization, n, for amylose (a) and cellulose (b), in water

Figure 8 shows an example of the chain extension and conformational freedom of amylose and cellulose in water, outlined by plots of the characteristic ratio, C_n,

Table 1 Values of C_∞ and $n(95\%)$ of amylose and cellulose under 'standard conditions' and in three solvents

	Standard conditions	Water	Dioxane	DMSO
Amylose ($L = 4.25$ Å)				
C_∞	4.32	5.56	4.45	4.89
$n(95\%)$	185	328	255	293
Cellulose ($L = 5.47$ Å)				
C_∞	96.22	48.85	89.39	68.50
$n(95\%)$	1070	538	990	760

and the correlation function, f_n, as functions of the degree of polymerization, n. The behaviours of these functions at low n values show the remarkable pseudo-helical character of the amylosic chain in comparison with the more extended cellulosic one. However the plots of *Figure 8* do not make it clear that, as already discussed in the literature[13,29,30], from the statistical point of view, the amylosic chain is characterized by a lower configurational entropy in terms of allowed conformational states, despite the more coiled and seemingly disordered trajectory. The lower entropy becomes evident from inspection of *Figures* 6 and 7. In addition, these figures show that the solvent effect is larger, the greater the variation in each solvent of the G_{solv} contribution among the conformational states available to the dimers.

Very interestingly, the values of C_∞ and $n(95\%)$ for amylose are larger in water than in 'standard conditions' and the values for other solvents lie in between, being roughly a function of the dielectric constant of the media. It should also be mentioned (see *Table 1*) that DMSO, which is known to be a 'good' solvent for amylose, favours those conformational states which apparently determine a more compact coiled shape of the chain although only slightly less helicoidal. The small but perceptible decrease of unperturbed dimensions of amylose in DMSO solvent, compared with those in water, is in qualitative agreement with the findings of Jordan and Brant[9]. These authors observed a decrease of about 20% in the unperturbed dimensions of amylose in passing from pure water to a DMSO/water mixture (60:40, v/v).

Undoubtedly, the most important result concerns the extension of the cellulosic chains (*Table 1*); the values of C_∞ decrease only slightly in dioxane, more significantly in DMSO and particularly in water, where a reduction of about one-half is obtained in comparison with the previously determined chain dimension in 'standard' calculations (see previous footnote). This change seems almost exclusively due to the solvation contribution of water, which plays an essential role in favouring those conformational states characterized by smaller cavity volumes. Although unmodified cellulose is insoluble in water, the theoretical prediction compares well with literature data[31], which indicate for cellulose derivatives the range of $30 \leqslant C_\infty \leqslant 60$.

The experimentally observed solvent dependence of the unperturbed dimensions of amylose and cellulose can therefore be settled on a theoretical basis. However, one must be aware of the fact that, beside all the approximations used, only in the case of cellulose is there almost absence of intra-chain excluded-volume effects; the theoretical results, therefore, can be considered realistically close to the experimental results. The peculiar pseudo-helical conformation of amylose studied by Monte Carlo methods[29] disclosed a non-negligible amount of middle-range interactions, which are not taken into account by classical conformational energy calculations. Refinements of the amylose chain conformation free of these (middle-range) excluded-volume effects have since been carried out using Monte Carlo approaches[32,33]. It has been found that no correlation exists between the collision probability and the *a priori* conformational probability, i.e. the elimination of colliding units does not significantly alter the conformational distribution function calculated from all non-colliding conformations[32]. Therefore the solvent perturbation calculated here can safely be assumed to be independent of the long-range interactions.

CONCLUSIONS

Solvent effects have been recognized in many thermodynamic and conformational phenomena (e.g. in Henry constants, the activity of biological components, etc.). In the case of carbohydrates it has been shown that the solvation free energy difference between the α- and β-form of glucose (1.42 kJ mol^{-1}) displaces the equilibrium in water towards the β-form (63% at 25°C). Theoretical calculations of the solvation energies agree with the experimental findings that the β-form is more stable in water, and slightly less stable in DMSO (56%). These results support the applicability to carbohydrate molecules of methods that have been used successfully in the literature to calculate the effects of solvents on conformational equilibria.

On a molecular level, the interaction between polymer and solvent is complex. The degree of perturbation caused by the solvent on the local conformation has not yet been quantitatively evaluated. In this paper we have offered a simple way to consider solvent effects on conformational equilibria, which are also seen to play an important role in determining chain conformation.

One has to be aware of the approximations made in calculation of the macromolecular properties. It is, however, noteworthy that the calculations refer in all cases to 'unperturbed states' in different solvents, since only short-range interactions are evaluated. In other words, the results presented here indicate that, if conditions can be experimentally found in which the polymer exists in an unperturbed state in different solvents, then chain dimensions will differ in the different solvents, according to the perturbations calculated here. This has been shown to originate from the changes of the conformer probabilities, which determine changes of the persistence length in different Θ solvents.

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