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Investigation of the conformational behaviour of permethylated cyclodextrins by molecular modelling

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Abstract

Conformations of manually built native and permethylated α-, β- and γ-cyclodextrins (CD) were investigated using various computer assisted molecular modelling methods. Calculations were carried by applying the MM + and the Tripos force field. The influences of atomic charges on the macrocyclic conformations during the optimization procedure were analyzed. The permethylation of hydroxyl groups of cyclodextrins changes bond and torsion angles between the glucose monomers and of the primary substituents. A method to determine the diameters of the cyclodextrin cavity by a modelling approach is described. It is shown that due to permethylation the larger cavity opening is increased and the primary substituents are canted outwards. As a consequence, the torus shape of the molecule changes, which is an important feature for docking and fitting studies. © 1996 Elsevier Science Ltd.

Keywords: Molecular modelling; Molecular mechanics calculations; Quantum mechanics calculations; Native α-, β- and γ-cyclodextrins; Permethylated α-, β- and γ-cyclodextrins

1. Introduction

Native and derivatized cyclodextrins (CD) are common components in chromatography as mobile phase additives or as stationary phases [1,2]. However, little is known about the mechanism of chiral recognition of the derivatives [3]. In the last few years attempts have been made to study the interaction by NMR [4], thermodynamic interac-

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tion energies [5,6] and other methods [7,8]. As recently shown [9–11], a new methodical potential may be a molecular modelling approach. In this paper we describe the results obtained by molecular and quantum mechanics of native and permethylated cyclodextrins using modelling programs which so far have not been tested on these substances. In particular the permethylated derivatives were of interest because they are almost universally employed as chiral selectors in enantioselective gas chromatography [12]. Only a single study was available with respect to modelling these derivatives [13]. In contrast, the conformations of native cyclodextrins have been investigated extensively [14–16]. For these compounds it was shown that C_n-symmetrical structures are not in the lowest energy states, because during optimization a twist of glucose monomers (one or more) around the glycosidic bonds is observed. This so-called 'symmetry-breaking' leads to conformations with lowered potential energy [17]. On the basis of computer simulations only few attempts were made to estimate diameters of the rim of glycosidic oxygen atoms [18]. But for investigations of host–guest interactions it is essential to know how deeply molecules can enter the cyclodextrin cavity. Therefore we developed a algorithm for a quantitative measure of the internal diameters of the torus, but also at the entrances of the cavity.

2. Experimental

Where available, the starting conformations of cyclodextrins for modelling approaches were obtained from X-ray crystal structures. Because, for the majority of cyclodextrin derivatives X-ray structures are not available, or they were obtained under conditions which are not comparable, we have developed a manual building procedure.

The $^4C_1$-conformation of α-D-glucopyranose units were refined using a conjugate gradient minimizer (Fletcher–Reeves) to RMS less than 0.001 kcal/Å · mol. To perform a suitable arrangement of the monomers, we defined a plane among the six ring atoms of the glucose with ordinary least-squares regression. The monomers were placed in such a way that the angles between two planes have values of 60° (α-cyclodextrin), 51.4° (β-cyclodextrin) or 45° (γ-cyclodextrin) and then linked by $\alpha(1,4)$ glycosidic bonds, before methyl group's substitution were necessary.

Computational methodology.—All calculations were performed on a 486 IBM-compatible PC with math coprocessor and 8 or 16 MB RAM, as well as on a workstation IBM-RISC/6000 model 360. The starting conformations were optimized using molecular mechanics calculations according to the *Aufbau* principle where the total energy E_{tot} is taken as a sum of local interactions found within and between separate molecular fragments. Two different force fields, the MM + [19] implemented in HyperChem™ 3.0 [20] and Tripos [21] in the program package Sybyl™ 6.0 [22], were used. The MM + force field is derived from Allinger's well-known MM2-program (1977). However, the last MM2 parameters (1991) and atom types are employed. For details see [23]. Dielectric permittivity $\epsilon = 1.5$ was used in all calculations, which simulated an apolar environment. The calculations did not take into account nonbonded cutoffs or constraints. Energy minimizations were carried out by the Newton–Raphson algorithm (50 cycles) followed by a conjugate gradient minimizer (Fletcher–Reeves), with a

maximum of 3000 cycles or an RMS-gradient of maximum 0.01 kcal/Å · mol. The aim was to approximate the electron distribution to investigate the influence of net atomic charges on each atom on the geometry of the CD obtained by minimization. The computations were carried out by two different semiempirical methods, AM1 [24] and CNDO/2 [25] (implemented in HyperChem 3.0), as well as by the connectivity-based iterative partial equalization of orbital electronegativity (Gasteiger method) which does not depend on a particular geometry optimization [26] and which is implemented in Sybyl 6.0. After an initial energy minimization of the starting conformations over 50 cycles the calculations of charges were made, followed by molecular mechanical optimization up to the convergence of the procedure. All calculations were repeated several times beginning with *different* starting structures. The noncharged and the charged systems were compared by ring diameters (D), glycosidic bond angles (ω) formed by the glycosidic O_1 oxygen atoms linking the glucose moieties, torsion angles (ϕ) O5–C5–C6–O6 for primary substituents, torsion angles O5–C1–O–C4 and C1–O–C4–O5 (θ_1 and θ_2) between adjacent glucose monomers and by the pseudo bond torsion angles O5–C1–C4–C5 (ϵ). In Fig. 1 all these parameters and the numbering used in the glucose units are shown.

The diameters of cyclodextrins were estimated approximately as an arithmetic mean of a great number of local minima obtained by a molecular dynamical simulation. The system was 'heated up' to a temperature of 20 K. After an equilibration time of 10 ps there followed a molecular dynamical simulation over periods of 100 ps with a step size

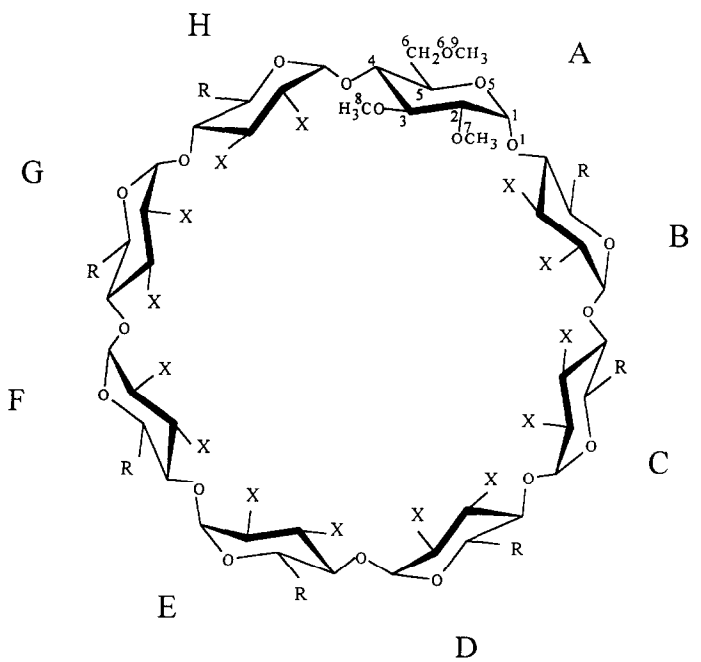

Fig. 1. Structural parameters and atom numbering used in the glucose units; example γ-CD; **A**–**H** labeling of glucose units position X = OCH_3, R = CH_2OCH_3.

of 1 fs. Every 100 fs the distance between relevant oxygen atoms was measured. The arithmetic mean of these values is time averaged and not influenced by thermal motions.

3. Results and discussion

The manually built structures were initially minimized using the Newton–Raphson algorithm (50 cycles). During this optimization step, all starting conformations changed their tube-like shape into a truncated cone with primary and secondary hydroxyl groups arranged on the narrow and the wide opening, respectively. Macrocyclic conformations obtained in such a way were nearly C_n-symmetrical before an exhaustive energy optimization was performed (n = 6–8 correspond to the number of glucose monomers for α-, β- and γ-cyclodextrins).

The various numerical data obtained were studied with respect to three issues. First the results were compared *methodologically*, i.e., the values were averaged over all structures (all native α-cyclodextrins, all permethylated α-cyclodextrins and so on) calculated by the same method. In a second step we compared the native and permethylated CD (e.g. *structural* comparing of the data was done). In the bar charts of the graphics 2 and 3, the share of the individual methods to the arithmetic means is displayed. It should be obvious that averaging in respect of the methods used is valid because the tendency from α- to γ-cyclodextrin will be the same for all structural elements.

The first structural element, the glycosidic bond angles (ω), differ by at most 0.55°. For molecules with more than 125 atoms such differences are not significant and are within the error limits. Therefore, the influence of force field and atomic charges on these structural elements is negligible. Although the bond angles of permethylated cyclodextrins (Fig. 2) are slightly modified compared with native compounds, one trend is obvious: in every case the angle ω is greater in permethylated derivatives than in the corresponding unsubstituted molecules. Furthermore, all glycosidic bond angles tend to have smaller values from α- to γ-cyclodextrin, both for native and for permethylated compounds. Considering the torsion angles (ϕ) of the primary substituents, there is a slightly modified picture. In contrast to the $g(-)$ conformation, for which all values of torsion angles are roughly comparable, differences of about 12° occur in the $g(+)$ conformations by using the Tripos force field.

The locations of primary substituents differ only slightly by using several methods of charge calculation. The conformational changes during the optimization on the CD investigated are small, from which it follows that the influence of atomic charges on the conformations of native and permethylated derivatives is not significant and can be neglected. In addition it is interesting to note that the torsion angles of the $g(-)$ conformations result in absolutely smaller values with increasing molecular size both for native and for permethylated structures (Fig. 3). With increasing size of cavities the primary substituents are directed more to the molecular axis passing the centre points of both cavity openings.

Due to permethylation, the torsional angles of $g(-)$ conformations have larger absolute values. The numerical differences are also small, compared with native

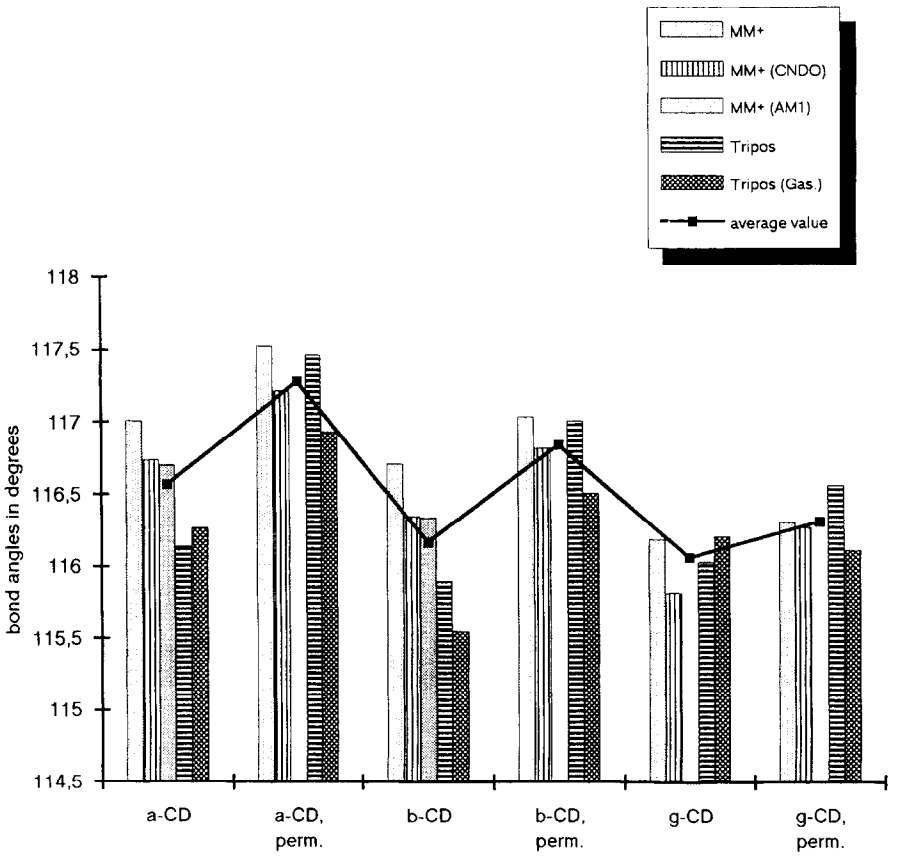

Fig. 2. Average magnitude of bond angle (ω) C1–O–C4 in dependence of structure (perm. = permethylated).

compounds. However, regarding glycosidic bond angles all alterations are in the same direction. Consequently, for the $g(+)$-conformations the angles tend to be smaller. The angles ϕ discussed above describe the position of primary substituents. For the torsion angles between glucose units (θ_1 and θ_2), which are a measure of rotation of the monomers against glycosidic bonds, we found a qualitative effect of the force field used which has a significant impact on the results. In contrast, the influence of atomic charges is rather small. Using the Tripos force field we obtained at least 8° smaller angles than calculated with MM + . Independently of this, a clear trend can be seen: The torsion angles will increase with molecular size. In permethylated derivatives these angles will be larger than in corresponding native cyclodextrins. Hydrogen bridges between primary hydroxyl groups of adjacent glucose units (not observed in permethylated derivatives) lead to a stabilizing of the native host molecule conformations (low standard deviations of the values), whereas the permethylated nonstabilized structures are more flexible (standard deviations are larger). As regards the torsional angle θ_2, the behaviour is the same as in the case of torsional angle θ_1. But the dependence of the angle on the

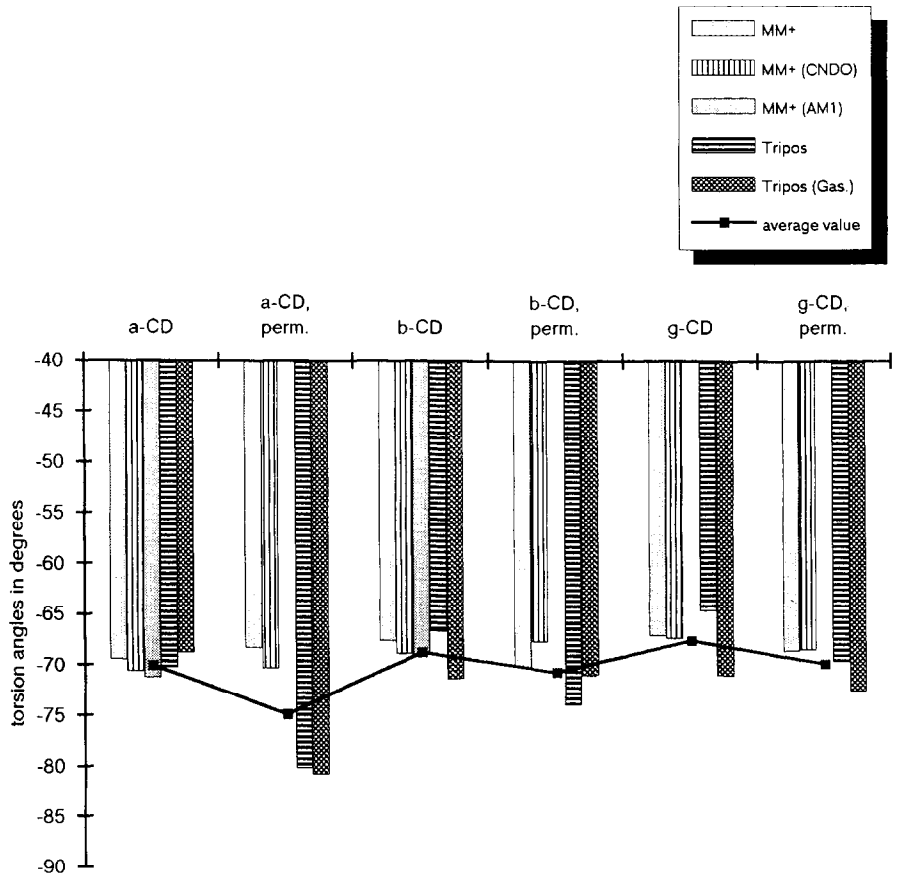

Fig. 3. Average magnitude of torsion angle (ϕ) O5–C5–C6–O6 (($g-$) conformation) in dependence of structure.

optimization shows the stronger impact of the force field. In contrast to θ_1 and θ_2, no significant dependence on the force field used is noticeable with respect to the pseudo torsional angle ϵ (see Table 1). The alterations of this angle with regard to molecular size and due to permethylation are essentially the same as found at θ_1 and θ_2.

Calculated standard deviations of bond angles ω and torsional angles ϕ are considerably smaller than those determined for all other structural elements, θ_1, θ_2 and ϵ underlying the extent of symmetry breaking. Furthermore, all standard deviations of torsional and pseudo torsional angles increase from α- via β- to γ-cyclodextrin. Thus, the existing flexibility of the structures becomes larger with increasing molecular size. This fact must be taken into account if the docking behaviour of molecules is to be studied.

As discussed in Section 1, the diameters of the cavity are important values. We have defined them as follows: The primary oxygens will be connected to each other. Now we have measured the distances between opposite oxygen atoms and the values obtained

Table 1
Influence of structure, force field, and charges on magnitude of pseudo torsion angle (ϵ) O5–C1–C4–C5 (in deg)

	MM+	MM+ (CNDO)	MM+ (AM1)	Tripos	Tripos (Gas.)	Average
α-CD	−18.54	−13.38	−13.05	−14.78	−18.68	−15.69
α-CD, perm.	−16.53	−9.65		−3.56	−12.06	−10.45
β-CD	−13.79	−11.65	−9.97	−16.34	−14.29	−13.21
β-CD, perm.	−8.68	−8.26		−11.41	−22.03	−12.60
γ-CD	−8.35	−7.63		−15.63	−11.94	−10.89
γ-CD, perm.	−7.77	−8.36		−3.12	−6.74	−6.50
average	−12.28	−9.82	−11.51	−10.81	−14.29	−11.64

will be averaged. The resulting arithmetic mean assists to construct a circle which fits well with hexa-, hepta- or octagons of the atoms. The magnitude of this circle serves as a *diameter* for the cavity opening which only needs to be reduced by the van der Waals radii of the atoms. To get the internal diameter of the host molecules the same procedure was applied to glycosidic oxygen atoms.

The diameters of the macrocycles on the side of secondary oxygen atoms were obtained by the arithmetic mean of distances between *O*-2 and *O*-3 oxygens. In the

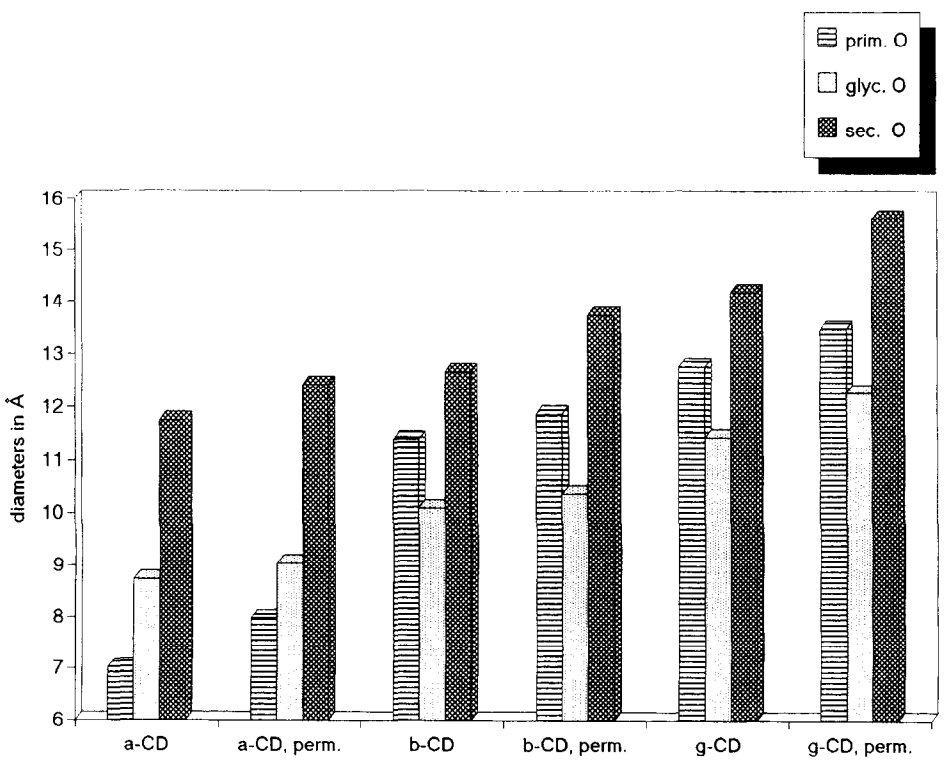

Fig. 4. Diameters (D) of cyclodextrins.

β-cyclodextrin the neighbouring *O*-2 and *O*-3 atoms of adjacent glucose units serve as location for the measurement of the distances. The averaged diameters obtained are shown in Fig. 4.

Three main issues are raised. *First*, complete methylation of the hydroxyl groups on cyclodextrins always leads to an increase of both *cavity opening* and of *internal diameter* (glycosidic oxygens). The greatest increase was found for γ-cyclodextrin, the lowest for β-cyclodextrin. *Second*, by means of permethylation the diameter on the side of secondary oxygens changes more than the diameter on the primary oxygen side (an exception is α-cyclodextrin). This feature corresponds to the fact that due to permethylation the shape of the molecule becomes more torus-like [3,4]. The diameters presented here are in good agreement with the results described in [18] as well as with experimental data established by X-ray structure analyses [27] and NMR-investigations [4]. *Third*, as a rule the cavity opening on the side of primary oxygens is greater than the internal diameter formed by the glycosidic oxygen atoms. Consequently, the methoxy groups are directed apart from the C_n-symmetry axis. This is in good agreement with the results for native cyclodextrins ('out structures' [17]) and shows that during theoretical studies of host–guest interactions also an entering of guest molecules into the cyclodextrin cavity from the primary side of the torus must be considered. The reader should keep in mind that the diameters discussed here are nucleus–nucleus distances of oxygen atoms. Sterical hindrance caused by hydrogen atoms and van der Waals radii must be taken into account.

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