

## Conformational Space of $\alpha$ 1-4 glycosidic linkage: A Molecular Dynamics Study

M.Prabhakaran

Department of Medicinal Chemistry and Pharmacognosy (M/C 781)  
University of Illinois at Chicago,  
P. Box 6998, Chicago, IL 60680

Received May 10, 1991

---

Molecular Dynamics simulations have been carried out for 100 ps on crystal structure of  $\beta$ -cyclodextrin in vacuo and with explicit inclusion of solvent at constant pressure and constant temperature using the GROMOS MD algorithm, with a time step of 0.005 ps. The conformational space of the glycosidic linkage was studied by calculating two virtual dihedrals connecting the successive glucose units for the 2000 structures saved during the two simulations. Three preferred regions for  $\alpha$  1-4 glycosidic linkage were found in both the simulations. The use of these virtual dihedral angles in representing the glycosidic linkage is also brought out from these studies. © 1991 Academic Press, Inc.

---

The nature of the glycosidic linkage is the most important feature in determining the different tertiary structural arrangements of polysaccharides. The nature of linkage is studied by both theoretical and experimental means (1). In this study, the polysaccharide linkage was investigated by molecular dynamics (MD) simulation, carried out in both vacuo and in water. Our earlier studies on  $\alpha$ -cyclodextrin (2) and the studies of Brady (3) have shown the large conformational space of the glucose ring in terms of the pucker angle of glucose. Hence, we followed the linkage between the polysaccharide molecules, defined in a unique way (4) without much dependence on the ring conformation. We choose  $\beta$ -cyclodextrin, a cyclic molecule consisting seven  $\alpha$ (1-4) linked glucose residues. Because of its annular structure, it can form inclusion complexes with a wide variety of guest molecules and has been studied as a model for enzyme activity and hydrogen bonding. The structure of the molecule was solved at a very high resolution by Saenger group (5).

### Method

The  $\beta$ -cyclodextrin coordinates refined by neutron diffraction are taken as the starting model (5). The MD simulations were carried out using the molecular dynamics package

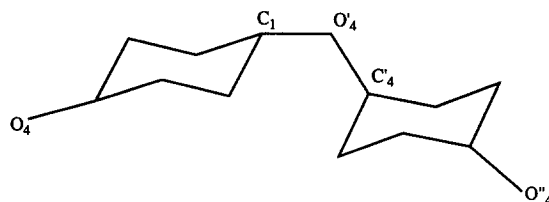


Figure 1. Dihedrals Definition:

$$\phi: O_4 \dots C_1 - O'_4 - C'_4$$

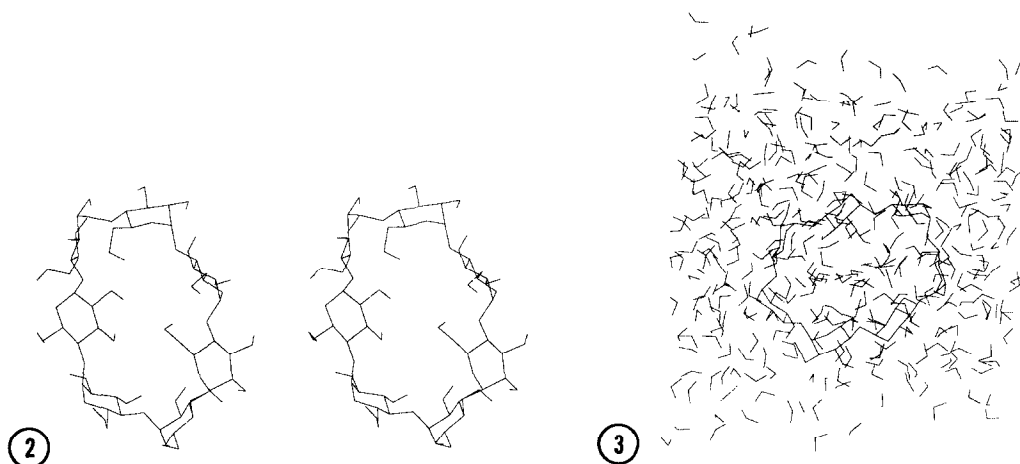
$$\psi: C_1 - O'_4 - C'_4 \dots O''_4$$

[ — denotes bonded atoms; ... denotes nonbonded atoms]

GROMOS (Groningen Molecular Simulation), using the parameters developed by us and published elsewhere (6). A 100 picosecond simulation was carried out on the system both in vacuo and in aqueous environment with a time step of 0.005 ps. 2000 structures were saved during the simulation. To scan adequate conformational space, the system was assigned new velocities at every 5 ps. After every 20 ps, the molecule was minimized with conjugate gradient minimization and a fresh simulation was started. To simulate aqueous environment, the minimized molecule was placed in a box of TIP3P water molecules with the dimensions 22.1 Å, 23.1 Å and 25.1 Å. The water molecules within 2.3 Å distance from the cyclodextrin atoms were removed. Further minimization was carried on the whole system, constraining the solute molecule in the starting configuration and adjusting the configuration of 407 water molecules. After adequate heating and equilibration, the simulation was carried out with suitable boundary conditions and at constant temperature and at constant pressure. Both the velocities of the solute and solvent molecules are scaled separately to maintain constant temperature. The lengths of the boxes were adjusted during the simulation. After every 20 ps, the simulation was restarted with a new box of molecules after adequate minimization. 2000 structures, saved from 100 ps run, were used for data analysis. The polysaccharide linkage as defined by Ramachandran's(4) group is shown in Fig 1. The virtual dihedral connecting O4, C1 of the first residue with O4 and C4 of the second residue is defined as  $\phi$  and the virtual dihedral connecting C1 of the first residue O4 and C4 of the second and O4 of the third residue is defined as  $\psi$ . As only one atom from the ring is chosen for these dihedrals, these dihedrals would be the representative of the tertiary linkage between the polysaccharides without much dependence on the local conformation. Each structure contains consecutive 7  $\phi$  and 7  $\psi$  dihedral angles. Our data base consists of two sets of 14000 values of  $\phi$  and  $\psi$  derived from in vacuo and in aqueous simulations.

### Results and Discussion

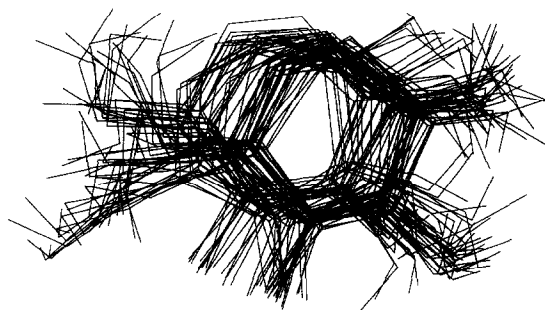
The stereo view of cyclodextrin molecule during the simulation is shown in Fig 2. The molecule placed in a box of water molecules is shown in Fig 3. The oscillations continued smoothly throughout the oscillations. To show the dynamic conformational space of the molecules the continuous 50 snap shots saved during the trajectory is depicted in Fig 4,



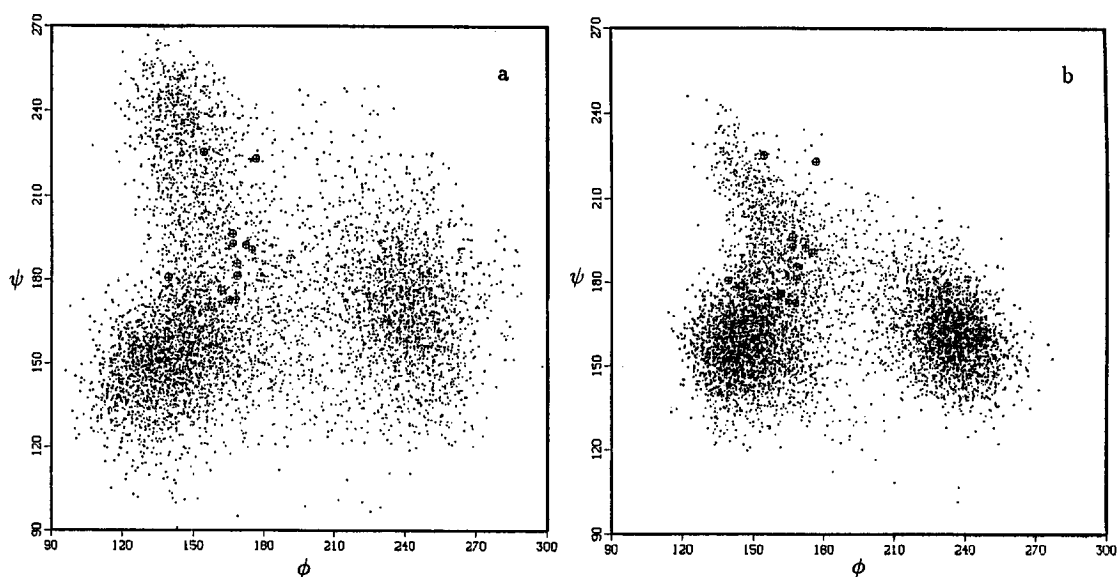
**Figure 2.** A stereo view of  $\beta$ -cyclodextrin during the simulation.

**Figure 3.**  $\beta$ -cyclodextrin in a box of dimensions 22.1 Å, 23.1 Å, 25.1 Å. The number of water molecules is 407.

showing the conformational movement of a single glucose unit within that 10 ps period. No superposition is attempted in this picture in order to show the dynamic movement of the molecule. We have carried out necessary steps to investigate the maintenance of the structure closer to the equilibrium. The fluctuation in glycosidic linkage dihedral is around  $10^\circ$  which is the same range as observed for  $\alpha$ -cyclodextrin in aqueous solution (7). The glucose linkage is measured by the dihedrals  $\phi$  and  $\psi$ . The dihedral values from  $-1^\circ$  to  $-180^\circ$  is added by 360 to have a continuous scale from  $0^\circ$  to  $360^\circ$ . The scatter plots (Fig 5) shows the distribution of  $\phi$  and  $\psi$  angles derived from the simulation. The circles denote the values of crystallography structures derived from both  $\alpha$  (8) and  $\beta$  (5) cyclodextrins. The plots display three specific occupancy regions. Three features are immediately noticeable: 1) the high density points are shifted from crystallographic structures. 2) The distribution near  $\phi=240$  and  $\psi=170$  is not crystallographically observable. 3) the conformational space is limited during the simulation in water due to damping as



**Figure 4.** The dynamic trajectory of glucose unit during 10 ps period. These 50 continuous snap shots were saved at every 0.2 ps.



**Figure 5.** Scatter plot of  $\phi$  and  $\psi$ :

a) In vacuo. b) In water during 100 ps simulation. 7000 points are shown in each plot.

The angles between  $-1^\circ$  and  $-180^\circ$  are added by  $360^\circ$ . Circles denote crystal structure values.

observed in similar studies (9). In Homans' (10) calculations on  $\beta$ 1-4 linkage, the minimum energy conformations were also observed other than that found in crystal structure. We have also calculated the linkage as defined by Tvaroshat et al (11) and find three similar regions of occupation as shown in these scatter plots. The dampening of torsional oscillations, when water molecules were included, could be due to the hydrogen bonds around the solute, as indicated by Edge et al (9). We have also plotted the frequency of occurrence of  $\phi$  and  $\psi$  during both the simulations (Fig 6). The maximum occurrence of  $\phi$  occurs near 165 in both the oscillations. As shown from the scatter plot, the distribution is limited in water in contrast to in vacuo simulation. It is interesting to note that the conformational space is limited between 120 and 240 in both the simulations, whereas the middle region is heavily populated in water simulation. The distribution of  $\psi$  shows a slight bimodal distribution, having peaks at 145 and at 225. In water, the distribution is limited to 225, whereas the distribution in vacuo extends beyond 280. A central minimum at 200 is noticeable at both the simulations. Pullman's group from their quantum mechanical calculations (12) have observed the global minimum for  $\phi$  90, and 180 and  $\psi$  between near 110 and 240. Allowed conformations with higher energy are also found for  $\phi$  between 180 to 240 and  $\psi$  between 180 and 240. Similar conformational space was observed earlier by Rao et al (4). By using this virtual dihedral, the anomalous linkage found in a glycosidic linkage of  $\alpha$ -cyclodextrin was brought out by Manor and Saenger (13). Thus our dynamics simulation exhibits the dynamic space of the glucose  $\alpha$  1-4 linkage and the use of this virtual dihedrals to represent the tertiary linkage of the polysaccharides. The conformational space of further linkage could be scanned by this method.

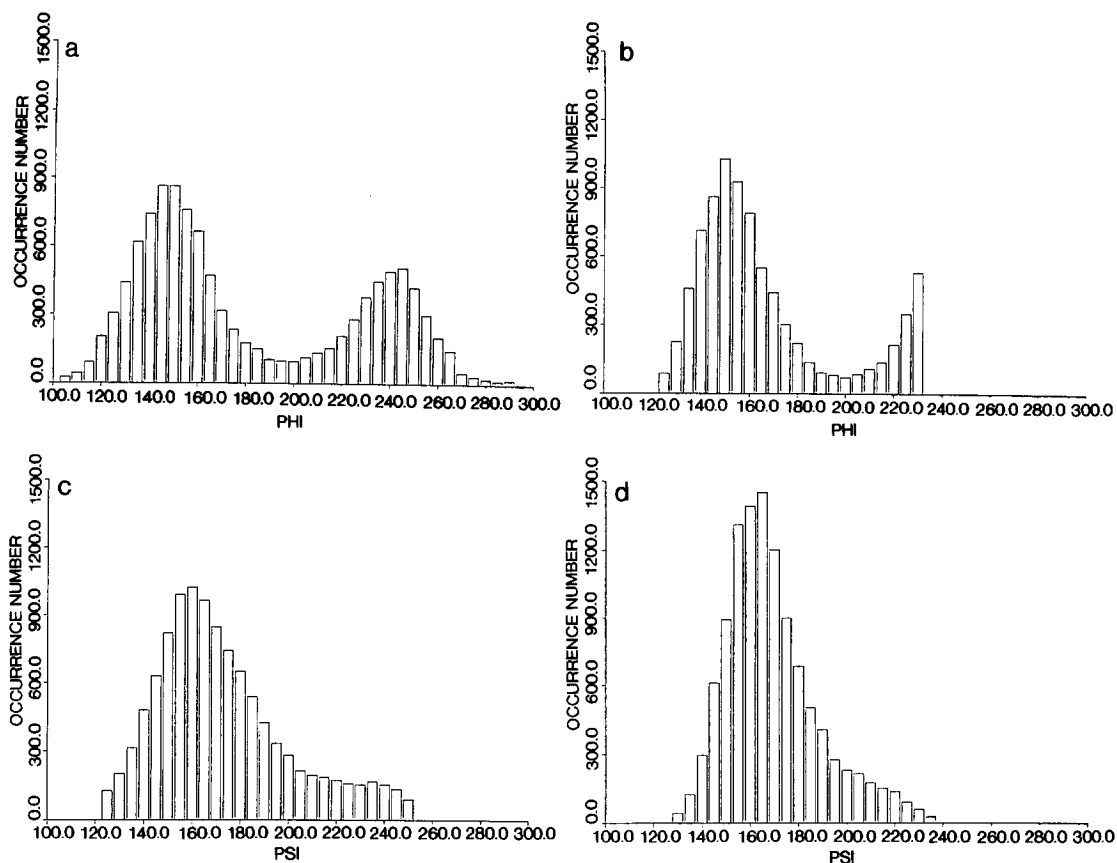


Figure 6. Bar diagram of the  $\phi$  and  $\psi$  distribution during the 100 ps simulation.

a)  $\phi$  in vacuo, b)  $\phi$  in water, c)  $\psi$  in vacuo, d)  $\psi$  in water.

### Acknowledgments

I am thankful to Profs Stephen C. Harvey of University of Alabama at Birmingham and Michael E. Johnson for their support. This work was supported in part by American Heart Association of Chicago Senior Fellowship and grant.

### References

- 1) Bender, M. L and Komiyama, M. (1978) Cyclodextrin Chemistry. Springer Verlag.
- 2) Prabhakaran, M. and Harvey, S. C. (1987) *Biopolymers* **26**, 1087-96.
- 3) Brady, J. W (1986) *J. Am. Chem. Soc.* **108**, 8153-8160.
- 4) Rao, V. S. R., Sundararajan, P. R., Ramakrishnan, C. and Ramachandran, G. N (1967) Conformation of Biopolymers Vol II Academic Press NY p 721-737.
- 5) Betzel Ch., Saenger, W., Hingerty, B. E and Brown, G. M. (1984) *J. Amer. Chem. Soc.* **106**, 7545-7557.
- 6) Ramakrishnan, N., Bo-young, C., Prabhakaran, M., Ekbery, G. C., Roden, L. and Harvey, S. C. (1990) *J. Biol. Chem* **265**, 18256-62.

- 7) Koehler, J. E. H., Saenger, W. and van Gunsteren, W. F. (1988) *J. Mol. Biol.* **203**, 241-250.
- 8) Lindner, K. and Saenger, W. (1981) *Acta Crysta* **B38**, 203-210.
- 9) Edge, C. J., Singh, U. C., Bazzza, R., Taylor, G. L., Dwek, R. A., and Rademacher, T. W. (1990) *Biochemistry* **29**, 1971-74.
- 10) Homans, S. W. (1990) *Biochemistry* **29**, 9910-9118.
- 11) Tvaroska, I., Perez, S., Noble O. and Taravel, F. (1987) *Biopolymers* **26**, 1499-1508.
- 12) Giacomini, M., Pullman, B. and Maigret, B. (1970) *Theoret. Chim. Acta* **19**, 347-364.
- 13) Manor, P. C and Saenger, W (1974) *J. Am. Chem. Soc.* **96**, 3630-3639.