J Incl Phenom Macrocycl Chem (2007) 57:29–33
DOI 10.1007/s10847-006-9165-3

ORIGINAL ARTICLE

Molecular dynamics simulations and quantum chemical calculations on β-cyclodextrin–spironolactone complex

Philipp Weinzinger · Petra Weiss-Greiler · Walter Snor · Helmut Viernstein · Peter Wolschann

Received: 15 May 2006 / Accepted: 20 October 2006 / Published online: 24 January 2007

Abstract Molecular dynamics simulations on β-cyclodextrin in vacuo, with water and complexed with spironolactone (SP) were performed at a temperature of 300 K over a period of 1 ns. Two different orientations of SP in the cavity were considered. Along with conformational parameters, the formation of hydrogen bonds has been monitored during the whole simulation time. Cyclodextrins have the capability to form hydrogen bonds with the surrounding water molecules or intramolecular ones. The incorporation of ligands into the hydrophobic interior of β-cyclodextrin changes the preference of hydrogen bonds significantly and results in a contribution to the decrease of flexibility. Quantum chemical calculations on SP–β-CD inclusion complex were performed to determine the interaction energy and to prove the applicability of various methods. Although all applied methods describe reasonable geometries for the association complex, higher level methods (e.g., B3LYP/6-31G(d,p)) seem to be necessary to determine reliable interaction energies.

Keywords β-Cyclodextrin · Molecular dynamics · DFT calculations · Inclusion complex · Hydrogen bonding · Spironolactone · Solubility enhancement

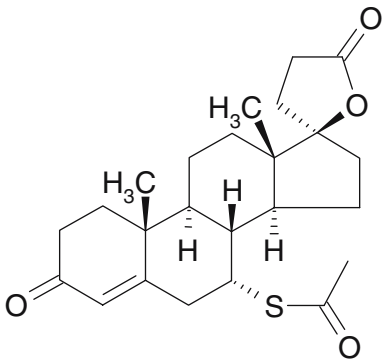
P. Weinzinger · P. Weiss-Greiler · W. Snor · P. Wolschann
Institute of Theoretical Chemistry, University of Vienna, Währinger Straße 17, 1090 Vienna, Austria

H. Viernstein (✉)
Department of Pharmaceutical Technology and Biopharmaceutics, University of Vienna, Althanstraße 14, 1090 Vienna, Austria
e-mail: Helmut.Viernstein@univie.ac.at

Introduction

The affinity of host molecules to the cavity of cyclodextrins (CDs) depends on the agreement of the molecular surface of the guest and the complementary surface of the host. Moreover, the complementarity of electrostatic potentials, hydrogen bond donor and acceptor properties are features which influence the stability of the inclusion complexes significantly. Therefore, investigations on the geometry of the inclusion complexes are very helpful to get some insight into the detailed reaction mechanism, the driving forces of complexation and the related geometrical conditions. Molecular calculations are useful methods, which contribute to answer these questions. Empirical, ab initio and DFT methods can be used to obtain energetically minimized structures. Molecular dynamics (MD) and Monte-Carlo (MC) simulations allow to explore the energy hypersurfaces and to find local and global conformational minima distinct from a certain starting geometry. Particularly, MD simulations are widely used to explore the structural features of CDs and cyclodextrin complexes, as exemplified by some recent publications [1–8].

In continuation of our research concerning the reaction mechanisms of CD inclusion complexation [1, 9, 10] we have performed molecular calculations on spironolactone (SP). This compound (Scheme 1) was selected because of its exceptionally high affinity to β-CD, which results in an extensive enhancement of its solubility. SP is a partial synthetic steroid-analogue of aldosterone, which works as competitive aldosterone-antagonist. The solubility of SP in water is rather low (2.8 mg/100 mL at 25°). For that reason the solubility



Scheme 1

enhancement by complexation is of high importance for its practical application.

Methods of calculations

Starting geometries of β-CD and SP were obtained from the Cambridge database. The ligand was placed in two orientations (in complex A the lactone ring of SP is located close to the more narrow rim of β-CD, in complex B SP is turned 180° along the β-CD-entry axis) and energy-minimized with Gaussian03 [11] using the HF/6-31G(d) basis set. The AMBER 7 program package [12] was used to generate the MD trajectories using the general amber force field (gaff) and a step size of 1fs. The gaff is a practical force field for organic molecules such as the ligands and was used uniformly for all molecules, including water and β-CD. AMBER tools were used in part for the subsequent analysis. The simulations in aqueous environment were performed with approximately 2000 water molecules of the TIP3P type in a periodic cell. The system was heated to a temperature of 300 K and afterwards held at constant pressure and temperature using a weak coupling algorithm [13]. A 12 Å cutoff was used for the Lennard-Jones interactions. In order to evaluate the quality of the simulations the root mean square deviation (RMSD) of the atom positions have been estimated for the CDs (Fig. 1) in reference to the starting structure.

The trajectory was recorded at 0.5 ps intervals thus resulting in 2000 frames for the 1ns simulation. In order to estimate the hydrogen-bonding properties of the β-CD molecule all frames were searched for hydrogen bonds with the following characteristics: a maximum heavy atom distance of 3.2 Å and a maximum angle deviation of 45° from linearity. Although the simulations could be considered equilibrated after 50 ps, data for further analysis were collected after 100 ps.

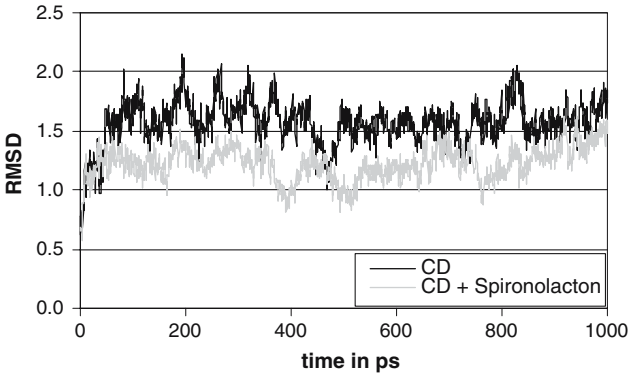


Fig. 1 RMSD values of cyclodextrin atom positions. In the simulation without a ligand an average of 1.57 was estimated and 1.22 in the simulation with spironolactone as ligand. Both set-ups included a periodic water box

To calculate the binding energy different energy contributions have been determined (e.g., the change in entropy for the binding process). These calculations were enabled by the nmode module of AMBER. Since this is a time consuming procedure only 100 snapshots at regular intervals were picked up for this calculation.

The interaction energy between β-CD and SP was also calculated by quantum chemical methods using Gaussian03 [11]. A semiempirical (PM3) and two ab initio methods (Hartree Fock with basis sets 3-21G and 6-31G(d,p)) and a DFT method (B3LYP with the basis set 6-31G(d,p)) were used for the full geometry optimization of β-CD, SP and the complexes. The starting geometries for the complexes were taken from MD calculations using the two different orientations of SP. The binding energies were calculated from the energy differences between both isolated compounds and the complexes.

Results and discussion

One of the most obvious observations during the MD simulations was the bend in the C1 backbone of the β-CD molecule. In the minimized structure β-CD rim is not completely planar, but shows a slight bent to the side of the O2/O3 groups. The RMS average of all seven C1-C1-C1-C1 torsion angles is 14°. During the simulation and the effects of kinetic energy this angle increases as the β-CD is allowed to occupy conformations farther away from the minimum. The root-mean-square of the angle was chosen in order to get a general parameter for the flexion of the β-CD, which is not modified by the algebraic sign of each individual torsion angle. In the summation of all seven torsion angles, without RMS, the torsions cancelled each other

out to approximately 0° in all frames and all simulations and hence did not provide any information for the flexibility of the ring structure.

Obviously, for the unliganded β-CD a larger bend is allowed than for complexes suffering from steric restrictions induced by the bound guest molecules. The in vacuo simulation of β-CD alone shows the effects of motion induced by temperature at 300 K. Although temperature inflicted deviations from planarity occur, the average structure remains the same as after minimization. Table 1 gives an overview of all simulations and the RMS torsion averaged over the full trajectory. The investigated simulations include β-CD without a ligand in water and in vacuo, β-CD complexed with SP in two different orientations in water and one of them in vacuo.

In the presence of water β-CD undergoes conformational changes. After the initial stages of the simulation two opposing glucose units turn their O2/O3 side inwards, resulting in higher torsional deviations from planarity. The flections of β-CD are a result of an effort to minimize the volume of the hydrophobic interior and the formation of a hydrogen bond network, which includes more or less seven water molecules. All O2/O3 of both bent glucose units are involved in this network.

Steric hindrances and a modified hydrogen bond network provided by a large ligand like SP let the β-CD assume a vacuum-like conformation. After complexation the water network in the interior is removed and β-CD can adopt its planar vacuum structure again. The hydrogen bonds are not simply broken, but shifted from the inside to the area on the rim of the ring and the exterior (esp. O6). So no energetic penalties occur after ligand binding. This supports the claim that desolvation is the driving force of complexation. β-CDs hydroxyl groups are encouraged to form an intramolecular hydrogen-bonding network, which involves the whole O2/O3 side of the ring.

Since each glucose unit contains one hydroxyl group (O6-H) on one side and two (O2-H and O3-H) on the other side of the donut shaped structure of β-CD the hydrogen bonds formation during the simulations involves O2 and O3 in the role of donor and acceptor (both intramolecular and to water molecules) and O6 in association mostly with water, although some hydrogen bonds to O6 of the neighboring glucose unit or an O5 can be formed from time to time (Table 2).

If water is present in the core of the macrocycle, O1, the linker between glucose units which points inwards, can accept hydrogen bonds to some amount. This ability gets lost after the incorporation of a guest molecule. O2-Hs tend to favor other cyclodextrin acceptors above water especially after ligation. On the other hand O3s prefer hydrogen bonds to water molecules, a preposition which is reduced by complexation. The acceptor properties of O2/O3 act vice versa to their donor activities. In this case O3 prefers more hydrogen donors from other cyclodextrin hydroxyl groups compared to O2 as a result of O3's donor preference to water. But again, the occurrence of intramolecular hydrogen bonds is increased after the interior water is removed by a ligand. The glucosidal O5 can act only as hydrogen acceptor and since it is fairly accessible by water because of its position on the rim of the tube-like structure it accepts preferably water hydrogens in comparison to CD hydrogens.

Table 1 Average RMS C1-C1-C1-C1 backbone torsion of neighboring glucose units of β-CD

	CD in water	Complex A in water	Complex B in water	CD in vacuo	SP in vacuo
Average RMS torsion	62.02	39.90	35.48	39.19	38.01

The standard deviation for these values is 6.90

Table 2 Occurrence of hydrogen bonds

	CD	Complex A	Complex B
Donors			
O2 to water	0.439	0.407	0.323
O3 to water	0.823	0.600	0.650
O6 to water	0.913	0.867	0.863
O2 to CD	0.563	0.605	0.687
O3 to CD	0.185	0.389	0.341
O6 to CD	0.082	0.121	0.131
All O2	1.001	1.012	1.010
All O3	1.008	0.989	0.990
All O6	0.995	0.989	0.994
Acceptors			
O1...H–OH	0.228	0.035	0.042
O2...H–OH	1.230	0.908	0.961
O3...H–OH	0.679	0.649	0.570
O5...H–OH	0.912	0.747	0.744
O6...H–OH	1.290	1.282	1.314
O1...H–CH	0.006	0.025	0.031
O2...H–CD	0.182	0.382	0.331
O3...H–CD	0.560	0.589	0.669
O5...H–CD	0.077	0.114	0.126
O6...H–CD	0.004	0.005	0.001
Lig from water	–	1.090	1.128
Lig from CD	–	0.101	0.085

The numbers represent the total count of hydrogen bonds divided by the number of frames and glucose residues (seven). In the case of SP the average of its four oxygen acceptors is given. All of them are sufficiently apart from the hydrophobic core of the β-CD to accept hydrogen bonds from the solvent

Throughout the simulations the capabilities of hydrogen bond formation are always covered even though the preferences for donors and acceptors shifts. Thus no negative influence of the formation could be found in this matter. The entropic effects of the loss of the hydrogen bond network inside the ring core are highly favorable since β-CD would otherwise put up with unfavorable bending measures to minimize this volume and increase the hydrophilic inside by turning two glucose units.

The calculated binding energies show a high affinity for both complexes (A and B). The nmode module of AMBER allows the calculation of the entropic energy contribution using a normal mode method. Its drawback is that it cannot take water molecules explicitly into account whose hydrogen bond network has a substantial contribution to this energy. The energies in Table 3 represent the β-CD–ligand complex formation alone without respect to this phenomenon.

There are some differences of the energies for both orientations of SP. First, the size of the entered side of complex A is larger than in complex B and it establishes more VdW contacts to β-CD's hydrophobic interior core. Although in A more hydrogen bonds to β-CD can be formed, its tight packing does not allow an orientation for optimal electrostatic interaction, which results in the slightly worse overall binding energy (Table 3, electrostatic energy contribution).

The results of quantum chemical calculations on the interaction energy show rather different results depending on the methods and basis set used. Complex B possesses a lower energy throughout. Although in all cases a more or less complete inclusion of SP is observed (Fig. 2), the interaction energies differ significantly.

Applying the semiempirical method PM3 low, but quite diverse energies are obtained (Table 4). The low-level HF methods overemphasizes the association affinity, whereas HF/6-31G(d,p) results in low, but somewhat more similar energy values. The probably most reliable method (B3LYP/6-31G(d,p) delivers interaction energies, which are rather similar for both orientation of SP.

Table 3 Binding energies and their contributions of both SP complexes with β-CD simulated in water environment

	Complex A	Complex B
Electrostatic	–11.48	–18.71
VdW	–33.66	–31.78
$-T\Delta S$	15.33	15.38
$\Delta G_{formation}$	–29.81	–35.11

Energies in kcal mol^{-1}

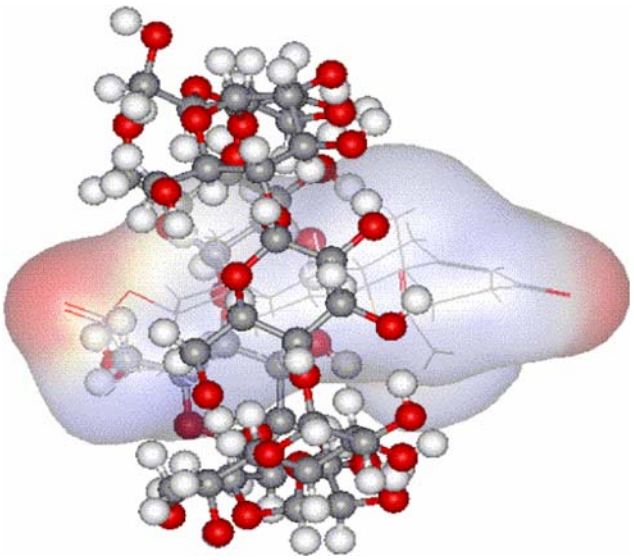


Fig. 2 Inclusion complex between SP and β-CD in lowest-energy orientation (complex B) fully minimized by B3LYP/6-31G(d,p)

Conclusion

Molecular dynamics simulations on β-CD and SP/β-CD complexes show that the formation of a rigid hydrogen bond network in the hydrophobic interior is responsible for large deformations of the β-CD ring. This flexion is energetically unfavorable for the ring itself and is a contributing factor for complex formation. After a ligand is placed hydrogen bonds are formed only on the rim and outside the ring structure. Furthermore, this study supports the idea that solvation is responsible for the excellent ligand binding properties of β-CD. The β-CD hydrate MD simulations are interesting model systems to investigate how conformational flexibility and dynamic disordering can influence inclusion complexes of a variety of compounds.

Molecular calculations on the geometry of SP/β-CD complexes show that the molecule is inserted completely into the cavity. For both possible orientations of SP, there is good agreement between the molecular surface of the guest molecule and the complementary β-CD surface, which explains to some extent the high

Table 4 Binding energies of complexes A and B in gas phase, obtained from various quantum chemical calculations

Complex	PM3	HF/3-21G	HF/6-31G(d,p)	B3LYP/6-31G(d,p)
A	–2.86	–17.06	–4.80	–9.51
B	–7.94	–20.66	–5.37	–10.86

Energies in kcal mol^{-1}

equilibrium constant of the inclusion complex in aqueous solution (K = 29,000 L/mol). The relative large dependency of the calculated interaction energy shows that higher level ab initio or DFT methods have to be used to obtain reliable results.

Acknowledgements This investigation was supported by the Hochschuljubiläumsstiftung der Stadt Wien (Project P H-778/2005). Technical assistance of Ms. Martina Ziehengraser is gratefully acknowledged.

References

1. Lawtrakul, L., Viernstein, H., Wolschann, P.: Molecular dynamics simulations of β-cyclodextrin in aqueous solution. Int. J. Pharm. **256**, 33–41 (2003).
2. Bea, I., Jaime, C., Kollman, P.: Molecular recognition by β-cyclodextrin derivatives: molecular dynamics, free-energy perturbation and molecular mechanics/Poisson-Boltzmann surface area goals and problems. Theor. Chem. Acc. **108**, 286–292 (2002).
3. Choi, Y.H., Yang, C.H., Kim, H.W., Jung, S.: Molecular modeling studies of the β- cyclodextrin in monomer and dimer form as hosts for the complexation of cholesterol. J. Incl. Phenom. Macro. Chem. **39**, 71–76 (2001).
4. Naidoo, K.J., Chen, J.Y.J., Jansson, J.L.M., Widmalm, G., Maliniak, A.: Molecular properties related to the anomalous solubility of β-cyclodextrin. J. Phys. Chem. B **108**, 4236–4238 (2004).
5. Choi, Y., Jung, S.: Simulations for the prediction of chiral discrimination of *N*-acetylphenylalanine enantiomers by cyclomaltoheptaose (β-cyclodextrin, β-CD) based on the MM–PBSA (molecular mechanics–Poisson–Boltzmann surface area) approach. Carbohydr. Res. **339**, 1961–1966 (2004).
6. Winkler, R.G., Fioravanti, S., Ciccotti, G., Margheritis, C., Villa, M.: Hydration of β-cyclodextrin: a molecular dynamics simulation study. J. Comput.-Aid. Mol. Des. **14**, 659–667 (2000).
7. Yu, Y., Chipot, C., Cai, W., Shao, X.: Molecular dynamics study of the inclusion of cholesterol into cyclodextrins. J. Phys. Chem. B **110**, 6372–6378 (2006).
8. Jara, F., Mascayano, C., Rezende, M.C., Tirapegui, C., Urzua, A.: A spectral and molecular dynamics simulation study of β-cyclodextrin inclusion complexes with solvatochromic dyes derived from barbituric acid. J. Incl. Phenom. Macro. Chem. **54**, 95–99 (2006).
9. Viernstein, H., Weiss-Greiler, P., Wolschann, P.: Solubility enhancement of low soluble biologically active compounds—temperature and cosolvent dependent inclusion complexation. Int. J. Pharm. **256**, 85–94 (2003).
10. Charumanee, S., Titwan, A., Sirithunyalug, J., Weiss-Greiler, P., Wolschann, P., Viernstein, H., Okonogi, S.: Thermodynamics of the encapsulation by cyclodextrins. J. Chem. Technol. Biotechnol. **81**, 523–529 (2006).
11. Frisch, M.J., Trucks, G.W., Schlegel, H.B., Scuseria, G.E., Robb, M.A., Cheeseman, J.R., Montgomery, J.A. Jr., Vreven, T., Kudin, K.N., Burant, J.C., Millam, J.M., Iyengar, S.S., Tomasi, J., Barone, V., Mennucci, B., Cossi, M., Scalmani, G., Rega, N., Petersson, G.A., Nakatsuji, H., Hada, M., Ehara, M., Toyota, K., Fukuda, R., Hasegawa, J., Ishida, M., Nakajima, T., Honda, Y., Kitao, O., Nakai, H., Klene, M., Li, X., Knox, J.E., Hratchian, H.P., Cross, J.B., Bakken, V., Adamo, C., Jaramillo, J., Gomperts, R., Stratmann, R.E., Yazyev, O., Austin, A.J., Cammi, R., Pomelli, C., Ochterski, J.W., Ayala, P.Y., Morokuma, K., Voth, G.A., Salvador, P., Dannenberg, J.J., Zakrzewski, V.G., Dapprich, S., Daniels, A.D., Strain, M.C., Farkas, O., Malick, D.K., Rabuck, A.D., Raghavachari, K., Foresman, J.B., Ortiz, J.V., Cui, Q., Baboul, A.G., Clifford, S., Cioslowski, J., Stefanov, B.B., Liu, G., Liashenko, A., Piskorz, P., Komaromi, I., Martin, R.L., Fox, D.J., Keith, T., Al-Laham, M.A., Peng, C.Y., Nanayakkara, A., Challacombe, M., Gill, P.M.W., Johnson, B., Chen, W., Wong, M.W., Gonzalez, C., Pople, J.A., Gaussian, Inc., Wallingford CT, (2004).
12. Pearlman, D.A., Case, D.A., Caldwell, J.W., Ross, W.R., Cheatham, T.E., DeBolt, S., Ferguson, D., Seibel, G., Kollman, P.: AMBER, a package of computer program for applying molecular mechanics, normal mode analysis, molecular dynamics and free energy calculations to simulate the structural and energetic properties of molecules. Comp. Phys. Commun. **91**, 1–41 (1995).
13. Berendsen, J.C.J., Postma, J.P.M., van Gunsteren, W.F., DiNola, A., Haak, J.R.: Molecular dynamics with coupling to an external bath. J. Chem. Phys. **81**, 3684–3690 (1984).