THE JOURNAL OF PHYSICAL CHEMISTRY B

Article

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Taste for Chiral Guests: Investigating the Stereoselective Binding of Peptides to β-Cyclodextrins

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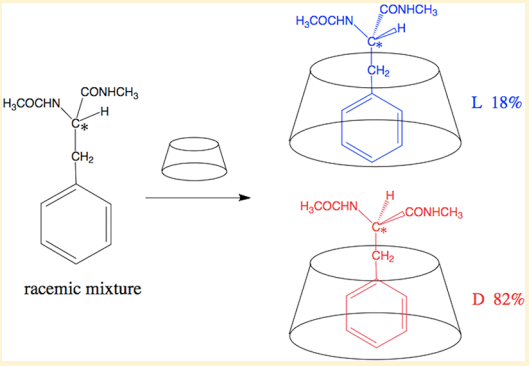
Supporting Information

ABSTRACT: Obtaining compounds of diastereomeric purity is extremely important in the field of biological and pharmaceutical industry, where amino acids and peptides are widely employed. In this work, we theoretically investigate the possibility of chiral separation of peptides by β-cyclodextrins (β-CDs), providing a description of the associated interaction mechanisms by means of molecular dynamics (MD) simulations. The formation of host/guest complexes by including a model peptide in the macrocycle cavity is analyzed and discussed. We consider the terminally blocked phenylalanine dipeptide (Ace-Phe-Nme), in the L- and D-configurations, to be involved in the host/guest recognition process. The CD−peptide free energies of binding for the two enantiomers are evaluated through a combined approach that assumes: (1) extracting a set of independent molecular structures from the MD simulation, (2) evaluating the interaction energies for the host/guest complexes by hybrid quantum mechanics/molecular mechanics (QM/MM) calculations carried out on each structure, for which we also compute, (3) the solvation energies through the Poisson−Boltzmann surface area method. We find that chiral discrimination by the CD macrocycle is of the order of 1 kcal/mol, which is comparable to experimental data for similar systems. According to our results, the Ace-(D)Phe-Nme isomer leads to a more stable complex with a β-CD compared to the Ace-(L)Phe-Nme isomer. Nevertheless, we show that the chiral selectivity of β-CDs may strongly depend on the secondary structure of larger peptides. Although the free energy differences are relatively small, the predicted selectivities can be rationalized in terms of host/guest hydrogen bonds and hydration effects. Indeed, the two enantiomers display different interaction modes with the cyclodextrin macrocavity and different mobility within the cavity. This finding suggests a new interpretation for the interactions that play a key role in chiral recognition, which may be exploited to design more efficient and selective chiral separations of peptides.



1. INTRODUCTION

The separation of stereoisomers of organic molecules has become increasingly important in the last years for the preparation of biologically active drugs to be used for pharmaceutical applications. The literature in this field is thus very wide, as it is reported in recent reviews.[1−3] In this paper, we focus on the chiral separation of peptides by means of selective complexation with cyclodextrins (CDs). On the one hand, the use of CDs as chiral selectors to separate drug enantiomers has attracted much attention in the context of high-performance liquid chromatography (HPLC), gas chromatography, or capillary electrophoresis.[4,5] On the other hand, the chiral separation of peptides has been developed owing to the increasing interest on these molecules as therapeutic agents.[6−9] CDs, in particular, have been shown to be very promising,[10−24] as they are able to separate enantiomers of aminoacid derivatives.[25−30] It is worth noting that CDs enantioselectivity is also important from the point of view of biotechnologies.[31]

However, the mechanism of chiral recognition is not yet completely understood, although some authors have stressed the importance of weak, short-range interactions,[25,26] of geometrical factors,[25] and of host−guest hydrogen-bond interactions[28] in the selective binding of enantiomers. Quite a few theoretical studies have been devoted to this topic[32−36] and some of them have considered amino acid derivatives[36−38] or molecules containing only one peptide bond (Ala-Phe and Ala-Tyr)[23] as possible guests. In these theoretical works, the techniques to evaluate the selectivity have been based on molecular docking and molecular mechanics/dynamics calculations and the interpretations have been made in terms of

Received: November 27, 2012
Revised: January 30, 2013
Published: January 31, 2013

energetic (electrostatic, van der Waals)[37] and structural[38] factors. However, to the best of our knowledge the CD-enantioselectivity of peptides of greater complexity has not yet been modeled, in spite of the pharmacological importance of such compounds. For instance, possible changes in the peptide conformation upon binding, through an analysis of the backbone angles (ψ and ϕ), have not been inspected so far, to investigate how they can influence the host/guest interactions leading to selectivity. The present study represents the first attempt to explore this topic and has been motivated in part by recent theoretical results[39] obtained for the binding modes of peptides to β-CDs that emphasized (1) the preference for a complexation mode where the peptide lies on the narrow rim of the CD cavity, and (2) the modification of the average backbone angles favoring values consistent with an α-helix.

To this aim, we have carried out molecular dynamics (MD) simulations followed by energy calculations using a combined quantum mechanics and molecular mechanics (QM/MM) method. We have considered a native β-CD and a simple model peptide Ace-(L,D)Phe-Nme, the terminally blocked phenylalanine dipeptide (Figure 1). The Ace-Phe-Nme model was

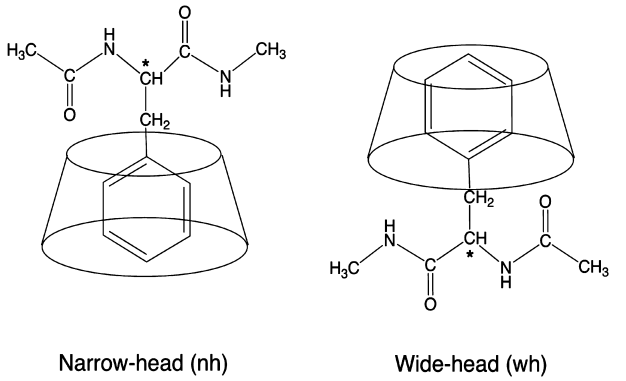


Figure 1. Model peptide and possible structures of the β-CD−peptide complex considered in this work.

used previously in a study of peptide−CD interactions[39] and can be considered as the simplest one allowing us to discuss CD-selectivity for peptide diastereoisomers in which one L-Phe residue is mutated into a D-Phe one. Phe has been chosen here because peptide-encapsulation by CDs usually occurs via the aromatic side chains. For simplicity, we shall refer below to L- or D-peptide for the Ace-(L)Phe-Nme and Ace-(D)Phe-Nme isomers of the model dipeptide, respectively.

2. COMPUTATIONAL DETAILS

We summarize here the computational procedure that we followed to analyze the different complexes. More details can be found in ref 39. MD simulations were run using AMBER 9.0[40] for the peptide in the L- and D-configurations, both in aqueous solution and in the solvated cyclodextrin environment. We used the AMBER03[41] force field and the TIP3P model for water.[42] Simulations were run with periodic boundary conditions in the *NPT* ensemble at 1 atm and 300 K, using the particle mesh Ewald method to treat long-range electrostatic interactions.[43] After a 2 ns-long equilibration, 8 ns were used for the analysis of the structure and the dynamics of the system, as well as the host−guest and complex-solvent interactions. A total of 500 snapshots were extracted along the simulation to be used for QM/MM calculations in which the peptide is described at the density functional theory (DFT) level using the B3LYP functional[44−46] together with the 6-311+G(d,p) basis set; the environment is described through the MM force field used in the MD simulations. On each snapshot, we performed a QM calculation in the presence of the electrostatic potential given by the surrounding point charges (cutoff at 10 Å), and the Lennard-Jones potential was used for nonelectrostatic interactions between the QM and the MM regions. QM calculations were run by using Gaussian03.[47]

The free energy of binding (ΔG_{bind}) was obtained in aqueous solution using the QM/MM variant[39] of the Poisson−Boltzmann surface area (PBSA) method[48] using the following relationship:

$$\Delta G_{bind} = \Delta E_{int} + \Delta E_{solv} - T\Delta S \quad (1)$$

The term ΔE_{int} represents the energy change upon complex formation, ΔE_{solv} represents the change in the solvation energy, and finally ΔS is the entropy change at constant temperature, T.

3. RESULTS AND DISCUSSION

The shape of a β-CD can be roughly described as a hollow truncated cone, thus the two different orientations of the peptide within the cavity will be referred to as narrow-head (nh) and wide-head (wh) (Figure 1). In this work, we calculated complexes of the CD with the two optical isomers of the model peptide; some results for the L-isomer were reported before.[39] The binding energies of the complexes are calculated and compared, to inspect the possibility of molecular recognition of one of the two chiral forms by the β-CD in water. Our results are discussed in terms of the mobility of the guest inside the CD cavity, the local host−guest interactions as well as the specific interactions with the solvent. We note here that, along the different simulation runs that we carried out, we did not observe any dissociation event for the CD−peptide complex.

The free energy of binding and the different terms on the right-hand side of eq 1 are shown in Table 1. Values reported in parentheses correspond to the standard deviation of mean

Table 1. Free Energy of Binding for the Inclusion Complexes between the Ace-L,D-Phe-Nme and β-CD in Water Computed at the QM/MM-PBSA Level Using Eq 1[a]

	L-configuration		D- configuration	
	CD (nh)	CD (wh)	CD (nh)	CD (wh)
ΔG_{bind}	−1.77 (±0.35)	−0.78 (±0.47)	−2.78 (±0.32)	0.55 (±0.41)
ΔE_{int}	−36.41 (±0.27)	−37.85 (±0.37)	−37.96 (±0.25)	−36.06 (±0.31)
ΔE_{solv}	16.60 (±0.19)	19.85 (±0.24)	16.88 (±0.17)	19.22 (±0.24)
$T\Delta S$	−18.04 (±0.10)	−17.22 (±0.16)	−18.30 (±0.11)	−17.39 (±0.12)
population (%)	15	2.8	82	0.2
tot. population (%)	17.8		82.2	

[a]The different terms in the equation, obtained by averaging over 500 structures extracted from an 8 ns-long MD simulation, are also reported (the corresponding standard deviations of mean are shown in parentheses). Energies and free energies are expressed in kcal/mol. The percentage of each complex obtained from the Boltzmann populations corresponding to the ΔG_{bind} values is shown in the last rows of the table.

dx.doi.org/10.1021/jp311671w | J. Phys. Chem. B 2013, 117, 3091−3097

(SDM) defined as SDM = $s/\sqrt{N}$, where s is the standard deviation and N is the total number of snapshot for which the energy contributions were evaluated.

For both L- and D-configurations, the nh complex is more stable than the wh complex by about 1 and 3 kcal/mol, respectively. The orders of magnitude of the free energy changes under complex formation evaluated through our calculations are consistent with experimental measurements on similar systems.[4,49,50] When we compare the results for the two nh complexes in solution, the one resulting from the D-configuration of the peptide is more stable by about 1 kcal/mol compared to the one corresponding to the L-configuration. Using the free energy differences in Table 1, one can make an estimation of the population of each complex. Hence, according to our calculations, the β-CD cavity selectively binds the D-isomer of the Ace-Phe-Nme dipeptide over the L-isomer, with populations of roughly 82% and 18%, respectively. Although the entropic contribution seems slightly less favorable for the D-dipeptide nh complex compared to the one formed by the L-isomer, the solvation energy change upon binding and the interaction energy provide a better stabilization in the former case.

As is commonly observed in problems related to enantio- or diastereoselectivity, the energy differences involved are small and close to (or even smaller than) the computational errors. Thus, it is difficult to conclude definitively on the chiral recognition capability of the CD only on the basis of the free energy results, which have to be employed with some caution. Nevertheless, the trend suggested by values in Table 1 can be rationalized in terms of differences of the specific peptide−CD interactions for each isomer, and we analyze this issue in more detail in the following.

To gain further insight into the CD−peptide binding, let us first look at the geometry differences of the peptide isomers in the complexes, and more particularly at the backbone angles ϕ and ψ. The Ramachandran plots of the two (most stable) nh complexes are compared in Figure 2. As shown, the plots for the L- and D-configurations are roughly symmetric. Thus, in the complex involving the peptide with L-configuration, the most populated area of the Ramachandran plot corresponds to ϕ and ψ angles around −78° and −22°, respectively, whereas for the complex involving the peptide in D-configuration the corresponding values are 77° and 20°. This basin is associated

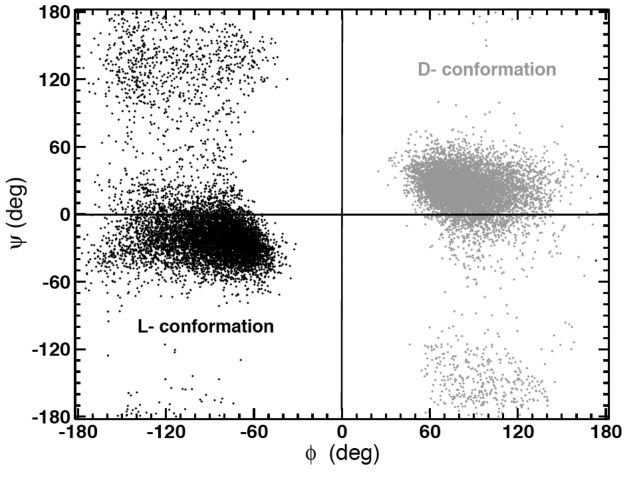


Figure 2. Ramachandran plot for the CD−peptide nh complexes (L-isomer on the left; D-isomer on the right).

with values of the backbones angles that are most likely observed in the α helical secondary structures of peptides and proteins, whereas the one corresponding to β sheets is the one displaying a smaller population. Some quantitative differences between the L- and the D-isomer are apparent, the distribution of points within the most populated region being denser in the latter case. The root-mean-square of the (ϕ, ψ) distribution around these values was evaluated to be about 167° in the case of the L-isomer and 46° for the D-isomer. Thus, though the average geometries of the two isomers are comparable, the above results indicate a lower flexibility of the D-peptide in the CD cavity, possibly induced by specific host−guest interactions. Before analyzing this hypothesis more in depth, we would like to discuss the connection between the peptide conformation and the CD−peptide interaction free energy.

We divided the (ϕ, ψ) plan into two regions arbitrarily defined to distinguish between structures in the α basin and those in the β basin: independently of ϕ, we consider as α structures those falling in the $\psi = [-120°, +60°]$ interval, and β structures all the remaining points. We tested other possible choices, finding that the discussion presented in the following is not significantly affected. If we use the populations from the MD simulations and assume Boltzmann statistics, we can estimate the difference in the free energy of binding for the α and β conformations of the peptide. Not surprisingly, for both peptide isomers (L- and D-), the CD-binding energies for α conformations are larger than for β conformations (in absolute value). However, the difference is more pronounced in the case of the D-isomer (1.72 kcal/mol) than in the case of the L-isomer (1.09 kcal/mol). According to this trend, we may conclude that the chiral selectivity of the CD macrocycle predicted for the simple model peptide Ace-(L,D)Phe-Nme (values in Table 1), should be higher for Phe inside an α-helix.

The role of specific interactions between the macrocavity and the peptides has been investigated by calculating the radial distribution functions (RDFs) describing the intermolecular hydrogen bonds, which are shown in Figure 3. Two types of H-bonds have been found. In one case, the hydrogen atoms of the −NH groups of the peptide (H^{Nme} and H^{Phe}) behave as donors, with the oxygen atoms of the OH groups of the CD being the acceptors. In the second case, the carbonyl oxygen atoms of the peptide bonds (O^{Phe} and O^{Ace}) behave as acceptors, the proton donors being the −OH groups of the CD. As shown, the RDF curves involving H^{Phe} or O^{Phe} atoms are quite similar in the two complexes. In contrast, some clear differences appear for hydrogen bonds involving atoms in the Nme or Ace groups. Thus, in the case of the D-peptide, the complex with the CD displays a much stronger $H^{Nme}\cdots O^{CD}$ hydrogen bond and a much weaker $O^{Ace}\cdots H^{CD}$ hydrogen-bond compared with the complex for the L-peptide.

Further analysis of the MD trajectories, and in particular of the relative peptide−CD movements, shows that the anchoring of the host to the guest cavity is tighter in the case of the D-isomer of the peptide. Figure 4 displays the analysis of H(peptide)···O(CD) distances as a function of time. We have considered the H atoms of the two NH donor groups in the peptide (H^{Phe}, H^{Nme}) and all the O atoms of the primary OH groups of the seven glucopyranose units in the CD. The points in the figure indicate structures at which the corresponding H(peptide)···O(CD) distance is lower than 2.5 Å, thus completing the average information given by the total RDFs depicted in Figure 3 (where all glucopyranose units are taken into account at once). We report the results obtained for all the

dx.doi.org/10.1021/jp311671w | J. Phys. Chem. B 2013, 117, 3091−3097

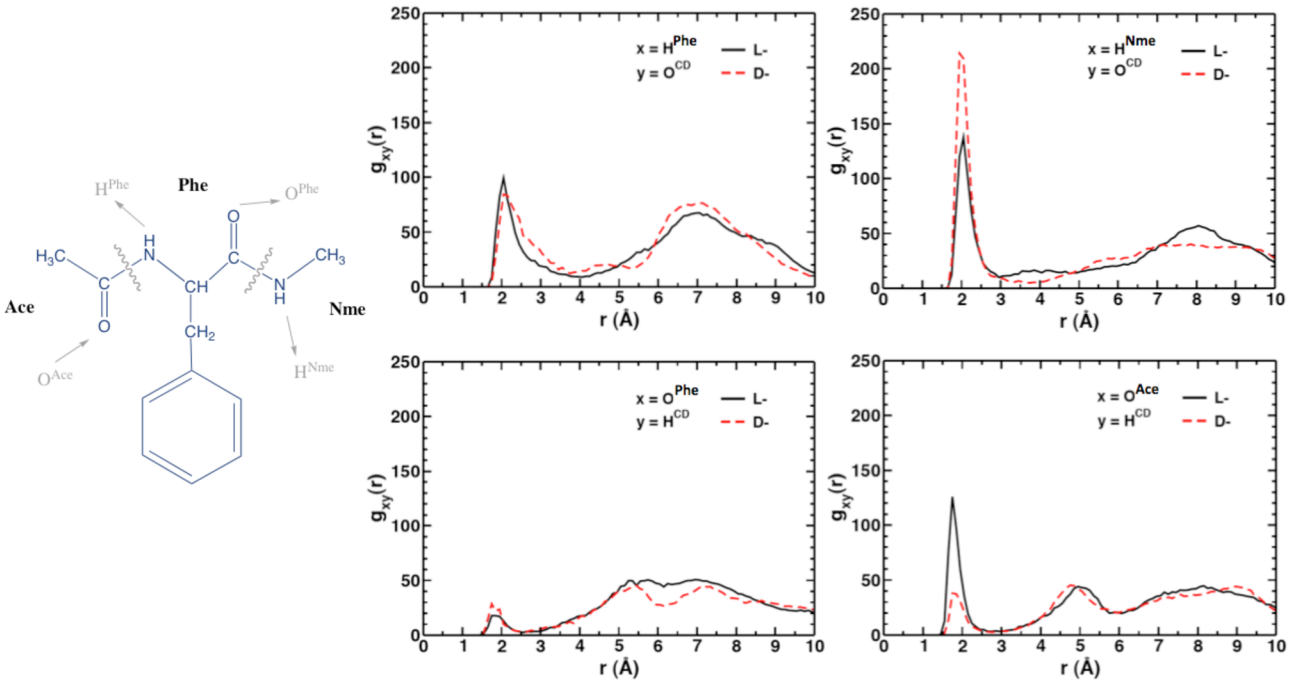


Figure 3. Radial distribution functions describing the interaction between the model peptides and the CD hydroxyl groups on the narrow side (L-configuration, solid black line; D-configuration, dashed red line).

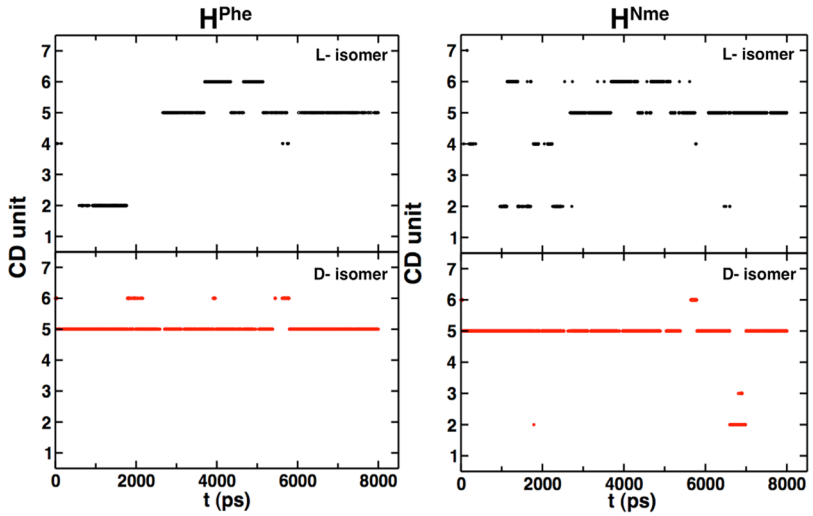


Figure 4. Short H(peptide)···O(CD) distances (<2.5 Å) as a function of time for the nh complexes showing the larger mobility of the L-isomer in the complex compared with the D-isomer.

CD units: the numbering that we used is arbitrary but it follows the ordering of adjacent units. Considering a top view of the structure in Figure 1, unit numbering follows the clockwise direction. As shown, along our simulation, the H^{Phe} and H^{Nme} atoms of the D-peptide spend most of the simulation time in front of the same OH group (glucopyranose unit 5). In the case of the L-peptide, the H^{Phe} and H^{Nme} atoms spend also a significant part of the total simulation time interacting with one specific glucopyranose unit but some interactions with other units regularly occur along the trajectory. In other words, one observes a larger mobility of the L-isomer compared to the D-isomer inside the CD cavity. It is worth emphasizing the fact that (most often) the H^{Phe} and H^{Nme} atoms are simultaneously involved in H-bonds with the same O atom, which is in agreement with the conclusions of previous simulations reported for the L-isomer nh complex (Figure 8 in ref 39). The lower mobility of the D-isomer in the complex is consistent with its larger interaction energy (Table 1) and also with the decrease of the internal flexibility induced by the CD binding (Figure 2, Ramachandran plots).

For the sake of completeness, it is interesting to comment on the role of the solvation energy. On one hand, the ΔE_{solv} values in Table 1 exhibit small differences for the CD−(L,D)peptide complexes of the same type (about 0.3 kcal/mol for the nh complexes; note that the differences between the nh and wh complexes are on the contrary significant, about 3 kcal/mol). On the other hand, the calculations show that the H-bond interactions between the peptide and the surrounding water molecules in the complexes are not very strong (in contrast with the free peptide in water solution[39]) and do not depend

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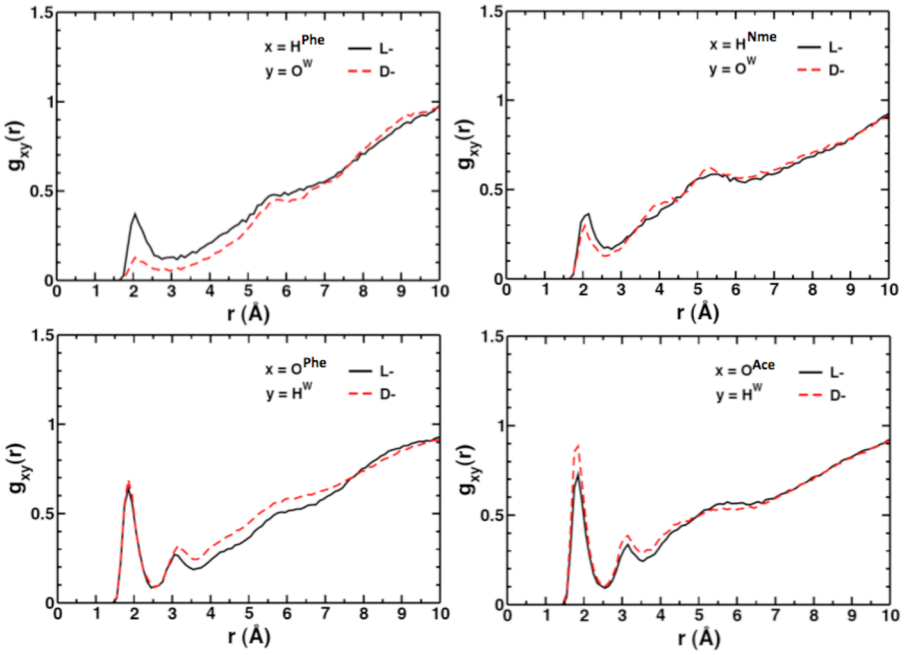

Figure 5. Radial distribution functions of hydrogen bonds between the model peptide and water for configurations L (solid line) and D (dash line).

much on the isomer type. The RDFs illustrating this point are drawn in Figure 5. As shown, the H(peptide)···O(water) H-bonds are rather weak, though for the H^{Phe} atom a somewhat stronger interaction with the L-isomer occurs. The O(peptide)···H(water) H-bonds are slightly stronger but again L- and D-isomers present similar characteristics. All these results suggest that there are no main differences in terms of solvation contributions for the two complexes and that the CD chiral selectivity is mainly based in CD−peptide interactions.

4. CONCLUSIONS

In this work, we discussed the structure and the energetics of the chiral separation of the two enantiomeric forms of the model dipeptide Ace-(L,D)Phe-Nme by β-CD. The results showed that the narrow complexation mode is preferred for both L- and D-configurations, and that the free energy of the complex formed by the D-isomer is lower by about 1 kcal/mol compared to the one formed by the L-isomer. To gain deeper insight on the interactions leading to enantioselectivity in our model system, we analyzed the structure and the intermolecular interactions within the complexes. We found that the D-isomer is more strongly anchored to the CD cavity, and that it forms more stable hydrogen bonds with the hydroxyl groups of the macrocycle. On the other hand, the interactions with the solvent are similar in the two cases. This is consistent with a larger rigidity of the D-isomer once the complex is formed, which is further confirmed by a more confined basin on the Ramachandran plot around the values corresponding to an α conformation, compared to the case of the L-isomer. This latest finding confirms some previous results pointing to a preferential interaction between the β-CD and a dipeptide in an α conformation. The link between the peptide conformation and the interaction with the CD could have some important implications in the design of improved procedures for the chiral separation of peptides and proteins. Simulations using larger peptides as models, thus including more generally the effect of different conformations, would be particularly interesting to further explore this topic.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Full refs 40 and 47. This information is available free of charge via the Internet at http://pubs.acs.org/

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Financial support by the French National Research Agency (CREAM project, ref. ANR-09-BLAN-0180-01) and computational facilities by the CINES (project lct2550) are gratefully acknowledged.

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