Application of Free Energy Perturbation Calculations to the Enantioselective Binding of Peptides to C_3-Symmetric Synthetic Receptors

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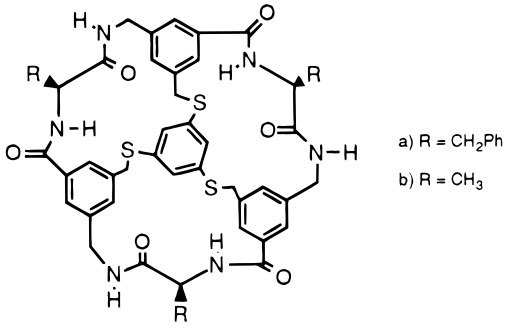
Abstract: Free energy perturbation (FEP) is applied to the calculation of the enantioselectivity in binding of three peptide guest molecules to certain C_3-symmetric synthetic receptors. The calculations are able to reproduce the observed trends in enantioselection with errors ≤0.7 kcal/mol, and the agreement with experiment is the best with those guests which form stronger complexes. We find that the weakly bound guests sample more conformational space in the receptor complex than do the more strongly binding guests. These results indicate that obtaining the necessary converged ensemble averages is more challenging for the more weakly binding guests because their complexes are structurally more poorly defined. Our simulations also suggest that the high enantioselectivities observed with our receptors arise in large part from the ability of the preferred guest peptides to form, on average, a greater number of hydrogen bonds with the receptor.

Introduction

In recent years the technique of free energy perturbation (FEP) has been applied to calculate differences in binding free energy for a range of biological and synthetic systems.[1] FEP has also been applied successfully in the design of highly enantioselective synthetic receptors for peptide-derived ammonium ions.[2] While the use of FEP as a predictive tool in molecular design appears promising, it is important to perform calculations for systems where experimental results are already available in order to validate such methods for a wide range of molecular systems.

Here we report the application of FEP to computation of the difference in binding energies of enantiomeric (L and D) alanine-derived peptides **3**−**5** with the C_3-symmetric host molecules **1b** and **2b**. The synthesis of C_3 hosts **1a** and **2a** and their binding energies with peptide guests **3**−**5** have been described elsewhere, and host **1a** in particular was reported to bind certain peptides with remarkably high enantioselectivity (>2 kcal/mol).[3] Hosts **1** and **2** are actually atropisomers, conformational isomers that are separated by large energy barriers. These hosts arise from different macrocyclization pathways during synthesis, and conformer **2** was isolated in 5% yield during the synthesis of **1**. No interconversion between **1** and **2** has been observed.

The three-dimensional structures of **1** and **2** have been discussed previously. Both molecular mechanics conformational searching and ^{1}H NMR studies indicate that these molecules form well-defined cavities in organic solvents. Representative low-energy conformations of **1b** and **2b** are shown in Figure 1. In receptor **2**, molecular mechanics suggests some interconversion between γ-turn and extended conformations of the alanine fragments whereas the corresponding fragments of the more rigid receptor **1** are calculated to occupy largely only γ-turn conformations. Receptor **2** has a broader and shallower binding cavity than **1**.



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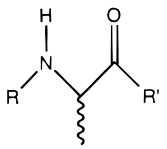

Although **1** was designed to be preorganized for binding peptide-derived guests, there is considerable intermolecular conformational flexibility in the bimolecular complexes of these systems. Hence the FEP studies described here constitute important tests of our ability to generate the converged ensembles of states that are required for reliable free energy calculations.

In addition to using FEP to calculate the relative binding energies of enantiomers, we also perform simulations in order to elucidate the structures of the complexes formed by peptides **3**−**5** with hosts **1a** and **2a**. In particular we wish to learn how the binding energies and selectivity depend on such factors as the conformational flexibility and intermolecular hydrogen bonding between host and guest. Such simulations should provide us with information that is not accessible from experiment and that should be useful in the design of other selective receptors.

[⊗] Abstract published in *Advance ACS Abstracts,* January 1, 1996.

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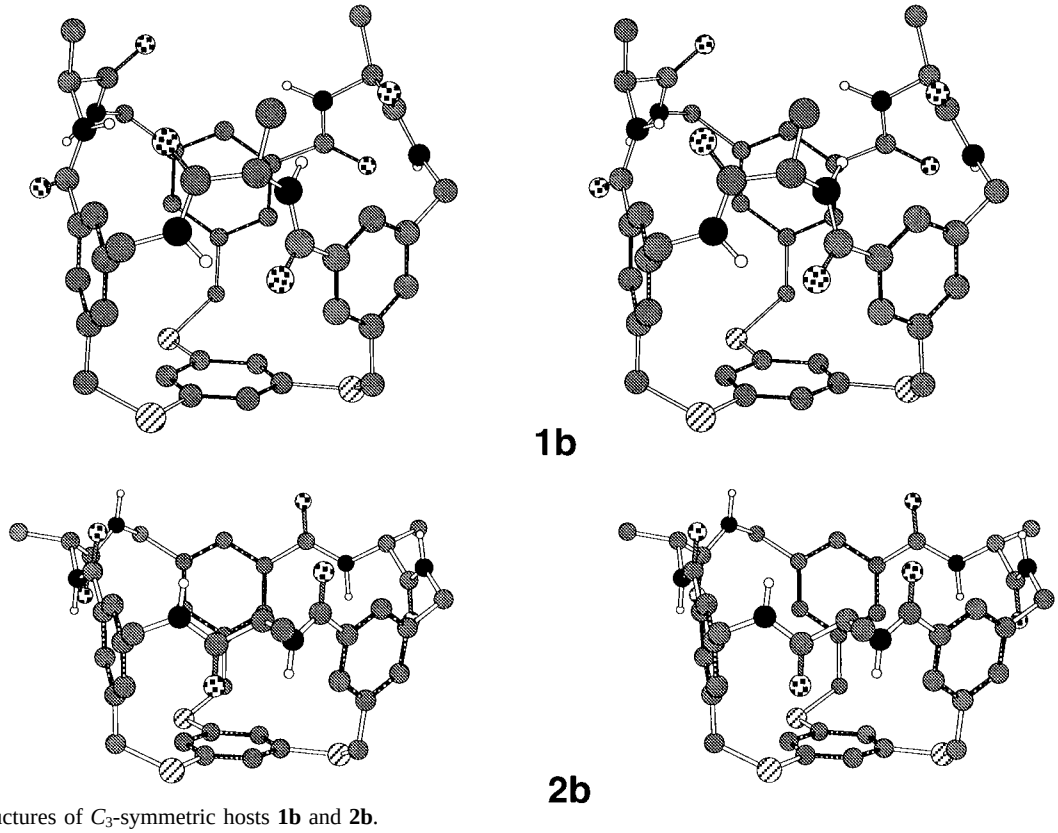

Figure 1. Structures of C_3-symmetric hosts **1b** and **2b**.

Methodology

All simulations were performed using version 5.0 of our MacroModel/BatchMin molecular modeling program. Though experimental enantioselectivies were measured on chloroform-soluble phenylalanine-derived hosts **1a** and **2a**, our calculations were performed on the computationally simpler alanine analogs **1b** and **2b**. To compute the difference in binding energies for the two enantiomers of peptides **3**−**5** with **1b** and **2b**, we used the standard free energy perturbation formula:[4]

$$\Delta\Delta G_{(\mathrm{L-D})} = \Delta G_{\mathrm{L}} - \Delta G_{\mathrm{D}} = -k_{\mathrm{B}}T \ln\langle \exp[-(H_{\mathrm{L}} - H_{\mathrm{D}})/k_{\mathrm{B}}T]\rangle_{\mathrm{L}}$$

in which the two states L and D designate the D and L forms of peptides **3**−**5** complexed with **1b** and **2b**. The perturbation we used changes the L-complex into the diastereomeric D-complex by interchanging guest peptide α-substituents (*i.e.* Me → Du, Du → Me) over 21 stages (*i.e.* $\lambda = 0.00, 0.05, 0.10, ...$) with double-wide sampling at each stage. Because the energies of enantiomeric guests are identical in the unbound states, the binding enantioselectivity studied here depends only upon the relative free energies of the diastereomeric complexes. A time step of 1.5 fs and a temperature of 300 K was used in all simulations. An equilibration period of 10 ps was used at each stage before data sampling for the free energy calculation. The data sampling time at each stage was 500 ps for the stronger binding complexes (**1·3** and **1·4**). For the more weakly binding complexes (**1·5** and **2·3**), 1000 ps sampling time was required to obtain reasonable convergence (overall $\sigma < 0.5$ kcal/mol). To generate the required ensemble averages (symbolized by < > in the above equation), we used the Monte Carlo/stochastic dynamics (MC/SD)[5] method at 300 K. The MC/SD method greatly speeds simulation convergence relative to traditional dynamical methods with conformationally heterogeneous systems. MC/SD operates by interleaving large, conformation-hopping Monte Carlo internal coordinate moves and stochastic dynamics time steps in a single simulation. Degrees of freedom used in the Monte Carlo moves of the peptidic guest molecule included torsion rotation around its ϕ and ψ angles and molecular rotations and translations centered on α and carbonyl carbon atoms (made relative to all three Cartesian axes). Guest torsional moves were in the range ±20° to ±180°. Guest rotational moves were in the range ±20° to ±180°, and translations ranged from 0.0 to 1.0 Å. Standard deviations of computed free energies were evaluated from block averages taking the quarters of each simulation as the blocks. All simulations started from geometries for the complexes that were previously proposed to be the most stable.[3]

The force field used was AMBER* force field[6] and our GB/SA solvation model[7] for chloroform as implemented in V5.0 MacroModel. GB/SA treats solvent as an analytical dielectric continuum that starts near the van der Waals surface of the solute and extends to infinity. The model includes both generalized Born-based (GB) solvent polarization terms and surface area-based (SA) solvent displacement terms. Partial atomic charges for **1**−**5** and new, quantum mechanically derived torsional parameters for α-amido ester derivatives are given as supporting information. Cutoffs for nonbonded interactions were set at 25 Å, which resulted in all nonbonded interactions being included. To prevent the host and guest from drifting apart during the lengthy simulations, flat-bottomed harmonic restraints were applied between the α-carbon of the guest and each of the three α-carbons of the host's alanine fragments. The restraints had force constants of 200 kJ/mol Å and were centered at 5 Å with a width of ±6 Å. These constraints allowed the α-carbon of the guest to move freely up to ∼10 Å from the center of the host before encountering a restoring force from the restraints. Additional flat-bottomed restraints (width = ±60°) were used to prevent the host's three C(sp^2)−S torsions from wandering far from their original (global minimum) angles. These restraints prevented the various host C−C−S−C conformers from slowly interconverting during the course of the simulations and thus providing a source of long-period free energy drift.

In addition to the above FEP calculations, we performed 1000 ps MC/SD simulations of complexes of the D and L forms of **3**-**5** with **1b** and **3** with **2b**. These simulations employed the same the force field, solvent model, and temperature described above. During each of these simulations, 250 structures were saved for subsequent geometrical analyses. Ensemble-averaged potential energies were also accumulated during these runs and were taken as average enthalpies of the complexes.

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Table 1. Calculated and Experimental $\Delta\Delta G$'s of Association (kcal/mol) for **1b** and **2b** with Alanine-Derived Peptides.

host	guest	$\Delta\Delta G_{expt}$[a] (kcal/mol)	$\Delta\Delta G_{calc}$[b] (kcal/mol)	$\Delta\Delta H_{calc}$ (kcal/mol)	sampling time (ns)[d]
1	**3**	2.2 ± 0.1	2.36(0.4)	3.21	0.5
1	**4**	2.5 ± 0.1	2.22(0.3), 2.56(0.4)[c]	3.27	0.5
1	**5**	0.3 ± 0.2	0.99(0.3), 1.09(0.3)[c]	0.75	1.0
2	**3**	0.0 ± 0.2	0.43(0.3)	2.42	1.0

[a] Reference 3. [b] Values in parentheses are the standard deviations of free energy. [c] The second values were computed using a modified parameter set for α-amino esters (see text). [d] Sampling time per window.

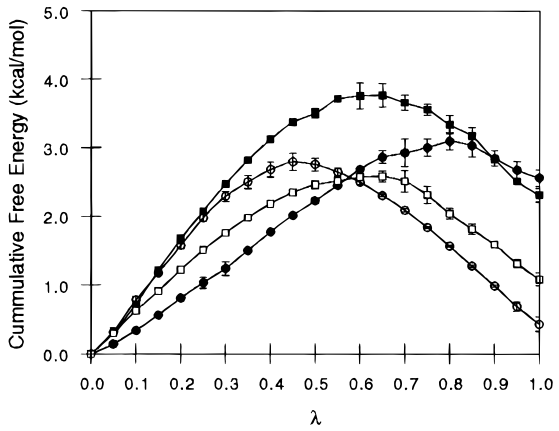

Figure 2. Cumulative free energy profile for free energy perturbation calculations of host−guest complexes: (filled circles) **1b·4**; (filled squares) **1b·3**; (open squares) **1b·5**; (open circles) **2b·3**. Error bars are shown at 1σ.

Results

Free Energy Perturbation. The FEP-calculated and experimentally determined $\Delta\Delta G$ values for enantioselective binding of alanine-derived guest molecules are given in Table 1. For the complexes associated with the highest enantioselectivity (**1·3** and **1·4**), the calculated values for the enantioselection are in excellent agreement with the experimentally determined differences in binding energies between the enantiomeric peptides. For the complexes having lower enantioselectivity (**1·5** and **2·3**), the FEP calculations were somewhat less successful in reproducing the observed enantioselectivity. With those complexes, errors of approximately 0.7 and 0.4 kcal/mol, respectively, were found relative to experiment. On the other hand, only in the case of **1·5** does experiment with error bars lie outside one standard deviation of the computed free energy of enantioselection, and even in that case these error limits are separated by only 0.2−0.3 kcal/mol. Also shown in Table 1 are calculated differences in average potential energy ($\Delta\Delta H$) taken from 1 ns simulations of the C_3 hosts with the D and L forms of the guests. While the values of $\Delta\Delta H$ significantly overestimate the extent of enantioselectivity observed, both the selectivity for the L guest and the rank ordering of enantioselectivity are reproduced by $\Delta\Delta H$ alone. The fact that the $\Delta\Delta H$ energies generally overestimate the experimentally observed free energy differences is consistent with the expectation that the complex of the more tightly bound guest will be more structurally defined and thus entropically disfavored, an example of the well known principle of enthalpy-entropy compensation. Figure 2 shows the free energy profiles for the conversion of the L into the D guest (shown left to right in the figure). Free energy error bars corresponding to one standard deviation (1σ) for each window are also shown in the figure. In each case, the free energy profile shows a generally smooth transition as the L guest is mutated into the D guest, and this is consistent with good convergence.

Though all of our simulations give similar statistical uncertainties in overall $\Delta\Delta G_{calc}$'s as measured by overall standard deviations, the bulk of the energetic uncertainties result from those parts of the simulations in which the host and guest are weakly bound. This effect may be seen in Figure 2 by noting that the largest error bars are associated with structures that are relatively high in energy (*e.g.* $\lambda = 0.5$). Around such points, many more (relatively high potential energy) geometries appear to be contributing to the ensemble average, and the simulation is having difficulty in sampling them all on a 100 ps time scale (one block in the standard deviation evaluation). To further illustrate the greater free energy fluctuations in the simulations of the more weakly bound structures, Figure 3 (circle points and solid lines) shows the cumulative free energy differences for forward and reverse direction sampling (and their average) as a function of simulation time for the $\lambda = 0.2$, 0.4, 0.6, and 0.8 windows of simulations for **1b·3**. When $\lambda = 0.2$ and 0.4, the simulations are sampling structures resembling the more tightly bound L-alanine complex and the free energies are stable within 0.01 kcal/mol from 100 to 1000 ps. When $\lambda = 0.6$ and 0.8, the simulations are sampling structures resembling the more weakly bound D-alanine complex, and the free energies show much greater fluctuations and also greater variations in the results of the forward and reverse data sampling. This greater fluctuation in average free energy is a reflection of the larger number of conformations which are being sampled in the case of the weaker complexes; this is supported by geometrical analysis of structures taken from the simulations of these systems as discussed below.

Figure 3 (as square points and dashed lines) also gives analogous free energies for the $\lambda = 0.2$, 0.4, 0.6, and 0.8 windows but computed using pure stochastic dynamics (SD, time constant $\tau = 0.2$ ps) at 300 K. The free energy results with pure SD are very close to those of MC/SD at $\lambda = 0.2$ and 0.4 where a relatively small number of different conformers are being sampled. However, at $\lambda = 0.6$ and 0.8 were more diverse structures contribute, the SD and MC/SD results differ because the SD simulation does not sample as many of these available states as does the MC/SD. Indeed, the SD simulations at $\lambda = 0.6$ and 0.8 spend the full 1000 ps exploring the initial conformational well.

Parameters for α-Amido Esters. The FEP calculations described above were initially carried out using the standard MacroModel V5.0 AMBER* parameter set. Although our FEP results reproduced experiment rather well, there were no specific torsional parameters for esters of α-amino acids in our force field. Instead, the parameters were taken from those developed for simple NH-Boc compounds and simple esters. Though using simple functional group parameters in polyfunctional molecules is common practice in molecular mechanics, it is likely to result in highly inaccurate conformational energies when the functional groups closely connected (*e.g.* by ≤3 bonds). In such highly interacting polyfunctional systems, new torsional parameters usually need to be developed and quantum mechanics is commonly employed to obtain data for the parameterization. To see how such reparameterization might effect our FEP results, we carried out the following calculations on *N*-acetylalanine methyl ester.

We first tested the performance of the existing AMBER* parameters for amides and esters in the context of α-amido esters after performing *ab initio* molecular orbital calculations[8] for a

(8) Calculations were performed with Gaussian 92: Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Gill, P. M. W.; Johnson, B. G.; Wong, M. W.; Foresman, J. B.; Robb, M. A.; Head-Gordon, M.; Replogle, E. S.; Gomperts, R.; Andres, J. L.; Raghavachari, K.; Binkley, J. S.; Gonzalez, C.; Martin, R. L.; Fox, D. J.; Defrees, D. J.; Baker, J.; Stewart, J. J. P.; Pople, J. A. Gaussian, Inc., Pittsburgh, PA, 1993.

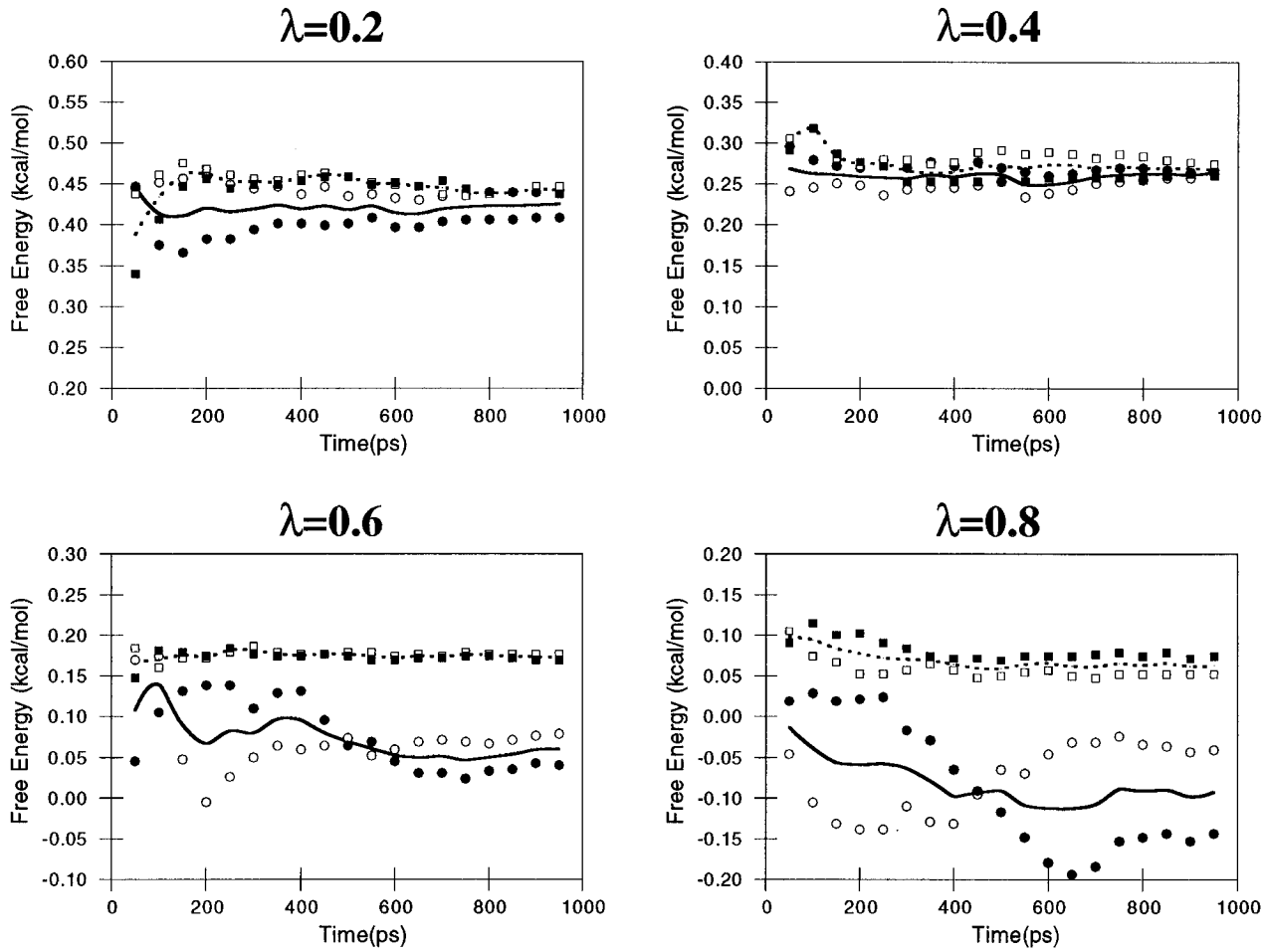

Figure 3. Free energy profiles from selected windows (λ = 0.2, 0.4, 0.6, 0.8) in the simulation of **1b·3** with MC/SD and SD simulation methods: (filled circles) "reverse" sampling with MC/SD; (open circles) "forward" sampling" with MC/SD; (solid line) average of "forward" and "reverse" sampling with MC/SD; (filled squares) "reverse" sampling with SD; (open squares) "forward" sampling" with SD; (dashed line) average of "forward" and "reverse" sampling with SD.

Table 2. Relative Energies of Four Conformations of *N*-Acetylalanine Methyl Ester by AMBER* and at the MP2/6-31+G**/HF/6-31+G** Level of Theory

conformation	original AMBER* (kcal/mol)	modified AMBER* (kcal/mol)	MP2/6-31+G**// HF/6-31+G** (kcal/mol)
C1	0.00	2.02	2.00
C2	0.27	0.00	0.00
C3	4.43	3.14	2.60
C4	5.75	3.18	3.00

number of conformations of *N*-acetylalanine methyl ester. Each conformation was optimized at the HF/6-31+G** level of theory, and single point energies were obtained using the MP2/6-31+G** level of theory.[9] In the molecular mechanics calculations, a constant dielectric of 1.0 was used to allow comparison with the *in vacuo* quantum mechanics energies. The results are shown in Table 2 and the geometries are shown in Figure 4. Detailed geometries and total energies for the *N*-acetylalanine methyl ester conformations are given as supporting information.

The four conformations found result from combinations of two rotomers of the HN−C_α and two rotomers of the C_α−CO bonds. As shown in Table 2, the original AMBER* parameters correctly ordered the NH−C_α rotomeric conformations (C3 and C4 less stable than C1 and C2), but the energy difference was ~2 kcal/mol higher than given by our *ab initio* results. Furthermore, the lowest energy conformers C1 and C2 were C_α−CO rotomers to which our original parameter set assigned approximately equal energies while quantum mechanics favored C2 by 2 kcal/mol. Thus there are substantial errors in conformational energies when α-amido esters are modeled using parameters taken from simple amides and esters.

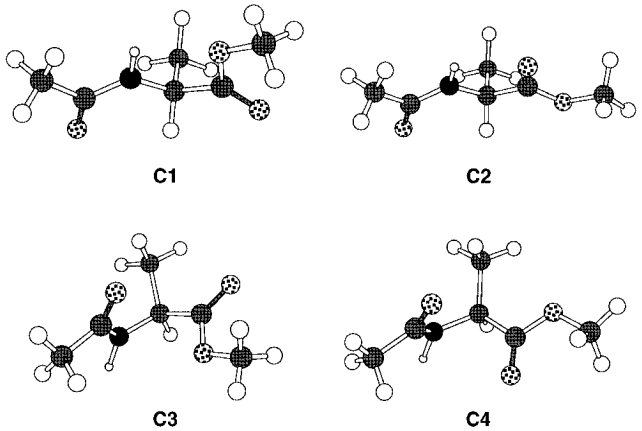

Figure 4. Structures of *N*-acetylalanine methyl ester conformations C1−C4.

We next developed new AMBER* torsion parameters specifically for α-amido esters that better reproduce the *ab initio* results (modified AMBER* in Table 2, the new torsional parameters are given as supporting information). Using these

(9) A recent study has concluded that calculations at the MP2/6-31G*//HF/6-31G* level give relative energies for conformations of amino acids which are in good accord with those obatined at the MP2/6-31+G*//MP2/6-31+G* level: Gronert, S; O'Hair, R. A. J. *J. Am. Chem. Soc.* **1995**, *117*, 2071.

Table 3. Number of Different Conformations Found after Energy Minimization of Structures from MC/SD Simulations

		number of structures		
host	guest	L guest	D guest	ratio D/L
1b	**3**	15	36	2.4
1b	**4**	8	25	3.1
1b	**5**	26	54	2.0
2b	**3**	14	34	2.4

new torsional parameters, we repeated our FEP calculations for the complexes which included alanine ester guests (**1b·3** and **1b·4**). The results of these new FEP calculations are shown as the second entries under $\Delta\Delta G_{calc}$ in Table 1. Though the new α-amido ester parameters result in significantly altered conformational preferences for the guests **3** and **4**, the calculated binding enantioselectivities with these guests are little changed relative to results with the original parameter set. For the weakly bound complex of **1b·4**, the calculated $\Delta\Delta G$ is only 0.1 kcal/mol higher than that calculated with the original parameters, and for the more tightly bound complex of **1b·3**, the $\Delta\Delta G$ is 0.3 kcal/mol higher than before. Although the latter result is closer to experiment, both new results are less than one standard deviation from the results obtained with the original parameters. Thus our calculations indicate that, *for this system*, binding enantioselectivity is not very sensitive to the detailed conformational preferences of the guest.

Though we obtain similar free energies of enantioselection with both parameter sets with **1b·3** and **1b·4**, our results should not be construed as indicating that careful force field parameterization is unnecessary for free energy calculations on molecular complexes. Indeed, force field validation and, if necessary, parameterization should be considered an essential step in any molecular modeling effort. In the case at hand, the insensitivity of enantioselection to guest torsional parameters may follow from the fact that C1-like and C2-like guest conformations appear electrostatically and sterically very similar to one another from points outside their surfaces.

Analysis of Simulation Trajectories. To determine how the structures of the complexes depend upon the chirality and nature of the guest, we analyzed the structures generated by 1 ns, 300 K MC/SD simulations of the diastereomeric complexes of peptides **3**−**5** with **1b** and peptide **3** with host **2b**. To estimate the amount of conformational space that was explored, 250 structures were sampled from each simulation and then subjected to energy minimization. The numbers of different[10] structures (minima) found for each complex during these simulations are summarized in Table 3.

Table 3 reveals a number of interesting trends. In all cases the enantiomer calculated to be more weakly bound (D) populates more than twice as many conformations as the more strongly bound enantiomer (L). This finding suggests that the enantioselection is enthalpically based, and an obvious source of an enthalpic driving force in a relatively nonpolar organic solvent (chloroform) is hydrogen bonding.

In each of the host−guest complexes, as many to five hydrogen bonds can be formed simultaneously between the host and the guest. To investigate a possible relationship between the number of intermolecular hydrogen bonds and the degree of binding enantioselectivity, trajectory-sampled structures of both diastereomers of **1·3**, **1·4**, **1·5** and **2·3** were analyzed for occurrence of intermolecular hydrogen bonds. Hydrogen-bonding populations were estimated by measuring the geometries around acceptor (O) and donor (H) atoms: a hydrogen bond was counted as present if the (N−)H···O distance was <2.5Å, if the N−H···O angle was >120° and (in the case of carbonyl acceptors) if the (N−)H···O═C angle was >90.[11] We found that most of the structures sampled during these simulations had either two or three intermolecular hydrogen bonds, but the stronger binding complexes, **1·(L)3** and **1·(L)4**, also had a considerable population (20−25%) of structures having four hydrogen bonds between the host and the guest. These same tightly bound complexes also had a significantly larger average number of intermolecular hydrogen bonds (3.1 and 2.8 hydrogen bonds for **1·(L)3** and **1·(L)4**, respectively) than the other complexes (2.0−2.6 hydrogen bonds). Thus calculated enantioselectivity of host **1** for L peptide guests **3** and **4** is largely a result of their ability to form more hydrogen bonds with **1**.

Conclusion

These results demonstrate that FEP calculations, using a simple force field potential and a continuum model for solvation, are able to give a good account of the experimentally observed enantioselective binding of peptides to two structurally well-defined synthetic receptors. Thus peptides found by experiment to be bound with high enantioselectivity (for L) were also highly enantioselective (for L) by calculation and poorly enantioselective peptides were also poorly enantioselective by calculation. We found that the ability to obtain converged ensemble averages was one of the most difficult and important factors that is necessary to obtain high-accuracy FEP results. Thus the weaker bound complexes, which were shown by analysis of simulation trajectories to explore more conformational space, have larger statistical uncertainties in the computed free energies of enantioselection than do the less flexible, more strongly bound complexes. It should be noted that these simulations were performed with our MC/SD method which is considerably more efficient in obtaining converged results than traditional dynamics methods.[12] Even so, the simulations for these relatively simple systems required up to 1 ns of simulation time per window to obtain results with an acceptable degree of convergence. Clearly, as others have emphasized, carrying out fully converged simulations is one of the major challenges to the successful application of FEP calculations.[13]

The driving forces for binding between receptors like **1** and **2** and peptide guests in organic solvents appear to be the formation of hydrogen bonds between the host and the guest molecules and, though the point was not addressed here, the ability of the guest to engage in attractive van der Waals contacts with the interior of the receptor cavity. Analysis of our simulations of these complexes indicates that the ability of one enantiomer of the guest to form, on average, a greater number of hydrogen bonds with the host than the other enantiomer is a major factor in determining the binding enantioselection.

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Supporting Information Available: HF/6-31+G* coordinates and energies for four conformations of *N*-acetylalanine methylamide, AMBER* torsional parameters for α-amido esters, and atomic partial charges for **1b**−**5** (6 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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(10) Unique structures where determined by checking each of the three possible positions for the C_3-symmetric guest.

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