Can Proteins and Crystals Self-Catalyze Methyl Rotations?

Jerome Baudry*,† and Jeremy C. Smith‡

School of Chemical Sciences, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801, and Computational Molecular Biophysics, IWR, University of Heidelberg, Heidelberg, Germany

Received: June 9, 2005; In Final Form: August 25, 2005

The χ ($C_\alpha-C_\beta$) torsional barrier in the dipeptide alanine (*N*-methyl-L-alanyl-*N*-methylamide) crystal was investigated using ab initio calculations at various levels of theory, molecular mechanics, and molecular dynamics. For one of the two molecules in the asymmetric unit the calculations suggest that rotation around the χ dihedral angle is catalyzed by the crystal environment, reducing by up to $\sim 2kT$ the torsional barrier in the crystal with respect to that in the gas phase. This catalytic effect is present at both low and room temperature and originates from a van der Waals destabilization of the minima in the methyl dihedral potential coming from the nonbonded environment of the side chain. Screening of a subset of the Protein Data Bank with a pharmacophore model reproducing the crystal environment around this side chain methyl identified a protein containing an alanine residue with an environment similar to that in the crystal. Calculations indicate that this χ torsional barrier is also reduced in the protein at low temperature but not at room temperature. This suggests that environment-catalyzed rotation of methyl groups can occur both in the solid phase and in native biological structures, though this effect might be temperature-dependent. The relevance of this catalytic effect is discussed in terms of its natural occurrence and its possible contribution to the low-frequency vibrational modes of molecules.

Introduction

Small peptides or peptide-like organic molecules, such as the alanine dipeptide, *N*-methylacetamide (NMA), and acetanilide, are well-suited for the study of the effects of nonbonded interactions on the structure, dynamics, and thermodynamics of protein constituents and are amenable to both experimental and high-level theoretical studies. Of particular interest are low-frequency conformational dynamics in biomolecular crystals, as they involve collective movements of atoms that are strongly influenced by nonbonded interactions. In previous work, methyl group energetics and dynamics were investigated using quantum chemical and empirical force field calculations and inelastic neutron scattering experiments. A cooperative effect of hydrogen bonding to the peptide groups on the torsional barrier around the ϕ and ψ dihedrals of NMA was found in ab initio calculations[1] and in molecular mechanics calculations.[2] The dynamics of the terminal (ϕ and ψ) methyl groups was further investigated in acetanilide[3] and the alanine dipeptide[4,5] by comparing empirical force field calculations of the density of states with experimental inelastic neutron scattering spectra. These studies showed that the low-frequency (<500 cm^{-1}) region of the density of states was correctly reproduced by normal mode calculations and molecular dynamics simulations and suggested that a relatively modest (less than ~ 1 kcal/mol) barrier was needed on the ψ dihedral angle to optimize the theoretical/experimental agreement.

In the above work, the focus was on terminal methyl groups which are chemically related to peptide rotations around the ϕ and ψ dihedral angles. In the present work, we investigate the alanine side chain dihedral, i.e., the χ ($C_\alpha-C_\beta$) torsional barrier. Comparison of experimental neutron scattering spectra with the calculated vibrational density of states indicated that rotations of the side chain methyl group contribute significantly to the low-frequency normal modes of the alanine dipeptide.[4,5] The same studies indicated also that rotations around the χ dihedral angle are coupled to rotations of the terminal methyl groups. We calculate here the ab initio χ torsional barrier of the alanine dipeptide in its crystal environment and compare the results with two different empirical force fields and simulation engines (CHARMM[6,7] and MOE/MMFF94s[8,9]) widely used to model the structure and dynamics of peptides and proteins. Subsequently, a protein environment around an alanine residue is identified that is similar to that of the crystal and where a catalytic effect on χ rotation is identified at low temperature. The potential of mean force for the rotations is calculated from molecular dynamics simulations, thus addressing the effect of the temperature on the χ barrier in the crystal and the protein environments.

Methods

Ab Initio and Empirical Calculations of Torsional Barriers. Atomic coordinates and symmetry operations for the crystal structure of the alanine dipeptide (*N*-methyl-L-alanyl-*N*-methylamide)[10] were obtained from the Cambridge Structural Database,[11] version 1.7 (CSD entry "LAANMA"). The crystal environment was built by applying the symmetry operations to the coordinates of the asymmetric unit and duplicating the corresponding unit cell in all dimensions using either the CHARMM,[6] version 22, or MOE,[8] version 2004, simulation engines. To limit CPU time, ab initio calculations were restricted to only the local environment of the side chain methyl groups. From the full crystal model, two different submodels were built, each including the two alanine dipeptide molecules of the

* To whom correspondence should be addressed. Phone: (217) 244 3210. E-mail: jerome@scs.uiuc.edu.
† University of Illinois at Urbana-Champaign.
‡ University of Heidelberg.

10.1021/jp053111z CCC: $30.25
Published on Web 10/08/2005

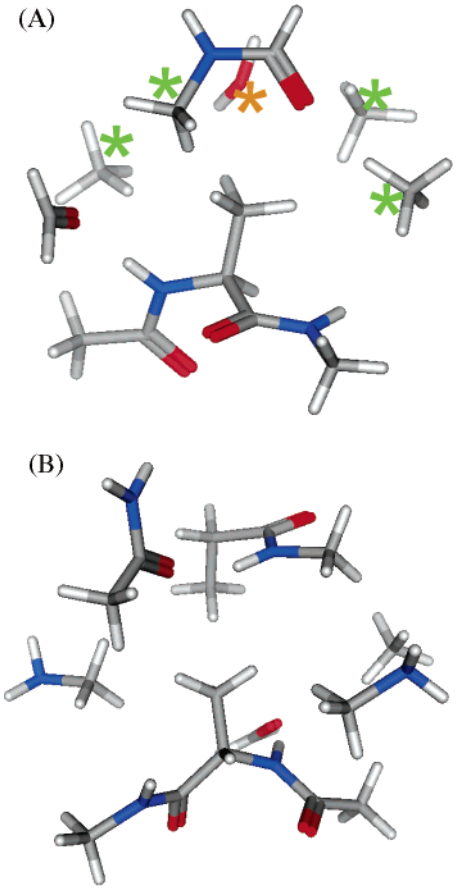

Figure 1. Alanine dipeptide and its environment as used in the ab initio calculations. (A, top) Molecule I. Green stars indicate methyl groups kept in the hydrophobic environment calculations. The orange star indicates the water molecule deleted from the protein pharmacophore screening. (B, bottom) Molecule II.

asymmetric unit and their respective methyl group environments. To build these two submodels, all atoms located within 4.5 Å of the β carbon of either side chain methyl group of the two dipeptide molecules were kept, together with the corresponding alanine dipeptide molecule, while all other atoms from the full crystal model were deleted. Hydrogen atoms were added to the unfilled valences created by the deletion of these atoms. These two models are shown in Figure 1. These two models explicitly include the local atomic environment of the methyl groups for each of the two alanine dipeptide molecules from the unit cell and at the same time are small enough to allow ab initio calculations to be performed in a reasonable time.

To calculate the χ torsional angle energy (the torsional angle of the methyl rotor), the two models were used in ab initio calculations as follows: Semiadiabatic calculations were run at the STO-3G level of theory using the program Spartan,[12] version 2004, with all atoms kept fixed except the (β) side chain methyl (carbon and hydrogen atoms), the α carbon and α hydrogen atoms, and the C═O and NH peptide groups bonded to the α carbon. The χ methyl dihedral angle (N−Cα−Cβ−H) was varied from −180° to −110° in 11 steps while allowing relaxation of the unfrozen atoms at each step. Calculations of this profile were performed for both models shown in Figure 1 and also for the isolated dipeptide molecule (with also only the central methyl, α carbon/hydrogen, and neighboring carbonyl and NH atoms free to move during the calculations). Other atoms are kept fixed to maintain the atomic positions around the methyl group in their crystalline structure. The structures corresponding to the minimum (−180°) and maximum (−118.75°) of the STO-3G-calculated profiles were used in single-point ab initio calculations at higher levels of theory using Spartan04. In addition to the ab initio calculations, adiabatic energy profiles (i.e., without fixing any atomic coordinates) of the χ torsional barrier were calculated in the entire crystal using the CHARMM22 empirical molecular mechanics force field and simulation engine[6,7] and also using the MMFF94s force field[9] within the program MOE,[8] version 2004. In both cases, the unit cell was constructed using periodic boundary conditions and duplicated in the *b* direction to fulfill the minimum image convention, i.e., to prevent an atom from interacting with its own image in periodic boundary conditions. For both CHARMM22 and MOE/MMFF94s, a cutoff switching function between 10 and 12 Å was applied to electrostatic and van der Waals interactions. In both the ab initio and empirical force field calculations, the χ methyl rotational barriers (ΔE) were calculated as the energy of the eclipsed conformation of the χ methyl minus that of the staggered conformation. $\Delta\Delta E$, a measure of the catalytic effect of the environment, was calculated by subtracting the ΔE of the isolated alanine dipeptide from that obtained with the crystal environment.

Molecular dynamics simulations were performed on the entire unit cell (duplicated in the *b* direction to fulfill the minimum image convention) using periodic boundary conditions. The simulations were performed in the canonical ensemble at an average temperature of 300 K using the program MOE and the MMFF94s force field. $\Delta A(\chi)$, the potential of mean force as a function of the χ dihedral angles for each of the two side chain methyls was calculated from $\rho_{(\chi)}$, the histogram distribution of the values of χ during a 10 ns simulation, using the formula $\Delta A(\chi) = -kT \ln[\rho_{(\chi)}]$, where k is the Boltzmann constant and T the temperature of the simulation. This relatively long simulation time ensured an efficient sampling of the reaction coordinate without having to rely on enhanced sampling methods such as umbrella sampling. All bins (3.6° width) in the histogram distribution of χ were populated during the molecular dynamics simulation. The free energy profiles calculated from the full 10 ns simulation and from the first 5 ns yield identical free energy barrier values, indicating converged free energy profiles.

Molecular dynamics simulation was also performed on an alanine dipeptide molecule in vacuo using the same force field, and the potential of mean force was calculated as in the case of the crystal simulations.

Protein "Pharmacophore" Search and Protein Calculations. "Pharmacophore models" of the alanine dipeptide and its environment as in "molecule I" (see below) were created using the pharmacophore facility of the program MOE[8] from the submodel used in the ab initio calculations (see Figure 1A). First, a 12-point pharmacophore model comprising all H-bond donor, H-bond acceptor, and hydrophobic features of the methyl environment of molecule I was used. Second, an 11-point pharmacophore model was obtained by deleting one H-bond acceptor feature from the 12-point pharmacophore model. These pharmacophore models were used to screen a subset of the Protein Data Bank[13] comprising 10823 protein structures, to identify alanine residues having all the environmental features of the alanine dipeptide in its crystalline environment. This subset included all protein structures with at least one nonion ligand and with a molecular weight of less than 180000. In proteins identified as positive from the 11-point pharmacophore search, hydrogen atoms were added to the PDB coordinates, and the structures were energy minimized with all heavy atoms fixed. Following this optimization of the hydrogen positions,

TABLE 1: Ab Initio and Empirical χ Rotational Barriers[a]

	STO-3G	HF/3-21G*	HF/6-31G*	B3LYP/6-31G*	CHARMM22	MMFF94s
			Molecule I			
ΔE^b	3.40	4.07	3.85	3.46	4.03	3.0
ΔE^c	2.91	3.77	2.69	2.65	2.63	2.4
$\Delta\Delta E$	−0.49	−0.30	−1.16	−0.81	−1.4	−0.6
			Molecule II			
ΔE^b	3.3	3.98	3.73	3.34	4.03	3.0
ΔE^c	4.2	4.78	4.45	3.78	5.75	6.36
$\Delta\Delta E$	0.9	1.05	0.72	0.44	1.72	3.36

[a] All values in kilocalories per mole. [b] In vacuo calculations. [c] Crystal environment as in Figure 1.

the adiabatic energy profile along the χ torsional angle for the alanine residue identified as the center of the pharmacophore model was calculated. In these energy profile calculations all atoms belonging to residues with at least one atom within 12 Å of any atom of the alanine residue were allowed to move during the minimization, with the exception of the heavy atoms of the protein backbone, which were held fixed.

A 5 ns molecular dynamics simulation of the C115A mutant of the MurA protein (PDB entry 1A2N) was performed using the CHARMM27 force field and the simulation engine NAMD2.[14] This protein was selected because it was identified as a positive for the 11-point pharmacophore search described above and one of its alanine residues exhibited environment-catalyzed methyl rotation (see the Results below). The ligand atoms were deleted from the crystal structure, and the protein structure was hydrated using a 10 Å thick layer of TIP3P water molecules. All atoms were allowed to move freely during the molecular dynamics simulation. The simulation was run in the canonical ensemble at a temperature of 300 K. Potentials of mean force for rotation around the χ dihedral angle of several alanine residues were calculated from the molecular dynamics trajectory as in the case of the crystal simulations. As in the case of the alanine dipeptide crystal, all bins (20° width) in the histogram distribution of χ were populated in the molecular dynamics simulation without having to use enhanced sampling methods.

Results

Ab Initio and Empirical Calculations of Torsional Barriers. Table 1 shows the ΔE and $\Delta\Delta E$ values calculated as described in the Methods for both dipeptide alanine molecules in the asymmetric unit at various levels of theory. One of the molecules in the crystal asymmetric unit (molecule I; see Figure 1A) possesses negative calculated ab initio $\Delta\Delta E$ values, indicating rotational barriers around the χ dihedral angle that are lower in the crystal environment than in the isolated molecules, the reduction being ~0.6 to ~1 kcal/mol, depending on the level of theory. In this case, then, the crystal environment catalyzes the χ torsional angle rotation. This effect is also obtained by empirical force field calculations using both the CHARMM22 and MMFF94s potential energy functions. In contrast to molecule I, the other molecule in the asymmetric unit (molecule II) exhibits $\Delta\Delta E$ values that are higher than those of the isolated molecule, indicating that the crystalline environment hinders rotation around this molecule's χ dihedral angle. As was the case for molecule I, this effect is also reproduced by the empirical force field calculations, though the amplitude of the barrier appears to vary significantly with the force field/molecular mechanics engine used.

To examine the relative influence of hydrophobic and hydrophilic moieties in the alanine environment, ab initio calculations of the torsional barriers were performed on a subset of the molecule I model comprising only the four methyl groups located "above" the alanine central side chain (i.e., the four methyl groups indicated by a green star in Figure 1), together with the full alanine dipeptide. In addition to this "hydrophobic" environment, the same calculations were also performed using only the "hydrophilic" environment of the alanine dipeptide, i.e., in which the four hydrophobic methyl groups were deleted. The ΔE and $\Delta\Delta E$ values listed in Table 2 show that a catalytic effect of the χ torsional barrier is found at most levels of calculation in this hydrophobic-only environment but that this effect is of smaller magnitude than in the "full environment" calculation shown in Table 1. Calculations using a hydrophilic environment do not exhibit a catalytic effect (with the exception of a small effect in the 6-31G* level calculations). This indicates that, while the hydrophobic environment appears to be mainly responsible for the catalytic effect on the χ rotation, a balance between hydrophobic and hydrophilic features of the environment must exist for the "full" catalytic effect of the environment on the χ torsional barrier to appear. The contributions of the hydrophobic and hydrophilic environments are, however, not additive: the effect of the hydrophilic environment can be thought to amplify the effect of the hydrophobic environment.

TABLE 2: Ab Initio χ Rotational Barriers Due to Hydrophobic and Hydrophilic Components of the Methyl Environment[a]

	STO-3G	HF/3-21G*	HF/6-31G*	B3LYP/6-31G*
ΔE^b	3.80	4.58	3.72	3.45
$\Delta\Delta E$	0.4	0.51	−0.13	−0.01
ΔE^c	2.80	4.01	3.07	3.14
$\Delta\Delta E$	−0.6	−0.06	−0.78	−0.32

[a] All values in kilocalories per mole. [b] Hydrophilic environment only. [c] Hydrophobic environment only.

The origin of the catalytic effect in molecule I was investigated by calculating the dihedral, electrostatic, and van der Waals contributions to the total potential energy barrier using the MMFF94s force field. The results are shown in Figure 2A,B. In Figure 2A, corresponding to the alanine dipeptide in vacuo, the dihedral and van der Waals contributions to the total barrier are in phase and the total barrier is the sum of these two contributions. The contribution to the total barrier from the electrostatic term is negligible. In contrast, in Figure 2B (corresponding to molecule I in the crystal), the van der Waals and dihedral profiles are nearly in opposite phases, yielding a partial cancellation of these two terms. Thus, the catalytic effect observed in the side chain of molecule I does not originate from reduced contributions of dihedral and van der Waals terms to the total potential energy barrier. Indeed, the magnitudes of these contributions are higher in the case of molecule I in the crystal (dihedral contribution, ~2.3 kcal/mol; van der Waals contribution, ~1.7 kcal/mol) than in the case of the dipeptide in vacuo (dihedral contribution, ~2 kcal/mol; van der Waals contribution, ~0.7 kcal/mol). Rather, the catalytic effect of the crystal

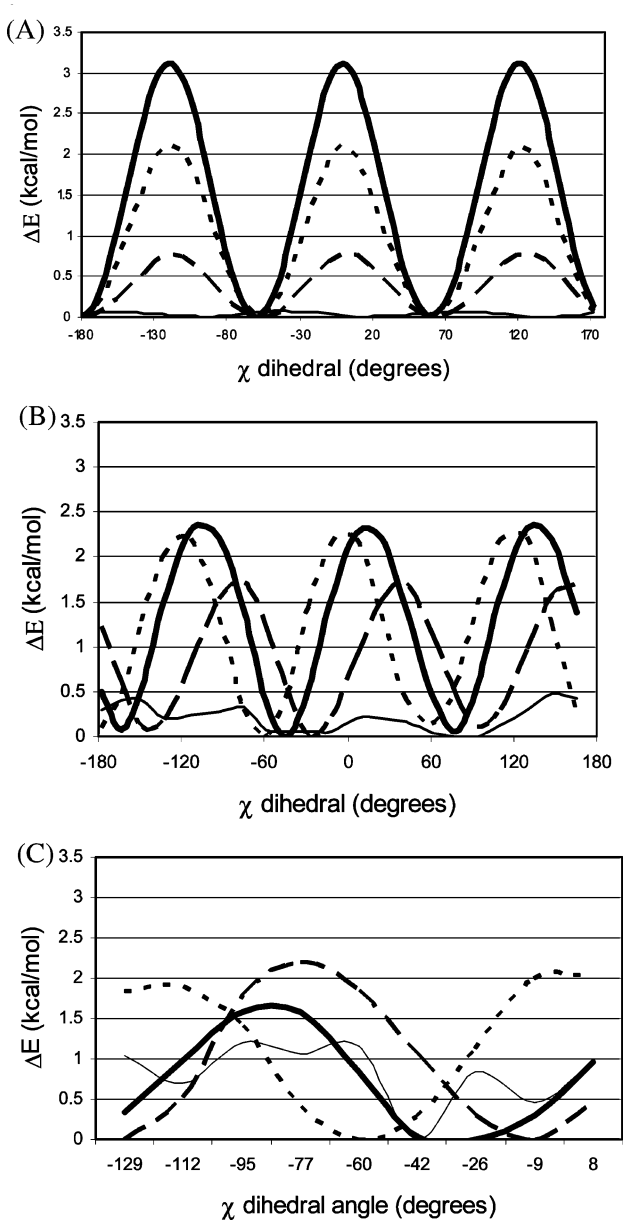

Figure 2. Potential energy profile: (thick line**)** total energy, (···) dihedral contribution, (---) van der Waals contribution, (thin line) electrostatic contribution, (A, top) dipeptide in vacuo, (B, middle) molecule I in the crystal, (C, bottom) Ala 195 in protein 1A2N.

environment on the χ rotation originates from a nonbonded (van der Waals) destabilization of the dihedral minima. In the case of rotations around molecule II's χ dihedral angle, the relatively high barriers (5.75−6.36 kcal/mol; see Table 1) originate from a large van der Waals contribution of ∼3.4 kcal/mol, in phase with a dihedral term contribution of ∼2.5 kcal/mol and a contribution from the angle term (∼0.8 kcal/ mol) of the potential energy function. For this molecule II, where the methyl rotation is not catalyzed by the environment, additive (as in the case of alanine dipeptide in vacuo) van der Waals and torsional terms contribute to the relatively high barrier. The high van der Waals contribution, and the presence of a contribution from the "angle" term of the potential energy function that was not present in the case of the in vacuo and molecule I profiles, suggest that relatively important steric effects appear to hinder rotations around the χ dihedral angle. The adiabatic calculations performed here do not allow this steric hindrance to relax, unlike in the case of the molecular dynamics simulation at room temperature (see below).

Protein Pharmacophore Search. Screening of the subset of the Protein Data Bank using a 12-point pharmacophore model did not identify any protein with exactly the same environment as that of the molecule I methyl group in the dipeptide crystal. However, deleting an H-bond acceptor feature (the "water molecule" atoms indicated with an orange star in Figure 1A) gave an 11-point pharmacophore that resulted in the identification of four proteins with alanine residues and environments that fulfill the pharmacophore description. The corresponding PDB entries (and in parentheses the alanine positions in the protein sequences) are 1V4V (Ala 358), 1I1Q (Ala 434), 1UEA (Ala 195), and 1A2N (Ala 195). Calculations of the corresponding χ torsional barriers, as described in the Methods, identified the Ala 195 residue of PDB entry 1A2N as having a barrier of 1.6 kcal/mol, i.e., less than the 3.3 kcal/mol χ torsional barrier of an isolated alanine residue. In contrast, the three other alanine residues investigated in the other PDB entries had barriers of 5.9 kcal/mol (1V4V), 4.5 kcal/mol (1I1Q), and 3.5 kcal/mol (1UEA). Thus, in the case of the Ala 195 residue of PDB entry 1A2N the protein environment has a catalytic effect comparable to that of the crystal environment of the molecule I methyl group of the alanine dipeptide in the crystal. This effect originates, as in the case of crystalline dipeptide molecule I, from a van der Waals destabilization of the dihedral minima, as shown in Figure 2C.

As shown in Figure 3B,C, the alanine methyl environments in 1A2N (displaying a catalytic effect) and 1V4V (not displaying a catalytic effect) both possess the characteristics defined by the 11-point pharmacophore model, as required by the pharmacophore screening procedure. However, there are also additional functional groups that render the environments different from each other and from that of the alanine dipeptide crystal. Since the alanine residues of interest in both proteins in Figure 3B,C belong to α helices (unlike the backbone of the alanine dipeptide), the hydrophilic feature surrounding the α carbon in Figure 3A originates in part from the alanine backbone but also from the carbonyl and NH groups of other residues within the α helix. Furthermore, other functional groups located in the vicinity of the alanine side chain methyl were also not present in the crystal structure, and these groups create a different nonbonded environment in each of the proteins identified though the screening procedure.

Potential of Mean Force for Rotations around the χ Dihedral Angle. Figure 4A shows the free energy profile for rotations around both side chain methyl groups in the crystal, obtained from a 10 ns molecular dynamics simulation, as described in the Methods. The free energy profile for the χ rotation in molecule I exhibits free energy barriers on the order of ∼2.5 to ∼3 kcal/mol, i.e., close to the 2.4 kcal/mol potential energy barriers calculated from adiabatic calculation (Table 1). The free energy profiles for rotation around χ in molecule II as well as for the in vacuo alanine dipeptide exhibit barriers that are ∼3.4 kcal/mol. This barrier is somewhat similar to the adiabatic barrier calculated for the isolated alanine dipeptide and is significantly lower than the adiabatic (i.e., energy minimized at each point of the calculation, corresponding to low-temperature behavior) potential energy barrier of 5.75−6.36 kcal/mol calculated for molecule II, suggesting that molecular dynamics at 300 K relieved the steric hindrances responsible for the adiabatic potential energy barrier. However, this barrier of ∼3.4 kcal/mol is higher than the free energy barrier of molecule I. The difference in barrier height between molecules I and II (and the alanine dipeptide in vacuo) is slightly more than k_BT (k_BT = 0.6 kcal/mol at 300 K). These results

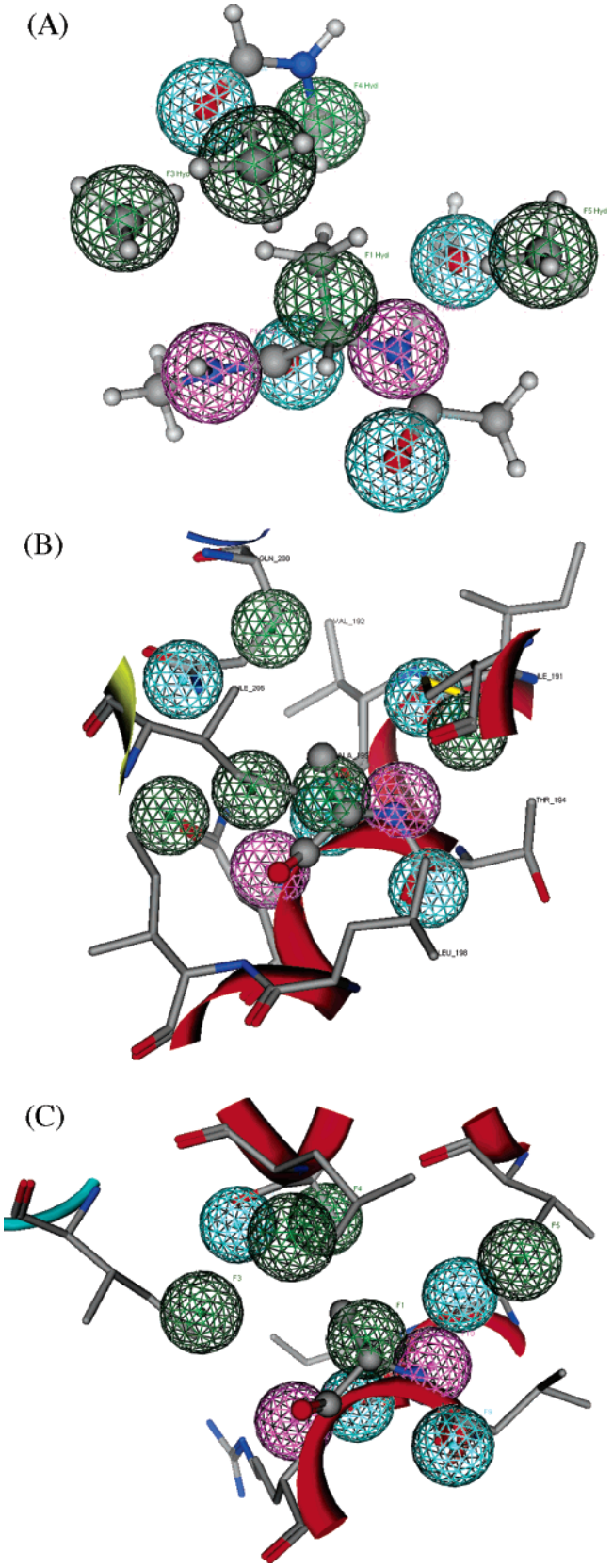

Figure 3. Pharmacophore models. (A, top) From molecule I's environment in the crystal (green, hydrophobic; blue, H-bond acceptor; purple, H-bond donor). (B, middle) In 1A2N. The Ala residue is rendered in ball-and-stick format. Hydrogens have been omitted for clarity. (C, bottom) In 1V4V. The Ala residue is rendered in ball-and-stick format.

indicate that, in the case of the alanine dipeptide crystal, the catalytic effect obtained from the adiabatic (corresponding to

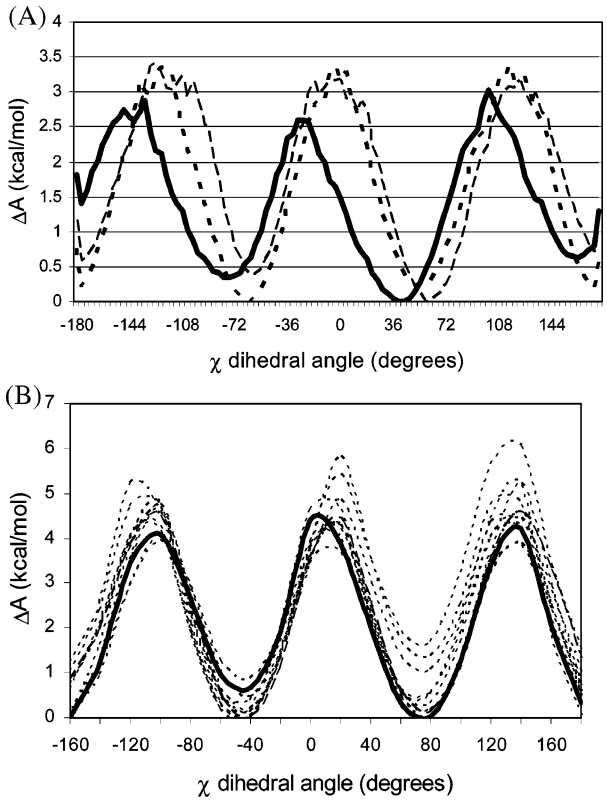

Figure 4. Potential of mean force profiles for rotation around the χ dihedral angle from molecular dynamics simulations. (A, top) Alanine dipeptide simulations: (thick line) dipeptide molecule I, (···) dipeptide molecule II, (---) dipeptide in vacuo. (B, bottom) Protein 1A2N simulation: (thick line) Ala 195, (···) several randomly chosen alanine residues.

low temperature) calculations is also present at room temperature, and by approximately the same magnitude ($\Delta\Delta E \approx 0.6$ kcal/mol from the adiabatic MMFF94s results as indicated in Table 1), though molecule II's methyl group appears to be significantly less hindered at room temperature than at low temperature ($\Delta\Delta E \approx 0$ kcal/mol, compared to $\Delta\Delta E = +3.36$ kcal/mol from the adiabatic MMFF94s results as indicated in Table 1).

Figure 4B shows the free energy barriers for rotation around several alanine χ angles in the protein 1A2N, calculated from the 5 ns molecular dynamics simulation of the hydrated protein at 300 K. The thick line corresponds to the profile calculated for alanine 195. While the χ barrier for this side chain is slightly lower than several other profiles calculated for various alanine residues, it is not systematically lower and is significantly above the 1.6 kcal/mol potential energy barrier obtained from adiabatic calculations and above the 3.0−3.3 kcal/mol adiabatic and free energy barriers calculated for the isolated alanine dipeptide. Therefore, in the protein, the free energy barrier at a temperature of 300 K does not exhibit the same clear catalytic effect as the (low-temperature) adiabatic calculations.

Discussion and Perspectives

Ab initio calculations suggest that barriers for rotations around the χ torsional angle of the alanine dipeptide molecule can be ~0.8 to ~3 kcal/mol higher when the molecule is crystallized than in the isolated molecule (molecule II) but also that rotation around the same dihedral angle can be catalyzed when the methyl environment is favorable (e.g., molecule I), reducing the rotational barrier by as much as $2kT$ ($kT \approx 0.6$ kcal/mol at 300 K), depending on the level of theory of the calculation.

This effect is reproduced in empirical (molecular mechanics) force field adiabatic (low-temperature) energy minimization calculations. Previously, empirical force fields have been shown to correctly reproduce certain subtle effects in peptides, such as the cooperative effect of multiple peptide group hydrogen bonds on the rotational barrier of the ϕ and ψ torsions in model organic molecules.[1,2] Here, we have shown that the two different empirical force fields used in the calculations, used in two different simulation engines, qualitatively agree in reproducing the catalytic effect observed in the ab initio calculations. This effect may be important for understanding vibrational spectra of the alanine dipeptide and, more generally, for understanding solid-state peptide molecular dynamics.

In earlier comparisons between experimental and calculated inelastic neutron scattering vibrational spectra of the alanine dipeptide, it was shown that the alanine methyl torsions vibrate predominantly in the low-frequency region (below $\sim$500 cm^{-1}) of the vibrational inelastic neutron scattering spectrum.[4] A detailed analysis of the contribution of the individual methyl groups to the low-frequency region of the spectra indicated that methyl torsional vibrations give rise to multiple peaks and that the peak positions are sensitive to the effective force constant of the methyl rotors.[3] The present catalytic effect would, a priori, result in lowering the frequencies of the corresponding central methyl group with catalyzed rotations (molecule I), while the hindered rotation of the other central methyl (molecule II) would result in shifting to higher frequencies. As observed in the detailed experimental/theoretical comparison of the neutron scattering spectra, the different methyl groups in the alanine dipeptide are coupled to each other[3] and vibrations of the χ dihedral angle contribute significantly to the high-intensity region between 230 and 500 cm^{-1}. It is therefore possible that the catalytic effect described here has an influence, through coupling with the terminal methyl, on the lowest frequency vibrational modes of the molecule in its solid-state form. Potential of mean force calculations suggest that the dynamics of the crystal at 300 K also exhibit a lower energy barrier for molecule I than for molecule II and the dipeptide alanine in vacuo. This suggests that the catalytic effect identified in low-temperature adiabatic calculations can probably exist at room temperature in the alanine dipeptide crystal, even though the magnitude of this affect is close to *kT*.

A comparable catalytic effect, identified using molecular dynamics simulations, was reported earlier for the alanine side chain of the crystalline cyclic peptide cyclo(Ala-Pro-D-Phe)$_2$.[15] The catalyzed rotation of the χ torsion seen in ref 15 thus demonstrates that the alanine dipeptide crystal is not the only material in which this catalytic effect can exist. As shown in the pharmacophore screening and the MMFF94s/MOE calculations, the protein environment of an alanine residue can also lead to the effect. The environments of the alanine side chain are different and more diverse in the proteins identified here through pharmacophore screening than in the alanine dipeptide crystal. It was found that only one out of four of these environments could lead to a catalytic effect, which suggests that, while different environments are capable of reducing the χ torsional barrier, this effect is sensitive to minute structural details. This is also exemplified by the fact that it was not possible to identify a protein with all the pharmacophore features of the crystal and that one H-bond acceptor had to be deleted from the pharmacophore query to obtain protein hits by pharmacophore screening. The effect of temperature on the alanine χ torsional barrier in the protein is different from that in the crystal. The free energy profile calculated for rotation of 1A2N's alanine 195 in the 300 K molecular dynamics simulation does not appear to be significantly lower than that of other alanine residues in the same protein during the same molecular dynamics calculations. The profile for Ala 195 also exhibits barriers that are significantly higher than the 1.6 kcal/mol adiabatic potential energy barrier. In this case, unlike in the case of the crystal structure, the dynamics of the protein at room temperature allows the environment of the methyl to fluctuate such that the catalytic effect is not preserved. In the case of the protein, the catalytic effect seems to be present only in a specific environment and is stable at low temperature, as exemplified by the adiabatic calculations, but is disrupted upon heating. This translates into a (rather unusual) rotational hindrance that increases with the temperature.

Is such a catalytic effect a rare natural curiosity, with a limited role in protein dynamics, or is it a more common phenomenon that may play, when present, a role in low-frequency vibrational protein dynamics and molecular crystals? In the present study, the catalytic effect is reported only for an alanine in a particular crystal environment and in only one protein identified from a subset of the Protein Data Bank. It will be of great interest to search for additional occurrences (if any) of a similar environment-induced catalytic effect, including possibly nonbiological molecules. Here, all 11 pharmacophore points around, and including, the alanine residue were required to be present for the protein to be identified as a positive during the pharmacophore screening. A less stringent pharmacophore model or a pharmacophore model based on the cyclic peptide structure described in ref 15 could possibly identify other alanine (and non-alanine) environments of side chains in which a catalytic effect might exist and verify whether similar environments lead to similar catalytic effects.

It will be interesting to characterize the apparent temperature dependence of the catalytic effect, to possibly identify a potential transition temperature above which the structural fluctuations suppress the low-temperature catalytic effect, and its potential link to the $\sim$180−210 K transition in protein dynamics.[16] Finally, it has been shown that ligand binding induces low-frequency vibrational changes in the protein−ligand complexes involving frequency shifts from higher to lower frequencies.[17] It would thus also be of interest to examine whether a catalytic environment might be formed upon protein−ligand binding or protein folding/structural transitions and in such occurrences whether this might influence ligand binding thermodynamics and kinetics.

References and Notes

(1) Guo, H.; Karplus, M. *J. Phys. Chem.* **1992**, *96*, 7273.

(2) Baudry, J.; Smith, J. C. *J. Mol. Struct.: THEOCHEM* **1994**, *308*, 103.

(3) Hayward, J.; Smith, J. C. *Biophys. J.* **2002**, *82*, 1216.

(4) Kneller, G. R.; Doster, W.; Settles, M.; Cusack S.; Smith, J. C. *J. Chem. Phys.* **1992**, *95*, 8864.

(5) Baudry, J.; Hayward, R. L.; Middendorf, H. D.; Smith, J. C. *J. Mol. Struct. Dyn. (Biol. Macromol. Dyn.)* **1997**, 49.

(6) Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. *J. Comput. Chem.* **1983**, *4*, 187.

(7) Mackerell, A. D.; Bashford, D.; Bellott, M.; Dunbrack, R. L., Jr.; Evenseck, J.; Field, M. J.; Fischer, S.; Gao, J.; Guo, H.; Ha, S.; Joseph, D.; Kuchnir, L.; Kuczera, K.; Lau, F. T. K.; Mattos, C.; Michnick, S.; Ngo, T.; Nguyen, D. T.; Prodhom, B.; Reiher, I. W. E.; Roux, B.; Schlenkrich, M.; Smith, J. C.; Stote, R.; Straub, J.; Watanabe, M.; Wiorkiewicz-Kuczera, J.; Yin, D.; Karplus, M. All-atom Empirical Potential for Molecular Modeling and Dynamics Studies of Proteins. *J. Phys. Chem. B* **1998**, *102*, 3586.

(8) MOE, version 2004.03, The Chemical Computing Group, Montreal, Canada.

(9) Halgren, T. A. *J. Comput. Chem.* **1999**, *20*, 720.
(10) Harada, Y.; Iitaka, Y. *Acta Crystallogr.* **1974**, *30*, 1452.
(11) Allen, F. H. *Acta Crystallogr.* **2002**, *58*, 380.
(12) Spartan, version 04, Wavefunction, Inc., Irvine, CA.
(13) Berman, H. M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T. N.; Weissig, H.; Shindyalov, I. N.; Bourne. P. E. *Nucleic Acids Res.* **2000**, *28*, 235.
(14) Kalé, L.; Skeel, R.; Bhandarkar, M.; Brunner, R.; Gursoy, A.; Krawetz, N.; Phillips, J.; Shinozaki, A.; Varadarajan, K.; Schulten, K. *J. Comput. Phys.* **1999**, *151*, 283.
(15) Kitson, D. H.; Hagler, A. T. *Biochemistry* **1988**, *27*, 5246.
(16) Tournier, A. L.; Smith, J. C. *Phys. Rev. Lett.* **2003**, *91* (20), 208105.
(17) Balog, E.; Becker, T.; Oettl, M.; Lechner, R.; Daniel, R.; Finney, J. L.; Smith, J. C. *Phys. Rev. Lett.* **2004**, *93* (20), 2028103.