Solvation Properties of N-Substituted Cis and Trans Amides Are Not Identical: Significant Enthalpy and Entropy Changes Are Revealed by the Use of Variable Temperature ^{1}H NMR in Aqueous and Chloroform Solutions and ab Initio Calculations

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The cis/trans conformational equilibrium of *N*-methyl formamide (NMF) and the sterically hindered *tert*-butylformamide (TBF) was investigated by the use of variable temperature gradient ^{1}H NMR in aqueous solution and in the low dielectric constant and solvation ability solvent $CDCl_3$ and various levels of first principles calculations. The trans isomer of NMF in aqueous solution is enthalpically favored relative to the cis ($\Delta H° = -5.79 \pm 0.18$ kJ mol^{-1}) with entropy differences at 298 K ($298 \cdot \Delta S° = -0.23 \pm 0.17$ kJ mol^{-1}) playing a minor role. The experimental value of the enthalpy difference strongly decreases ($\Delta H° = -1.72 \pm 0.06$ kJ mol^{-1}), and the contribution of entropy at 298 K ($298 \cdot \Delta S° = -1.87 \pm 0.06$ kJ mol^{-1}) increases in the case of the sterically hindered *tert*-butylformamide. The trans isomer of NMF in $CDCl_3$ solution is enthalpically favored relative to the cis ($\Delta H° = -3.71 \pm 0.17$ kJ mol^{-1}) with entropy differences at 298 K ($298 \cdot \Delta S° = 1.02 \pm 0.19$ kJ mol^{-1}) playing a minor role. In the sterically hindered *tert*-butylformamide, the trans isomer is enthalpically disfavored ($\Delta H° = 1.60 \pm 0.09$ kJ mol^{-1}) but is entropically favored ($298 \cdot \Delta S° = 1.71 \pm 0.10$ kJ mol^{-1}). The results are compared with literature data of model peptides. It is concluded that, in amide bonds at 298 K and in the absence of strongly stabilizing sequence-specific inter-residue interactions involving side chains, the free energy difference of the cis/trans isomers and both the enthalpy and entropy contributions are strongly dependent on the N-alkyl substitution and the solvent. The significant decreasing enthalpic benefit of the trans isomer in $CDCl_3$ compared to that in H_2O, in the case of NMF and TBF, is partially offset by an adverse entropy contribution. This is in agreement with the general phenomenon of enthalpy versus entropy compensation. B3LY/6-311++G** and MP2/6-311++G** quantum chemical calculations confirm the stability orders of isomers and the ΔG decrease in going from water to $CHCl_3$ as solvent. However, the absolute calculated values, especially for TBF, deviate significantly from the experimental values. Consideration of the solvent effects via the PCM approach on NMF·H_2O and TBF·H_2O supermolecules improves the agreement with the experimental results for TBF isomers, but not for NMF.

1. Introduction

Peptide bond isomerization is important in many processes that require alternation of peptide and protein structure, e.g., transport of peptides through membranes, oligomerization, delayed chain folding, and biocatalysis of peptides and proteins.[1−3] Therefore, numerous studies have been reported on the hindered internal rotation of peptide bonds, and a variety of statistical, theoretical, X-ray, and spectroscopic techniques have been applied.[1−8] The presence of an N-alkyl group in the peptide bond is of particular interest because of the resulting increase in population of the cis isomer.

Intra- and intermolecular (hydration) effects might be the primary factors that contribute to the difference in stability between cis and trans peptides in aqueous solution. Peptide bonds interact strongly with water;[7,9] therefore, even a modest difference between the free energies of hydration of the cis and trans isomers would have a significant bearing on peptide and protein structure. The difference in enthalpy for the cis and trans isomers of X-Pro bonds in aqueous solution has been established to be zero for model peptides;[10−12] however, other investigators reported a variety of values up to 5.4 kJ/mol.[13−16] Radzicka et al.[17] have found that the cis/trans equilibrium of simple amides is almost completely insensitive to the solvent environment, and $\Delta G°$ of solvation of the cis and trans isomers appears to differ by less than 0.4 kJ mol^{-1}. It was concluded that the transfer of a peptide bond from the interior of a protein to an exposed position on its surface would not, in itself, be expected to change its intrinsic preference for the cis or trans configuration.

Nuclear magnetic resonance (NMR) and theoretical calculations are among the primary methodologies in investigating cis/trans peptide bonds with emphasis to the effects of solvents, temperature, pH, intra- and intermolecular hydrogen bonds, and

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determination of rates and barriers to conformational isomerization.[4,5,13−22] Detailed information, however, on the thermodynamic origin of the cis/trans peptide bonds is rather limited, and the results are often contradictory.

In this paper, we report the combined application of variable temperature gradient ^{1}H NMR in solution and theoretical calculations of *N*-methylformamide (NMF) and the sterically hindered *tert*-butylformamide (TBF). By carrying out variable temperature NMR experiments in water and in a low dielectric constant and solvation ability solvent, like $CDCl_3$, and quantum chemical calculations, we can explicitly investigate the thermodynamic origin of the peptide cis/trans conformational equilibrium. This study provides the first direct evidence that both enthalpy and entropy are strongly dependent on the N-alkyl substitution of the amide bond and the nature of the solvent. Comparison is also made with literature data of model peptides.

2. Materials and Methods

N-methylformamide and *tert*-butylformamide, D_2O, and $CDCl_3$ were purchased from Sigma and were used without further purification. ^{1}H NMR spectra of NMF and TBF in 90% H_2O/10% D_2O, concentration 20 mM, and $CDCl_3$, concentration 2mM, were recorded on a Bruker AMX-400 instrument (University of Ioannina) and on a Bruker AVANCE 800 instrument (University of Florence) equipped with a 5 mm z-gradient probe. Temperature calibration was achieved by the use of 4% methanol in methanol-d_4.

All calculations were carried out using the *Gaussian 03* code.[23] Geometry optimizations, without imposing symmetry constraints, were performed by the use of Becke's three-parameter hybrid functional[24] combined with the Lee, Yang, and Parr (LYP)[25] correlation functional, denoted as B3LYP; the 6-311++G(d,p) standard basis set was employed for all the involved atoms. Harmonic vibrational frequencies were computed using the analytical B3LYP/6-311++G(d,p) second derivatives, and zero-point energy corrections were determined and included in all the relative energies calculations.

The polarizable continuum model (PCM) approach[26,27] was used for the fully optimized calculations in water and $CHCl_3$ employing the same functional and basis set, as in the gas-phase study. The PCM tool allows one to work with cavities of realistic molecular shapes with the surface of the cavity subdivided into small portions (tessera).[28] The solute−solvent electrostatic interaction is represented by a set of polarization point charges, placed in the center of each tessera. The cavity in which the solute is placed, defined in terms of interlocking spheres centered on non-hydrogen atoms, was built up using the Gepol procedure[29] and the UAHF atomic radii.[30] The effect of outlying charge was taken into account by means of an additional effective charge, distributed according to solute electronic density. The solvation free energy includes the electrostatic, dispersion/repulsion, and cavitation contributions. The cavitation term was determined using Pierotti's scaled particle theory,[31] and the dispersion/repulsion term was calculated using semiempirical atom−atom parameters.[32]

3. Results and Discussion

Variable Temperature NMR Experiments. The cis/trans equilibrium of a peptide group can be described by the equilibrium constant K_{eq}

$$K_{eq} = \frac{[\text{trans}]}{[\text{cis}]} \tag{1}$$

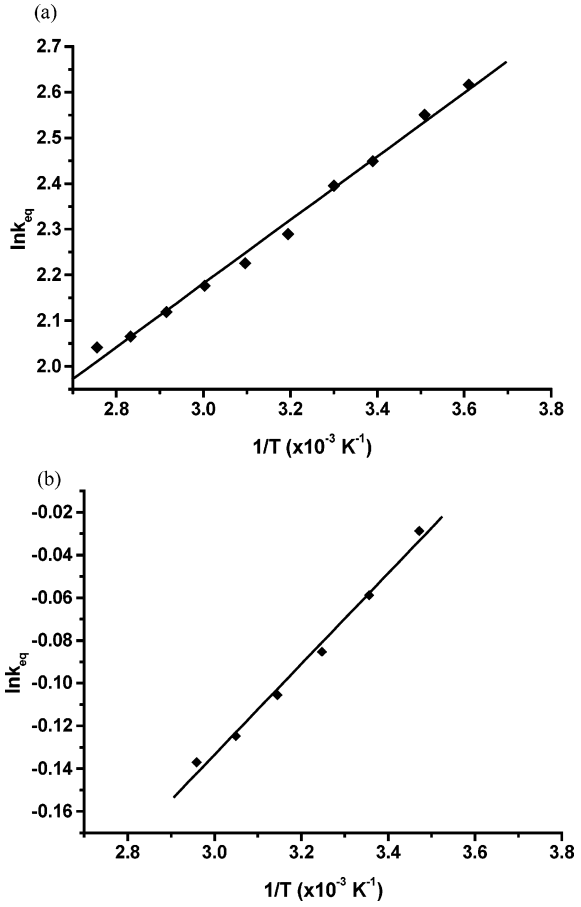

Figure 1. Representative Van't Hoff plots based on the cis/trans equilibrium NMR integration data of (a) $-CH_3$ protons of NMF and (b) $-C(CH_3)_3$ protons of TBF in 90% H_2O/10% D_2O, concentration 20 mM. The solid lines represent the best fits to eq 3.

which is related directly to the free energy $\Delta G°$

$$\Delta G° = -RT \ln K_{eq} \tag{2}$$

The thermodynamic parameters of the cis/trans isomerism in water can be obtained from variable temperature experiments, provided that the cis/trans forms are present simultaneously and the relative concentrations of the two species can be determined accurately. Once the equilibrium constant for this process has been determined at several temperatures, then, according to the Van't Hoff equation

$$\ln K_{eq} = -\frac{\Delta H°}{RT} + \frac{\Delta S°}{R} \tag{3}$$

a plot of ln K_{eq} versus $1/T$ provides $\Delta H°$ and $\Delta S°$. Because of the limited sensitivity of ^{1}H NMR, applications of this probe are, in practice, confined in the range of free energies between approximately 8.4 kJ mol^{-1} > $\Delta G°$ > −8.4 kJ mol^{-1}. One of the attractive features of this technique is that, within this range, small differences in $\Delta G°$ on the order of tenths of a kilojoule per mole are readily discernible.

The cis/trans equilibrium of NMF is most readily measured by the relative integrals of the $-CH_3$ proton resonances. For $-CH_3$, the relative chemical shifts between the cis and trans isomers are on the order of 0.15 ppm, and hence, the resonances of the two isomeric forms are well-separated at 400 MHz. Figure 1(a) shows a representative Van't Hoff plot in 90% H_2O/10% D_2O. The line represents the best fit to eqs 2 and 3 with a

TABLE 1: Apparent Enthalpy ($\Delta H°$), Entropy ($\Delta S°$, $T\cdot\Delta S°$ (298 K)), and Free Energy (ΔG (298 K)) Changes for the Conversion of the Cis to Trans Isomer for NMF, TBF, and Model Peptides

peptide	solvent	$\Delta H°$ (kJ mol^{-1})	$\Delta S°$ (J K^{-1} mol^{-1})	$T\cdot\Delta S°$ (298 K) (kJ mol^{-1})	ΔG (298 K) (kJ mol^{-1})
NMF	90% H_2O/10%D_2O	-5.79 ± 0.18	0.77 ± 0.57	0.23 ± 0.17	-6.02 ± 0.18
TBF	90% H_2O/10%D_2O	-1.72 ± 0.06	-6.28 ± 0.20	-1.87 ± 0.06	0.15 ± 0.06
NMF	$CDCl_3$	-3.71 ± 0.17	3.42 ± 0.65	1.02 ± 0.19	-4.73 ± 0.19
TBF	$CDCl_3$	1.60 ± 0.09	5.75 ± 0.33	1.71 ± 0.10	-0.11 ± 0.10
Gly-L-Pro[15]	D_2O	-4.2	-9.7	-2.91	-1.29
Ac-Gly-[β,δ-^{13}C]Pro-OMe[16]	90% H_2O/10%D_2O	-5.33	-1.05	-0.32	5.01
Ac-L-Pro-D-Ala-NHMe[38]	90% H_2O/10%D_2O	-5.14	-5.47	-1.64	-3.50

correlation coefficient of 0.96, $\Delta H° = -5.79 \pm 0.18$ kJ mol^{-1} and $\Delta S° = 0.77 \pm 0.57$ J K^{-1} mol^{-1} (Table 1).

The experimental cis/trans equilibrium of TBF in 90% H_2O/10% D_2O was also found to be temperature dependent. At 298 K, the amount of the trans isomer is about 48%. Unequivocal assignment of the trans isomer was achieved by a 2D $^1H-^1H$ NOESY experiment, which demonstrates the through-space proximity of the C(O)H and NH protons.[33,34] This assignment is further supported by the magnitude of the $^3J_{NHC(O)H}$ coupling constant.[4,33,34] For $-C(CH_3)_3$, the relative chemical shift difference between the cis and trans isomers is ~0.016 ppm, and hence, the resonances are strongly overlapped at 400 MHz. However, at 800 MHz, the $-C(CH_3)_3$ protons are well-separated, and thus, the cis/trans equilibrium of TBF is readily measured by their relative integrals. Figure 1(b) shows a representative Van't Hoff plot. The line represents the best fit to eq 3 with a correlation coefficient of 0.92, $\Delta H° = -1.72 \pm 0.06$ kJ mol^{-1} and $\Delta S° = -6.28 \pm 0.20$ J K^{-1} mol^{-1} (Table 1).

Figure 2 shows that the experimental data of the cis/trans equilibrium of NMF (a) and TBF (b) in $CDCl_3$ cannot be approximated by a linear Van't Hoff plot. It is well-known that eq 3 holds when the temperature range is small and the difference in heat capacities is approximately zero. To overcome this problem in the case of $CDCl_3$, we introduced the heat capacity under constant pressure in eq 3. Enthalpy and entropy changes are given as follows:

$$\frac{d(\Delta H)}{dT} = \Delta C_p \tag{4}$$

and

$$\frac{d(\Delta S)}{dT} = \frac{\Delta C_p}{T} \tag{5}$$

and the heat capacity can be approximated by

$$\Delta C_p = A_0 + A_1 T + ... \tag{6}$$

where A_0, A_1, and so forth are constants. Assuming $\Delta C_p = A_0$, the Van't Hoff eq 3 can, therefore, be rewritten as

$$\ln k_{eq} = -\frac{\Delta H°_{298}}{RT} + \frac{\Delta S°_{298}}{R} + \frac{A_0}{R}\left(\ln\frac{T}{298} - \frac{T-298}{T}\right) \tag{7}$$

The three-variable eq 7 was used for the simulation of the experimental data in $CDCl_3$.

Table 1 shows the apparent enthalpy ($\Delta H°$), entropy ($\Delta S°$, $T\Delta S°$ (298 K)), and free energy ($\Delta G°$ (298 K)) changes for the conversion of the cis to trans isomers for NMF and TBF in 90% H_2O/10% D_2O and $CDCl_3$ solution. It is strikingly apparent that $\Delta H°$ and $\Delta S°$ differ markedly in the two amides. For NMF in 90% H_2O/10% D_2O, the trans form is strongly favored enthalpically and very weakly favored entropically relative to the cis form. The entropy difference for the cis/trans equilibrium is only 0.77 ± 0.57 J K^{-1} mol^{-1}, which demonstrates that the free energy difference $\Delta G°$ at 298 K (-6.02 ± 0.18 kJ mol^{-1}) should be attributed to steric effects, with hydration differences playing a minor role. These results are in agreement with detailed NMR investigations of ^{17}O shieldings of NMF in a variety of solvents, which suggested that the cis and trans amide oxygens are fully exposed to the solvent and, in aqueous solution, are equally solvated by two molecules of water.[33] For NMF in $CDCl_3$, the trans form is also strongly favored enthalpically ($\Delta H° = -3.71 \pm 0.17$ kJ mol^{-1}) with a small favorable contribution of entropy ($\Delta S° = 3.42 \pm 0.65$ J K^{-1} mol^{-1}; $T\Delta S°$ (298 K) $= 1.02 \pm 0.19$ kJ mol^{-1}). This demonstrates that, at room temperature, the free energy difference $\Delta G°$ should be attributed to steric effects with solvation differences playing a minor role.

For TBF both in 90% H_2O/10% D_2O and $CDCl_3$, the enthalpy difference for the cis/trans equilibrium is significantly reduced compared to that of NMF ($\Delta H° = -1.72 \pm 0.06$ and 1.60 ± 0.09 kJ mol^{-1} for H_2O and $CDCl_3$, respectively), and the entropy

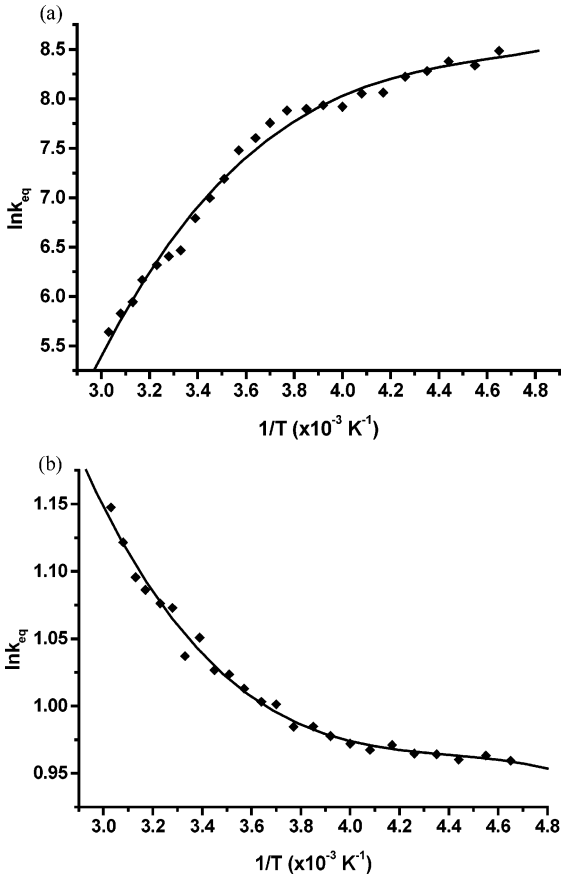

Figure 2. Representative Van't Hoff plots based on the cis/trans equilibrium NMR integration data of (a) $-CH$ protons of NMF and (b) $-CH$ protons of TBF in $CDCl_3$, concentration 2 mM. The solid lines represent the best fits to eq 7.

difference at 298 K ($T\Delta S° = -1.87 \pm 0.06$ and 1.71 ± 0.10 kJ mol^{-1}) becomes slightly larger, in absolute terms, than the effect of enthalpy (Table 1). This reduced enthalpy difference can be attributed to the strong steric effect of the bulky *tert*-butyl group and the reduced solvation of the CO group in the trans isomer. This result is in agreement with one-dimensional steady-state selective intermolecular ^{13}C and ^{1}H Overhauser effect studies of *tert*-butylformamide, which provided experimental evidence of differential hydration of the trans isomer, compared to the cis isomer, due to the presence of the bulky *tert*-butyl group.[33] Furthermore, ^{17}O NMR studies of N-substituted amides[34,35] indicated a deshielding of the ^{17}O resonance by 20 ppm of *trans-tert*-butylformamide compared to the cis isomer. This was attributed to reduced solvation and/or significant out-of-plane (torsion angle) deformation of the amide bond of the trans isomer.

Interestingly, a decreasing enthalpy benefit of the trans isomer of NMF in $CDCl_3$ compared to that in H_2O is partially offset by an adverse entropy effect. This phenomenon is even more pronounced in the case of TBF. In $CDCl_3$, the trans isomer is enthalpically less stable than the cis ($\Delta H° = 1.60 \pm 0.09$ kJ mol^{-1}); however, this enthalpic benefit of the cis isomer is offset by an adverse restricted solvation entropy. This phenomenon of enthalpy−entropy compensation, originally suggested by Jenks et al.,[36,37] is of fundamental importance to molecular associations in situations where the binding enthalpy is comparable to the thermal energy *RT* (~2.5 kJ mol^{-1} at 298 K). Several thermodynamic studies, especially those of Williams and collaborators,[38,39] have highlighted a relationship between the enthalpy of association and the corresponding entropy change in solution associations and in biological recognition studies.

Table 1 lists the experimental enthalpy ($\Delta H°$), entropy ($\Delta S°$, $T\Delta S°$ (298 K)), and free energy $\Delta G°$ (298 K) changes for the cis and trans isomers of some model peptides in aqueous solution. Cheng and Bovey[15] investigated by the use of ^{13}C NMR glycyl-L-proline and concluded that the trans isomer is favored by enthalpy but disfavored by entropy (Table 1). The latter was attributed to greater solvent immobilization by the trans isomer. More recently, Eberhardt et al.[16] investigated the thermodynamic origin of prolyl peptide bond isomers of the racemic dipeptide Ac-Gly-[β,δ-^{13}C]Pro-OMe in aqueous and toluene solutions. It was concluded that the difference in $\Delta G°$ for the X-Pro isomers originates almost entirely from enthalpic differences ($\Delta H° = -5.33$ kJ mol^{-1}) both in aqueous buffer and in toluene solution. Differences in entropy, though small, favor the cis isomer in both aqueous and toluene solutions. This entropic preference was found to be lower in aqueous ($\Delta S° = -1.05$ J K^{-1} mol^{-1}) than in toluene solution ($\Delta S° = -2.97$ J K^{-1} mol^{-1}). This was interpreted by assuming a lower solvent accessibility of the amide CO group in the trans isomer, which diminishes the ability of this group to restrict H_2O molecules through hydrogen bonding.

Variable temperature ^{1}H NMR studies in H_2O of the blocked model dipeptide Ac-L-Pro-D-Ala-NHMe (Ac is the amino-terminal blocking group $-COCH_3$ and $-NHCH_3$ is the carboxyl-terminal blocking group) indicated also that the trans form is enthalpically favored and entropically disfavored relative to the cis form.[40] This model dipeptide is one of the simplest molecules compatible with β-turn folding containing an intramolecular hydrogen bond of the ($i + 3$) → i type[41−43] in a variety of organic solvents and in the crystalline state.[44] The difference in $\Delta G°$ for the cis and trans isomers of the Ac-L-Pro bond originates primarily from enthalpic differences, in agreement with Eberhardt et al.[16] However, the contribution of entropy was found to be appreciably larger in absolute terms. This was attributed to the significant folding of the trans isomer and, thus, reduced disorder or configurational freedom.[40]

From the experimental data of model peptides (Table 1), it is evident that the entropy term indicates significant variation, which is not easy to interpret because of the complex interplay of peptide−solvent conformational, rotational, and translational changes. Investigation, therefore, of model amides that are conformationally more restricted than peptides could be more informative. Thus, the experimental reduced enthalpy difference between the two isomers of TBF in H_2O ($\Delta H° = -1.72 \pm 0.06$ kJ mol^{-1}) is accompanied by a significant contribution of entropy ($\Delta S° = -6.28 \pm 0.20$ J K^{-1} mol^{-1}), which further destabilizes the trans isomer. In $CDCl_3$, the trans isomer of TBF is further destabilized enthalpically compared with the cis ($\Delta H° = 1.60 \pm 0.09$ kJ mol^{-1}); however, there is a stabilizing contribution of entropy ($\Delta S° = 5.75 \pm 0.33$ J K^{-1} mol^{-1}). The present data, therefore, provide evidence for the thermodynamic significance of the release of bound water molecules around the polar but uncharged peptide group. These results seems not to be in agreement with the widespread notion that water dissolvation of polar groups is entropically favorable.[45−48] Presumably, this is due to the fact that nonionic hydrophilic groups, such as amide bonds, do not normally form hydrogen bonds as easily as water.

This adverse entropic effect of the release of bound molecules of water around polar groups, such as amide bonds, is, therefore, worthy of further notice. Rossky and Karplus[49] made a short (1.5 ps) molecular dynamics (MD) simulation of a dipeptide in water. The rotational correlation times were found to increase by a factor of 3 for the first layer of water next to the hydrophobic groups of the amino acids, whereas the water next to the hydrophilic parts of the peptide was found to be much less affected (correlation times increased by about 50%). Berendsen et al.[50] made a 40 ps simulation of a hydrate crystal of pancreatic trypsin inhibitor. They concluded that water molecules remained about 10 ps in the ordered water positions found in the X-ray structure. Wong and McCammon[51] simulated trypsin in aqueous solution and found the diffusion coefficients to vary from 1.6×10^{-9} to 5×10^{-9} m^2/s for water molecules 3−15 Å from the protein surface, respectively. This is to be compared with 3.6×10^{-9} m^2/s from a bulk simulation of water. Ahlstrom et al.[52] performed a 106 ps molecular dynamics simulation of the calcium-binding protein parvalbumin in water. It was concluded that structure and dynamics of interfacial water are only marginally affected by the presence of the protein. More recently, elegant NMR experiments based on 2D and 3D homonuclear NOE- and ROE-type dipolar cross-relaxation processes between water protons and polypeptide protons indicate that there is no evidence for preferentially ordered solvent molecules around charged or polar groups on the surface of proteins.[53,54]

Quantum Chemical Calculations. To get further information on the cis/trans equilibrium, for both NMF and TBF, we have performed B3LYP/6-311++G** quantum mechanical calculations in vacuo and in the liquid phase, considering the free molecules as well as their complexes with one and two water molecules.

As is evident from the results collected in Table 2, in vacuo both molecules prefer the trans form ($\Delta E_{cis-trans} = -4.54$ and -6.67 kJ/mol for NMF and TBF, respectively). Ab initio calculations at the CCSD(T) level confirm the preference for the trans isomers for both species. If we take into consideration

TABLE 2: Cis/Trans Isomerization Energies (kJ/mol) at Different Levels of Theory Referred to the Most Stable Complexes Depicted in Figures 3 and 4

system	method	$\Delta E^{(SCF)}$ cis−trans	ΔG cis−trans
NMF	B3LYP/6-311++G** in vacuo	−4.54	
NMF	CCSD(T)/6-311++G**//B3LYP/6-311++G** in vacuo	−6.15	
NMF·H_2O	B3LYP/6-311++G** in vacuo	6.60	
NMF·H_2O	CCSD(T)/6-311++G**//B3LYP/6-311++G** in vacuo	3.13	
NMF·$2H_2O$	B3LYP/6-311++G** in vacuo	7.08	
NMF·$2H_2O$	CCSD(T)/6-311++G**//B3LYP/6-311++G** in vacuo	4.46	
NMF	B3LYP/6-311++G**PCM (solvent = H_2O)		−3.57
NMF·H_2O	B3LYP/6-311++G** PCM (solvent = H_2O)		−0.61
NMF·$2H_2O$	B3LYP/6-311++G** PCM (solvent = H_2O)		3.39
NMF	B3LYP/6-311++G**PCM (solvent = $CHCl_3$)		−3.43
TBF	B3LYP/6-311++G** in vacuo	−6.67	
TBF	CCSD(T)/6-311++G**//B3LYP/6-311++G** in vacuo	−9.63	
TBF·H_2O	B3LYP/6-311++G** in vacuo	2.09	
TBF·H_2O	CCSD(T)/6-311++G**//B3LYP/6-311++G** in vacuo	3.76	
TBF·$2H_2O$	B3LYP/6-311++G** in vacuo	8.67	
TBF	B3LYP/6-311++G** PCM (solvent = H_2O)		−3.39
TBF·H_2O	B3LYP/6-311++G** PCM (solvent = H_2O)		−1.91
TBF·$2H_2O$	B3LYP/6-311++G** PCM (solvent = H_2O)		1.03
TBF	B3LYP/6-311++G**PCM (solvent = $CHCl_3$)		−3.03

the bulk solvent effects through PCM, the stability order of the two forms of the isolated NMF and TBF molecules is confirmed both in water and in $CHCl_3$ solution. However, since reaction field theory does not account for specific interactions between the solute and the solvent, such as hydrogen bonding, these effects have been considered explicitly by adding one or two molecules of water to NMF and TBF.

The different NMF·H_2O and NMF·$2H_2O$ complexes are depicted in Figure 3. With the addition of one water molecule, the more stable complex of the cis form of NMF appears to be that having a bifurcated hydrogen bond between the carbonyl oxygen and the water hydrogen and between the amidic hydrogen and the water oxygen (Figure 3(a)). The complex (b), which is characterized by a single hydrogen bond, lies 12.1 kJ/mol higher in energy. The more stable complex of the trans form is that having one hydrogen bond between the carbonyl oxygen and the water hydrogen followed by the complexes (d) and (e) of Figure 3, which are higher in energy by 1.26 and 6.74 kJ/mol, respectively. The cis/trans isomerization energy becomes positive and equal to 6.60 kJ/mol, which is the opposite of what is observed experimentally. The CCSD(T) value of 3.13 kJ/mol confirms the higher stability of the cis form. The systems with two water molecules give rise to many different geometrical arrangements. The most stable ones for the cis and trans forms are presented in Figure 3. The addition of the second water molecule stabilizes the cis form both at the B3LYP level ($\Delta E_{cis-trans}$ = 7.08 kJ/mol) and at the CCSD(T) ($\Delta E_{cis-trans}$ = 4.46 kJ/mol) level. When the energy of the NMF·H_2O supermolecule is calculated via PCM (see Table 2), the trans isomer becomes more stable than the cis one by only 0.61 kJ/mol, whereas the cis isomer is preferred in the NMF·$2H_2O$−PCM computations.

For the TBF·H_2O complex, as in the case of NMF, we have explored several topological situations. The most stable supermolecules are those in which TBF interacts with water through its carbonyl moiety, as shown in Figure 4. The more stable cis form is stabilized in vacuo by a bifurcated hydrogen bond, whereas the trans one shows a single hydrogen bond (see Figure 4). $\Delta E_{cis-trans}$ values were found to be 2.09 and 3.76 kJ/mol at B3LYP and CCSD(T) levels, respectively. The lowest-energy isomers obtained by adding two water molecules are depicted in Figure 4; the cis form is again preferred over the trans one by 8.67 kJ/mol at the B3LYP level.

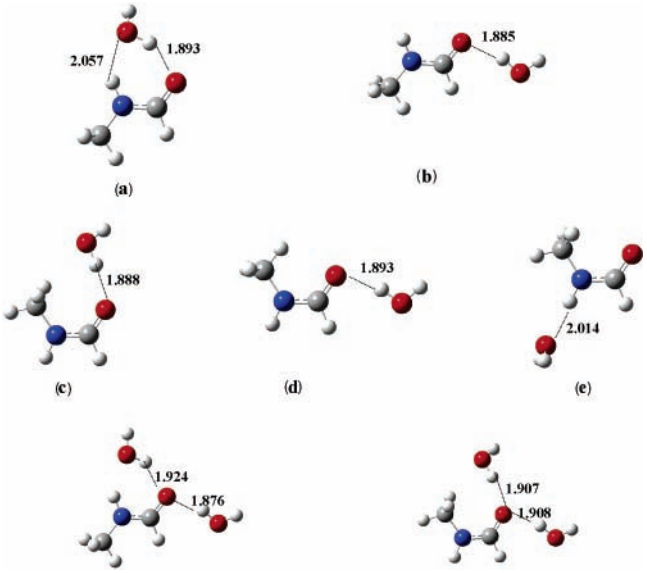

Figure 3. Specific interactions of a single H_2O molecule with cis (a, b) and trans (c, d, e) NMF in vacuo. Bond lengths are in angstroms.

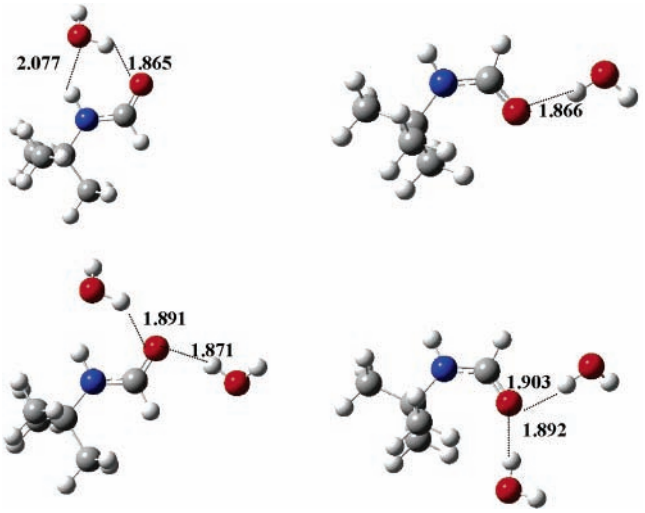

Figure 4. Specific interactions of a single H_2O molecule with the most stable cis (left) and trans (right) TBF isomers in vacuo. Bond lengths are in angstroms.

For TBF·H_2O when bulk solvent effects are taken into account through the PCM approach, the Gibbs free energy difference decreases (−1.91 kJ/mol) compared to that for NMF, in agreement with the experimental values (0.16 kJ/mol). Finally, when the TBF·$2H_2O$ is placed in the bulk solvent, $\Delta E_{cis-trans}$ of 1.03 kJ/mol was obtained. In these cases, it seems that the combination of supermolecule and PCM approaches gives better agreement with the experimental data.

In any case, these results indicate that the two forms have practically the same energy and are equally populated. Our theoretical value of the free energy difference is at the limit of the theoretical accuracy, and it can be assumed that the two isomers are degenerate in energy.

The PCM computations in $CHCl_3$ solution gives $\Delta G_{cis-trans}$ values of −3.43 and −3.03 kJ/mol for NMF and TBF, respectively, following the experimental trends. In general, we can underline that the absolute difference between the values of free energy of solvation is too small and falls into the range of the theoretical uncertainty.

Finally, it is worth noting that the cis and trans isomers of TBF and TBF·H_2O are planar in vacuo as well as in solution. The same planarity was obtained for NMF, in agreement with previous MP2/6-311G* results.[55] On the other hand, the gas electron diffraction experiments (GED) are reproduced equally well with a planar equilibrium structure or with a pseudo-planar skeleton.[56] In the case of *N*,*N*-dimethylformamide and *N*,*N*-dimethylcarbamyl chloride, the GED technique indicates a slight deviation from planarity (about 3°), which was attributed to the large-amplitude out-of-plane vibrations of an exactly planar structure. It is worth noting that microwave and infrared spectra of gaseous formamide account for a planar structure[57] confirmed by theoretical computations.[58]

In conclusion, variable temperature ^{1}H NMR studies of the cis/trans conformational equilibrium of N-substituted amides in aqueous and $CDCl_3$ solutions demonstrate that $\Delta H°$ and $\Delta S°$ values indicate significant variations. In planar amides at 298 K and in the absence of bulky N-substitution, the thermodynamic difference of the cis/trans isomers is mainly due to enthalpic differences in both H_2O and $CDCl_3$ solution with a significant contribution of entropy. In sterically hindered amides, the contribution of entropy at 298 K becomes equal to the effect of enthalpy both in H_2O and $CDCl_3$ solution. A decreasing enthalpic benefit of the trans isomer in $CDCl_3$ compared to that in H_2O, in the case of NMF and TBF, is partially offset by an adverse entropy contribution, in agreement with the general phenomenon of enthalpy versus entropy compensation. The release of bound molecules of water around sterically hindered trans amide bonds is entropically unfavorable and provides a significant driving force for the thermodynamic origin of the cis/trans isomerism in aqueous solution. B3LY/6-311++G** and CCSD(T) calculations agree among them in the energy difference between cis and trans isomers in vacuo. Consideration of solvent effects via the PCM approach on NMF·H_2O and TBF·H_2O supermolecules improves the agreement with the experimental data for TBF isomers, while this approach does not work properly for the NMF isomers. The same results were obtained when two water molecules were explicitly considered in the supermolecules. Further investigations, therefore, of model systems in a wide range of solvents with different dielectric constants and solvation abilities with particular emphasis on sterically hindered amides are needed. These studies would provide useful insights that guide estimates of enthalpy and entropy changes in the cis/trans isomerism.

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