Conformational Stability of Furfural in Aqueous Solution: The Role of Hydrogen Bonding

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The importance of hydration on the equilibrium involving internal rotation around carbon-carbon single bond is investigated for the furfural molecule dissolved in water. To analyze the solvent effects in stabilizing any preferred conformation of furfural, we perform Monte Carlo (MC) *NPT* simulations corresponding to different rotation angles of the carbonyl group. The hydrogen bonds formed between solute and solvent are also analyzed along the conformational equilibrium. They are found to be equivalent, both in number and in binding energy, for all rotation angles. These results give a strong evidence that the conformational stability and the rotation barrier of the furfural molecule in water relate to the bulk properties rater than solute-solvent hydrogen bonds.

1 Introduction

Biochemical processes are transformations that occur in, or by means of, living organisms. They involve a great variety of proteins, carbohydrates, lipids, steroids, and other substances which have important and specific activities. Yet all these complex substances make up only a minor portion of the total weight of biochemical systems. In fact the main constituent is water, present in amounts far exceeding the total of all the other components [1]. Furthermore, water is known to be the indispensable matrix for the structural components and the activities of living organism. Thus, an important characteristic feature of water is the property of hydration. Because of its high dielectric constant [2], water can dissolve better ionic compounds like salts, acids, and bases than any other medium. Also in aqueous environment compounds containing hydroxyl, carboxyl, amino, or keto groups are extensively hydrated [3]. All these groups can become interconnected with water molecules by hydrogen bonds [4, 5]. In virtue of these power to dissolve many different substances water is considered a universal solvent.

The importance of hydration for conformational stability of organic molecules and for the specificity and affinity of folding and binding events has long been recognized [3, 6]. It is clearly important to obtain a detailed understanding of the origin of the hydration effects so that observations can be correctly interpreted and that accurate predictions can be made in the course of designing biological processes. For instance, the hydrophobic effect [7] is considered to be the major driving force for the folding of globular proteins and the formation of membranes and micelles in aqueous solution [8]. Such phenomenon is related to the fact that water has no attraction by non polar groups held in any molecule [9]. Thus, hydrophobic substances tend to be concentrated by decreasing entropy [8], with the non polar groups dipped into the bulk, and the non polar groups clustered together in the surface of water.

Therefore, it is an increasing consensus that there is a considerable difference between studying the behavior of isolated molecules and their reactions in solution. Indeed, even the most basic characteristic of the molecules, their structures, may be strongly influenced by the solvent. The effects of solvents on the properties of organic and biological molecules have been successfully described by using different and complementary theoretical models [10-12]. In this direction a useful tool to investigate the solvation mechanism and the specific role of the solute-solvent interactions is provided by statistical mechanics simulation techniques [13]. Particularly, applications of the Monte Carlo (MC) method [13, 14] have permitted to obtain statistical averages of thermodynamic properties and solute-solvent configurations as well. Also, supermolecular structures can be efficiently extracted from the MC simulations to be used in subsequent quantum mechanical (QM) calculations [15-17].

Here, we direct attention to the study of the conformational equilibrium [18] of furfural (Fig. 1) in aqueous solution using MC simulations. The furfural molecule may rotate the carbonyl ($-$CHO) group around the carbon-carbon single bond and the specific equilibrium conformation is solvent-dependent. This single-bond rotation to some extension mimics the rotation around the peptide bond that is crucial for protein folding. Then, our major interest is to investigate the importance of water (a strong protic solvent) in stabilizing any specific rotation of the carbonyl group in

furfural. In doing this, we give a detailed statistical analysis of the hydrogen bonds formed between solute and solvent as a function of the rotational angle for the OO-cis $\rightleftarrows$ OO-trans interconversion [19]. To asses the effects of hydrogen bonding in stabilizing any preferential conformer of furfural along the conformational equilibrium, we also perform MC simulations of furfural in chloroform (a weaker protic solvent) and compare the results.

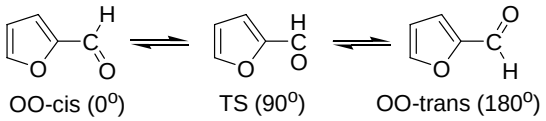

Figure 1. Conformational equilibrium of furfural. The carbonyl group ($-$CHO) rotates along the furan ring.

2 Computational Details

The conformational analysis of the furfural solutions is made using Monte Carlo (MC) statistical mechanics simulations with Metropolis sampling technique [13, 14]. The conformers are analyzed rotating the dihedral angle ($O_4C_5C_cO_c$, shown in Fig. 2) by 9° from OO-cis (0°) to OO-trans (180°). The MC simulations are carried out with one specific conformation of furfural plus 450 molecules (in the case of water) and 343 (in the case of chloroform). All systems are contained in cubic boxes that are surrounded by images of themselves to remove the edge effects. The calculations are performed in *NPT* (isotermal-isobaric) ensembles at temperature of 298.15 K and pressure of 1 atm. The intermolecular interaction is given by the three-parameter Lennard-Jones plus Coulomb potential [20] (ε_i, σ_i, and q_i) for each site i, j on molecules α and β:

$$U_{\alpha\beta}(r_{ij}) = \sum_{i}^{\text{on }\alpha}\sum_{j}^{\text{on }\beta}\left\{4\varepsilon_{ij}\left[\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}}\right)^{6}\right] + \frac{q_i q_j}{r_{ij}}\right\}, \quad (1)$$

where the geometric combining rules for the parameters in Eq. (1) are given by $\varepsilon_{ij} = (\varepsilon_i\varepsilon_j)^{1/2}$ and $\sigma_{ij} = (\sigma_i\sigma_j)^{1/2}$.

For the water molecules we use the SPC potential parameters [21], with C_{2v} symmetry, OH distance of 1.00 Å, and HOH angle of 109.47°. The chloroform molecules are modeled by the five-site potential parameter suggested by Barlette and Freitas [22]. For the furfural molecule we employ the OPLS-AA [23] set, but the atomic charges are calculated for each conformation with the CHELPG fitting method [24], at the MP2/6-311+G(d) level of theory [25]. We can notice that the oxygen atom in the carbonyl group (O_c) has always a larger negative charge than the ring oxygen (O_4). Thus, as we shall see further, the occurrence of hydrogen bond formation is more likely in O_c than in O_4 (Fig. 2).

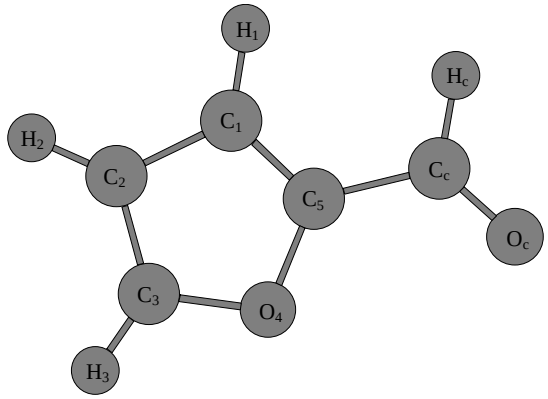

Figure 2. Symbols of the atomic sites in the furfural molecule showing the dihedral angle $O_4C_5C_cO_c$.

The MC simulations are carried out with the DICE code [26] and consist of a thermalization stage of 2.0×10^6 MC steps, followed by an averaging stage of 27.0×10^6 MC steps in the case of water; and 20.6×10^6 MC steps in the case of chloroform. The intermolecular interactions are spherically truncated for a center of mass separation larger than the cutoff radius, which is half of the cubic box, and long-range corrections are calculated beyond this distance. To select statistically uncorrelated configurations we use the autocorrelation function of the energy [15, 16, 27] that provides the correlation interval. In total, 60 configurations are obtained with less than 10% of statistical correlation. Using the radial distribution function we separate the first solvation shell, composed of one furfural molecule surrounded by 34 water molecules or one furfural molecule surrounded by 13 chloroform molecules.

3 Results and Discussion

Before discussing the solvent effects on the conformational equilibrium of furfural, it is interesting to mention that this molecule may exist in two possible conformations: OO-cis and OO-trans (Fig. 1). The OO-trans form is known to be more stable in the vapour, whereas the OO-cis form is more stable in polar media [18]. Recently, Baldridge *et al.* [12] showed that the conformer with larger dipole moment, OO-cis, prevails in solvents with dielectric constants higher than 5. However, the stability between both conformers does not obey a simple relationship with the dielectric constant [28]. This, of course, indicates that the solvent effects are more complex than could be inferred by a simple reaction field. The solvent dependence of the activation barrier for the cis-trans interconversion of furfural is really an important topic in biophysical chemistry.

Bain and Hazendonk [28] have used NMR measurements to determine the thermodynamic parameters for the rotation of the carbonyl group in furfural dissolved in toluene, acetone, and methanol. They have found large entropy effects for the non protics solvents, toluene and ace-

tone, and none for the protic solvent methanol. In toluene the activation entropy is obtained to be positive, indicating that the transition state is disordered compared to the planar form. Conversely, in acetone it is observed a negative activation entropy, which means that the transition state is ordered with respect to the planar form. However, in the case of methanol there is no preferred interaction between the solvent and any conformation of furfural.

This analysis is of interest because one normally expects that in protic solvents like water the hydrogen bonds formed with the oxygen atoms in the furfural molecule should favor one conformation along the internal rotation of the carbonyl group. To understand the specific role of solute-solvent interactions, we perform a detailed investigation of the hydrogen bonding along the OO-cis $\rightleftarrows$ OO-trans interconversion in water, a strong protic solvent [19]. We also compare our results performing MC simulations for furfural in a weaker protic solvent, chloroform. Then, we perform an structural analysis, as obtained via converged atomistic MC simulations.

3.1 Hydrogen Bonding Patterns

The number of hydrogen bonds formed between furfural and water, as well as the corresponding binding energies, are obtained as a function of the rotation angle $O_4C_5C_cO_c$ described in Fig. 2. Our results correspond to 20 different MC simulations, one for each conformation of furfural. These provide a microscopic description of the specific interaction involving the proton acceptor furfural and the proton donor water during the cis-trans interconversion. The hydrogen bond is defined using the geometric and energetic criteria [15, 29, 30], which are $R_{\rm OX} \leq 3.7$ Å, $\theta_{\rm OXH} \leq 36^\circ$, and positive binding energy, as given in Fig. 3. Along the carbonyl rotation in water, we have obtained the geometric parameters as given in Figs. 4 and 5, i.e., about 3.0 Å for the intermolecular O−O distance and 19° for the hydrogen bonding angle. Also, in chloroform, a very similar result is found, i.e., about 3.3 Å and 21°, respectively.

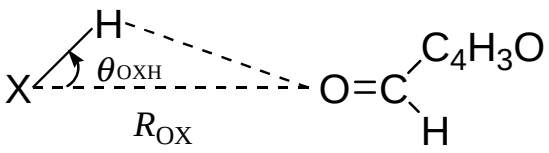

Figure 3. Geometric parameters for the hydrogen bond formation. The X atom represents oxygen in the case of water and carbon in the case of chloroform.

In aqueous solution, by considering only the most representative conformers of furfural, OO-cis, OO-90° (transition state), and OO-trans, we calculated that for the OO-cis 23% of the configurations make no hydrogen bond, 58% make one, 17% make two, and 2% make even three hydrogen bonds. Then, out of the 60 configurations selected there exist 58 hydrogen bonds in the OO-cis conformer, making

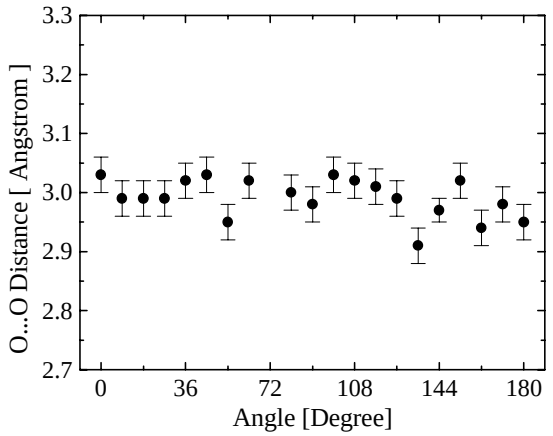

Figure 4. Intermolecular O−O distance ($R_{\rm OO}$) between furfural and water calculated as a function of the rotational angle $O_4C_5C_cO_c$.

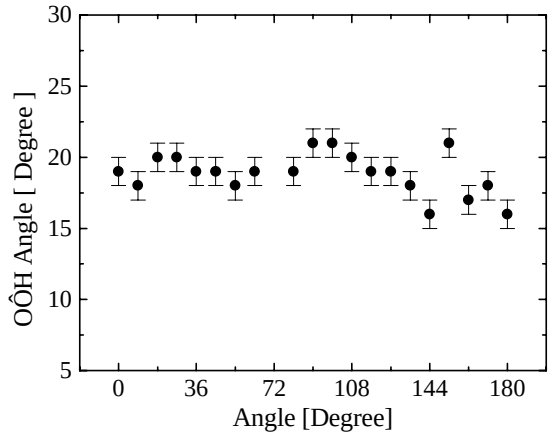

Figure 5. Hydrogen bonding angle ($\theta_{\rm OOH}$) between furfural and water calculated as a function of the rotational angle $O_4C_5C_cO_c$.

therefore, 0.97 hydrogen bond on average. This is the statistics of the hydrogen bonding for the solution of furfural in water. In fact, most of the hydrogen bonds have the carbonyl oxygen (O_c) as the proton acceptor. Similar results are obtained for the OO-trans conformer and transition state, with an average number of 1.13 hydrogen bonds in the first case and 0.84 for the transition state.

The total number of hydrogen bonds is remarkably stable, varying between a minimum of 0.75 hydrogen bond for the 135°−conformer, and a maximum of 1.25 hydrogen bonds for the 45°−conformer. This indicates that the hydrogen bonds do not contribute strongly to any conformation of furfural in water. These results lead to a small change in the binding energy along the rotational interconversion angle. For instance, in the OO-cis case the binding energy is calculated as 3.36 ± 0.70 kcal/mol, whereas the maximum value lies in 3.58 ± 0.80 kcal/mol for the 162°−conformer. In comparison, the minimum value lies in 2.83 ± 0.80 kcal/mol for the 63°−conformer. The binding energy for the OO-trans conformer is 3.45 ± 0.80 kcal/mol, i.e., 0.1 kcal/mol stronger than in the case of the OO-cis conformer. Indeed, as the standard deviation in the calculations

is 0.8 kcal/mol, we cannot distinguish the conformers. For the transition state the binding energy is only 0.5 kcal/mol weaker than in the planar forms. The main conclusion is that hydrogen bonds between furfural and the water are essentially equivalent for all conformations and therefore they make no significant contribution for any rotamer.

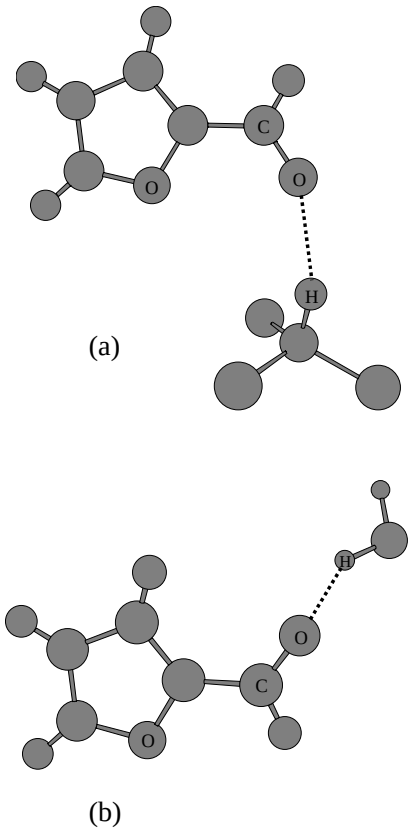

Figure 6. Hydrogen bonds formed between furfural and chloroform (a) and furfural and water (b) along the conformational equilibrium.

Now we compare the statistical analysis for the hydrogen bond formation of furfural dissolved in water by considering the conformational equilibrium of furfural in chloroform, a weaker protic solvent than water. In this case we consider the three more important conformers of furfural, OO-cis, transition state, and OO-trans. The statistics for the other conformations are very similar to that for water. In Fig. 6 we show two types of hydrogen bonds formed with arbitrary conformers of furfural in both solvents. Our results are summarized in Tables 1 and 2. As we can observe, a similar hydrogen bonding pattern is obtained dissolving furfural in water or chloroform. Here, the average number and the strength of hydrogen bonds formed between the solute and the solvent are essentially constant along the internal rotation, giving again no contribution to the activation entropy. These results are in fair agreement with previous observations from Bain and Hazendonk [28] for the investigation of furfural in methanol.

3.2 Stabilization of the Solute

Although the carbonyl group in furfural appears to make the hydrogen bonds less significant to stabilize any specific conformation of the solute, the entire dipole moment of the molecule is relatively high to allow an electrostatic stabilization. In gas phase the dipole moment of furfural is 3.97 D for the OO-cis form, 2.93 D for the transition state, and 3.23 D for the OO-trans form [18]. Thus, the difference in dipole moment for two species in equilibrium is large enough to drive the conformational stability. In polar solvents the more polar conformer is expected to be preferentially stabilized, as predicted by the reaction field theory [31]. For instance, in water the OO-cis form is predominant. However, in the case of chloroform (a low polarity solvent with dielectric constant of about 5) a simple reaction field analysis seems to be inappropriate in predicting which is the more stable conformer of furfural [12, 31]. In this case, the energy difference between the OO-cis and OO-trans forms tends to approximately zero [18]. As given in Table 1, the hydrogen bonding energy contribution predicts qualitatively that the higher dipole moment conformer, OO-cis, is more stable when dissolved in water. In fact, a dielectric constant around 5 (e.g. chloroform) seems to be the turning-point for the OO-cis $\rightleftarrows$ OO-trans change in the conformational equilibrium of furfural [12].

The polarization effects thats occur in the furfural molecule when dissolved in chloroform or water are better described by calculations of the dipole moment for the three more important conformers embedded in solvent and comparing them with the calculated dipole moments in the gas pase. Having obtained the solvation shells of furfural using the radial distribution functions, we treat the solvent as discrete molecules surrounding the solute. Then, dipole moment calculations are performed as averages over 60 MC configurations. To obtain these values we have employed a Monte Carlo/quantum mechanics (MC/QM) procedure with the *ab initio* MP2/6-31+G(d) level of theory [25], treating the molecules in the first solvation as point charges. We also compare our results, presented in Table 3, with the dipole moments calculated using the reaction field, where the solvents are characterized by their dielectric constants $\epsilon = 4.8$ (for chloroform) and $\epsilon = 78.5$ (for water). All calculations are carried out at the MP2/6-31+G(d) level with the same geometries of the furfural conformers. Using this method our calculated dipole moments for the gas phase are about of 0.2 D higher than the corresponding experimental values [18] of the more stable conformer, and only 0.08 D for the transition state.

Here, it is interesting to notice that the back polarization of the solute due to the reaction field appears to overestimate the solvent effects. As can be seen in Table 3, in the case of chloroform the reaction field predicts a dipole moment increase of 21% for the OO-cis form, and 23% for the OO-trans. Using our MC/QM procedure, we obtain polarizations 10% less than the reaction field for these cases. This

Table 1. Statistics of the hydrogen bond formation in water. The distance $R_{\rm OO}$ is given in Å, the angles $\theta_{\rm OOH}$ in degree, and binding energy $-\Delta E$ in kcal/mol. The standard deviations are also given.

Conformer	$\langle$No. of H-bonds$\rangle$	$\langle R_{\rm OO}\rangle$	$\langle\theta_{\rm OOH}\rangle$	$\langle -\Delta E/$H-bond$\rangle$
OO-cis	0.97	3.03 ± 0.23	19.5 ± 8.0	3.36 ± 0.70
Transition state	0.84	2.98 ± 0.23	20.8 ± 8.0	3.08 ± 0.60
OO-trans	1.13	2.95 ± 0.23	16.5 ± 8.0	3.45 ± 0.70

H-bonding energy contribution in H_2O: $\Delta E_{\text{cis-trans}} = -0.64$ kcal/mol.

Table 2. Statistics of the hydrogen bond formation in chloroform. The distance $R_{\rm OC}$ is given in Å, the angle $\theta_{\rm OCH}$ in degree, and the binding energy $-\Delta E$ in kcal/mol. The standard deviations are also given.

Conformer	$\langle$No. of H-bonds$\rangle$	$\langle R_{\rm OC}\rangle$	$\langle\theta_{\rm OCH}\rangle$	$\langle -\Delta E/$H-bond$\rangle$
OO-cis	1.07	3.26 ± 0.23	21.7 ± 8.0	3.26 ± 0.70
Transition state	0.97	3.24 ± 0.23	20.9 ± 8.0	3.21 ± 0.50
OO-trans	0.92	3.27 ± 0.23	20.7 ± 8.0	3.19 ± 0.60

H-bonding energy contribution in $CHCl_3$: $\Delta E_{\text{cis-trans}} = +0.56$ kcal/mol.

Table 3. Calculated dipole moments (in debye) of furfural in different media at the MP2/6-31+G(d). The statistical averages are performed over 60 uncorrelated MC configurations considering only the first solvation shell.

Dipole moment	OO-cis	Transition state	OO-trans
$\mu_{\rm gas}$	4.17	3.01	3.46
$\mu_{\rm SCRF/CHCl_3}$	5.06	3.49	4.26
$\langle\mu\rangle_{\rm MC/CHCl_3}$	4.64 ± 0.30	3.32 ± 0.19	3.91 ± 0.30
$\mu_{\rm SCRF/H_2O}$	5.50	3.71	4.67
$\langle\mu\rangle_{\rm MC/H_2O}$	5.37 ± 0.55	3.46 ± 0.40	4.26 ± 0.55

analysis is more interesting in the case of water, when we use only a dielectric constant to model the solvent effects. Going from the gas phase to water in the OO-cis conformation, the polarization is of 32%, whereas the MC/QM procedure gives an value of 29%. On the other hand, considering the less stable OO-trans form in water, the reaction field predicts a higher polarization of 35%. As expected, this conformer is found to be less polarized, with a dipole moment increase of 23%, when we use the MC/QM scheme.

4 Concluding Remarks

Water is the most important solvent in nature. Because of its high dielectric constant and ability to form hydrogen bonds, the hydration effects can control many different molecular processes. In this work we have investigated the mechanism of hydration on the conformational equilibrium of furfural. It is well known that this molecule can exist in two stable conformations, OO-cis and OO-trans, separate by a relatively large interconversion barrier. In aqueous solution, the conformer with the higher dipole moment (OO-cis) is the most stable. Comparing this stability in chloroform the reverse occurs, i.e., the conformer with lower dipole moment (OO-trans) is the most stable. However, considering both solvents, we have demonstrated that the hydrogen bonding patterns along the rotational equilibrium are very similar. For instance, the number of the hydrogen bonds formed and the binding energies do not vary much along the OO-cis $\rightleftarrows$ OO-trans interconversion. This suggests that the hydrogen bond interaction makes no preference for any particular rotamer. These results are in agreement with experiments obtained for protic solvents.

This investigation might be important to understand more complicated processes that occur for biomolecules in aqueous solutions. An interesting observation about the conformational analysis in a medium is that the equilibrium process can be limited by the potential energy barrier or the organization of the solvent molecules around the solute. In non protic solvents the molecular orientation around the solute seems to be important, possibly stabilizing certain forms of the solute due to steric interactions. In water, if the solute is a strong donor-acceptor system, the hydrogen bonds become important to stabilize one specific conformation in detriment of another. This is not, however, the case of furfural that is a weaker proton acceptor and its rotational equilibrium appears to be dominated by the polarity of the solvent instead of any specific solute-solvent interaction. In

other words, the bulk properties of water are responsible for keeping specific conformers of the solute.

Acknowledgments

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