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Structures and Hydrogen Bonding Interactions in Urea-water System Studied by All-atom MD Simulation and Chemical Shifts in NMR Spectrum

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The interactions and structures of the urea-water system are studied by an all-atom molecular dynamics (MD) simulation. The hydrogen-bonding network and the radial distribution functions are adopted in MD simulations. The structures of urea-water mixtures can be classified into different regions from the analysis of the hydrogen-bonding network. The urea molecule shows the certain tendency to the self-aggregate with the mole fraction of urea increasing. Moreover, the results of the MD simulations are also compare with the chemical shifts and viscosities of the urea aqueous solutions, and the statistical results of the average number hydrogen bonds in the MD simulations are in agreement with the experiment data such as chemical shifts of the hydrogen atom and viscosity.

Key words: All-atom simulation, Chemical shift, Viscosity, Urea-water system, Hydrogen bond

I. INTRODUCTION

Urea is often used as protein denaturation for analyzing protein stability, but the mechanism of urea-induced protein denaturation is not well understood [1−5]. Two models of urea denaturation are mainly considered. One is that urea weakens the intramolecular hydrogen bonds of proteins by binding directly to the proteins, and the other is that urea weakens hydrophobic interaction between proteins [6,7]. The latter model is related to the surroundings of the solvent around the protein. The urea aqueous solution is the special associated system that show some remarkable properties, such as enhanced solubility of hydrocarbons [8], decreased micelle formation [9], and most importantly, the ability to denature proteins. A variety of experimental and theoretical methods have been carried out to study the hydrogen bonding of urea-water system [10−15]. Molecular dynamics (MD) simulation has been proven to be particularly valuable for studying structures and interactions in mixtures system [16−18]. Spectral measurements such as NMR spectra are highly powerful techniques which can be used to investigate structures and interactions in the mixtures [19−22]. Burton and co-workers use neutron scattering to study the structure of aqueous urea at a supersaturated solution [11]. The results reveal that in moving into the supersaturated state the solutions show a significant and unexpected change in the self-aggregation of urea

molecules. Fong and co-workers found that a new group of self-assembling urea surfactant possesses the simplest peptide containing headgroup which has been found to have sufficient hydrophilicity to result in lyotropic liquid crystalline phase formation [12].

We have investigated the interactions and structures in the special associated system such as amide-water mixtures [23−25]. Some interesting phenomena were observed in the mixture, such as the weak $C-H\cdots O$ contacts and the additional properties. For instance, the two methyl groups in amide molecule are found to show different capabilities in forming weak $C-H\cdots O$ contacts in the mixtures from the radial distribution functions (RDFs). Furthermore, the temperature-dependent NMR results of the different methyl groups also show excellent agreement with the MD simulations [23,24]. We also found that in N-methylacetamide (NMA)-water system there was a special point at mole fraction of NMA $x_{\mathrm{NMA}}\approx 0.33$ in the hydrogen-bonding network analysis which presents excellent agreement with the excess enthalpy and other excess properties in the concentration dependence [25].

In this work, we adopted an all-atom MD simulation, chemical shifts to investigate the structures and interactions in the urea-water system. The radial distribution functions, statistics of hydrogen bonding networks, and the viscosities were also used.

II. COMPUTATIONAL METHODS

A. Molecular models

Simple potential models were used for urea and water. The nonbonded interactions are represented by a sum

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TABLE I Potential parameters and symbol of the atoms for SPC water and urea.

Model	Atom	$\sigma/$Å	$\varepsilon/$(kJ/mol)	$q/$e
SPC	OW[a]	3.1656	0.1554	−0.8200
	HW[b]	0.0000	0.0000	0.4100
Urea	C (C=O)	3.7500	0.1050	0.1420
	O	2.9600	0.2100	−0.3900
	N	3.2500	0.1700	−0.5420
	H	0.0000	0.0000	0.3330

[a] OW: oxygen atom of water.
[b] HW: hydrogen atoms of water.

of the Coulomb and Lennard-Jones terms with Eq.(1),

$$E_{\mathrm{ab}} = \sum_i^{\mathrm{on\ a}} \sum_j^{\mathrm{on\ b}} \left[\frac{q_i q_j e^2}{r_{ij}} + 4\varepsilon_{ij} \left(\frac{\sigma_{ij}{}^{12}}{r_{ij}{}^{12}} - \frac{\sigma_{ij}{}^{6}}{r_{ij}{}^{6}} \right) \right] f_{ij} \quad (1)$$

where E_{ab} is the interaction energy between molecules a and b. Standard combining rules are used via Eq.(2).

$$\sigma_{ij} = (\sigma_{ii}\sigma_{jj})^{1/2}, \qquad \varepsilon_{ij} = (\varepsilon_{ii}\varepsilon_{jj})^{1/2} \quad (2)$$

The same expression is used for intramolecular non-bonded interactions between all pairs of atoms ($i<j$) separated by three or more bonds. In Eq.(1), f_{ij}=1.0 except for intramolecular 1,4 interactions for which f_{ij}=0.5.

For the urea molecules and the water molecules the simulations details should be consistent. The similar force field terms should be used and their force field parameters should have been calibrated similarly. Simple-piont-charge (SPC) model [26] for water molecule and optimized potentials for liquid simulations-all atom (OPLS-AA) model [27,28] are used for water and urea molecules respectively. The potential parameters and symbol of the atom in the molecules is given in Table I. The structure of urea molecule is shown in Fig.1.

B. Simulation details

MD calculations were performed by a modified TIN-KER 4.2 molecular modeling package [29]. The simulations were carried out in the *NPT* ensemble at *T*=298 K and *P*=101 kPa with a total of 512 molecules with the mole fraction of urea molecules at 0.05, 0.1, 0.15, 0.2, and 0.25. The saturation limit at 300 K is at a mole fraction of 0.27 [30]. Periodical boundary conditions were adopted together with a sphere cutoff. The SET-TLE algorithm was used to constrain the water geometry, and the SHAKE algorithm was applied to constrain the bond length of the urea molecules. The energies of the initial configurations were minimized using the MINIMIZE program in TINKER 4.2 package. The time step was 1 fs and the configurations were saved
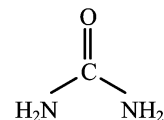
FIG. 1 Structure of urea molecule.

every 0.1 ps for analysis. The mixtures were sufficiently equilibrated to ensure that there were no systematic drifts in the potential energies with time. The equilibrations were followed by monitoring the RDFs as well as the fraction of molecules of each species which had a given number of hydrogen bonds. The statistics were collected during the last 100 ps.

C. Definition

An analysis of hydrogen bonds was used to gain a deeper insight into the aqueous structures. Although there are a great number of researches, defining the hydrogen bond is still somewhat arbitrary. Here a geometric criterion that is the same as that used by Luzar and Chandler has been performed [31], such as the typical criteria of neat water: R(OW$\cdots$HW)$\leq$2.45 Å, R(OW$\cdots$OW)$\leq$3.60 Å, and the angle ϕ(HW−OW$\cdots$OW)$\leq$30°.

III. RESULTS AND DISCUSSION

A. $g(r)$ in urea-water system

The structure of the liquid can be characterized well by the RDF, $g(r)$[x−y], which gives the probability of finding an atom of type y at a distance r from an atom of type x. In view of the inconclusive results about the liquid structure of urea, the all-atom simulation further give an insight into the local structure of liquid urea. The $g(r)$ of the urea aqueous solutions are given in Fig.2. Based on the structure of the urea molecule, there are just the traditional strong hydrogen bonds existing in the aqueous solutions such as OW−HW$\cdots$OW, N−H$\cdots$O hydrogen bonds. The distinct peaks near 1.8 Å are observed in $g(r)$[OW−HW] which reflects the hydrogen bonds of the water self-association. The short distances and strong intensities exhibit that the hydrogen bonds of OW$\cdots$HW are very strong. The peaks of the second hydration shell are observed near 3.2 Å which imply that the structures of the water molecules are more ordered. The first peaks near 1.9 Å are also found in $g(r)$[O−HW] which indicates the cross-association of hydrogen bonds C=O$\cdots$HW are obvious. In $g(r)$[OW−H], the larger distance and the weaker intensities compared with the other two $g(r)$ imply that the capability of forming the N−H$\cdots$OW is weaker than forming the hydrogen bonds
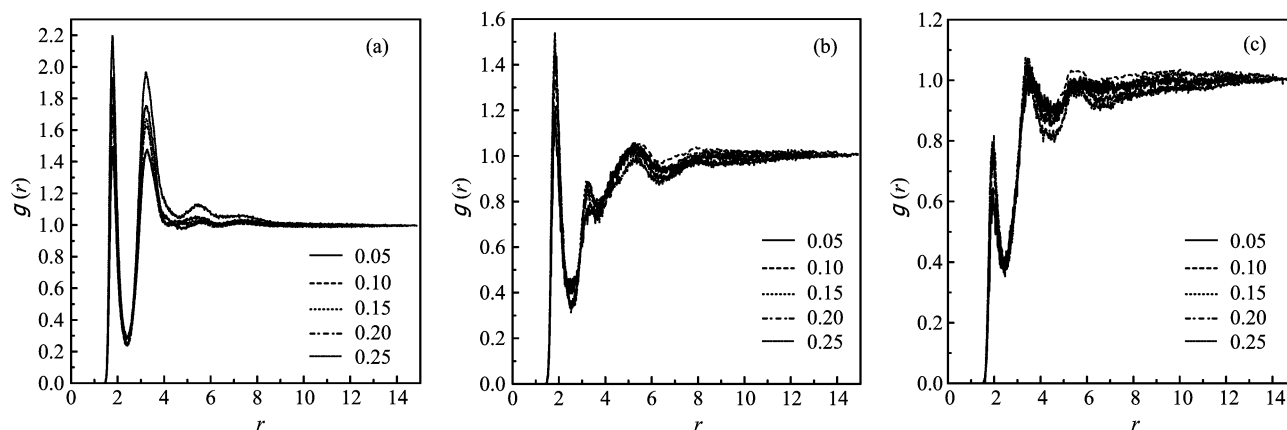
FIG. 2 RDF in the urea-water mixtures. (a) $g(r)$[OW−HW], (b) $g(r)$[O−HW], and (c) $g(r)$[OW−H]. Distances r are in Å.

of C=O$\cdots$HW and HW−OW$\cdots$HW. These also indicate that in the water rich region, the water molecules show the tendency to self-aggregates and the hydrogen bonds of N−H$\cdots$OW are not dominant. The broad peaks of the RDFs also imply that the intermolecular interactions are likely to include the weak hydrogen bonds as well as other weak interactions such as dipolar interactions and dispersions.

B. Hydrogen-bonding network

The traditional hydrogen bonds of OW$\cdots$HW and C=O$\cdots$HW play the essential role in aqueous solutions. One basic aspect, the hydrogen bonding network is the probability distribution, describing the number and type of hydrogen bonds. The carbonyl and the water oxygen atoms compete as acceptors of hydrogen bonds. Moreover, the water and the amide hydrogen atoms in urea compete as donors of forming hydrogen bonds. It is the competition of these hydrogen bonding interactions which leads to hydrogen bonding networks in the urea-water mixtures. The hydrogen bonds are determined by a geometrical criterion of pure water as defined. A summary results of the statistics are given in Fig.3.

The strong hydrogen bonds of HW−OW$\cdots$HW are given in Fig.3(a). The number of free oxygen atom in water increases with the increasing of mole fraction of urea (x_{urea}). The fractions of cluster for the OW atom accepting one or two hydrogen atoms of water are in high level in the water rich region. So in the water rich region, the water molecules prefer to form hydrogen bonds with themselves. The stable clusters of water self-aggregates predominate. For another hydrogen bond of C=O$\cdots$HW shown in Fig.3(b), it is found that the free carbonyl oxygen atoms of urea (O) increase with x_{urea} becoming large. The clusters of carbonyl oxygen atom accepting one proton of water are also in the high level. And the aggregates of carbonyl oxygen

atom accepting two protons are high in the water rich region, and then decrease with the x_{urea}. These indicate that the carbonyl oxygen is easy to form the hydrogen bonds with hydrogen atom of water molecule. And it's a good proton acceptor.

In Fig.3 (c) and (d), the aggregates of O and OW atom accepting amide proton are both in a low level. These imply that the hydrogen bonds of N−H$\cdots$O and N−H$\cdots$OW are not dominated in the water rich region. However, with the increase of x_{urea}, the fraction of hydrogen bonds N−H$\cdots$O which is related to the self-aggregate of urea become higher than that of the hydrogen bonds N−H$\cdots$OW. The fractions of the accepted H-bonds for these different hydrogen bonds at x_{urea}=0.25 are shown in Table II. For the hydrogen bonds N−H$\cdots$OW and N−H$\cdots$O, the fraction of the oxygen atom accepting one proton is 14.74% and 27.80% respectively. The values are much smaller than those in the hydrogen bonds of C=O$\cdots$HW and HW−OW$\cdots$HW, which show agreement with the results of RDF's. However, the numbers of N−H$\cdots$O hydrogen bonds are larger than those of the N−H$\cdots$OW hydrogen bond, which imply that the urea self-aggregation become dominant with the increase of urea molecules. In summary, the oxygen atoms of water prefer its own hydrogen atom as acceptors. And the atom of HW can form the hydrogen bonds with atom of OW or O. With the increasing number of urea molecules, the structure of the water cluster is broken down, and the urea self-associate clusters become dominant.

C. Comparision with NMR experiment

Since the chemical shift is a measurement of the electron density about the probe nuclei, it gives the information state for the atoms. Chemical shifts will move to low field and the values will become smaller after the hydrogen bond formation [21,22]. It is well known that the effects on the chemical shift of hydrogen bond are much
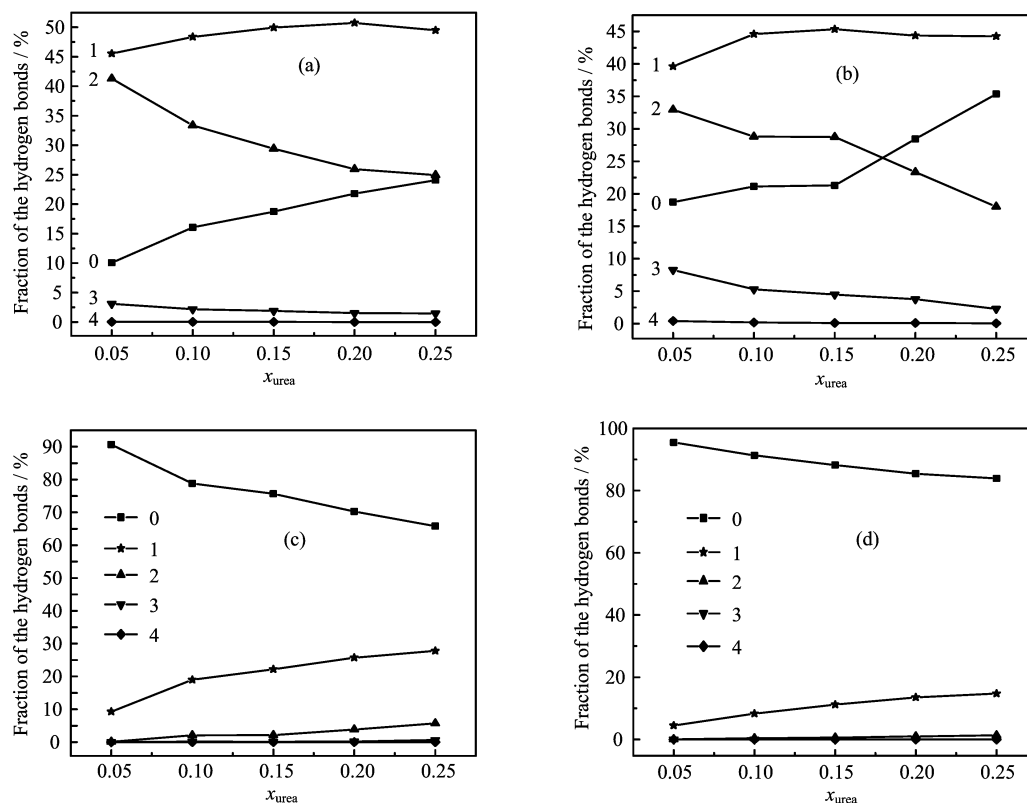
FIG. 3 Hydrogen-bonding network in the urea-water system. (a) HW−OW···HW, (b) C=O···HW, (c) N−H···O, and (d) N−H···OW.

TABLE II The fraction (%) of the accepted H-bonds for different hydrogen bonds at mole fraction of urea x_{urea}=0.25.

Accepted H-bonds	HW−OW···HW	C=O···HW	N−H···OW	N−H···O
0	24.08	35.37	83.89	65.82
1	49.49	44.25	14.74	27.80
2	24.94	18.03	1.33	5.71
3	1.47	2.29	0.03	0.64

larger than the other intermolecular interaction effects such as van del Waals interaction. So NMR spectra are often used to investigate intermolecular interactions in solution. However, there is still a little spectral data over the entire composition range for aqueous mixtures. Finer and co-workers have adopted the NMR spectra to get the chemical shifts of the hydrogen atom in urea-water system [32]. The comparison between the average number hydrogen bonds in MD simulation and chemical shifts of hydrogen atoms with the concentration are given in Fig.4.

The chemical shift of water hydrogen atom, δ(HW), is a measure of the electron density for the water protons, and it can reflect the polarization of water molecules dissolving solutes. The atom of HW can form strong hydrogen bonds of O···HW and OW···HW which will lead to the variety of the chemical shifts with the concentration dependence. In Fig.4(a), it is found that the average numbers of hydrogen bonds for O···HW and

OW···HW both decrease with the increasing of x_{urea}. On the other side, the values of δ(HW) are also becoming smaller with the increasing of x_{urea}. In Fig.4(b), the average hydrogen bonds in MD and the chemical shifts which are related to the amide hydrogen atom (H) also presents agreement with the concentration dependence. And the varieties of the chemical shifts show good agreements with the MD simulations.

D. Comparision with the results of viscosities

Viscosity is a measurement of the resistance to flow. The shear viscosity of solution is the sum of two contributions. One of them is the natural properties of the simple liquids, and another is caused by effects of the hydrogen bonds. In solutions, the surface tension and viscosity of water are also related to the strength of the hydrogen bonds between the two molecules [33,34]. In the urea aqueous solution, the urea molecule exhibits
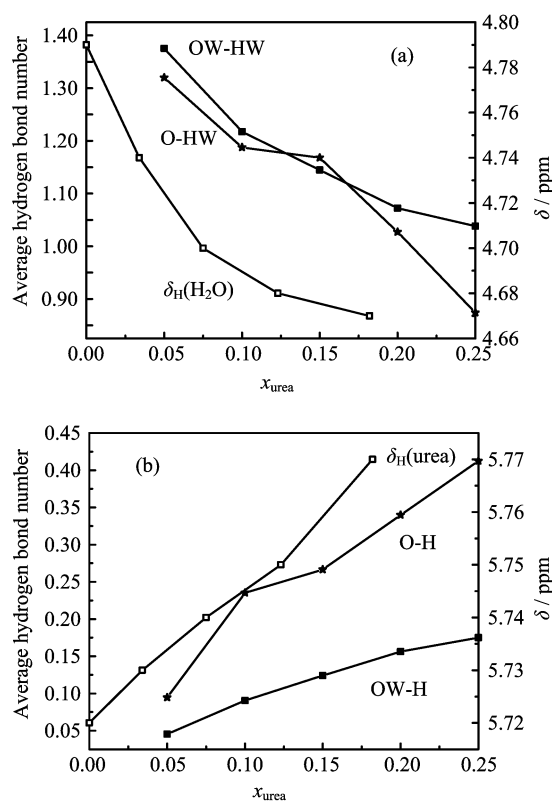
FIG. 4 Comparison between the average number of hydrogen bonds and the chemical shifts for hydrogen atom in the water and urea molecules at 293 K [32].

FIG. 5 Comparison between the average number of hydrogen bonds $N-H\cdots O$ and the relative viscosity data [35] at 298 K in urea aqueous solution.

a certain tendency to self-aggregate [6]. The formation of the urea self-aggregates will lead to the higher viscosity. The comparison between the average number of $N-H\cdots O$ hydrogen bonds by the MD simulations and the relative viscosity data at 298 K [35] are shown in Fig.5. With the increasing of x_{urea}, the statistics of average number of $N-H\cdots O$ hydrogen bonds become larger which imply that the clusters of self-associate become dominant. These results present agreement with the results of the viscosity.

IV. CONCLUSION

An all-atom MD simulation combined results of the experiment are performed to investigate the structures and interactions in the urea-water system. The RDF's indicate that different strong hydrogen bonds exist in the urea-water mixture. Different capabilities are found in forming these hydrogen bonds. Through the analysis for hydrogen-bonding network, the mixtures can be classified into different regions. In the water rich region, the stable self-aggregates of water molecules are dominant. With the x_{urea} increasing, the structure of the water cluster is broken down, and there are aggregates of the across association between the water and urea in the mixture. And the urea molecule exhibits the ten-
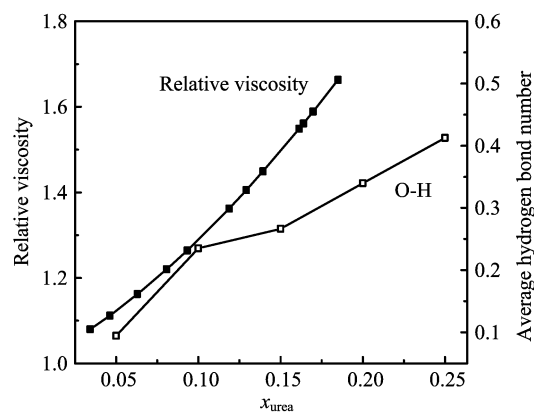
dency to self-aggregate. The statistical average number of hydrogen bonds and chemical shifts in NMR and viscosity represent the consistent concentration dependence. The all-atom MD simulations and experiment such as chemical shifts and viscosity are successful in revealing the structures and interactions in the urea-water mixtures.

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