

# Molecular Dynamics Simulation of the Hydration of the Alanine Dipeptide

S. G. Kalko,<sup>†</sup> E. Guàrdia,<sup>‡</sup> and J. A. Padró\*

*Departament de Física i Enginyeria Nuclear, Universitat Politècnica de Catalunya, Campus Nord, Mòdul B4–B5, Sor Eulàlia d'Anzizu s/n, 08034 Barcelona, Spain and Departament de Física Fonamental, Universitat de Barcelona, Diagonal 647, 08028 Barcelona, Spain*

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Molecular dynamics computer simulations of an alanine dipeptide molecule in aqueous solutions have been carried out. Three different molecular conformations ( $\alpha$ ,  $\beta$ , and  $C_7^{ax}$ ) have been considered. Structural and dynamical properties of the hydration water molecules have been calculated separately for solvent molecules surrounding different atoms or atomic groups of such dipeptide, i.e., two carbonyl oxygens, three methyls, and two amide hydrogens. The results for the three conformations have been compared for possible differences. The study includes molecule sites—water radial distribution functions and coordination numbers as well as residence times, hydrogen bonding, and reorientational times of water molecules in the hydration shells. The little differences between the properties of water around the polar and nonpolar atomic groups have been carefully analyzed. The discrepancies between the hydration water properties on the different conformations are not significant.

## I. Introduction

The solvation of biomolecules has been proved to be a determinant factor on their structure and functionality. So, the conformation adopted by a macromolecule in solution depends strongly on the solute–solvent interactions. The study of the hydration of small peptides could provide us with basic information for understanding the conformation and folding of proteins. The ordering of water molecules around polar and nonpolar solutes is different and the behavior of a solute molecule nontotally polar or nonpolar should be the result of a balance between the opposite effects of their hydrophobic and hydrophilic groups. A microscopic study of the influence of the relevant components of a molecule on the surrounding water can give us useful information on the solute–solvent interaction.

The alanine dipeptide (AD) has served as a paradigm for studying the thermodynamics of protein conformations and folding. Experimental work on AD by CD as well as NMR measurements by Madison and Kopple<sup>1</sup> combined with conformational energy calculations indicate that the  $\alpha$ -helical and polyproline II-like ( $\approx\beta$ ) conformations are dominant in water. Besides, the alanine-based 3K(I) peptide was reported to be very helical in aqueous solution in an experimental work done by Miick et al.<sup>2</sup> using electron spin resonance. There are also many free energy calculation works that look for the more stable structure of AD in aqueous solution.<sup>3–5</sup> However, little has been done on the characterization of the hydrating water of AD. To our knowledge, the only study of this type is that carried out by Rossky and Karplus 20 years ago.<sup>6</sup> In that work, the properties of AD in the  $C_7^{eq}$  conformation were analyzed from a very short molecular dynamics simulation (1.5 ps).

The results of a molecular dynamics (MD) simulation study of AD in water solution are presented in this paper. Three

different conformations of the AD molecule have been considered, i.e., the  $\alpha$ ,  $\beta$ , and  $C_7^{ax}$  conformations. In order to discuss the possible differences in the behavior of water molecules that surround the polar and nonpolar groups of the AD molecule, the structural and dynamical properties of water around seven different molecular groups have been calculated separately. The two carbonyl oxygens and the two amide hydrogens are the polar groups whereas the three methyl groups are the nonpolar groups. The methodology in this work is similar to that used for the study of the hydration of single ions with different electric charges.<sup>7–10</sup>

The outline of this paper is as follows. In section II, the details of the simulation are given. Section III is devoted to the analysis of the solute–solvent structure. The dynamics of hydration water molecules is presented in section IV. Section V deals with hydrogen bonding of water close to the different AD groups. The differences between the results for the three AD conformations are discussed in section VI. Finally, some concluding remarks are summarized in section VII.

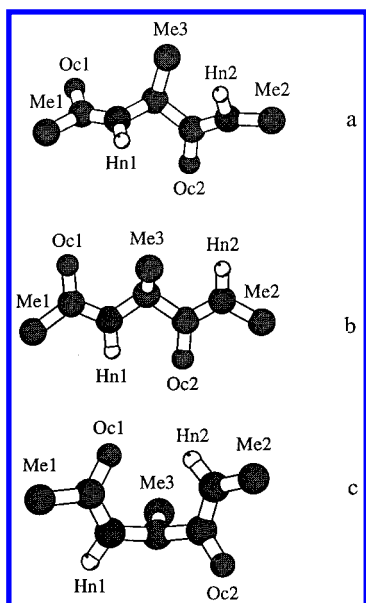
## II. Computational Details

Molecular dynamics simulations at constant volume were performed using the GROMOS package,<sup>11</sup> where the equation of motion is integrated by using a leapfrog Verlet algorithm with a time step ( $\Delta t$ ) of 2 fs. The temperature was kept at 298 K with a coupling time constant of 10  $\Delta t$ .<sup>12</sup> A total number of 221 water molecules and the blocked alanine dipeptide (*N*-acetylalanyl-*N'*-methylamide) molecule were included in a box of dimensions: 1.76 nm  $\times$  1.91 nm  $\times$  2.08 nm. The SHAKE<sup>13</sup> method was used to keep the bond lengths of both solute and solvent molecules fixed. Short-range interactions were truncated at half of the minimum box length with a spherical molecule based cutoff, and the Ewald summation was used for the electrostatic interactions.<sup>14</sup> A modified version of GROMOS program was then used, where the Ewald methodology was incorporated. Additional calculations at similar conditions were performed for pure water.

\* To whom the correspondence should be addressed. E-mail: joan@ffn.ub.es.

<sup>†</sup> Present address: Departamento de Biología Molecular, Centro de Investigación y Desarrollo (C.I.D.), C.S.I.C. Jordi Girona, 18-26, 08034 Barcelona, Spain. E-mail: skacri@cid.csic.es.

<sup>‡</sup> E-mail: guardia@fen.upc.es.



**Figure 1.** Alanine dipeptide in the  $\alpha$  (a),  $\beta$  (b), and  $C_7^{\text{ax}}$  (c) conformations, respectively (Molscript representation<sup>15</sup>).

**TABLE 1: Interaction Potential Parameters**

molecule	site	$q$ (e)	$C_6^{1/2}$ (kcal $\text{\AA}^6/\text{mol}$ ) <sup>1/2</sup>	$C_{12}^{1/2}$ (kcal $\text{\AA}^{12}/\text{mol}$ ) <sup>1/2</sup>
alanine dipeptide	Oc	-0.38	23.35	421.0
	Me	0.0	46.06	2500.0
	Hn	0.28	0.0	0.0
water	Ow	-0.848	25.01	421.0
	Hw	0.424	0.0	0.0

We studied the properties of the hydration water molecules for seven sites (atoms or atomic groups) of each dipeptide conformation separately (Figure 1). As polar sites, we considered the carbonyl oxygens (Oc1, Oc2) and the amide hydrogens (Hn1, Hn2). As nonpolar sites, the methyl groups (Me1, Me2, Me3). A water molecule was considered as pertaining to the first hydration shell of a tagged site if its distance to the site was lower than the radius of that shell. This radius was defined as the position of the first minimum of the corresponding site-water radial distribution function.

The interaction potentials assumed in this work are given by the function

$$U_{ij} = \frac{q_i q_j}{r_{ij}} + \frac{C_{12_i} C_{12_j}}{r_{ij}^{12}} - \frac{C_{6_i} C_{6_j}}{r_{ij}^6}$$

Water molecules were modeled according to the extended point charge SPC/E model of Berendsen et al.,<sup>16</sup> whereas the model in GROMOS<sup>11</sup> was assumed for the alanine dipeptide. The potential parameters for the different sites in both kinds of molecules are gathered in Table 1.

The alanine dipeptide can adopt several minimum conformations without a high energy cost. This molecule appears preferentially in the areas of the Ramachandran plot called  $\beta$ ,  $\alpha_R$ ,  $C_7^{\text{eq}}$  and  $C_7^{\text{ax}}$ , corresponding to angles  $\phi \approx -180.0^\circ$ ,  $-60.0^\circ$ ,  $-60.0^\circ$ ,  $60.0^\circ$ , and  $\psi \approx 180.0^\circ$ ,  $-60.0^\circ$ ,  $60.0^\circ$ ,  $-60.0^\circ$ , respectively. Rossky and Karplus performed MD simulations on the  $C_7^{\text{eq}}$  conformation of such a molecule. We decided to carry out calculations on the  $\alpha$ ,  $\beta$ , and  $C_7^{\text{ax}}$  conformations. Since only the distances between adjacent sites were kept fixed by the SHAKE method, we used a very high force constant to model the AD molecules according to the desired dihedral angles  $\phi$  ( $1\text{C}-2\text{N}-2\text{C}_\alpha-2\text{C}$ ) and  $\psi$  ( $2\text{N}-2\text{C}_\alpha-2\text{C}-3\text{N}$ )<sup>17</sup> (see Figure

**TABLE 2: Dihedral Angles ( $\phi$  and  $\psi$ ) and Hydration Enthalpies Obtained from the MD Simulations for the Three AD Conformations**

dialanine conformation	$\phi$ (deg)	$\psi$ (deg)	hydration enthalpy (kcal/mol)
$\alpha$	-116	-115	-0.10311E+05
$\beta$	-172	-172	-0.10272E+05
$C_7^{\text{ax}}$	77	-77	-0.10317E+05

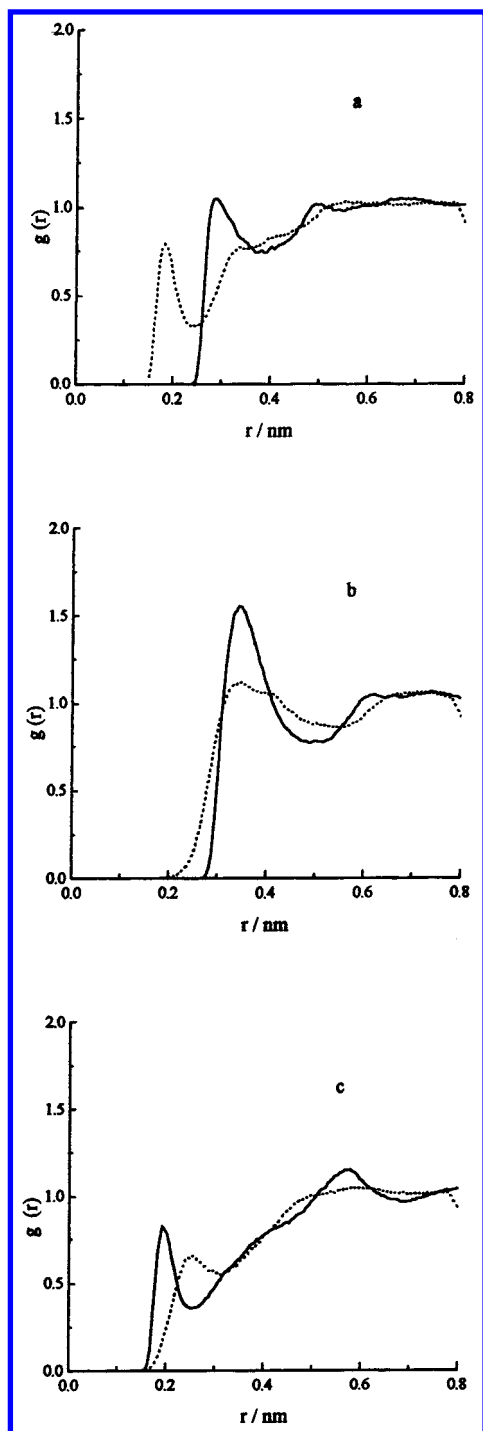
1 of ref 6 for details). It was verified that this procedure allows us to keep these angles approximately constant along the MD trajectories.

We carried out three MD simulation runs. At the beginning of each run we located the AD molecule with dihedral angles corresponding to one of the three conformations under study surrounded by water molecules. After an equilibration period of 30 ps, the trajectories were collected every five time steps for further analysis up to 200 ps of total time for each AD conformation. The average dihedral angles for the three conformations, together with the corresponding hydration enthalpy are shown in Table 2. Even though we started with the desired AD conformation and a high force constant was applied, the results in Table 2 show that only the  $\beta$  conformation was strictly kept during the MD runs. For the helical case, the resulting dipeptide angles do not correspond to the  $\alpha_R$  zone though the dipeptide is still at the helical zone of the Ramachandran plot. Analogously, the angles corresponding to the  $C_7$  conformation are lightly far from the strict  $C_7^{\text{ax}}$ . These deviations of the desired conformations must be considered as a consequence of the particular force field used. Henceforth, in this work we will give results on a strict  $\beta$  conformation on the one hand, and on a helical conformation not restrained to the narrow region corresponding to the  $\alpha$ -helices ( $\alpha_R$ ), but called by us  $\alpha$  conformation, and on a slightly distorted  $C_7^{\text{ax}}$  conformation.

Since we have only observed a very weak dependence of the studied properties on the AD molecular conformation, the results presented in sections III–V are an average over those obtained for the three conformations under study. Furthermore, we have not found significant differences between the properties for the analogous sites in the AD molecule. So, the properties for Oc1 and Oc2 have been averaged and will be named generically as Oc. Analogously, Me corresponds to Me1, Me2, and Me3 while Hn corresponds to Hn1 and Hn2. The emerging differences between findings for different conformations and between distinct sites of the same class in the AD molecule will be analyzed in section VI.

### III. Structure

**III.1. Radial Distribution Functions.** The resulting radial distribution functions ( $g(r)$ ) between the different sites of the AD molecule and both the oxygen (Ow) and the hydrogen (Hw) of the surrounding water molecules are shown in Figure 2. According to earlier findings,<sup>18,19</sup> in the case of the nonpolar sites (Me), the  $g(r)$  functions corresponding to Ow and Hw show a first peak at approximately the same position (Figure 2b). On the contrary, in the case of polar sites (Oc, Hn) the position of the first peak of the  $g(r)$  for Ow and Hw shows marked shifts. The Ow atoms are closer to the positive site (Hn) than Hw atoms whereas Hw atoms are closer to the negative site (Oc) than Ow atoms. The relatively well-defined first minima of the  $g(r)$  between Ow and Hn and between Hw and Oc reflect the existence of hydrogen bonds between the polar AD sites and neighboring water molecules.



**Figure 2.** AD site-Ow (—) and AD site-Hw (····) radial distribution functions: (a) Oc sites; (b) Me sites; (c) Hn sites.

The differences between the properties of water molecules in the first hydration shells of the different AD sites will be analyzed throughout this paper. We have considered the first minimum of the  $g(r)$  between Ow and a given AD site as the radius of the hydration shell of this site. So, it has been assumed that water molecules with their oxygen atom whose distance to a site is smaller than the radius for this site belong to its hydration shell. The radii ( $r_0$ ) of the different hydration shells are given in Table 3. It should be noted that in the case of Oc and Me the hydration radii could not be unambiguously determined because the positions of the corresponding  $g(r)$  first minima are poorly defined. This fact can have some influence on our quantitative findings but should not affect the conclusions

**TABLE 3: Hydration Shell Radii ( $r_0$ ), Coordination Numbers (cn), Mean Values of the Angle between the Dipole Vector of Water Molecules and the Position of Ow in Relation to the Site ( $\theta_m$ ) and Mean Residence Times of Water Molecules in the First Coordination Shells ( $\tau_n$ )**

site	$r_0$ (nm)	cn	$\theta_m$ (deg)	$\tau_n$ (ps)
Oc	0.40	4.5	80	4.6
Me	0.50	12.9	92	7.7
Hn	0.26	0.8	122	3.9

of this work, which are basically deduced from the most relevant qualitative features.

The coordination numbers have been calculated from the integrals until the radius of the hydration shell of the  $g(r)$  functions between the AD site and Ow. It may be observed in Table 3 that the longer the radius of the shell is, the larger is the coordination number. Moreover, the higher peak of the  $g(r)$  for the nonpolar site as compared with those for the polar sites also contributes to the higher coordination number obtained for Me.

**III.2. Orientation.** For the study of the orientation of the water molecules around the different sites of AD, we considered the angle ( $\theta$ ) between the dipole vector of the water molecule ( $\mu$ ) and the position vector ( $r$ ) of Ow in relation to the site. The mean values ( $\theta_m$ ) of that defined angle  $\theta$ , are shown in Table 3. Since a high dispersion of the  $\theta$ -values was observed, we also determined the probability distributions of  $\cos \theta$ . The resulting curves, which are consistent with the  $g(r)$  results, are shown in Figure 3a. The probability distributions for Oc and Hn show marked maxima at positive and negative  $\cos \theta$  values, respectively. This corroborates that water molecules around Oc prefer H-bond donor orientations whereas molecules around Hn prefer H-bond acceptor orientations. This is in accordance with previous findings for aqueous solutions of negative and positive ions.<sup>7</sup> The probability distribution for Me only shows a broad maximum around  $\cos \theta \approx -0.3$ , reflecting that there are no significant preferences in the orientation of water molecules around the nonpolar sites. However, it should be noted that our results have been averaged over the rotation of water molecules about their dipole moments and there are some possible orientational correlations that cannot be observed by this procedure.

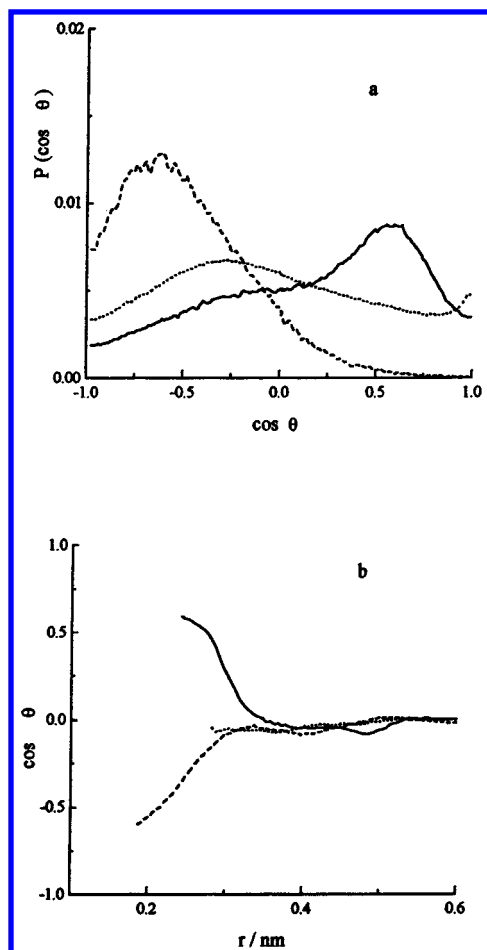
Figure 3b shows the ensemble average of  $\cos \theta$  ( $\langle \cos \theta \rangle$ ) as a function of  $r$ . The main trends of these curves coincide with those in previous studies on single  $F^-$  or  $Na^+$  ions in water.<sup>7</sup> However, the orientation of water molecules close to the AD sites disappears much faster than in the case of isolated ions, which can be related to the rather low residual charge values in the AD sites.

## IV. Dynamics

**IV.1. Residence Times.** We have calculated the residence time correlation function  $N(t)$  of water molecules close to the different sites of the AD molecule during the MD simulations. This function is defined as the number of molecules that lie initially within the first coordination shell and are still there after a time  $t$  has elapsed

$$N(t) = \langle \sum_j P_j(t_0, t, t^*) \rangle$$

where  $P(t_0, t, t^*)$  takes the value 1 if a water molecule  $j$  lies at a distance shorter than  $r_0$  of the site at times  $t_0$  and  $t$  without having left the coordination shell for any continuous period longer than  $t^*$ .  $P(t_0, t, t^*)$  takes the value 0 in any other case.



**Figure 3.** Orientation of water molecules in the first hydration shell of the AD sites (according to the definitions given in the text): (a) probability distribution of  $\cos \theta$ ; (b) mean value of  $\cos \theta$  as a function of the AD site—Ow distance. In both figures: Oc sites (—), Me sites (···), and Hn sites (---).

The distance  $r_0$  corresponds to the radius of the hydration shell of the site, and  $t^*$  has been chosen equal to 2 ps according to the usual criteria.<sup>20</sup> The symbol  $\langle \dots \rangle$  means that an average over all the molecules in the shell and over different time origins  $t_0$  during the run was taken.  $N(t)$  turns out to decay exponentially at long times, and the corresponding characteristic time ( $\tau_N$ ) gives a simple definition of the mean residence time of the water molecules in the shell around the site, i.e., the time over which the molecules in the first coordination shell exchange identity with molecules in the bulk. So, we determined  $\tau_N$  by fitting  $N(t)$  to exponential functions and the results are shown in Table 3. The  $N(0)$  values coincide with the coordination numbers also shown in Table 3. It is interesting to point out that the  $\tau_N$  values for the three sites do not show large differences but is clearly greater for Me. This shows that the H-bonds between the polar Oc and Hn sites and water molecules in their hydration shell do not increase significantly the residence time of molecules in the shell. The longer hydration radius for Me can also contribute to the larger value of  $\tau_N$  for this site.

**IV.2. Reorientational Motions.** Information on reorientational motions of water molecules may be obtained through a set of reorientational time-correlation functions, defined as:<sup>21</sup>

$$C_l(t) = P_l(\vec{u}_i(t) \cdot \vec{u}_i(0))$$

where  $P_l$  is the  $l$ th Legendre polynomial and  $\vec{u}_i$  is a unit vector that characterizes the orientation of the molecule. We have

**TABLE 4: Characteristic Times (in ps) for the Reorientational Motions of Different Unit ( $\vec{u}_i$ ) Vectors Fixed to the Water Molecules in the First Hydration Shells of the AD Sites (More Details Are Given in the Text)**

site	$\vec{u}_1$		$\vec{u}_2$		$\vec{u}_3$		$\vec{u}_4$	
	$\tau_1$	$\tau_2$	$\tau_1$	$\tau_2$	$\tau_1$	$\tau_2$	$\tau_1$	$\tau_2$
Oc	4.1	1.9	3.5	2.2	2.6	1.6	9.1	4.0
Me	6.0	2.5	5.4	2.9	3.8	2.1	18.3	6.3
Hn	2.9	2.0	2.6	2.0	2.5	1.8	3.4	2.7
pure water	5.0	2.2	4.8	2.6	3.2	1.8		

obtained these functions for molecules in the different hydration shells considered in this work. Three different unit vectors for each molecule were taken into account; i.e.,  $\vec{u}_1$  in the direction of the dipole vector,  $\vec{u}_2$  in the intramolecular Hw—Hw direction, and  $\vec{u}_3 = \vec{u}_1 \times \vec{u}_2$  orthogonal to the plane of the water molecule. For the study of the orientation of the hydrated complexes, we also considered the unit vector  $\vec{u}_4$  along the site—Ow direction. The reorientational times ( $\tau_l$ ) were calculated by assuming an exponential decay of the  $C_l(t)$  functions at long times

$$C_l(t) \approx \exp(-t/\tau_l)$$

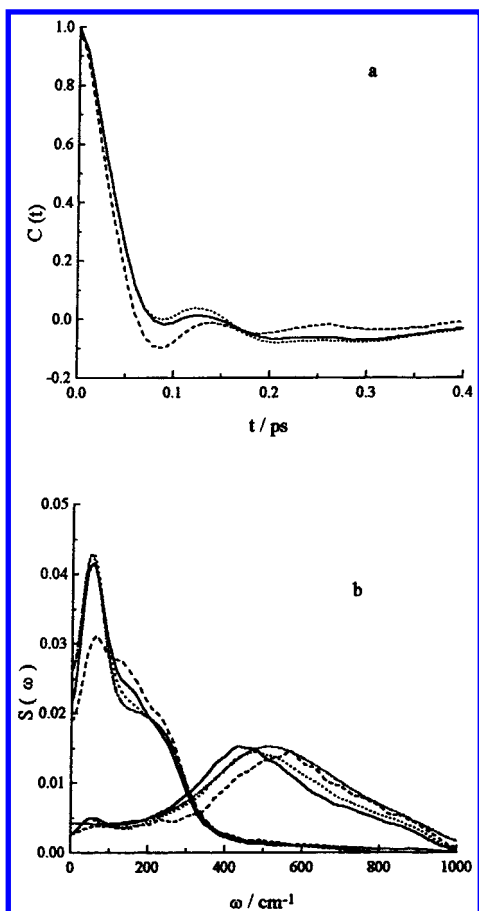
The results of  $\tau_l$  for  $l = 1$  and  $l = 2$  are summarized in Table 4. As can be seen from this table, the highest reorientational times correspond to the Me sites, which values are even slightly greater than those for pure water. These findings are in agreement with the earlier results by Rossky and Karplus.<sup>6</sup>

**IV.3. Translational Motions. IV.3.1. Velocity Autocorrelation Functions.** The translational motions of water molecules in the different site hydration shells were analyzed through the normalized velocity autocorrelation functions ( $C(t)$ ) for the Ow and Hw atoms and their corresponding power spectra  $S(\omega)$ , calculated as the Fourier transforms of the  $C(t)$  functions. The resulting functions are compared with those for pure water in Figure 4. It should be noted that the  $S(\omega)$  functions for the oxygen in pure water show some features related to the fact that molecular motions are not strictly diffusive but restricted by the cage effects characteristic of dense fluids as well as by H-bonding. So,  $S(\omega)$  shows a shoulder around  $\omega = 200 \text{ cm}^{-1}$  which is attributed to the intermolecular O—O stretching vibrations of pairs of H-bonded molecules. The maximum close to  $50 \text{ cm}^{-1}$  is associated with vibrations of water molecules in the cage formed by their neighbors.<sup>22</sup> The  $S(\omega)$  functions for water close to the different AD sites show the same trends.

The  $C(t)$  functions for the Ow atoms of molecules in the Me and Oc shells are quite close to those for pure water and only show noticeable differences at intermediate times between 0.05 and 0.2 ps (Figure 4a). Such differences are also reflected in the  $200 \text{ cm}^{-1}$  region of the corresponding  $S(\omega)$  functions (Figure 4b). In the case of Me the discrepancies with the results for pure water are rather small. This indicates that nonpolar sites have little influence on the H-bonding network of water molecules in their hydration shell. On the contrary, the results for water in the Hn hydration shell are markedly different from those for pure water and water near to the Oc sites, which indicates that the structure of the water network around the Hn sites is significantly distorted. These findings are consistent with earlier results for single  $\text{Na}^+$  and  $\text{Cl}^-$  in aqueous solution.<sup>23</sup> In this case it was also observed that  $S(\omega)$  for the Ow atoms of water molecules close to the ions are strongly dependent on the sign of the ionic charge,  $S(\omega)$  for the  $\text{Cl}^-$  hydration shell being markedly closer to  $S(\omega)$  for pure water than that for the  $\text{Na}^+$  hydration shell.

In the case of Hw the  $C(t)$  functions for the three kind of site hydration shells do not show important differences. So, the  $S(\omega)$





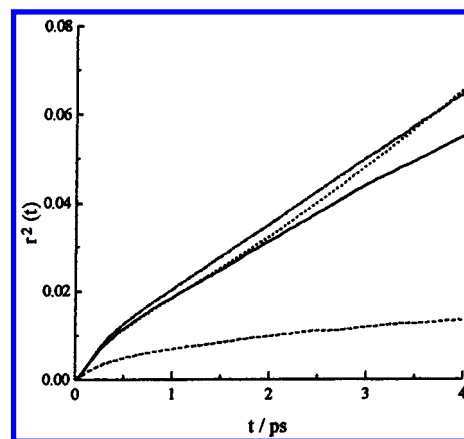
**Figure 4.** (a) Velocity autocorrelation functions of the Ow atoms in the first hydration shell of the different AD sites. (b) Power spectra of the velocity autocorrelation functions of the Ow and Hw in the first hydration shell of the different AD sites. In both figures: Oc sites (—), Me sites (···), Hn sites (---), and pure water results (-·-·-).

spectra (Figure 4b) show in all cases broad peaks between 400 and 600  $\text{cm}^{-1}$  which are associated with the libration motions of water molecules.<sup>24</sup> The only differences which may be observed are slight shifts in the positions of the  $S(\omega)$  peaks.

**IV.3.2. Mean Square Displacements.** The mean square displacements ( $r^2(t)$ ) as a function of time provide information on the characteristics of diffusive motions on the picosecond time scale. So, the self-diffusion  $D$  coefficient is frequently calculated from the long-time slope of  $r^2(t)$ . It may be observed in Figure 5 that  $r^2(t)$  for the Me and Oc shells do not show marked differences with that for pure water. However, the discrepancies in the case of Hn are important. This fact indicates that the influence of Hn on the characteristics of the molecular motions of neighboring water is not only important at very short time scales (as reflected by the  $C(t)$  and  $S(\omega)$  functions) but also produces a significant reduction of the water diffusion coefficient. This may be associated with a stronger bonding between AD and water in the Hn shell, as compared with water in the other shells.

## V. Hydrogen Bonding

Further information on the water behavior can be obtained from the study of the hydrogen bonds network from both static and dynamical points of view. In order to decide if two water molecules are hydrogen bonded, we adopted the following geometric criteria: (1) the distance between the oxygens of both molecules is smaller than a cutoff distance  $R_{OO}$ ; (2) the distance between the oxygen of the acceptor molecule and the hydrogen



**Figure 5.** Mean square displacement for water molecules translation around Oc atoms (—), Me groups (···), and Hn atoms (---). Results for pure water (-·-·-) are shown.

**TABLE 5: Percentage of Water Molecules with  $n$  H-Bonds ( $f_n$ ), Mean Number H-Bonds ( $\langle n_{hb} \rangle$ ) per Molecule in the First Hydration Shell of the Different AD Sites and Mean Lifetimes ( $\tau_{hb}^C$  and  $\tau_{hb}^R$ ) of the H-Bonds (As Defined in the Text)**

site	$f_0$	$f_1$	$f_2$	$f_3$	$f_4$	$f_5$	$\langle n_{hb} \rangle$	$\tau_{hb}^C$ (ps)	$\tau_{hb}^R$ (ps)
Oc	3.0	18.5	34.0	31.5	12.5	0.5	2.3	0.5	2.3
Me	0.5	6.5	24.0	40.0	27.0	2.0	2.9	0.6	3.2
Hn	0.5	35.0	33.0	26.5	5.0	0.0	2.0	0.7	2.4
pure water	0.0	2.0	13.5	37.5	42.0	5.0	3.3	0.5	2.5

of the donor is smaller than  $R_{OH}$ ; (3) the angle defined within the dimer geometry is smaller than  $\psi$ . As  $R_{OO}$  and  $R_{OH}$  we selected the position of the first minima of the OwOw and OwHw radial distribution functions in pure water, i.e.,  $R_{OO} = 0.36$  nm and  $R_{OH} = 0.24$  nm, respectively.  $\psi$  was chosen equal to 30°.<sup>22</sup>

**V.1. Number of H Bonds.** We calculated the mean number of H-bonds per molecule ( $\langle n_{hb} \rangle$ ) of water molecules in the first hydration shells corresponding to the different sites. We also determined for each hydration shell the percentage ( $f_n$ ) of molecules forming  $n$  H-bonds ( $n = 0, 1, 2, 3, 4, 5$ ). The  $\langle n_{hb} \rangle$  and  $f_n$  results are shown in Table 5. It should be emphasized that only the H-bonds between water molecules were considered whereas possible H-bonds between water and the polar sites of AD were not taken into account. For the sake of comparison, recent results for pure water<sup>25</sup> are also included in Table 5.

As could be expected, because of geometrical restrictions the number of H-bonds of water molecules neighboring the AD sites is lower than that for pure water. For the former, the majority of molecules form 2 or 3 H-bonds whereas for the second the majority of molecules form 3 or 4 H-bonds. Moreover, the values of  $\langle n_{hb} \rangle$  increase with the radius of the hydration shell. This may be attributed to the fact that all molecules in a hydration shell with small radius should be very close to the corresponding AD site which increases the geometrical difficulties of water molecules to be H-bonded to external water molecules.

**V.2. Mean Lifetime of H Bonds.** We have determined the survival probabilities and lifetimes of the H-bonds of water molecules by the following procedure.<sup>25–27</sup> We defined a variable  $\eta_{ij}$  that takes the values 0 or 1 depending on the H-bond state of a pair of water molecules, i.e.,  $\eta_{ij} = 1$  if molecules  $i$  and  $j$  are H-bonded at time 0 and time  $t$  and the bond has not been broken for any period longer than  $t^*$ ;  $\eta_{ij} = 0$ , otherwise. We calculated the normalized autocorrelation function  $C_{HB}(t)$

$= \langle \eta_{ij}(t) \cdot \eta_{ij}(0) \rangle / \langle \eta_{ij}(0)^2 \rangle$ . It represents the probability that a given H-bond existing between two molecules at the instant  $t = 0$  remains alive at the instant  $t$ , independently whether the H-bond has been broken for a time less than  $t^*$  during that time interval. If we assume an asymptotic exponential relaxation behavior, we can calculate the H-bond lifetime  $\tau_{HB}$ :

$$C_{HB}(t) \approx \exp(-t/\tau_{HB})$$

The H-bond lifetimes have been obtained by fitting an exponential function to  $C_{HB}$  in an interval between 0.5 and 2.5 ps. As in earlier studies of H-bonding,<sup>25</sup> we considered two values of  $t^*$ . On the one hand,  $t^* = 0$ , i.e.,  $C_{HB}^C(t)$  is calculated allowing  $\eta_{ij}(t)$  to make just one transition from 1 to 0. Then we can determine a continuous H-bond lifetime  $\tau_{HB}^C$ . On the other hand,  $t^* = \tau_{HB}^C$ , i.e., the transition of  $\eta_{ij}(t)$  from 0 to 1 is restricted to those cases in which the H-bond was broken during an interval smaller than  $\tau_{HB}^C$ . In this case, the correlation functions and lifetimes are named  $C_{HB}^R(t)$  and  $\tau_{HB}^R$ , respectively. The values of the resulting lifetimes for water molecules in the first hydration shells of the AD sites are compared with those for pure water<sup>25</sup> in Table 5.

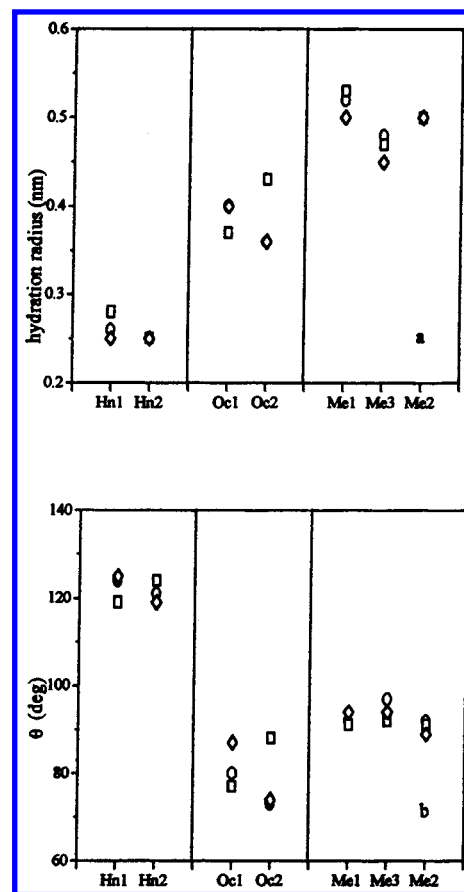
The  $\tau_{HB}^C$  values for different sites and for pure water do not show significant differences. Moreover,  $\tau_{HB}^R$  for the polar sites and for pure water are very similar and only  $\tau_{HB}^R$  corresponding to water in the nonpolar Me hydration site is noticeably higher. This finding together with the relatively high number of H-bonds obtained for Me support earlier suggestions,<sup>6,19</sup> which indicate that there could be some mechanism that makes the hydrogen network around nonpolar species more efficient than in pure water or around polar species.

## VI. Differences between Conformations

As was pointed out at the end of section II there were only little differences between the properties of water surrounding sites of the same species but located at different positions of the AD molecule. Moreover, the results for different AD conformations are very close. For a more detailed analysis of the differences among the properties for different sites and AD conformations, some structural and dynamical results are compared in Figures 6 and 7, respectively.

It may be observed in Figure 6a that the hydration radius corresponding to Oc2 is somewhat greater for the  $\beta$  configuration than for the other two. Besides the number of H-bonds for Oc2( $\beta$ ) is slightly larger than for Oc2( $\alpha$ ) and Oc2( $C_7^{ax}$ ) and the majority of water molecules in the hydration shell of the former site form three H-bonds (Table 4). We also found that the most noticeable differences concerning to the orientation of water in the hydration shell (Figure 6b) correspond to Oc2- $\beta$ . These findings may be attributed to the proximity of the Hn1 and Oc2 sites in the  $\beta$  configuration (see Figure 1b). So the proximity of the former site can perturb the water molecules in the hydration shell of the second. It should be pointed out that though the Hn1 and Oc2 sites are quite close, they do not have a disposition favorable for the formation of an H-bond since their associated dipoles are antiparallel.

The dynamical properties shown in Figure 7a,b show larger dispersion than the static ones. This finding should be mainly associated with a greater statistical uncertainty of these properties. However, it may be also noticed that both the residence and reorientational times for Oc2( $\beta$ ) are higher than for Oc2- $\alpha$ ) and Oc2( $C_7^{ax}$ ). This discrepancy corroborates the structural findings discussed in the last paragraph. It may be also observed that the residence time for Me3( $\alpha$ ) is noticeably larger than for



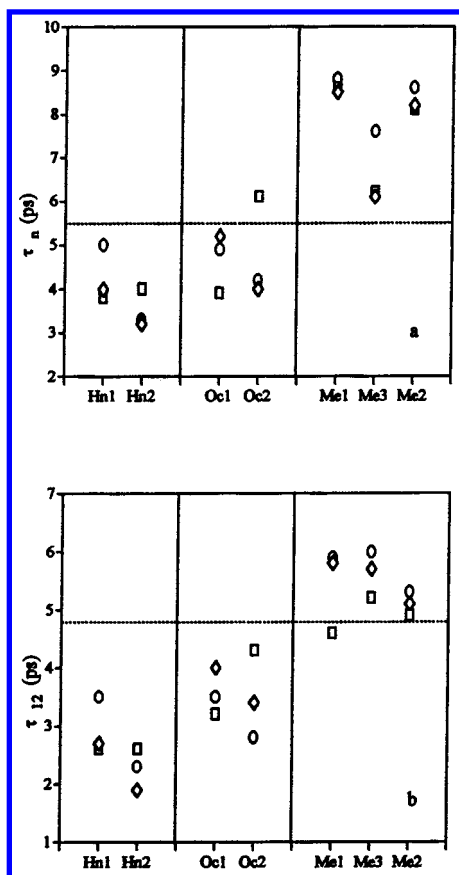
**Figure 6.** Comparison of some structural properties for different AD conformations and analogous AD sites located at different molecular positions: (a) hydration radius; (b) mean value of the  $\theta$  angle. In both figures:  $\alpha$  conformation ( $\circ$ );  $\beta$  conformation ( $\square$ ), and  $C_7^{ax}$  conformation ( $\diamond$ ).

the other two molecular and Me sites (Figure 7a). This could be related to the fact that, unlike Me1 and Me2, the Me3 site seems to be slightly blocked between the respective closest atoms (see Figure 1).

Tobias and Brooks,<sup>28</sup> and Anderson and Hermans,<sup>3</sup> have found that  $\beta$  conformation has a slightly lower free energy than  $\alpha$  or  $C_7^{ax}$  conformations. In turn, the initial experiment from Madison and Kople<sup>1</sup> gave as a result that  $\alpha$  and  $\beta$  conformations are predominant over the  $C_7$  one. In any case, the differences among the free energies for the different AD conformations are rather small. These facts together with the weak dependence of our results on the AD conformation suggests that the influence of the conformation of a protein on their properties is the result of the combination of the little differences of the properties of the hundreds and hundreds of amino acids forming the protein. To improve the analysis of these little effects, very costly computer simulations, using more refined potential models and longer computer than those in this work, would be required.

## VII. Concluding Remarks

We analyzed the hydration of the alanine dipeptide through structural and dynamical properties. We studied three conformations in order to consider, together with a previous work of Rossky and Karplus,<sup>6</sup> the more favorable conformations of such a molecule. At first, all the properties were studied separately for different molecular sites, i.e., the two oxygens, the two hydrogens, and the three methyl groups. Looking at the results, it seemed reasonable to average them between those of the oxygen on one hand, those of the hydrogen on the other and



**Figure 7.** Comparison of some dynamical properties for different AD conformations and analogous AD sites located at different molecular positions: (a) residence times ( $\tau_n$ ); (b) reorientation times ( $\tau_{12}$ ). In both figures:  $\alpha$  conformation ( $\circ$ );  $\beta$  conformation ( $\square$ ), and  $C_7^{ax}$  conformation ( $\diamond$ ). The values for pure water are also shown (— —).

the methyl results last. Furthermore, an average over the three conformation results was made because of the small differences obtained. At that level, the outstanding characteristic was the clear differentiation between the nonpolar (Me) and the polar (Oc, Hn) molecular sites. The differences for the distinct properties analyzed in this work have been discussed along this paper. Nevertheless, the influence of polarity was not large, unlike what was recently suggested in an experimental work by Luzar et al.<sup>29</sup> on a small organic molecule. Our findings suggest that the discrepancies between the properties of water

in the first hydration shells of the different AD sites are markedly dependent on the size of the site (i.e., on the hydration radius of the shell) whereas the influence of the polarity of the site (i.e. the sign of the electric charge) is rather weak.

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