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Site-specific solvation determined by intermolecular nuclear Overhauser effect—measurements and molecular dynamics

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Site-specific solvation has been determined by intermolecular NOE measurements between solvent and solute. The experimental effect is shown on the four compounds 2-butanol, L-alanyl-L-tryptophan (Ala-Trp), adenosine and the disodium salt of adenosine 5′-monophosphate (5′-AMP) in the two solvents water and dimethyl sulfoxide (DMSO). The strength of NOE transfer correlates with the average distribution of solvent molecules around the corresponding solvation sites represented by the number of solvent molecules in a first solvation sphere, which can be obtained from molecular dynamics simulations in water. Saturation transfer between exchanging protons explains some deviations from this correlation. The NOE transfer measurements provide information on specific solute–solvent interactions and contribute to a better understanding of solvation phenomena. On the basis of a distinct relationship between steric solvation hindrance and the strength of NOE transfer, the application of such measurements for conformational analysis has been demonstrated for the first time.

Introduction

Solvation is a fundamental process in chemistry and the classification of solvent properties has been investigated for decades.[1] The influence of solvents on chemical reactions and molecular properties can be significant. It is possible to define a first solvation sphere by the number of solvent molecules following the solute in its translational diffusion.[2,3] There is, however, little known on specific solute–solvent interactions with respect to the question whether a solvent interacts with solutes in a globular isotropic fashion or whether one can demonstrate a directed interaction at specific sites of a solute molecule. This could be of importance in understanding chemical reaction mechanisms, since a reagent might have to replace a solvent molecule at first. One method to study such problems is the measurement of *inter*molecular nuclear Overhauser effects (NOEs). The methodology and some applications of this kind of measurement have recently been reviewed.[4,5] In molecular biology this topic has gained attention because it is possible now to detect water in the interior of protein structures as has been outlined by Otting.[6] In a first experimental attempt on sucrose in water and DMSO we have shown that site-specific solvation can be detected experimentally.[7] The method was also used to indicate preferential solvation of a tetrapeptide by trifluoroethanol (TFE) in TFE–water mixtures.[8,9] The experimental results for a tetradecapeptide have been compared with extensive molecular dynamics (MD) calculations.[10] It was of interest for us to detect site-specific solvation in other representative molecules of bioorganic interest experimentally and to predict site-specificity by computational methods. We were also looking for a significant application of these effects.

Starting from 2-butanol (**1**) as a simple experimental standard to test the calculation methods, we show then on three more elaborate samples, namely L-alanyl-L-tryptophan (**2**) (Ala-Trp), adenosine (**3**) and the disodium salt of adenosine 5′-monophosphate (**4**) (5′-AMP), possible applications of the methods.

Results and discussion

2-Butanol

All NOEs were determined by measuring 1D build-up curves with a selective pulse sequence. The enhancement factors were calculated using the initial rate approximation as described in the experimental section.[11] The relative strength of the NOE effects for DMSO with 2-butanol is illustrated in the bar diagram of Fig. 1a. There is a decrease going from the polar OH proton *via* the methine proton to the remaining proton sites indicating the expected hydrogen bonds formed between DMSO and the OH group of 2-butanol. It is obvious, that the NOE transfer shows a site-specific solute–solvent interaction. Similar results were obtained in water (data not shown). However, the OH signal cannot be detected due to the rapid exchange in this case.

It could be assumed that the extent of the NOE transfer between the solvent and specific solvation sites of a solute correlates with the average distribution of the solvent molecules localised around them. The number of solvent molecules in a first solvation shell around a solute can be estimated on the basis of molecular dynamics (MD) simulations by integration over pair distribution functions. We have adapted this well-known procedure (see Experimental) for our purposes to estimate the number of solvent molecules in the vicinity of the various solvation sites of a solute (*cf.* Experimental). In Fig. 1b, the NOE values determined for 2-butanol are plotted *versus* the number of water molecules localised in definite distances from the various structures of the solute. There is a fair agreement with the exception of the considerable deviation from the correlation line for the methine group indicating a too strong experimental NOE result compared with the theoretical predictions. An enhanced NOE value stemming from saturation transfer after exchange of the irradiated water protons with the OH group and a subsequent intramolecular NOE to the methine group could be a possible explanation for this.

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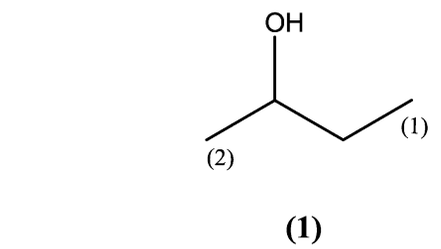

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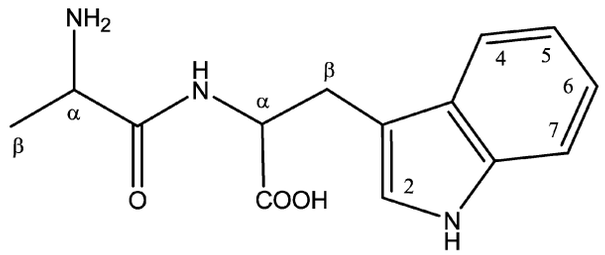

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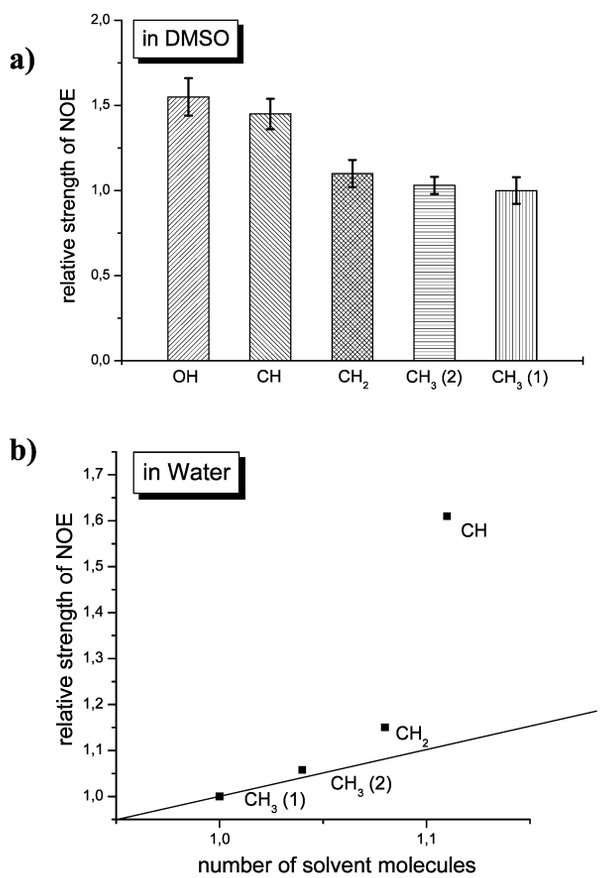

Fig. 1 (a) Normalised experimental intermolecular NOE enhancements between DMSO and 2-butanol (**1**) calculated from initial build-up rates. (b) Correlation between experimental NOE enhancements and the number of attached water molecules obtained from MD simulations.

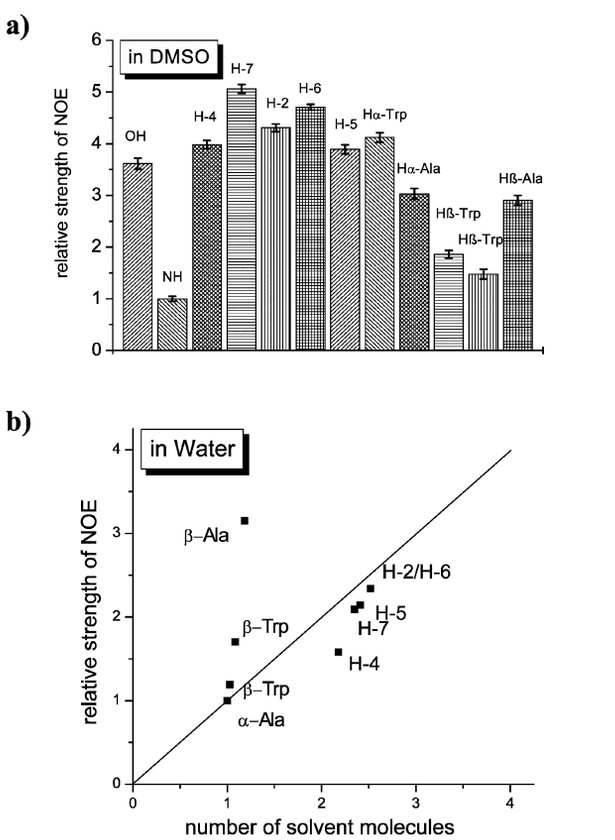

Fig. 2 (a) Normalised experimental intermolecular NOE enhancements between DMSO and Ala-Trp (**2**) calculated from initial build-up rates. (b) Correlation between experimental NOE enhancements and the number of attached water molecules obtained from MD calculations.

L-Alanyl-L-tryptophan

Ala-Trp (**2**) is a dipeptide with aliphatic and aromatic side chain protons. It was of interest to study their behaviour in both solvents. Again, the problem of exchange has to be considered for such a type of molecule. In Fig. 2a, the bar diagram for the normalised NOE interactions of DMSO with Ala-Trp is given. A qualitative inspection of this diagram reveals, surprisingly, that the aromatic protons H-2 and H-4–H-7 exhibit the strongest NOE interactions of the molecule. We interpret this result as a hydrophobic interaction between the methyl groups of DMSO and the aromatic ring protons. The NOE values for the methylene group of Trp are significantly lower than those of all other carbon-bound protons. Most likely this could be attributed to a steric hindrance which does not allow the solvent molecules to interact strongly with these protons. The experiments in water reveal a similar behaviour. A rather large NOE is detected for the methyl group of Ala which cannot be observed in DMSO. We presume that this comes from an exchange of water with the amino group and a subsequent intramolecular NOE transfer. Fig. 2b shows the comparison between the experimental and theoretical results for Ala-Trp in water. Again, a fair agreement between the calculated and experimental values can be seen with the exception of the alanine methyl group. A full explanation of these deviations especially in comparison with the behaviour of the methyl group in 2-butanol **1** will only be possible in terms of a complete relaxation matrix treatment.

Adenosine and adenosine 5′-monophosphate disodium salt: conformation at the glycosidic bond

The studies on 2-butanol and the dipeptide L-alanyl-L-tryptophan show unequivocally a site-specificity of the NOE transfer between solvent and solute and a correlation between the strength of NOE transfer and the average solvent distribution around the solvation sites as it can be estimated on the basis of MD simulations. Thus, NOE transfer measurements provide information on the arrangement of solvent molecules around a solute, which could be important for the understanding of its behaviour in solution. Another aspect of such studies will now be demonstrated on further bioorganic compounds. For instance, the very small NOE interaction between the methylene group of Trp in Ala-Trp obviously comes from a steric

solvation hindrance. This observation could be used to solve structure problems by the determination of the NOE transfer between solvent and solute, provided that the corresponding solvation sites in the structure differ significantly in their capacities to interact with solvent molecules.

We want to illustrate this aspect by the investigation of the conformation at the glycosidic bond (C-1′–N) of adenosine (**3**) and the disodium salt of adenosine 5′-monophosphate (5′-AMP) (**4**). This conformation problem has long been debated[12–14] and has been addressed by numerous different NMR techniques[15] and theoretical calculations.[16] Both compounds were measured in DMSO and water at 310 K. At this temperature, the proton H-2′ was shifted from the water resonance. To determine the conformational preference of the *anti* or *syn* orientation of the adenine group with the sugar ring, it may be sufficient to consider the solvation properties around the protons H-2 and H-8 of the heterocyclic base. For an *anti* arrangement, we expect in both molecules smaller intermolecular NOEs between the solvent and the solute at H-8 compared with H-2 due to steric hindrance of the solvent interaction in this orientation. The opposite effects should appear in the case of a *syn* orientation.

The relative spectral assignment of the two proton signals in both compounds was confirmed in this work by an HMBC spectrum using the ^3J(C,H) of H-8 to C-1′. Our NOESY measurements showed the H-8 directed toward the sugar ring in aqueous solution, thus confirming the preference of the *anti* arrangement. The intermolecular NOE build-up curves for adenosine in Fig. 3 indicate significant differences in the region of the aromatic protons with stronger interactions between H-2 and the solvent. There are no significant differences of the NMR results between **3** and **4**. The smallest solute–solvent interaction is found for the methylene group C-5′, which is barely accessible to the solvent molecules.

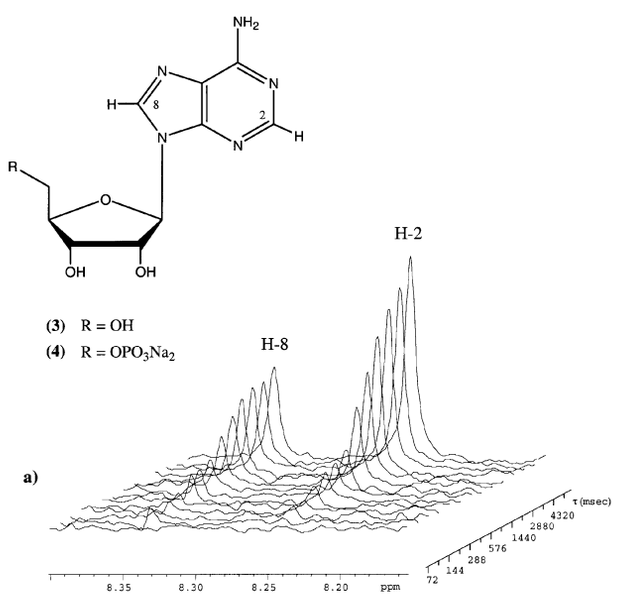

Fig. 3 (a) ^{1}H-NMR NOE enhancements between water and adenosine (**3**) in the aromatic proton region as a function of the mixing time.

Conclusions

We have shown that site-specific intermolecular NOE effects can be measured and compare well with solvation data obtained from molecular dynamics simulations. The agreement between calculated and experimental values is fair, thus giving the MD calculations predictive power. Exceptions are most likely due to additional experimental effects such as intramolecular NOE after saturation transfer due to exchange. In this way, NOE transfer measurements provide information on the arrangement of solvent molecules in the vicinity of various structural parts of a solute, which could be important for the understanding of its behaviour in solution and contribute to a better understanding of solvation phenomena. A distinct relationship between steric solvation hindrance and the strength of the NOE transfer was observed, which could be important for structure determination, if structure alternatives of a solute exhibit distinct differences of solvation at corresponding solvation sites. The application of this idea has been demonstrated for the first time in this work by a conformational analysis of nucleosides and nucleotides.

Experimental

NMR measurements

The NMR measurements were performed in typically 14–100 mM concentration in water and DMSO, employing a Bruker DRX-400 equipped with a multinuclear inverse gradient probe-head, z-gradient coils and a temperature controller, which was set to 300 K for **1** and **2** and to 310 K for **3** and **4**. To avoid radiation damping effects, the solvents were used in a mixture of 90% deuterated and 10% protonated form, DMSO was dried using molecular sieves. The DPFGSE-NOE method was used as a pulse sequence for the 1D intermolecular NOE measurements with a subsequent solvent suppression as described previously.[8] The selective pulse was adjusted to either the H_2O or the DMSO proton signals. For one mixing time typically 1k transients were recorded. The mixing times were in the range of 50 ms and 5 s. The method of initial rate approximations[11] was applied for the evaluation of the NOE experiments by linearising the NOE build-up between 50 ms and 0.5 s. The obtained slopes were normalised corresponding to the number of protons.

Molecular dynamics (MD) simulations

The MD simulations were performed on the basis of the CHARMM 24b2 package[17] employing the CHARMm 23.1 force field.[18,19] The global minimum structures of the molecules **1**–**3** obtained from systematic conformational searches were placed in a cubic box of about 30 Å containing 1000 water molecules. After heating and equilibration periods of 20 ps, trajectories of 200 ps evolution time were generated at 300 K considering periodic boundaries. The average distribution of the solvent molecules around a solute can well be described by pair distribution functions. To define solvation regions, it is sufficient to examine atomic pair distributions. We consider the distribution of water molecules W around a particular atom A of the solute. The pair distribution function is given by

$$g_{WA}(r) = (1/4\pi\rho_w r^2)\, \mathrm{d}N_{WA}(r)/\mathrm{d}r,$$

where $N_{WA}(r)$ is the average number of oxygen atoms within a sphere of radius r around the atom A and ρ_w is the density of water molecules in the system. The integration over the pair distribution functions leads to the number of water molecules localised in a definite distance from the atoms A. In our cases, the integration was performed until the first minimum of the pair distribution functions.[20,21]

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