Water hydration of macrocyclic systems in organic solvents: an NMR diffusion and chemical shift study

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NMR diffusion measurements and chemical shift data are used to study water hydration of 18-crown-6 1 and its potassium iodide complex (1.KI) in [^{2}H]chloroform suggesting different modes of solute–water interaction in the two cases.

Water has been shown to exert a dramatic influence on the conformations and hence on the function of biological macromolecules such as DNA and proteins.[1,2] Recently, efforts have been directed to characterize the hydration layers of macromolecules in bulk water using sophisticated NMR techniques.[3–6] The implications of macromolecule hydration to magnetic resonance imaging (MRI) have also been discussed.[7,8]

Water hydration in aqueous media is a very complex problem but some insight into the nature of solute–water interactions may also be gathered from studies of water hydration in organic solvents.[9] In addition, there are systems in which the hydration in the organic phase has a direct relevance to the process under investigation. One such process is carrier mediated transport through biological or chemical membranes in which the actual species involved in the process are, for example, the hydrated macrocycle and its complex. Nevertheless, it has been recently pointed out that reports on water hydration of various solutes in organic solvents are relatively scattered in the literature.[9] Pulsed gradient spin echo (PGSE) NMR has been used extensively to study the diffusion of complex systems in the last 30 years.[10–13] Recently we have demonstrated the utility of NMR diffusion measurements in the characterization of organic complexes and in the elucidation of the solution structure of some supramolecular systems.[14] Therefore, we decided to investigate the interaction of water molecules with macrocyclic systems, before and after complexation, in chloroform.

We chose to concentrate on a classical host–guest system, namely 18-crown-6 **1** and its potassium iodide complex, **1**.KI, in $CDCl_3$. We used simultaneous measurements of the changes in the diffusion coefficient and in the chemical shift of water molecules as a function of the macrocyclic system : water ratio in the $CDCl_3$ solution.‡ The changes in these parameters were used to estimate the average number of water molecules associated with each molecule of **1** or **1**.KI.§

Table 1 depicts the diffusion coefficients of **1**.KI and **1** as well as the diffusion coefficients and the chemical shifts of the water molecules in the $CDCl_3$ solution as a function of the solute : water ratios, *i.e.* **1** : H_2O and **1**.KI : H_2O. The data show that, when the ratios of **1**.KI : H_2O or **1** : H_2O are increased, the gradual decrease in the water diffusion coefficient is accompanied by a gradual shift of the water chemical shift to low field. The diffusion coefficients of the macrocyclic systems (**1** or

Table 2 Average number of water molecules (X_{H_2O}) associated with **1** and its potassium iodide complex (**1**.KI) in $CDCl_3$ calculated according to eqns. (1) and (2)

Substance	Equiv. of water	X_{H_2O} from *D*	X_{H_2O} from δ
1.KI	3.14	0.26	0.28
1.KI	2.42	0.26	0.29
1.KI	1.95	0.36	0.28
1.KI	1.01	0.32	0.28
1.KI	0.90	0.33	0.29
1.KI	0.72	0.28	0.27
1.KI	0.53	0.31	0.26
1	3.79	0.89	1.05
1	3.03	—	0.92
1	1.86	—	0.75
1	1.71	—	0.77
1	1.44	0.53	0.70
1	1.03	—	0.59
1	0.84	0.37	0.50
1	0.75	—	0.49

Table 1 Chemical shift (δ_{H_2O}) and diffusion coefficients (D_{H_2O}) of water in the presence of **1** and **1**.KI in $CDCl_3$[a]

Substance	Equiv. of H_2O	D_{Cr}/ 10^{-5} cm^2 s^{-1}	$D_{H_2O}{}^1$/ 10^{-5} cm^2 s^{-1}	$D_{H_2O}{}^2$/ 10^{-5} cm^2 s^{-1}	$\delta_{H_2O}{}^1$	$\delta_{H_2O}{}^2$
1.KI	3.14	0.77 ± 0.01	3.68 ± 0.03		1.74	
1.KI	2.42	0.86 ± 0.08	3.60 ± 0.08		1.78	
1.KI	1.95	0.72 ± 0.01	3.45 ± 0.06		1.81	
1.KI	1.01	0.82 ± 0.06	3.14 ± 0.03		1.99	
1.KI	0.90	0.89 ± 0.01	2.80 ± 0.01		2.05	
1.KI	0.72	0.76 ± 0.01	2.65 ± 0.01		2.11	
1.KI	0.53	0.78 ± 0.06	2.07 ± 0.09		2.26	
1.KI	2.12[c]	0.75 ± 0.02	3.41 ± 0.05	0.93 ± 0.08	1.87	4.73
1	3.79	0.99 ± 0.01	3.24 ± 0.04		1.99	
1	1.44	0.91 ± 0.01	2.76 ± 0.05		2.27	
1	0.84	0.97 ± 0.01	2.59 ± 0.16		2.41	
1	2.12[c]	0.87 ± 0.01	2.52 ± 0.04	0.89 ± 0.01	2.45	4.89
$CDCl_3$	Low[b]		3.77 ± 0.10		1.62	
$CDCl_3$	High[c]		3.92 ± 0.02	0.84 ± 0.01	1.62	4.88

[a] Values are means ± standard deviation of at least 3 experiments and corrected for $CDCl_3$ at 283 K. [b] Experiments performed on the water dissolved in water saturated $CDCl_3$ solutions. [c] Same solution as in *b* to which 3 μl of water were added to the NMR tube.

1.KI) were found to be unchanged within the experimental errors. The diffusion coefficient of the solvent ($CHCl_3$ in $CDCl_3$) was also constant as expected and therefore was used as an internal reference.

Table 2 shows the average number of water molecules bound per molecule of **1** or **1**.KI. Two major differences were observed: firstly, the number of bound water molecules in the case of **1** depends on the water–macrocycle ratio although such a dependency is not observed for **1**.KI. Secondly, more water molecules are bound to **1** than to **1**.KI. It was found that the average number of water molecules associated with each molecule of **1**.KI is *ca.* 0.3 with no dependency on the **1**.KI : H_2O ratio. Table 2 shows that there is a good agreement between the diffusion data and the chemical shift data in this case. Iwachido *et al.* have found that the average number of water molecules extracted into nitrobenzene per molecule of the potassium complex of **1** is 0.3.[15] The average number of water molecules associated with each molecule of **1** is higher and varies between around 1 to 0.5 water molecules per molecule of **1**. Here the agreement between the two methods is less satisfactory with the results from chemical shift data being 20–30% higher than the results obtained from diffusion measurements. This may be partially due to the relatively poor estimate of the chemical shift of bound water ($\delta^{b}_{H_2O}$)§ in the case of **1**. Iwachido *et al.* have found that the average number of water molecules extracted by **1** into nitrobenzene is 1.6.[15]

The above data demonstrate that 18-crown-6 acts as an effective hydration shell for potassium thus effectively reducing the number of water molecules which are associated with the potassium salt from 1.3[15] to 0.3. In the case of **1**, however, in addition to the hydration there is a possibility of water complexation in the empty cavity of **1**. This in line with the X-ray-structure of $\mathbf{1}\cdot(H_2O)_6$ which was only characterized recently and which shows that two water molecules interact directly with each macrocyclic ring.[16] When we repeated the experiment with the LiI complex of 12-crown-4 (**2**.LiI) it was found that the average number of water molecules associated with the complex was even smaller than in the case of **1**.KI (*ca.* 0.2). However, as expected the lithium cation was found to extract many more water molecules into nitrobenzene solution than potassium (5.6 *vs.* 1.3 water molecules per cation).[16] The explanation may be that **2** acts as a good substitution for the hydration shell of the highly hydrated Li^+ cation and since the number of water molecules that interact with **2** is intrinsically low, the **2**.LiI complex is only weakly hydrated.

In addition, to one sample of **1** and **1**.KI we added 3 μl of water. As a result an additional new peak was observed at *ca.* δ 4.7 which was found to have a very low diffusion coefficient. Both the chemical shift and the diffusion coefficient of this peak ($\delta_{H_2O}{}^2$ and $D_{H_2O}{}^2$, respectively) suggest that it represents water in water (Table 1).

Water hydration of macromolecules in aqueous media is very complex and many types of hydration spheres have been evoked[17] when the main criterion for distinguishing between these types is their average lifetime in the bound state.[3–5,17] In aqueous media one of the main problems is to avoid the signal of the bulk water which does not participate in any interaction with the macromolecule. In addition, one has to overcome the fast exchange between hydration water and bulk water. In organic solvents such complications do not exist making the study of solute–water interactions much more simple. Nevertheless, it is important to study the interactions of the water molecule with such systems. Our data clearly demonstrates that the polar water molecules in chloroform prefer to spend their life associated with the polar species in the organic solvent if such species are available. Our data shows that there is a good agreement between the two methods which assists interpretation of the experimental results. It should be noted, however, that both NMR methods used in this study give an average picture. Nevertheless, the complementarity between the methods and our data demonstrate the utility of PGSE NMR for studying water hydration of macrocyclic systems in organic solvents.

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Footnotes

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‡ NOE experiments were also run to confirm the existence of NOE cross peaks. The change in the chemical shift of water was calculated relative to $CHCl_3$ in the $CDCl_3$ solution. All NMR experiments were performed on a Bruker 500 MHz ARX NMR spectrometer equipped with a B-AFPA 10 pulsed gradient unit. Diffusion coefficients were calculated as described previously.[14]

§ The fraction of bound water $X^{b}_{H_2O}$ was calculated using eqns. (1) and (2)

$$\delta_{H_2O}{}^{obs} = \delta_{H_2O}{}^{f} * (1 - X^{b}_{H_2O}) + \delta_{H_2O}{}^{b} * X^{b}_{H_2O} \qquad (1)$$

$$D_{H_2O}{}^{obs} = D_{H_2O}{}^{f} * (1 - X^{b}_{H_2O}) + D_{H_2O}{}^{b} * X^{b}_{H_2O} \qquad (2)$$

where $\delta_{H_2O}{}^{f}$, $\delta_{H_2O}{}^{b}$, $D_{H_2O}{}^{f}$ and $D_{H_2O}{}^{b}$ are the chemical shifts of the water in the free and bound states and the diffusion coefficients in the free and bound states, respectively. $\delta_{H_2O}{}^{obs}$ and $D_{H_2O}{}^{obs}$ are the observed chemical shift and diffusion coefficient of the water peaks, respectively and $X^{b}_{H_2O}$ is the fraction of bound water in each case. $\delta^{b}_{H_2O}$ was obtained by separate titration experiments and $D_{H_2O}{}^{b}$ was assumed to be equal to the diffusion coefficient of the macrocyclic system.

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