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Biopolymers and Biomacromolecules Solvent Dynamics

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Summary: Biomacromolecules in solution modify the structure and the dynamics of the bulk water at the solute-solvent interface. The ordering effects of biomolecules, in particular proteins, are extended for several angstroms. The role of the hydration shells around a protein has yet to be completely understood. Hydrated proteins maintain more dynamic flexibility with respect to the dried system, which is an important property in protein-protein and/or protein-ligand recognition processes. In this paper we propose a method for analyzing the dynamical properties of the water molecules present in the hydration shells of proteins. The approach is based on analysis of the effects of protein-solvent interactions on water protons NMR relaxation parameters. The water proton spin-lattice relaxation rate in protein solution is analyzed considering all possible dipolar contributions from coupled protons environments. The analysis of both selective and non-selective water spin-lattice relaxation rates allowed the calculation of the average effective correlation time for the water molecules at the protein interface and the evaluation of the long range ordering effect of the protein surface.

Keywords: hydration shell; NMR; protein hydration; water dynamics; water proton relaxation rate

Introduction

Protein in solution modify the structure and the dynamics of the bulk water at the solute-solvent interface.[1–4] This process results in a protein hydration shell in which water molecules have restricted dynamics with respect to the bulk water. The extent of interaction can be monitored studying the solvent parameters mostly affected by the presence of a large, slowly reorienting biomacromolecule.[5–12] NMR relaxation parameters, especially the non-selective (R_1^{NS}) and selective (R_1^{SE}) spin-lattice relaxation rates of water protons are useful for investigating the solvent dynamics at the macromolecule-solvent interfaces as well as the perturbation effects caused by the ligand-macromolecule interactions on the solvent dynamical properties.[13–25] In this paper we developed a strategy, based on Nuclear Magnetic Resonance Spectroscopy, to define the dynamical contribution of the biomacromolecules to the water molecules belonging to their hydration shells. In a globular proteins solution, three different water environments are present, i.e., the buried water molecules (which are integrant part of the protein structure and cannot be removed even during protein crystallization),[3,4] the water hydration shell around the protein and the bulk water. The present investigation analyzes the dynamical properties of the water molecules present in the hydration shell around a protein system. Water proton relaxation rates have been used to investigate different systems and

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phenomena, and theoretical interpretations of the experimental results have been proposed.[26–30] Both the water proton spin-lattice relaxation rates R_1^{NS} and R_1^{SE} in solution are analyzed considering all possible sources of dipolar contributions arising from proton environments. From this analysis an equation for the calculation of ordering effect induced by the macromolecule on the hydration water was derived. In particular the average water rotational correlation time which characterizes water protons dynamics in the protein hydration shell was calculated. This information was then used for the calculation of the dimension of the long-range ordering effect caused by the protein molecules on the hydration water.

Theory

Dipolar non-selective R_1^{NS} and selective R_1^{SE} spin-lattice relaxation rates have the following expressions:

$$R_1^{NS} = \sum \rho_{ij} + \sum \sigma_{ij} \quad (1)$$

$$R_1^{SE} = \sum \rho_{ij} \quad (2)$$

For any i,j dipolar coupling R_1^{NS} and R_1^{SE} assume the explicit form:

$$R_1^{NS} = \frac{3}{10}\frac{\gamma_H^4\hbar^2}{r_{ij}^6}\left[\frac{4\tau_c}{1+4\omega_H^2\tau_c^2} + \frac{\tau_c}{1+\omega_H^2\tau_c^2}\right] \quad (3)$$

$$R_1^{SE} = \frac{1}{10}\frac{\gamma_H^4\hbar^2}{r_{ij}^6}\left[\frac{3\tau_c}{1+\omega_H^2\tau_c^2} + \frac{6\tau_c}{1+4\omega_H^2\tau_c^2} + \tau_c\right] \quad (4)$$

In pure water, the water non-selective wR_1^{NS} and selective wR_1^{SE} spin-lattice relaxation rates are:

$$wR_1^{NS} = \sum \rho_{ww} + \sum \sigma_{ww} \quad (5)$$

$$wR_1^{SE} = \sum \rho_{ww} + \sum \sigma_{ww} \quad (6)$$

where ρ_{ww} e σ_{ww} are the water direct and cross-relaxation rate contributions which result from water proton-proton intra and intermolecular interactions.

In pure water both wR_1^{NS} and wR_1^{SE} assume the same value as the cross-relaxation term σ_{ww} affects the selective and non-selective measurements equally.

In binary system (water-protein and/or polymer) we assume the distribution of water molecules as schematically represented by the model showed in Figure 1. Water molecules can be classified into three different categories accordingly to their dynamical properties: bulk water with a typical reorientational correlation time of the order of picoseconds; water present at the macromolecular surface which exhibits a partially restricted reorientational motion. The dynamical properties of the water molecules in these conditions can be well represented by a distribution of correlation time values. These molecules are in fast chemical exchange with the microenvironments present at the protein surface and with the bulk water molecules; buried water molecules. These long lived water molecules show dynamics which are mostly determined by the slow reorientation motion of the macromolecule with τ_c values typically of the order of 10^{-8} seconds. These molecules exhibit slow chemical exchange rate in the NMR time scale with the waters present at the macromolecular surface. The contribution of these water molecules to the observed spin-lattice relaxation rates is negligible due to their very low molar fraction.

Relaxometric studies have been used to determine the number and the dominant reorientational correlation time which is involved in the relaxation of water molecules buried in the macromolecular structure. Nevertheless relaxometric experiments cannot monitoring the dominant fluctuations which are involved in the relaxation of the water molecules present at the macromolecular surface. In fact this environment is characterised by water molecules which exhibit a distribution of the τc values and display fast chemical exchange with other waters of the same environment or with the

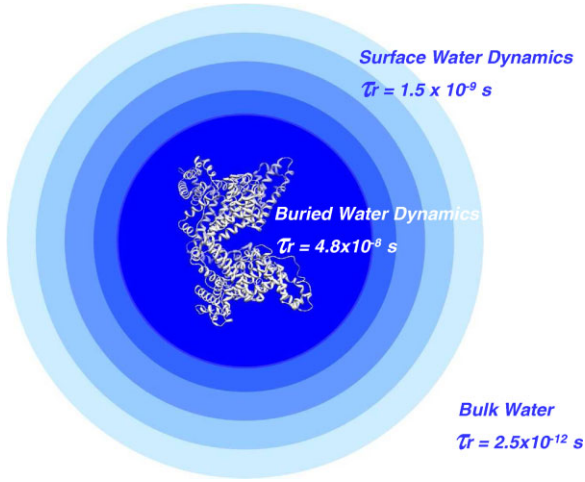

Figure 1.
Effect of the ordering effect of proteins on water. Three water environments defined by their dynamical properties can be observed: bulk, surface and buried water environments.

bulk molecules. These are in fact the appropriate conditions to apply the selective and non-selective water spin-lattice relaxation methodologies.

In water-protein binary systems, under fast chemical exchange conditions between the free (bulk) and bound water (the water molecules at the protein surface, where as bound water are considered), the changes observed in water spin-lattice relaxation rates with respect to the bulk water reflect the presence of water molecules in environments characterised by restricted dynamical reorientation. In these conditions non-selective (wR_1^{NS}) and selective (wR_1^{SE}) water spin-lattice relaxation rates assume different values as a consequence of a negative protein-water cross-relaxation contribution to wR_1^{NS} and wR_1^{SE} are defined as:

$$wR_{1exp} = \chi_b R_{1b} + \chi_f R_{1f} \tag{7}$$

where $wR_{1\ exp}$ is the experimental relaxation rate of water in the presence of the protein, R_{1b} and R_{1f} the water relaxation rates of the pure bound and free environments and χ_b and χ_f the molar fraction of water in bound and bulk conditions.

Considering the following equilibrium based on the model assumed in Figure 1:

$$\begin{aligned} &M(H_2O)_m^A(H_2O)_n^B + n(H_2O)^C \leftrightarrows \\ &M(H_2O)_m^A(H_2O)_n^C + n(H_2O)^B \end{aligned} \tag{8}$$

where M is the macromolecule, A are the non-exchangeable and B and C are the exchangeable water molecules respectively, χ_b, the bound water molar fraction assumes the form:

$$\begin{aligned} \chi_b &= \frac{n\left[M(H_2O)_m^A(H_2O)_n^B\right]}{[H_2O] + \left[M(H_2O)_m^A(H_2O)_n^B\right]} \\ &\approx \frac{n\left[M(H_2O)_m^A(H_2O)_n^B\right]}{[H_2O]} \end{aligned} \tag{9}$$

Considering that $[H_2O] >> \left[M(H_2O)_m^A(H_2O)_n^B\right]$, χ_f of the free water molar fraction is assumed to be: $\chi_f = 1 - \chi_b \cong 1$

Under these conditions $wR_{1\ exp}^{NS}$ and $wR_{1\ exp}^{SE}$ are described by:

$$\begin{aligned} wR_{1exp}^{NS} = &\sum \rho_{ww}^f + \sum \sigma_{ww}^f \\ &+ \chi_b \sum \rho_{ww}^b + \chi_b \sum \sigma_{ww}^b \\ &+ \chi_b \sum \rho_{wp}^b + \chi_b \sum \sigma_{wp}^b \end{aligned} \tag{10}$$

and

$$wR_{1\exp}^{SE} = \sum \rho_{ww}^{f} + \sum \sigma_{ww}^{f} + \chi_b \sum \rho_{ww}^{b} + \chi_b \sum \sigma_{ww}^{b} + \chi_b \sum \rho_{wp}^{b} \quad (11)$$

where ρ_{wp} and σ_{wp} represent the water-protein ^{1}H-^{1}H direct and cross-relaxation terms respectively and the indexes f and b consider the free and bound water.

At the bound site, in the presence of $D_2O > 95\%$, the residual water protons show a relaxation which is mainly dominated by the dipolar interactions with the non-exchangeable protein protons. Water-water interactions (both inter- and intra-) have a sufficient low frequency to be neglected and the Equations (10) and (11) are:

$$wR_{1\exp}^{NS} = \sum \rho_{ww}^{f} + \sum \sigma_{ww}^{f} + \chi_b \sum \rho_{wp}^{b} + \chi_b \sum \sigma_{wp}^{b} \quad (12)$$

$$wR_{1\exp}^{SE} = \sum \rho_{ww}^{f} + \sum \sigma_{ww}^{f} + \chi_b \sum \rho_{wp}^{b} \quad (13)$$

By combining Equations (5), (6) and (12), (13):

$$wR_{1\exp}^{NS} = wR_{1}^{NS} + \chi_b \left(\sum \rho_{wp} + \sum \sigma_{wp} \right) \quad (14)$$

$$wR_{1\exp}^{SE} = wR_{1}^{SE} + \chi_b \left(\sum \rho_{wp} \right) \quad (15)$$

The protein contribution to the water relaxation rates, ΔR_1, can be calculated as:

$$\Delta R_1^{NS} = wR_{1\exp}^{NS} - wR_1^{NS} = \chi_b \left(\sum \rho_{wp} + \sum \sigma_{wp} \right) = \chi_b R_{1b}^{NS} \quad (16)$$

$$\Delta R_1^{SE} = wR_{1\exp}^{SE} - wR_1^{SE} = \chi_b \left(\sum \rho_{wp} \right) = \chi_b R_{1b}^{SE} \quad (17)$$

where R_{1b}^{NS} and R_{1b}^{SE} are the relaxation rates of the water molecules present in the bound conditions.

Considering the dependence of the R_1^{NS}/R_1^{SE} ratio on τ_c (see Equations 3 and 4), $\Delta R_1^{NS}/\Delta R_1^{SE}$ ratio allows the calculation of the τc value resulting from the average contribution of the distribution of motions that characterises the water dynamics at the macromolecular surface. Equations (3) and (4) hold their own validity when a single correlation time value is replaced by a distribution function which considers all different fast exchanging microenvironments.

In fact:

$$\Delta R_1^{NS}/\Delta R_1^{SE} = \chi_b R_{1b}^{NS}/\chi_b R_{1b}^{SE} = R_{1b}^{NS}/R_{1b}^{SE} = \frac{\frac{12\tau_{c1}}{1+4\omega_H^2\tau_{c1}^2} + \frac{3\tau_{c1}}{1+\omega_H^2\tau_{c1}^2}}{\frac{6\tau_{c1}}{1+4\omega_H^2\tau_{c1}^2} + \frac{3\tau_{c1}}{1+\omega_H^2\tau_{c1}^2} + \tau_{c1}} \quad (18)$$

where τ_{c1} represents a distribution function which considers all individual dynamics which modulate the relaxation. The calculated τ_c value may be not direct related to a physical meaning as it is not demonstrated the presence at the macromolecular surface of a specific dynamics defined by this value. Nevertheless this experimentally determined parameter represents the average value which affects the dipolar water-protein interactions at the macromolecular surface. This parameter assumes a value which has to be in between the protein τc reorientational motion ($\sim 10^{-8}$ s) and the solvent free tumbling reorientation ($\sim 10^{-12}$ s).

Materials and Methods

^{1}H-NMR spectra were obtained on a Bruker AMX 400 spectrometer operating at 400 MHz. Spin-lattice relaxation rates were measured using the (180°-τ – 90°-t)n sequence. The τ values used for the selective and non-selective experiments were: 0.01, 0.02, 0.04, 0.06, 0.08, 0.1, 0.2, 0.4, 0.8, 1, 1.5, 2, 3, 4, 5, 7 and 10 seconds. The 180° selective inversion of the proton spin population was obtained with a selective perturbation pulse, generated by the decoupler channel. The selective spin-lattice relaxation rates were calculated

using the initial slope approximation and subsequent three parameter exponential regression analysis of the longitudinal recovery curves. The maximum experimental error in the relaxation rate measurements was 5%.

Human albumin (molecular weight 66200 Dalton) was purchased from Sigma Chemical Co. All the solution were obtained using D_20 with a minimum content of deuterium of 99.9%.

Results and Discussion

The theory presented in the previous section is supported by the experimental results obtained on human albumin system.

Water selective and non-selective spin-lattice relaxation rates as a function of protein concentrations, are reported in Table 1.

The proteins contribution to the water selective ΔR_1^{SE} and non-selective ΔR_1^{NS} relaxation for human albumin systems is shown in Figure 2. In this figure the fitting of the experimental results is also shown. As required by the theory, the calculated straight lines pass through the origin in the system under study. As shown in Figure 2, water selective spin-lattice relaxation rates assume a larger value with respect to the water non-selective spin-lattice relaxation rates, which results affected by the negative protein-water cross-relaxation contributions.

The ratios calculated from the proteins contribution to the water non-selective and selective relaxation rates, $\Delta R_1^{NS}/\Delta R_1^{SE}$, assumes a value of 0.36. The behavior of the $\Delta R_1^{NS}/\Delta R_1^{SE}$ ratio as a function of τc is reported in Figure 3.

Using the previously computed $\Delta R_1^{NS}/\Delta R_1^{SE}$ ratio of 0.36, an average reorientational correlation time of 1.5×10^{-9} s was calculated for the water molecules in the protein hydration shell. In Figure 1 a summary of the water environments typical of protein systems in the case of human albumin is shown: bulk, buried and hydration water. In the same figure the rotational correlation time values typical of each water environments are reported. The average water hydration correlation time previously computed was used to calculate the ordering effects of the protein on water molecules in the hydration shells at different distance from the protein surface. Assuming a spherical shape with a diameter of 70 Å, the volume of ten hydration spheres around human albumin was calculated. The number of water molecules in each hydration sphere was computed as well as the number of the total water molecules contained in the first ten hydration spheres. Assuming an exponential decay of the water correlation time from its value at the protein surface to the bulk

Table 1.
Water non-selective and selective proton spin-lattice relaxation times as a function of the human albumin content at 298 K. In the same table the protein contribution to the selective and non-selective proton spin-lattice relaxation rates ΔR_1^{SE} and ΔR_1^{NS} is also reported.

Albumin concentration mol/L	Albumin concentration mg/ml	T_1^{NS} s	T_1^{SE} s	R_1^{NS} s^{-1}	R_1^{SE} s^{-1}	ΔR_1^{NS} s^{-1}	ΔR_1^{SE} s^{-1}
0	0	10.10	10.30	0.099	0.097	0	0
1.6×10^{-5}	1.0	8.30	6.45	0.120	0.155	0.021	0.058
3.2×10^{-5}	2.0	7.10	4.55	0.141	0.220	0.042	0.123
4.8×10^{-5}	3.0	6.15	3.60	0.163	0.278	0.064	0.181
6.5×10^{-5}	4.0	5.50	2.90	0.182	0.345	0.083	0.248
7.3×10^{-5}	4.5	5.10	2.70	0.196	0.370	0.097	0.273
8.1×10^{-5}	5.0	4.70	2.45	0.213	0.408	0.114	0.311
8.9×10^{-5}	5.5	4.45	2.30	0.225	0.435	0.126	0.338
9.7×10^{-5}	6.0	4.15	2.10	0.241	0.476	0.142	0.379
1.3×10^{-4}	8.0	3.55	1.66	0.282	0.602	0.183	0.505
1.6×10^{-4}	10.0	3.10	1.40	0.323	0.714	0.202	0.559

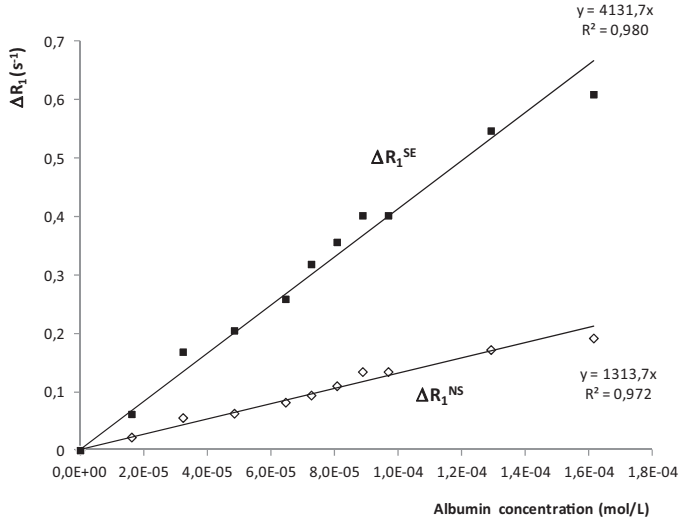

Figure 2.
Non-selective and selective proton spin-lattice relaxation rates ΔR_1^{SE} and ΔR_1^{NS} as a function of the human albumin concentration.

conditions, the following equation was developed:

$$\tau_{c(1,2,\ldots 10)} = a + be^{-kd} \tag{19}$$

where τ_c (1,2 . .,10) are the calculated correlation time values of the water molecules present from the first to the tenth hydration shell $a = 2.5 \times 10^{-12}$ s the bulk water rotational τ_c, $b = 4.8 \times 10^{-8}$ s the buried water rotational τc, d the hydration shell distance from the protein surface assumed here to be from 1 to 10 Å, k (d^{-1}, Å) a constant which define how strong is the ordering effect of the protein on the water molecules in the protein surrounding. In Figure 4 the computed correlation times (calculated from Equation 19), of the water molecules in each of the

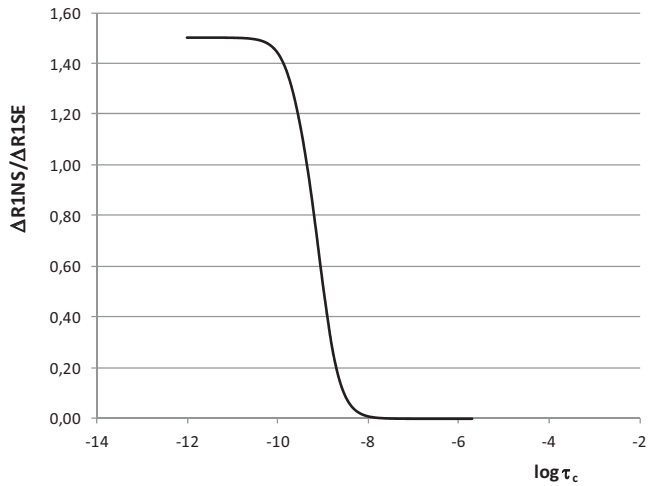

Figure 3.
Computed values of $\Delta R_1^{NS}/\Delta R_1^{SE}$ ratio as a function of τ_c at a proton frequency of 400 MHz.

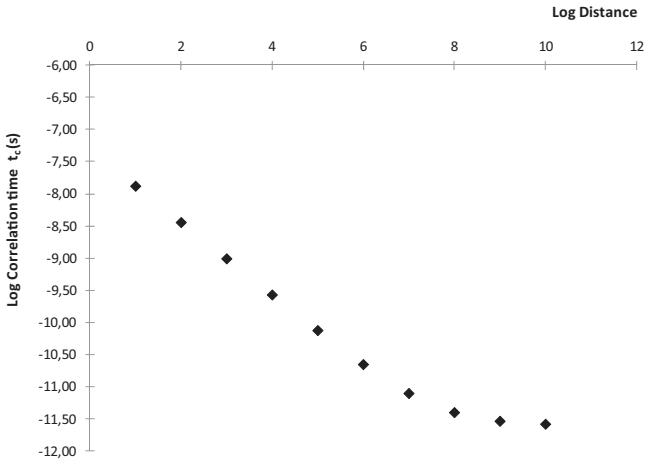

Figure 4.
Computed values of the reorientational water correlation times typical of water molecules in the first tenth hydration shells around human albumin. Correlation time was calculated using equation (19) with $a = 2.5 \times 10^{-12}$, $b = 4.8 \times 10^{-8}$, $k = 1.3$. The average correlation time over the ten shells was calculated using the equation $\tau_{c\ average} = \sum_{i=1}^{10} \chi_i \tau_{ci} = 1.5 \times 10^{-9}$s.

first tenth hydration shell as a function of the distance d is reported. The convergence between the experimental average reorientational correlation times of the water molecules in the protein hydration shells of 1.5×10^{-9}s with the value computed on the basis of Equation 19, was obtained for a K equal to 1.3 ($Å^{-1}$). The long range ordering effect of the protein on the hydration water is extended at least to 8 Å (Figure 4).

Conclusion

In diluted protein solutions, the bulk water proton relaxation shared the contributions from the water molecules in the protein hydration shell. These water molecules differ from the bulk water, mainly because of their correlation times, which is are short for bulk water and longer for the protein hydration waters. In slow motion conditions ($\omega_0\tau_c >> 1$, typical of the slow tumbling of protein molecules, these contributions are different: large and positive to wR_1^{SE} and negligible or absent to wR_1^{NS}. This process makes wR_1^{SE} larger than wR_1^{NS} as showed in Figure 2. The analysis of both the selective and non-selective water spin-lattice relaxation rates allowed the calculation of the average effective correlation time for the water molecules at the water-protein interface. Moreover, using the assumption of an exponential decay of the rotational correlation time of the hydration water from its value at the protein surface to the bulk conditions, the long range ordering effect of the protein surface on the surrounded water molecules was calculated.

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