Molecular Dynamics in Biological Systems Observed by NMR Relaxation in a Rotating Frame

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NMR relaxation provides powerful tools for obtaining information on three-dimensional structures, dynamic properties and intermolecular interactions of biological macromolecules. One of these methods, called dispersion profile, is based on measuring the field dependence of spin-relaxation rates in the rotating frame, $R_{1\rho} = 1/T_{1\rho}$, in the presence of a low magnetic field B_1. In the presented study we use this method for investigation of molecular dynamics in protein samples. Dispersion profiles can be predicted theoretically and using two models, assuming either dipolar interaction between protons or power law dispersion, we have evaluated some molecular dynamic parameters of water adsorbed on protein surface. Our researches are focused on the connections of obtained parameters of molecular dynamics with conformation changes of protein. We have calculated the correlation times and power parameters for samples of lyophilized powder of albumins (egg white and bovine and rabbit blood serum) and lysozyme, as well as its aqueous solutions. Analysis of these parameters yields valuable information on the molecular nature of investigated biological systems. We also used this method to analyze experimental data of $T_{1\rho}$ obtained by other authors for bovine serum albumin and we have found good accordance with their conclusions concerning molecular dynamics of proteins.

PACS: 76.60.−k, 76.90.+d

1. Introduction

Over the last decade, there has been an increase of interest on molecular dynamics in biological systems investigated by nuclear magnetic resonance (NMR) relaxation methods. One of these methods is nuclear magnetic relaxation dispersion [1–7], that is the measurement of nuclear magnetic relaxation rates $R_i = 1/T_i$ ($i = 1, 2, 1\rho$) as a function of the magnetic field strength. In particular, there are some of interesting publications on molecular dynamics of proteins based on the spin–lattice relaxation rate, R_1, dependence on B_0 [1–5] and the spin–lattice relaxation rate in the rotating frame, $R_{1\rho}$, dependence on B_1 [6–9].

The motion of the water adsorbed on protein surface (hydration shell), which is very important to a number of processes in biochemistry, can be observed with dispersion methods. The relationship between bulk water dynamics and physical and chemical properties of proteins are useful for protein structural dynamic and protein function investigations. They are also important for understanding clinical magnetic resonance (MR) tomography image contrast, often determined by difference in tissue water proton relaxation behavior [6, 9]. One example to this end is the investigation of dispersion in cartilage at different levels of degeneration [10].

We report a study of molecular dynamics of water molecules adsorbed on protein surfaces (measured as samples of lyophilized powder and in solution) based on the dispersion profile in the rotating frame method. This technique tracks the relaxation of nuclear magnetization in the presence of the constant field $\boldsymbol{B}_0$ together with a time-dependent magnetic field $\boldsymbol{B}_1$. The field $\boldsymbol{B}_1$ rotates in the plane perpendicular to $\boldsymbol{B}_0$ at the Larmor frequency $\omega = \gamma B_0$ of the resonant nuclei. Under these circumstances, during holding pulse, the magnetization decays not only in the laboratory frame, but also in the rotating field $\boldsymbol{B}_1$, with time constant $T_{1\rho}$ (relaxation time in the rotating frame) [11]. Since the magnitude of $\boldsymbol{B}_1$ is always much smaller than the magnitude of $\boldsymbol{B}_0$, the relaxation time $T_{1\rho}$ can provide information on slow molecular motions [7]. Another advantage of this method is the possibility of changing the B_1 field strength relatively easily by an attenuation of the holding pulse magnitude. Thus, the measurements of $T_{1\rho}$ as a function of B_1 offers an excellent way of probing the motion of macromolecules characterized by frequencies in the mid-kHz region. In reported measurements the range of magnetic field B_1 strength was of 0.3 to 12 Gs [10^{-4} T], which corresponds to correlation times τ typically observed for molecular motion of water adsorbed on macromolecule surface [5, 6, 9].

Proteins, as a lyophilized powder as well as in aqueous solution, are representative of biological systems as they are always associated with water molecules and their pro-

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ton relaxation is determined by water–protein interaction influenced by molecular dynamics. To test the use of the method we have selected well examined proteins: albumins and lysozyme. Although proton relaxation in free water has been successfully explained in terms of classic dipole–dipole interaction theory, when water molecules are bounded to biological macromolecules, the relaxation mechanism becomes more complicated and understanding of the bounded water relaxation is highly speculative. Therefore the obtained $T_{1\rho}$ dispersion data are analyzed using two models: first one based on pure dipole–dipole interaction as a dominant mechanism in the relaxation of water protons and the second, simple power law, which is widely used in the interpretation of polymer relaxation [5].

2. Relaxation models

Assuming only dipole–dipole interactions of a pair of protons at constant inter-proton distance r, the relaxation rate $R_{1\rho}$ is given by the well-known formula [11]:

$$R_{1\rho} = \frac{1}{T_{1\rho}} = A r^{-6}$$
$$\times \left(\frac{5\tau}{1+\omega^2\tau^2} + \frac{2\tau}{1+4\omega^2\tau^2} + \frac{3\tau}{1+4\omega_1^2\tau^2} \right), \qquad (1)$$

where A is constant, $\omega = \gamma B_0$, $\omega_1 = \gamma B_1$ and the correlation time τ is dependent on temperature T according to the Arrhenius relation: $\tau = \tau_0 \exp(-E/kT)$, where E is activation energy.

If we measure $T_{1\rho}$ at constant temperature and established magnetic field B_0, the first two terms of Eq. (1) are constant and the above equation may be approximated as

$$R_{1\rho} = a_1 + \frac{a_2}{1+a_3 B_1^2} , \quad \text{where} \quad a_3 = 4\gamma^2\tau^2, \qquad (2)$$

and a_1, a_2 and a_3 are parameters, whose values are determined by fitting the experimentally obtained dispersion profile.

From the parameter a_3 in Eq. (2) the correlation time τ of water protons in protein can be simply calculated: $\tau = 1.869\sqrt{a_3} \times 10^{-5}$ s, when B_1 is in Gs.

Using Eq. (2) the simulations of dispersion profiles for different correlation times may be carried out [7]. For our experiments these simulations showed that if τ is outside of the range of 6×10^{-7} s to 2×10^{-4} s no dispersion is observed. Thus, for experimentally observed dispersion relationships $T_{1\rho}(B_1)$, the correlation times have to be in the range of 0.1 to 100 ns.

At this point it should be explained why using a single correlation time is appropriate here. In general, the water molecule dynamics in hydrated proteins is expected to be more complicated and in a number of biological systems a distribution of correlation times have been applied [12, 13]. The fact that Eq. (2) satisfactorily describes a major dispersion profiles in our data sets indicates that motions with the single correlation time, or with correlation times spread over a narrow distribution centered

around this correlation time, plays an important role in characterizing the protein dynamics found here. In other words, we consider the correlation time obtained from the $R_{1\rho}$ dispersion experiments to be the effective one.

It is known that proton exchange between protein side groups and bulk water substantially contributes to the relaxation in the rotating frame. The changing of the inherent motion of proteins in solution by denaturation or cross-linking can enhance nuclear spin relaxation dramatically and modify the shape of the $T_{1\rho}$ relaxation dispersion profile. These exchange effects may be also exploited in NMR studies of molecular dynamic aspects in protein solutions, but in the research presented here it is not possible to distinguish the relaxation caused by exchange from molecular dynamic relaxation [7, 9].

The second model applied here is the so-called power law model, often used in the interpretation of $T_{1\rho}$ relaxation dispersion in biopolymers [5]. The final formula for evaluation of relaxation rates $R_{1\rho}$ for uncorrelated homonuclear dipolar coupled two-spin systems has the form

$$R_{1\rho} = b_1 + b_2 B_1^{-b_3}, \qquad (3)$$

where the exponent b_3 is a parameter sensitive to changes in the spectrum of molecular motions and for polymers is in the range of 0 to 2/3. The physical origin of this law has been related to a relaxation mechanism, which correlate the value of power b_3 to some molecular dynamic parameters. In the case of immobilized proteins according to T_1 dispersion measurements, b_3 is evaluated to be near 0.75 and does not change with protein hydration [5]. In the case of proteins in aqueous solutions this parameter is greater, even exceeding 1 [6].

3. Materials and methods

Dry, lyophilized powder of egg white albumin (EWA) (MW 44 287 Da), bovine serum albumin (BSA) (MW 66 382 Da) and lysozyme (MW 14 500 Da) were purchased from the Sigma Aldrich Company. Rabbit serum albumins as lyophilized powder were obtained from Wytwórnia Surowic i Szczepionek, Kraków, Poland. Hydrated protein was obtained by keeping the protein powder in humid air. Protein solutions at concentrations ranging from 4% to 25% (by weight) were prepared by dissolving protein lyophilized powder in double distillated and de-ionized water. Some samples of protein solution were denatured thermally at 80 °C for at least 15 min.

All samples were measured with a Minispec Bruker spectrometer working at a resonant proton frequency of 60 MHz. T_1 was measured using the inversion recovery (IR) sequence and the standard spin-locking pulse sequence ($\pi/2$-τ-spin-lock-FID) was used to measure $T_{1\rho}$ dispersion profiles. By changing the attenuation for the locking pulse from 20 to 40 dB the rotating magnetic field B_1 has been varied from 0.3 to 12 Gs and the exact value of B_1 was verified by measurement of the $\pi/2$ pulse duration.

Measurements were performed at room temperature 23 °C, which was stabilized with accuracy ±1 °C.

4. Results and discussion

Figure 1 illustrates, as an example, a dispersion profile $R_{1\rho}(B_1)$ obtained for a sample of lyophilized powder of EWA at +23 °C. The curves represent fits of two models described by Eq. (2) and Eq. (3). Both fits are satisfactory in limits of experimental errors.
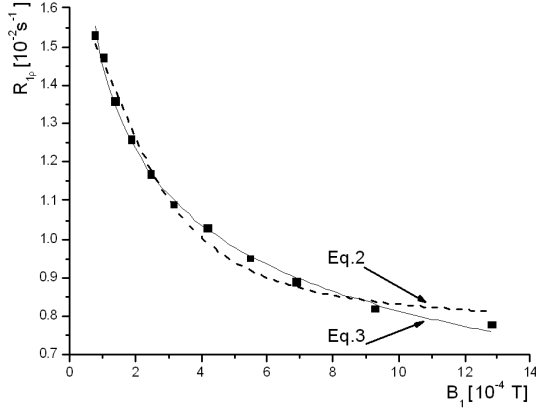

Fig. 1. Dispersion profile for EWA lyophilized powder fitted by two models: Eq. (2) (dashed line) and Eq. (3) (solid line).

Dispersion profiles of BSA aqueous solution, 7% and 20% (by weight), fitted to Eq. (2) are depicted in Fig. 2. Both of them give very similar fitting parameters which means that there is no dependence of correlation time on the amount of water in the solution.
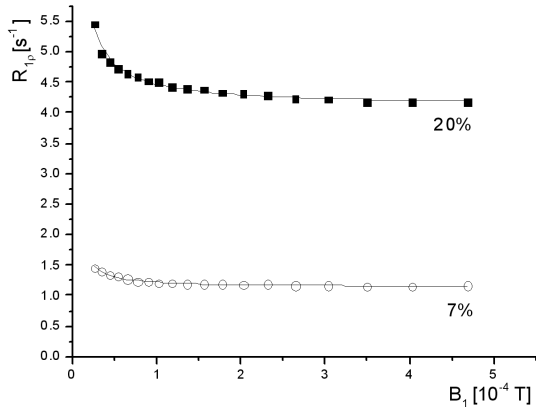

Fig. 2. Dispersion profiles of BSA aqueous solutions, fitting curves are obtained according to Eq. (2) with correlation times at protein concentration (by weight): 7% $\tau = (1.9 \pm 0.5) \times 10^{-5}$ s and 20% $\tau = (1.8 \pm 0.5) \times 10^{-5}$ s.

Figure 3 shows the dispersion profile, before and after thermal denaturation, for a sample of BSA solution. The denaturation process changed the character of dispersion
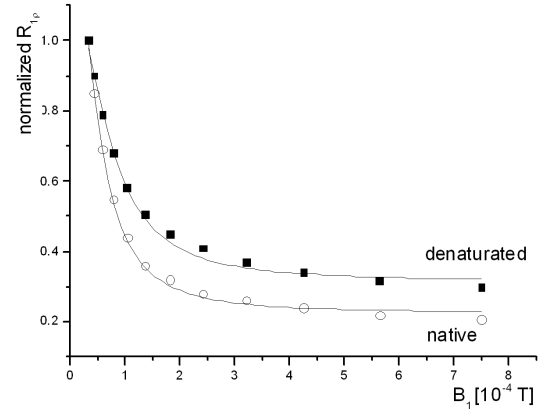

Fig. 3. Comparison of dispersion profiles for native and thermally denatured BSA solutions (13.5% weight). The values of $T_{1\rho}$ are normalized by the assumption that the maximum value of $T_{1\rho} = 1$ [arb.u.].

profiles, as well as shortened spin–lattice relaxation times T_1 and correlation times.

Table I presents the spin–lattice relaxation times T_1, correlation times τ obtained using Eq. (2) and power law parameters b_3 obtained using Eq. (3) for different protein samples, via non-linear least squares fit of the experimental $T_{1\rho}$ dispersion profile data (see Fig. 1).

Correlation times τ obtained from $R_{1\rho}$ dispersion profiles, are comparable with results obtained using other methods for investigations of molecular motion in proteins [1, 3, 6]. We found that these correlation times for proteins are not strongly dependent on hydration level (see Table II and Fig. 2). On the other hand, the values of $T_{1\rho}$ (at estimated B_1), as well as T_1, are strongly controlled by water content. It likely reflects the fact that the slow, local dynamics of interfacial water does not vary much between different proteins. Furthermore, the differences in the amount of water closely associated with the proteins relative to those less associated, lead to the observed large differences in the T_1 and $T_{1\rho}$ values.

For examining usefulness of our method, we have analyzed the results of $T_{1\rho}$ measurements obtained for BSA solutions and published by Chen and Kim [6]. Variations in BSA crosslink density were produced in a chemical reaction of 10% or 20% solutions of BSA with glutaraldehyde (from 10 to 200 mM GA). However, addition of GA alters the BSA dispersion. Analysis of parameters obtained using our fittings to dipole–dipole model (Eq. (2)) and power law model (Eq. (3)) (see Table III) confirmed that addition of 60 mM causes the formation of large BSA polymers. It is clearly seen in correlation time values, which between 40 mM and 60 mM GA shorten about 8 times, in parallel to observed changes of T_1. The power law model notices also this change in protein structure by decreasing of power parameters 1000 times. We expect that presented dispersion profile method will be able to show also the other changes of protein molecular structure and dynamics.

Values of parameters for fits of Eq. (2) and Eq. (3) to dispersion profilesof different protein samples.

Sample	T_1 [ms]	Correlation time τ [10^{-5} s]	Power parameter b_3
lysozyme (lyophilized powder)	191 ± 3	1.8 ± 1.0	1.1 ± 0.1
rabbit serum albumins (lyophilized powder)	148 ± 1	2.4 ± 1.5	1.4 ± 0.1
BSA (lyophilized powder)	160 ± 0.5	0.42 ± 0.18	1.7 ± 0.0
BSA hydrated	201 ± 0.8	0.42 ± 0.19	2.1 ± 0.7
EWA (lyophilized powder)	161 ± 0.8	0.37 ± 0.19	1.5 ± 0.2
EWA hydrated	360 ± 7	0.44 ± 0.32	2.4 ± 0.2

TABLE II

Concentration dependence of correlation times obtained using Eq. (2) for native and thermally denatured aqueous solutions of EWA.

Sample	Albumin concentration [% by weight]	T_1 [ms]	Correlation time τ [10^{-5} s]
native aqueous solutions of egg white albumin	4.0%	2210 ± 10	9.2 ± 3.5
	6.1%	2040 ± 20	6.7 ± 3.1
	8.1%	1820 ± 20	6.5 ± 1.1
	10.1%	1650 ± 20	4.9 ± 1.2
	12.8%	1530 ± 20	7.3 ± 3.1
	17.3%	1200 ± 20	5.7 ± 0.5
	24.5%	776 ± 2	7.0 ± 2.3
	dry powder	335 ± 10	2.9 ± 2.3
thermally denaturated solutions	13.5%	1148 ± 2	2.5 ± 0.1
	12.2%	1410 ± 2	1.3 ± 0.1
	6.4%	1672 ± 2	1.1 ± 0.1

TABLE III

Correlation times and power law parameters calculated using our method for data from work of Chen and Kim [6] for BSA solutions with different cross-link.

Sample	T_1 [ms] at 2 T	Correlation time τ [10^{-5} s]	Power law parameter b_3
BSA	1873	35.5 ± 14.8	0.20 ± 0.05
BSA+GA 20 mM	1793	45.8 ± 19.4	1.03 ± 0.13
BSA+GA 40 mM	1746	35.3 ± 30.0	1.24 ± 0.11
BSA+GA 60 mM	1656	4.5 ± 1.9	0.0024 ± 0.020
BSA+GA 80 mM	1559	6.8 ± 2.7	0.0065 ± 0.018
BSA+GA 100 mM	1617	8.7 ± 3.1	0.0095 ± 0.015

5. Conclusions

The current study shows that the used models satisfactorily describe a major dispersion slope and indicates that both of them can be used to reasonably estimate molecular dynamic parameters. However, the parameter b_3 from the power model Eq. (3) is not as clearly defined as correlation time and its connection with molecular dynamics of proteins has no explicit physical interpretation. Therefore, we think that the understanding of the relationship between correlation times and physicochemical properties of proteins using the dipolar model of Eq. (2) is more clear.

The dispersion profile measurements, in comparison with fast field cycling method or temperature dependence of T_1 measurements, are cheaper, faster and simple methods. We hope that dispersion profile methods will be widely applicable in molecular research of biological systems in the future.

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