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Hydrogen Exchange Rates in Proteins from Water ^{1}H Transverse Magnetic Relaxation

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Hydrogen exchange (HX) analysis is a powerful tool for studying macromolecular dynamics, stability, and folding.[1] High-resolution NMR methods[2] have provided a wealth of data on amide hydrogen exchange in proteins, with fewer studies of faster exchanging side-chain hydrogens.[3] Whereas slowly exchanging hydrogens are exploited in saturation-transfer MRI experiments,[4,5] fast HX between protein side chains and water can be a major source of relaxation contrast in MRI of biological tissues.[6−8]

Fast HX kinetics in proteins can be studied by the chemical shift modulation (CSM) mechanism of transverse relaxation of solvent protons, provided that the HX rate is brought into the intermediate exchange regime (on the chemical shift time scale) by varying the Larmor frequency and/or pH.[9] Figure 1 shows the water ^{1}H relaxation rates as a function of the Larmor frequency in solution of the small protein bovine pancreatic trypsin inhibitor (BPTI).

Whereas the R_1 dispersion has been previously accounted for by rapidly exchanging labile hydrogens and four internal water molecules in BPTI,[8] the dramatic increase of R_2 at high frequencies is due to the water CSM by HX with the labile protein hydrogens, whereby each hydrogen contributes to R_2 (estimated from the water line width or measured by the spin−echo) according to[9,10]

$$\Delta R_{2i} = f k_i [R_{2i}(R_{2i} + k_i) + \Delta\omega_i^2]/[(R_{2i} + k_i)^2 + \Delta\omega_i^2] \quad (1)$$

where $2f$ is the protein/water mole ratio, R_{2i} and k_i are intrinsic transverse relaxation and exchange rates of a labile hydrogen, and $\Delta\omega_i = 2\pi\nu_0(\delta_i - \delta_{bulk})$ is the chemical shift difference between the hydrogen and the bulk solvent, the total line broadening being $R_{ex} = \sum(\Delta R_{2i})$, with the sum going over all labile protein hydrogens. Due to aqueous acid/base catalysis of the HX rates, $k_i = k_{0i} + k_{1i}[H^+] + k_{2i}[OH^-]$, the individual hydrogen contributions in eq 1 are strongly pH-dependent (cf. Figure 1A,B), reaching their maximum values,

$$(\Delta R_{2i})_{max} = f(\Delta\omega_i^2 + R_{2i}^2)/(2|\Delta\omega_i|) \quad (2)$$

at $k_i = (\Delta\omega_i^2 + R_{2i}^2)/(|\Delta\omega_i| - R_{2i})$, provided that $|\Delta\omega_i| > R_{2i}$. If the exchange rates k_i are all fast with respect to both R_{2i} and $\Delta\omega_i$, eq 1 predicts a quadratic frequency scaling of R_{ex} (via the chemical shifts), with the scaling parameter[11] $\alpha = (\delta R_{ex}/R_{ex})/(\delta\nu_0/\nu_0) = 2$. The data in Figure 1 exhibit weaker frequency dependence of R_{ex}, with an apparent α approaching 0.5 (pH 3.9) and 1.5 (pH 7.5) at high frequencies. This reflects mixing of CSM contributions from several labile groups in BPTI with different values of k_i and $\Delta\omega_i$.

To separate the contributions, we measured R_2 (at $\nu_0 = 360$ MHz, and using the CPMG pulse sequence[12]) in the pH range of ca. 1−11, exploiting the extreme stability of BPTI.[13] The resulting pH variation (Figure 2) exhibits several unresolved peaks from different

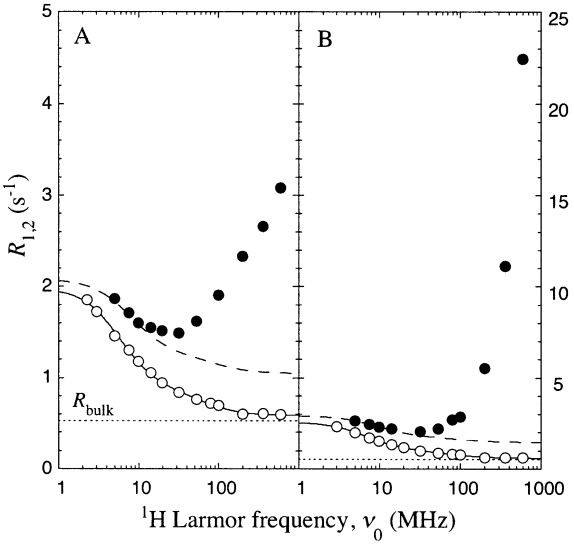

Figure 1. ^{1}H longitudinal (R_1, open symbols) and transverse (R_2, filled symbols) relaxation dispersion in 19 mM BPTI solutions, pH 3.9 (A) and pH 7.5 (B), at 4 °C. R_1 and R_2 rates were measured using inversion−recovery and spin−echo pulse sequences, respectively. The solid curve is a fit of the bi-Lorentzian function[9] to the R_1 data. The dashed curve represents the expected R_2 dispersion (based on the R_1 fit) in the absence of the CSM contribution.

labile groups (except carboxyls, which exchange rapidly at all pH values). Further separation may be achieved by measuring the CPMG dispersion, i.e., by varying the π-pulse spacing, $\tau_{\pi\pi}$ (Figure 2 insert), or by measuring at different Larmor frequencies or temperatures. On the other hand, using the CPMG dispersion alone (at fixed pH) is not sufficient to separate the contributions.[14]

To analyze the data, the total exchange line broadening was calculated analytically using the Carver−Richards formulas[14,15] and numerically by standard matrix techniques[16] starting from the extended Bloch equations for multisite exchange. The intrinsic relaxation rates R_{2i} were calculated[8] on the basis of the BPTI crystal structure and using the protein tumbling time of 5 ns. We used published data for the chemical shifts,[17] exchange rate constants,[19] and ionization constants.[13] Since HX from the N-terminal NH_3 may be catalyzed[3] by salt-bridging to the C-terminal COO^- (pK_a = 2.9),[24] its rate was modeled as a weighted average (assuming fast exchange on the HX time scale) of the bridged and nonbridged conformations at each pH.

A least-squares fit of the analytical expressions to the combined pH and CPMG data was made with the Levenberg−Marquardt algorithm (Figure 2). The following 10 parameters were adjusted in the fit: R_2 offset (from the bulk water and internal water molecules in BPTI[8]), k_1 and k_2 for Tyr OH (except Tyr 35), k_2 for Tyr-35 OH,[25] k_2 for the Arg ϵ-NH, Arg η-NH_2, and amino protons,

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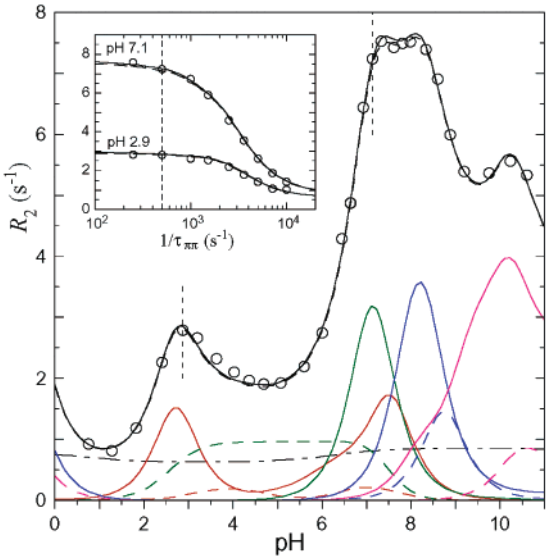

Figure 2. ^{1}H R_2 in 10 mM solution of (extensively dialyzed) BPTI, measured at 360 MHz, 27 °C, using the CPMG pulse sequence ($\tau_{\pi\pi} = 2$ ms). The inset shows the CPMG dispersion at two pH values (vertical dashed lines). The black dashed curves represent an analytical fit to the combined pH and CPMG data. The (nearly coincident) black solid curves are calculated numerically with the same parameters as obtained from the fit. The colored curves show contributions from Tyr OH (orange, solid), Ser and Thr OH (orange, dashed), Lys NH_3 (green, solid), N-term NH_3 (green, dashed), Arg η-NH_2 (blue, solid), Arg ϵ-NH (blue, dash), peptide amides (magenta, solid), and primary amides of Gln and Asp (magenta, dash). The dash−dot curve shows R_2 in the absence of CSM (i.e., with all $\Delta\omega_i$ set to zero).

k_0 for the N-terminal NH_3 in the salt-bridge conformation, and protection factors for the primary amides of Asn and Gln and fast exchanging ($k_2 > 5 \times 10^7$ M^{-1} s^{-1}) peptide amides. The protection factors incorporate any differences from the published rate constants for surface NH groups due to salt effects and protein association.[20]

The main features of the pH variation of R_2 in Figure 2 are due to dominant contributions from Lys NH_3 groups (pH 7.2) and Tyr OH groups (pH 2.8 and 7.5), Arg side-chain hydrogens (pH 8.2), and amide hydrogens (pH 10.2). For example, the peak height at pH 2.8 from the three Tyr OH protons ($\Delta\omega_i/2\pi \approx 5$ ppm) is close to 1.6 s^{-1}, as obtained[27] using eq 2 (valid in the long $\tau_{\pi\pi}$ limit). The second Tyr peak (at pH 7.5) is skewed due to the contribution from the fourth (anomalous[25]) Tyr 35, with a fitted k_2 of 2.3×10^8 $M^{-0.57}$ s^{-1}. For the other three Tyr OH, we get $k_1 = 5.9 \times 10^6$ M^{-1} s^{-1} and $k_2 = 2.8 \times 10^{10}$ M^{-1} s^{-1}, corresponding to an activation energy[3] of 18−19 kJ/mol. At pH 4−6, high R_2 values are due to internal catalysis of the salt-bridged N-terminal NH_3, whose exchange becomes slow at pH < 2 only.[3] Its k_0 value of 6×10^3 s^{-1} obtained from the fit may reflect intramolecular proton transfer via several intervening water molecules, despite ca. 5 units difference in the pK_a values of the involved groups.[28] The rate constants $k_2(\eta\text{-}NH_2) = 2.1 \times 10^9$ M^{-1} s^{-1}, $k_2(NH_3) = 3.9 \times 10^{10}$ M^{-1} s^{-1} are close to the literature values for model amino acids,[7] whereas $k_2(\epsilon\text{-NH}) = 9 \times 10^8$ M^{-1} s^{-1} corresponds to a protection factor of ca. 3.5 with respect to a free arginine.[7] Although less accurately determined by the data,[29] an average protection factor of 3.8 (with respect to free amino acids) for primary amides of Asn 24 and Gln 31 is consistent with their surface location,[30] whereas the protection factor of 2.3 for the surface peptide amides (with respect to the published BPTI data) may be due to self-association of BPTI.

The present approach allows HX rates to be determined in protein solutions and biomaterials. It can also be used to study HX catalysis by cosolutes and buffers,[7] of relevance for magnetization transfer in biological MRI,[31] metabolite imaging,[5] and development of HX-based contrast agents.[32]

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