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Residence Times of Molecular Complexes in Solution from NMR Data of Intermolecular Hydrogen-Bond Scalar Coupling

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Abstract

The residence times of molecular complexes in solution are important for understanding biomolecular functions and drug actions. Here we show that NMR data of intermolecular hydrogen-bond scalar couplings can yield information on the residence times of molecular complexes in solution. The molecular exchange of binding partners via the breakage and reformation of a complex causes self-decoupling of intermolecular hydrogen-bond scalar couplings, and this self-decoupling effect depends on the residence time of the complex. For protein–DNA complexes, we investigated the salt-concentration dependence of intermolecular hydrogen-bond scalar couplings between the protein side-chain ^{15}N and DNA phosphate ^{31}P nuclei, from which the residence times were analyzed. The results were consistent with those obtained by $^{15}\text{N}_z$ -exchange spectroscopy. This self-decoupling-based kinetic analysis is unique in that it does not require any different signatures for the states involved in the exchange, whereas such conditions are crucial for kinetic analyses by typical NMR and other methods.

Graphical Abstract

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Supporting Information: The following file is available free of charge: Experimental details (PDF)

The authors declare no competing financial interests.

In solution, molecular complexes formed via noncovalent interactions typically undergo dynamic equilibria involving dissociation and association. The residence times of molecular complexes are important for our understanding of biomolecular functions and for the development of effective drugs.^{1–3} Various methods, such as fluorescence and NMR spectroscopy and surface plasmon resonance, can be used to determine the residence time of a complex. Regardless of the method used, the determination of residence times usually requires the observation of a transition between distinct states (e.g., free and bound states or two different complexes) that exhibit distinct physiochemical signatures (e.g., different NMR chemical shifts). In this paper, we demonstrate a unique NMR approach that does not require distinct states with different NMR chemical shifts, yet does provide information on the residence times of molecular complexes.

This approach utilizes NMR scalar couplings across intermolecular hydrogen bonds. Hydrogen-bond scalar couplings were discovered in the late 1990s, initially for the hydrogen bonds of nucleic-acid base pairs and protein secondary structures (e.g., as reviewed in ref. 4). This type of scalar coupling represents direct evidence of the presence of hydrogen bonds and provides structural and dynamic information on hydrogen bonding. Hydrogen-bond scalar couplings have also been observed for the intermolecular hydrogen bonds formed at the molecular interfaces of nucleic acids (as reviewed in refs 5–6), protein–nucleic acid complexes,^{7–11} and other protein–ligand complexes^{12–13}.

First, we consider how the residence time of a complex is related to the apparent values of the intermolecular hydrogen-bond scalar coupling constants measured by quantitative J -modulation spin-echo difference experiments.^{7,14} This type of experiment involves two sub-experiments, with (B) and without (A) intensity modulation by evolution of the hydrogen-bond scalar coupling hJ during the constant-time period $2T$ (an example is shown in the Supporting Information [SI]). From the signal intensities I_A and I_B in the spectra of sub-experiments A and B, the magnitude of the coupling constant is determined by:

$$^hJ_{\text{app}} = \arccos(I_B/I_A)/(2\pi T) \quad [1]$$

The following consideration shows that this yields the intrinsic magnitude $|^hJ|$ only when the “self-decoupling” effect^{15–17} is negligible. The evolution of the density operator ρ is given by:

$$\rho_A(2T) = \exp\{-\mathbf{L}(T - t_1/2)\} \mathbf{S}_N \exp(-\mathbf{L}T) \mathbf{S}_P \exp(-\mathbf{L}t_1/2) \rho(0) \quad [2]$$

for the sub-experiment A and

$$\rho_B(2T) = \exp\{-\mathbf{L}(T - t_1/2)\} \mathbf{S}_N \mathbf{S}_P \exp\{-\mathbf{L}(T + t_1/2)\} \rho(0) \quad [3]$$

for the sub-experiment B, where t_1 represents the time for chemical shift evolution for the F_1 dimension; $\mathbf{L}$, a Liouvillian matrix; $\mathbf{S}_N$ and $\mathbf{S}_P$ the rotation matrices for 180° pulses for coupled nuclei. In the case of intermolecular $^hJ_{NP}$ coupling between the ^{15}N and ^{31}P nuclei,

a basis for the density operators can be defined as a column vector of $[N_x, 2N_yP_z]$. For this basis, the Liouvillian matrix $\mathbf{L}$ and the rotation matrices $\mathbf{S}_N$ and $\mathbf{S}_P$ are as follows:

$$\mathbf{L} = \begin{pmatrix} R_{2,N} & \pi J \\ -\pi J & R_{2,N} + R_{1,P} + k \end{pmatrix} \quad [4]$$

$$\mathbf{S}_N = \mathbf{S}_P = \begin{pmatrix} 1 & 0 \\ 0 & -1 \end{pmatrix} \quad [5]$$

, where $R_{2,N}$ represents the ^{15}N transverse relaxation rate; J , the intrinsic hydrogen-bond scalar coupling; $R_{1,P}$ the ^{31}P longitudinal relaxation rate; and k , a first-order rate constant for molecular exchange. Eq. 5 assumes 180° pulses along x . The mean residence time τ of the molecular complex is given by:

$$\tau = k^{-1} \quad [6]$$

Figure 1 shows the apparent constant $^hJ_{app}$ calculated with Eqs. 1–6 as a function of the residence time of a complex. When the residence time is short, making $k \gg 2\pi|J|$ (i.e., fast exchange on a scalar coupling timescale rather than a chemical shift timescale), the apparent $^hJ_{app}$ constant approximates zero (Figure 1). This represents self-decoupling due to molecular exchange.

We have examined this theoretical relationship between the intermolecular hydrogen-bond scalar couplings $^hJ_{app}$ and the residence times for protein–DNA complexes. We can alter the residence times of the complexes by changing the ionic strength. Figure 2a shows the residence times for the specific protein–DNA complexes of the Antp homeodomain (with C39S mutation)¹¹ and the Egr-1 zinc-finger (with T23K/Q60E mutations)¹⁸ proteins with their target DNA at various concentrations of salt. The residence times were measured by $^{15}\text{N}_z$ -exchange spectroscopy^{19–20} (for Antp) or a fluorescence-based kinetic assay (for Egr-1), as described in the SI. For these systems, the residence time is defined as the inverse of the apparent first-order rate constant for translocation of the protein from one target DNA duplex to another. This rate constant does not necessarily correspond to a dissociation rate constant because proteins can transfer from one DNA duplex to another via the “intersegment transfer” (also known as the “direct transfer”) mechanism without going through the intermediary of free protein.²¹ It is known that homeodomains can undergo intersegment transfer between specific DNA duplexes.²² For Egr-1, the intersegment transfer between two target DNA duplexes is virtually negligible,²³ though this protein can undergo extremely efficient intersegment transfer between nonspecific DNA sites via the “search mode” during the target search process.^{24–27} Due to this difference, the residence time of the Antp–DNA complex is far shorter than that of the Egr-1–DNA complex, although these complexes exhibit a comparable dissociation constant (K_d) at the same ionic strength.^{11,27} Figure 2a shows that for each complex, the logarithm of the residence time is linearly dependent on the logarithm of the salt concentration, as expected from previous theoretical and experimental studies.^{20,25,28}

For these complexes, we measured the intermolecular hydrogen-bond scalar couplings between ^{15}N and ^{31}P nuclei across the contact ion pairs of lysine (Lys) side-chain NH_3^+ and DNA phosphate groups at various ionic strengths (Figure 2b). Owing to the extremely slow ^{15}N transverse relaxation of the Lys side-chain NH_3^+ groups (typically, $R_{2,N} < 3 \text{ s}^{-1}$), the magnitude of the $^hJ_{NP}$ coupling constants can be measured precisely for the Lys side chains that are in contact with DNA phosphate groups.^{7,9,11,29} The $^hJ_{NP}$ coupling constants for the Antp–DNA complex exhibited a strong dependence on the salt concentration between 20 and 150 mM, whereas those for the Egr-1–DNA complex exhibited no significant dependence on the salt concentration up to 300 mM (Figure 2b). Importantly, these observations are at least qualitatively consistent with the theoretical expectation from Figures 1 and 2a.

For the Egr-1–DNA complex, the $^hJ_{NP}$ data and Figure 1 qualitatively suggest that the residence time of this complex is longer than $(2\pi|^hJ_{NP}|)^{-1}$ in the range of 20–300 mM KCl. For the Antp–DNA complex, the salt-dependent $^hJ_{NP}$ data provide more quantitative information on the residence time of the Antp–DNA complex. Assuming that the residence time τ satisfies $\log \tau = a\log[\text{Salt}] + b$ (as observed in Figure 2a), we conducted a nonlinear least-squares fit of the salt-dependent $^hJ_{NP}$ data using Eqs 1–6. For the Antp–DNA complex, the calculations were performed via a global fit of the experimental data for three residues (with Lys46, Lys55, and Lys57), as described in the SI. The parameters $R_{I,P}$ and hJ , which were individually defined for each residue, and the global parameters a and b were optimized. The best-fit curves (red solid lines in Figure 2b) showed excellent agreement with the experimental salt-dependent $^hJ_{NP}$ data. Furthermore, as shown in Figure 3, the residence times from this analysis were in good agreement with those from the $^{15}\text{N}_2$ -exchange experiment in which the exchange process of two complexes with different DNA duplexes was analyzed.

Theoretically, the self-decoupling effect should become significant when the interconversions between the α and β spin states of the coupling partners (in the current case, ^{31}P nuclei) occur at a rate faster than $2\pi|^hJ|$.^{15–17} Such interconversions can be caused by longitudinal relaxation of the coupling partner or by molecular exchange (i.e., large $R_{1,P}$ or k in Eq. 4). Intramolecular dynamics that transiently break the hydrogen bond within the residence time should certainly attenuate hJ coupling,^{4,30} but do not necessarily cause self-decoupling because the α and β spin states of the coupling partner remain the same. In fact, our previous NMR and molecular dynamics studies showed that the Lys $^{15}\text{NH}_3^+$ groups that exhibit sizable $^hJ_{NP}$ coupling with the DNA ^{31}P nuclei undergo dynamic transitions on a sub-nanosecond timescale between the contact ion-pair and solvent-separated ion-pair states.^{7,9,31}

In conclusion, our current work demonstrates that intermolecular hydrogen-bond scalar coupling data can provide information on the residence times of molecular complexes in solution. The observation of intermolecular hydrogen-bond scalar couplings is possible only if the residence time is sufficiently long ($> \sim 10^{-2} \text{ s}$); otherwise, complete self-decoupling occurs as shown in Figure 1. Qualitatively, when an intermolecular hydrogen-bond scalar coupling is observed with a magnitude comparable to that of the intrinsic coupling constant hJ , the residence time of the complex should be longer than $(2\pi|^hJ|)^{-1}$. The intrinsic

values of hydrogen-bond scalar couplings can be calculated from structural information by quantum chemical (e.g., DFT) calculations or from the empirical relationship between the coupling constants and the hydrogen-bond geometry.^{4,32–33} As demonstrated above, detailed analysis of the self-decoupling of intermolecular hydrogen-bond scalar couplings can provide more quantitative information about the residence time of the complex. For quantitative applications of $^hJ_{NP}$, the analyzable range of residence time is $\sim 0.001 - 1$ s, provided that $|^hJ_{NP}| \sim 4$ Hz (as estimated by DFT calculations^{7,33}). For systems for which the ionic-strength dependence cannot be used, temperature dependence could be used under the assumption that the molecular exchange rates obey the Arrhenius equation (or the Kramers theorem on mean first passage times³⁴). Thus, although self-decoupling is typically regarded as a nuisance in NMR scalar coupling measurements,^{15–17} the quantitative analysis of self-decoupling presents a useful tool for kinetic analysis. This self-decoupling-based method is unique because it does not require different signatures for the states involved in the exchange, although such conditions are typically crucial for other methods. For example, kinetic analyses by other NMR methods (e.g., relaxation dispersion, CEST, exchange spectroscopy)^{35–36} require different chemical shifts for the interconverting states. The self-decoupling-based method can provide kinetic information even if the exchange is not accompanied by any change in the chemical shifts.

Experimental Methods

Chemically synthesized DNA oligonucleotides were purchased from the Integrated DNA Technologies, Inc. (Coralville, IA) and purified by anion-exchange chromatography. Isotopically labeled proteins were expressed in *E. coli* and purified by chromatographic methods. The protein–DNA complexes were prepared as previously described.^{11,18} Intermolecular hydrogen-bond scalar couplings between protein side-chain ^{15}N and DNA phosphate ^{31}P nuclei were measured using a Bruker Avance III 600 MHz spectrometers equipped with a $^1\text{H}/^{13}\text{C}/^{15}\text{N}/^{31}\text{P}$ QCI cryogenic probe, as described.⁷ The $^{15}\text{N}_z$ -exchange spectroscopy data were obtained using a Bruker Avance III 800 MHz spectrometer equipped with a $^1\text{H}/^{13}\text{C}/^{15}\text{N}$ TCI cryogenic probe. Further experimental details are included in the Supporting Information.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Exchange-induced self-decoupling of intermolecular hydrogen-bond scalar coupling hJ . The graph shows the theoretical relationship between the residence time of a complex and the apparent hJ constants (on a logarithmic scale for each axis). The curves were obtained with Eqs. 1–6 together with $R_{I,P} = 1 \text{ s}^{-1}$ and the intrinsic hJ constants indicated.

Figure 2.

Salt concentration-dependence data for the sequence-specific DNA complexes of the Antp homeodomain (C39S mutant) (red) and the Egr-1 zinc-finger (Q32E/T23K mutant) (green). (a) Residence times measured for the protein–DNA complexes. The residence times in these graphs were measured by $^{15}\text{N}_z$ -exchange spectroscopy for the Antp complex and by a fluorescence anisotropy-based kinetic method for the Egr-1 complex, as described in the SI. The data are shown on logarithmic scales in both axes. (b) Intermolecular hydrogen-bond scalar coupling h^3J_{NP} between protein side-chain NH_3^+ and DNA phosphate groups. The red

data points are Lys46 (square), Lys55 (circle), and Lys57 (triangle) of the Antp homeodomain–DNA complex. The green points are Lys23 (square) and Lys79 (circle) of the Egr-1 zinc-finger(Q32E/T23K mutant)–DNA complex. The solid curves represent the best-fit curves with Eqs. 1–6 and $\log \tau = a\log[\text{Salt}] + b$. Further details of the curve-fitting calculations are included in the SI.

Figure 3.

Comparison of the residence times determined from the salt-dependent $^3J_{NP}$ data with those from the $^{15}N_z$ -exchange data for the Antp homeodomain-DNA complex. Assuming that the residence time τ satisfies $\log \tau = a \log[\text{Salt}] + b$, the parameters a , b , $R_{I,P}$ and hJ were optimized via nonlinear least-squares fitting with Eqs 1–6 to the salt-dependent $^3J_{NP}$ data (Figure 2b). In the $^{15}N_z$ -exchange experiment, the exchanges between two DNA complexes (one with the duplex I and the other with duplex II; the sequences are shown) were

investigated through the analysis of the time-dependence of auto and exchange cross peaks, as previously described.²² The signals from the Arg-5 Nε-Hε groups are shown.

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