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Coupled protein domain motion in Taq polymerase revealed by neutron spin-echo spectroscopy

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Long-range conformational changes in proteins are ubiquitous in biology for the transmission and amplification of signals; such conformational changes can be triggered by small-amplitude, nanosecond protein domain motion. Understanding how conformational changes are initiated requires the characterization of protein domain motion on these timescales and on length scales comparable to protein dimensions. Using neutron spin-echo spectroscopy (NSE), normal mode analysis, and a statistical-mechanical framework, we reveal overdamped, coupled domain motion within DNA polymerase I from *Thermus aquaticus* (Taq polymerase). This protein utilizes correlated domain dynamics over 70 Å to coordinate nucleotide synthesis and cleavage during DNA synthesis and repair. We show that NSE spectroscopy can determine the domain mobility tensor, which determines the degree of dynamical coupling between domains. The mobility tensor defines the domain velocity response to a force applied to it or to another domain, just as the sails of a sailboat determine its velocity given the applied wind force. The NSE results provide insights into the nature of protein domain motion that are not appreciated by conventional biophysical techniques.

**Protein domain motions are critical for proteins to coordinate precise biological functions.** For example, coupled domain motions occur in genome regulatory proteins, motor proteins, signaling proteins, and structural proteins (1–6). Structural studies have documented the conformational flexibility in proteins accompanying their activities (7). Results from macroscopic studies, such as biochemical kinetics and single molecule detection studies, have also shown the importance of conformational dynamics and Brownian thermal fluctuations within proteins (5, 8–10). However, the time-dependent, dynamic processes that facilitate protein domain rearrangements remain poorly understood.

The function of DNA polymerase I from *Thermus aquaticus* (Taq polymerase) (see Fig. 1) requires coordinated domain and subdomain motions within this protein to generate a precise ligatable nick on a DNA duplex (11–13). *Taq* polymerase performs nucleotide replacement reactions in DNA repair and RNA primer removal in DNA replication (14). During such processes, *Taq* polymerase utilizes a DNA polymerase domain to catalyze the addition of dNTP to the 3’ hydroxyl terminus of an RNA primer and a 5’ nuclease domain to cleave the downstream, single-stranded 5’ nuclease displaced by the growing upstream strand (11). Because the structure of *Taq* polymerase possesses an extended conformation with the polymerase and the 5’ nuclease active sites separated by ∼70 Å (15–17), the DNA needs to be shuttled between these two distant catalytic sites when switching from the DNA synthesis mode to the nuclease cleavage mode. This scenario is similar to that which occurs when the DNA needs to be shifted from the polymerase active site to the 3’–5’ exonuclease catalytic center, which are ∼30 Å apart in the Klönenow fragment (the polymerase domain plus the 3’–5’ exonuclease domain) domain of polymerase I, to cleave an incorrectly incorporated dNTP (18). In addition, the polymerase domain can communicate with the 5’ nuclease domain, as evidenced by biochemical experiments that show that the presence of the polymerase domain affects the activity of the 5’ nuclease domain (12, 19). These studies imply that dynamic coupling among protein domains plays a very significant (if not well appreciated) role in their biological functions.

We have studied protein domain motions in *Taq* polymerase by neutron spin-echo spectroscopy (NSE). NSE is a quasielastic neutron scattering (QENS) technique that can study long-range relaxation processes in a macromolecule on timescales up to 100 ns and on length scales from 5 to 150 Å (20, 21). Conventional QENS techniques (e.g., time-of-flight and back-scattering) have been used to study the incoherent dynamics of hydrogen atoms within proteins on timescales of 0.1 s ≤ Q ≤ 2 Å⁻¹ (where Q = 4πsin(θ)/λ is the magnitude of the scattering vector, with θ the scattering angle and λ the wavelength of the neutrons) and on timescales of 10⁻¹² to 10⁻¹⁰ s (22–26). For a protein in D₂O buffer solution, NSE mainly measures coherent scattering in the Q region of 0.02 s ≤ Q ≤ 0.3 Å⁻¹ (in this study), as in small-angle neutron scattering (SANS). However, rather than the static correlation function obtained by SANS, NSE gives information about the time evolution of the correlation function. NSE is analogous to dynamic light scattering (DLS), but the timescales and length scales are better suited for the mesoscopic scale of the internal modes of macromolecules than DLS. Here, we show that NSE can resolve the domain mobility tensor of a protein, thus specifying how protein domains are dynamically coupled during global conformational changes.

**Methods**

**NSE Experiments.** The expression and purification of *Taq* polymerase have been described in ref. 17. Before NSE experiments, *Taq* polymerase was exchanged into 99.9% D₂O buffer containing 25 mM per-deuterated Tris-d₁l (Cambridge Isotope Laboratories, Cambridge, MA) (pH 8.0) and 75 mM NaCl repeatedly by using a Centriprep concentrator (Millipore). The protein concentration used for NSE experiment was ∼8 mg/ml in D₂O solution. This protein concentration is dilute to eliminate intermolecular interaction effects (17).

NSE experiments were conducted at the Institut für Festkörperforschung (27). The wavelength was 8.6 Å. The path length of the sample cell was 4 mm. The data were collected over the range of 0.039 Å⁻¹ ≤ Q ≤ 0.260 Å⁻¹. NSE experiments were performed at 30°C. The $S(Q,t)/S(Q,0)$ spectra can be approximated by the first cumulant representation as

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Abbreviations: NSE, neutron spin-echo spectroscopy; NMA, normal mode analysis; SAXS, small-angle x-ray scattering; DLS, dynamic light scattering.

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where the decay rate of the dynamic form factor is

\[
\Gamma(Q) = \lim_{t \to 0} \frac{\partial}{\partial t} \ln[S(Q, t)],
\]

and the effective diffusion coefficient is

\[
D_{\text{eff}}(Q) = \frac{\Gamma(Q)}{Q^2}.
\]

**DLS Experiments.** DLS experiments were performed with a DynaPro (Wyatt Technology, Santa Barbara, CA), using a laser of wavelength of 824.7 nm at a fixed angle of 90°, corresponding to \(Q = 0.00108 \text{ Å}^{-1}\). The Taq polymerase concentration was 0.25 mg/ml in 70 mM NaCl/35 mM Tris-HCl, pH 8.0, in which the intermolecular interaction effect is eliminated. The DLS experiments were performed at 30.0°C in D2O buffer. Because the size of Taq polymerase is much smaller than the wavelength of light used in a DLS experiment (\(Q R_g \approx 0.04\), with \(R_g\) being the radius of gyration of Taq polymerase), DLS measures the center-of-mass translational diffusion constant of Taq polymerase. DLS experiments show that Taq polymerase does not aggregate in D2O solution in which we conducted the NSE experiments (see Fig. 2).

**Solution Small-Angle X-Ray Scattering (SAXS) Experiment.** SAXS experiments were conducted with an in-house apparatus (28).
The effective $Q$ range covered was from 0.016 to 0.35 Å$^{-1}$. The Taq polymerase concentration was 5 mg/ml in 70 mM NaCl/35 mM Tris-HCl, pH 8.0/H$_2$O buffer. SAXS experiments were performed at 30.0°C. SAXS data reduction and analysis procedures have been described in ref. 28. Inverse Fourier transformation of $I(Q)$ gives the length distribution function $P(r)$, which is the probability of finding two scattering points at a given distance $r$ from each other in the measured macromolecule. Inverse Fourier transformation of $I(Q)$ gives the length distribution function $P(r)$, which is the probability of finding two scattering points at a given distance $r$ from each other in the measured macromolecule (29):

$$P(r) = \frac{1}{2\pi^2} \int I(Q)Qr \sin(Qr)dQ.$$  

Calculating $D_{el}(Q)$ for a Rigid-Body Model of Taq Polymerase. A formula derived by J. M. Schuff (personal communication) was used to calculate the first cumulant $D_{el}(Q)$ of a rigid-body model of Taq polymerase from the x-ray crystal structure coordinates (Protein Data Bank ID code 1TAQ) (15) by using the program HYDROPRO, created by Garcia de la Torre and coworkers (30, 31). The integration over the two Euler angles $(\beta, \gamma)$ of $Q$ was performed numerically by the trapezoid rule, with step sizes of 0.01 in both $\cos\beta$ and $\gamma$ proving to be adequate.

Results and Discussion

Unusual Internal Dynamic Behavior in Taq Polymerase Revealed by NSE. The NSE-measured dynamic form factor $S(Q, t)/S(Q, 0)$ from Taq polymerase can be fitted with single exponential decay functions as shown in Fig. 3A. However, the nonlinearity of the decay rate $I(Q)$ of $S(Q, t)/S(Q, 0)$ as a function of $Q^2$ shown in Fig. 3B indicates that NSE has revealed a dynamic behavior in Taq polymerase that is significantly different from the center-of-mass diffusion of a macromolecule. The effective diffusion coefficient $D_{el}(Q)$ oscillates, as a function of $Q$, around the center-of-mass translational diffusion constant measured by DLS ($D_{DLS}$) (see Fig. 4A). The oscillatory behavior of $D_{el}(Q)$ as a function of $Q$ indicates that NSE has detected the presence of internal dynamics in Taq polymerase.

We first examine the contributions of rigid-body translational and rotational diffusion to the oscillatory behavior of $D_{el}(Q)$ because the intramolecular interference in the static form factor of a rigid structure could, in principle, cause the oscillations in $D_{el}$ (32, 33). The $D_{el}(Q)$ calculated by the rigid-body model using Eq. 2 is shown in Fig. 4A. Although the experimental $D_{el}(Q)$ has maximums and minimums (see Fig. 4A) that correspond to the dip and rise in $I(Q)$ (shown in Fig. 4B), respectively, the experimental $D_{el}(Q)$ shows much more significant oscillations than the calculated $D_{el}(Q)$ using Eq. 2 of the rigid-body model. Fig. 4A shows that the $D_{el}(Q)$ derived from Eq. 2 only agrees with the experimental data in the region of $Q < 0.125$ Å$^{-1}$, suggesting that Taq polymerase behaves as a rigid body only on length scales longer than $2\pi/Q > 50$ Å. As $Q > 0.125$ Å$^{-1}$, the NSE-measured $D_{el}(Q)$ oscillates more markedly than that calculated by Eq. 2 of the rigid-body model. Thus, the dynamic behavior of Taq polymerase cannot be described by the rigid-body model when $Q > 0.125$ Å$^{-1}$.

Next, we compare the structure of Taq polymerase in solution by SAXS with the crystal structure to examine whether the existence of multiple static structures in solution could possibly cause the deviations of the NSE-measured $D_{el}(Q)$ from the rigid-body behavior. The static form factor $I(Q)$ calculated from the crystal structure coordinates is shown in Fig. 4B, together with that measured by solution SAXS, which is an ensemble average of all possible structures that can be adopted by Taq polymerase in solution. The length distribution functions $P(r)$ calculated from the crystal structure and solution SAXS data are also plotted in Fig. 4C. As shown in Fig. 4B and C, both $I(Q)$ and $P(r)$ from solution SAXS are very similar to those calculated.
from the crystal structure. The ensemble-averaged global structure of Taq polymerase in solution by SAXS is thus very close to the crystal structure. If there were distinct multiple structures, we would expect the SAXS results to be significantly different from the crystal structure. Thus, the oscillatory behavior of $D_{\text{eff}}(Q)$ measured by NSE shown in Fig. 4A must arise from the internal motions of Taq polymerase. In the following, we analyze the NSE results from Taq polymerase by using a normal mode framework and statistical mechanics to show how the oscillation in $D_{\text{eff}}(Q)$ can be explained by internal motion.

**Normal Mode Analysis (NMA) Suggests That the Lowest Frequency Normal Modes of Internal Motions in Taq Polymerase Are Interdomain Motions.** We have carried out NMA on Taq polymerase by using the program ELNEMO (34) to identify the type and direction of internal motion (35). In NMA, the first six modes of lowest frequency are the translational motion and rotational motion of the protein molecule as a whole. NMA suggests that the lowest frequency modes of internal motion, which are modes 7 and 8 (Fig. 1), are the en bloc relative motion between the 5' nuclease and the Klentaq domains. The direction of motion of modes 7 and 8, shown in Fig. 1, is consistent with our previous structural study by small-angle neutron scattering that finds the 5' nuclease domain to be in closer contact with the thumb subdomain when Taq polymerase binds to a structural specific overlap flap DNA (17). In higher normal modes, subdomain motions appear. Specifically, in normal modes 9 and 10, the relative motions of the 5' nuclease, the 3'-5' exonuclease, and the polymerase domains are apparent (see Fig. 1).

Thus, NMA predicts that the lowest frequency mode of internal motion in Taq polymerase involves the relative motions of two domains, the polymerase plus the 3'-5' exonuclease domain (together called the Klentaq domain) and the 5' nuclease domain, which are connected by a spring-like linker (see Fig. 5B). Higher normal modes display relative motion of three rigid domains, with the Klentaq domain split into its 3'-5' exonuclease and polymerase components (see Fig. 5C).

**NSE Can Determine the Domain Mobility Tensor That Defines the Degree of Dynamical Coupling Between Domains.** The above analysis shows that a rigid-body analysis of Taq polymerase is inadequate and that the ensemble-averaged solution structure is very close to the crystal structure. We therefore generate a progression of models that systematically include internal normal modes by considering (i) the lowest frequency internal modes in which the 5' nuclease and the Klentaq domains are treated as two oscillating lobes and (ii) the two lowest frequency modes that include the 5' nuclease domain but in which the Klentaq domain is now further subdivided into its polymerase and 3'-5' exonuclease domain components.

First, we treat the Klentaq domain and the 5' nuclease domains as separate rigid objects whose coordinates are assumed to vary little from the crystal structures (see Fig. 5B). The time evolution of the coordinates can be described by the Langevin equation for the two domains at center-of-mass coordinates $\tilde{r}_i$ ($i = 1, 2$) that comprise the protein (36):

$$\frac{d}{dt}\tilde{r}_i = \sum_k \tilde{H}_{ik}\left[ -\frac{dU_{ij}}{dt_k} + \tilde{F}_k(t)\right],$$

where $\tilde{H}_{ik}$ is the domain mobility tensor and is defined by $V_i = \sum_k \tilde{H}_{ik}\tilde{F}_k$ in terms of the velocities $V_i$ and forces $\tilde{F}_k$ for each domain. Here, $U$ is the potential of mean force between the two domains and $\tilde{F}$ is the usual random thermal force with ensemble averages.
These limits are (i) the limit in which a stiff spring becomes perfectly rigid and (ii) the limit of zero time in the first cumulant of the effective diffusion constant. If the second limit is taken first, very fast underdamped modes can appear (32). In this case, it is no longer reasonable to neglect inertial modes, and the usual derivations of Eq. 5 are invalid. These difficulties can be avoided by taking limit (i) first (32). Thus, such relations are correct for perfectly rigid bodies (Eq. 2) or for rigid bodies connected by soft spring linkers (Eq. 5), as we consider in this study. The existence of underdamped motion requires that the spring constant for a linker connecting domains of mass $m$ and friction constant $\zeta$ be $>\zeta^2/4m$ (9); explicit calculations as well as measurements (23) indicate that proteins are well within the overdamped soft spring regime.

Eqs. 2, 4, and 5 explicitly show that, given the structural coordinates of a protein, the NSE experiment essentially tests models of the domain mobility tensor $\mathbf{H}_{jk}$, which defines the velocity $\mathbf{v}_j$ of domain $j$ given the force $F_k$ applied to domain $k$. We construct the simplest possible model of a domain mobility tensor for internal motion

$$\mathbf{H}_{jk} = \zeta_j^{-1} \delta_{jk} \mathbf{T}$$

with friction constants $\zeta_j$, $j = 1, 2$, appropriate for each domain and evaluate $D_{\text{eff}}(Q)$ using Eq. 5 for the case in which Taq polymerase is separated into two domains, a 5' nucleic acid domain ($j = 1$) and a Klentaq domain ($j = 2$). The $D_{\text{eff}}(Q)$ for the two-domain model (see Fig. 5B) is then

$$D_{\text{eff}}(Q) = \frac{D_j S_j(Q) + D_2 S_2(Q)}{S_{\text{Taq}}(Q)}, \quad [7a]$$

where $D_j = (k_B T)/\zeta_j$ with $D_1 = D_{5'-\text{nuc}}$ and $D_2 = D_{\text{Klentaq}}$, and

$$S_j(Q) = \sum_{m,n,j} b_m b_n \frac{|Q| r_m - r_n|}{Q|r_m - r_n|} \quad [7b]$$

$$S_{\text{Taq}}(Q) = \sum_{m,n=1}^{N_1+N_2} b_m b_n \frac{|Q| r_m - r_n|}{Q|r_m - r_n|} \quad [7c]$$

are the rotationally averaged static form factors, which can be approximated by the crystal structure coordinates as we show by SANS (see Unusual Internal Dynamic Behavior in Taq Polymerase Revealed by NSE) and previous small-angle neutron scattering studies (17). In Eq. 7b, the sum is taken over the $N_j$ atoms in domain $j$, and $N_1$ and $N_2$ are the number of atoms in domain 1 and domain 2, respectively. The cross-term 1–2 in the numerator of Eq. 7a disappears because the mobility tensor is diagonal. Subtleties in using Eq. 7 arising when rigid constraints are applied (41–44) are avoided by explicitly separating the center-of-mass coordinate of each domain before performing the ensemble average and then subsequently folding in domain form factors. Eq. 7 was also verified by explicit calculation using Eq. 2 with $U$ taken as a harmonic oscillator potential. As per Eq. 7, the first cumulant is explicitly independent of the interdomain spring constant.

The calculated $D_{\text{eff}}(Q)$ using Eq. 7a, shown in Fig. 4A, also has peaks and dips in the intermediate $Q$ values as the NSE-measured $D_{\text{eff}}(Q)$. When using Eq. 7a to calculate the curves shown in Fig. 4A, we find that the friction constants $\zeta_1$ and $\zeta_2$ of both domains in Taq polymerase increase by a factor of $\approx 2$ compared with those of the separated individual domains as calculated by the Kirkwood–Riseman formula (45). As the domains are in close proximity, the friction constant for each domain is increased because of the fluid displaced between them by their motion. Quantitatively similar phenomena have been found when two circular disks or two spheres

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**Fig. 5.** Dynamic models of Taq polymerase. (A) Rigid-body model of Taq polymerase used in Eq. 2. (B) Two-rigid-domain model connected by a spring-like linker calculated by Eq. 7a. (C) Three-rigid-domain model connected by two spring-like linkers calculated by Eq. 8.
Repeating the analysis of Eqs. 8 and 9, reducing the data within a normal mode framework, we show that the internal motion can be systematically analyzed by mobility tensor analysis and that the systematic inclusion of higher normal modes consistently reveals coupled domain motion within Taq polymerase. Moreover, we show that the internal motion can be systematically analyzed by reducing the data within a normal mode framework.

The rms amplitude \( \langle \chi \rangle^{1/2} \) and the spring constant of interdomain motion can be estimated from the equipartition theorem, which states

\[
\langle \chi \rangle = \frac{k_B T}{k} = \left( \frac{k_B T}{\xi} \right) \left( \frac{\xi}{k} \right) = D \tau, \tag{9}
\]

where \( k \) is the spring constant, \( k_B \) is Boltzmann’s constant, \( D \) is the Stokes–Einstein diffusion constant, \( \tau \) is the relaxation time that can be estimated from the NSE results, and \( T \) is the temperature. Thus, for relaxation times of the order of 10 ns, the estimated amplitude \( \langle \chi \rangle^{1/2} \) of the normal mode is \( \sim 10^{-1} \) Å. For \( \langle \chi \rangle^{1/2} = 7 \) Å, the spring constant \( k \) for the linker region is \( \sim 8.5 \times 10^3 \) N/m. This value is less than one-third of the spring constant of myoglobin (23) but \( \sim 5.6 \) times larger than the reported spring constant of cross-linked polystyrene (49).

In summary, protein conformational changes are typically initiated through an ensemble of states that interconvert on picosecond to nanosecond timescales (50). These small-amplitude conformational changes (Eyring dynamics) can eventually encourage thermally activated (Kramers kinetics) events that lead to large-scale conformational changes on the nanosecond to microsecond timescale (9). Our NSE results have revealed coupled motion between protein domains that are separated by 70 Å. On the nanosecond timescales probed by NSE, this coupled domain motion is an overdamped, creeping motion rather than the harmonic oscillation expected for inertial motion (9, 51). We show how NSE can determine the domain mobility tensor of a protein and thus characterize dynamic interdomain coupling. The mobility tensor defines the velocity response of a given domain to a force applied to it or to another domain, much as the sails determine the velocity (direction and magnitude) of a sailboat’s travel when a given wind force is applied. NSE thus provides unique dynamic information about a protein that is functionally important and inaccessible by other methods.

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