Domain Flexibility in Ligand-Free and Inhibitor-Bound Escherichia coli Adenylate Kinase Based on a Mode-Coupling Analysis of $^{15}$N Spin Relaxation

Yury E. Shapiro,† Edith Kahana,‡ Vitali Tugarinov,†‡ Zhichun Liang,§ Jack H. Freed,*§ and Eva Meirovitch*†

Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan 52900, Israel, and Baker Laboratory of Chemistry and Chemical Biology, Cornell University, Ithaca, New York 14853-1301

Received December 7, 2001; Revised Manuscript Received March 5, 2002

ABSTRACT: Adenylate kinase from Escherichia coli (AKeco), consisting of a 23.6-kDa polypeptide chain folded into domains CORE, AMPbd, and LID catalyzes the reaction AMP + ATP ↔ 2ADP. The domains AMPbd and LID execute large-amplitude movements during catalysis. Backbone dynamics of ligand-free and AP$_5$A-inhibitor-bound AKeco is studied with slowly relaxing local structure (SRLS) $^{15}$N relaxation, an approach particularly suited when the global ($\tau_g$) and the local ($\tau_l$) motions are likely to be coupled. For AKeco $\tau_g$ = 15.1 ns, whereas for AKeco*AP$_5$A $\tau_g$ = 11.6 ns. The CORE domain of AKeco features an average squared order parameter, $<S^2>$, of 0.84 and correlation times $\tau_l$ = 5–130 ps. Most of the AKeco*AP$_5$A backbone features $<S^2>$ = 0.90 and $\tau_l$ = 33–193 ps. These data are indicative of relative rigidity. Domains AMPbd and LID of AKeco, and loops $\beta_3$/H, $\alpha_3$/$\alpha_3$, $\alpha_4$/$\beta_3$, $\alpha_3$/$\beta_4$, and $\beta_9$/$\alpha_3$ of AKeco*AP$_5$A, feature a novel type of protein flexibility consisting of nanosecond peptide plane reorientation about the C$_i$-$C_j$ axis, with correlation time $\tau_l$ = 5.6–11.3 ns. The other microdynamic parameters underlying this dynamic model include $S^2$ = 0.13–0.5, $\tau_g$ on the ps time scale, and a diffusion tilt $\beta_{MD}$ ranging from 12 to 21°. For the ligand-free enzyme the $\tau_l$ mode was shown to represent segmental domain motion, accompanied by conformational exchange contributions $R_{ex}$ ≤ 4.4 s$^{-1}$. Loop $\alpha_2$/$\beta_3$ and $\alpha_3$/$\beta_4$ dynamics in AKeco*AP$_5$A is related to the “energetic counter-balancing of substrate binding” effect apparently driving kinase catalysis. The other flexible AKeco*AP$_5$A loops may relate to domain motion toward product release.

The ability to interpret nuclear spin relaxation properties in terms of microdynamic parameters turned NMR into a powerful method for elucidating protein dynamics (1, 2). The amide $^{15}$N spin in proteins is a particularly useful probe, relaxed predominantly by dipolar coupling to the amide proton and $^{15}$N chemical shift anisotropy (CSA) (3). The experimental NMR observables are controlled by the global and local dynamic processes experienced by protein N-H bond vectors, which determine the spectral density function, $J(\omega)$. $^{15}$N relaxation data in proteins are commonly analyzed with the model-free (MF) approach, where the global and local motions are assumed to be decoupled (4–6). In a recent study (7), we applied the two-body slowly relaxing local structure (SRLS) approach developed by Freed and co-workers (8, 9) to $^{15}$N relaxation in proteins. SRLS accounts rigorously for dynamical coupling between the local and global motions, and treats the global diffusion, the local diffusion, the local ordering, and the magnetic interactions as tensors that may be tilted relative to one another, providing thereby important information related to protein structure (10–12). The MF spectral density functions constitute asymptotic solutions of the SRLS spectral densities (7, 8, 13). It was found that currently available experimental $^{15}$N relaxation data are sensitive to the coupling-induced mixed modes inherent in the SRLS model (7, 14).

AKeco is a particularly intriguing case in the context of protein dynamics in general, and the structure–function relationship associated with domain motions in enzymes (15), in particular. AKeco consists of a single 23.6-kDa polypeptide chain folded into domains CORE, AMPbd, and LID. It catalyzes the phosphoryl transfer reaction Mg$^{2+}$ATP + AMP ↔ Mg$^{2+}$ADP + ADP (16). The ribbon diagram of the “open” ligand-free form crystal structure (17) is shown in Figure 1a. CORE is the largest domain. It includes residues 1–29, 60–121, and 160–214, which form a five-stranded parallel $\beta$-sheet comprising strands $\beta_1$–$\beta_7$ and $\beta_9$ surrounded by helices $\alpha_1$ and $\alpha_4$–$\alpha_6$. Domain AMPbd includes helices $\alpha_2$ and $\alpha_3$ formed by residues 30–59. The LID domain includes residues 122–159, which form a four-stranded antiparallel $\beta$-sheet (strands $\beta_5$–$\beta_8$). Domains AMPbd and LID are displaced significantly upon substrate binding (17–20). The
active site is configured thereby, and the “closed” structure, illustrated in Figure 1b by the ribbon diagram of the complex of AKeco with the two-substrate-mimic inhibitor AP5A (21), is obtained. It is assumed that following the reaction the structure “opens up” again through reverse movements of AMPbd and LID to recover the original ligand-free enzyme (17). This mechanism has been inferred based on the crystal structures of ligand-free AK enzymes, and various molecular complexes with nucleoside monophosphates, nucleoside triphosphates, and inhibitors (19, 22). Time-resolved fluorescence energy transfer studies of fluorescent AKeco derivatives confirmed domain closure in solution upon inhibitor binding (23, 24). These studies also indicated that large-amplitude segmental mobility of AMPbd and LID is in effect in the ligand-free form (23). AKeco is the only adenylate kinase for which crystal structures corresponding to the extreme stages of the catalytic cycle are available (17, 21, 22), with the closed form represented by AKeco*AP5A. The ATP phosphates are bound to the enzyme partly through the so-called P-loop GXXGXGK (residues 7–13). This binding motif between proteins and nucleotides occurs in many proteins that bind nucleoside triphosphates, in all the nucleoside monophosphate kinases, and in the weakly homologous G-proteins (17). When bound to AKeco, the two-substrate mimicking inhibitor AP5A adopts a conformation close to the suggested transition state (25). Thus, we have at hand a prototype for a multidomain biological machine where mobility and function must be necessarily related. The SRLS concept of coupling between motions occurring on arbitrary time scales is particularly important for elucidating dynamic properties of proteins when segmental mobility is in effect.

Experimental 15N relaxation data of AKeco and AKeco*AP5A acquired at 14.1 T were studied previously (26) using the conventional MF approach. The SRLS approach was applied recently to AKeco data acquired at magnetic fields of 14.1 and 18.89 T (14). This study indicated that SRLS is significantly more accurate and discriminating than MF.

Herein we apply SRLS to AKeco*AP5A, complementing the relaxation data acquired at 14.1 T (26) with 15N T1, T2, and 15N-{1H} NOE data acquired at 18.79 T. The dynamic properties of the inhibitor-bound form were found to differ significantly from those of the ligand-free form. In general, two different dynamic models are experienced. These include the conventional rigid structure with rapid N–H bond fluctuations model, and a new intrinsically flexible model, where peptide planes move on the nanosecond time scale. The CORE domain of AKeco, and the majority of the AKeco*AP5A residues, comply with the conventional model. It was found that the catalysis-related domains AMPbd and LID of AKeco are engaged in collective nanosecond peptide-plane motion interpretable as domain motion, and microsecond–millisecond conformational exchange. Residues within the loops β1/α1, α2/β1, α3/β3, and β3/α3 of AKeco*AP5A experience nanosecond peptide plane motion which may be of a loop-swinging type. Loops α4/β1 and α5/β4 are rigid in AKeco and representative residues thereof are intrinsically flexible in AKeco*AP5A, in accordance with the “energetic counterweight balancing of substrate binding” concept (17, 26).

THEORETICAL BACKGROUND

We showed recently (7) that the two-body structural mode-coupling SRLS theory (8, 9), extended in recent years to spin-labeled proteins (10–12), can also be applied to N–H bond vector motions in proteins. A short description of fundamentals is presented below.

The N–H bond vector and the protein surroundings (the two “bodies”) are dynamically coupled by a potential U(QCM) that depends on their relative orientation QCM(t), where C denotes the global diffusion frame of the protein surroundings, and M denotes the local diffusion/local ordering frame of the N–H bond vector. The local coupling potential exerted by the protein surroundings tends to align the N–H bond vector along the principal axes of the ordering frame. In the case of axially symmetric (along ZM) local

![Figure 1: Ribbon diagram of the crystal structures of (a) AKeco and (b) AKeco in complex with the two-substrate-mimic inhibitor AP5A. The figures were drawn with the program Molscript (63) using the PDB coordinate files 4ake (complex II) for AKeco and 1ake (complex II) for AKeco*AP5A.](image)
Domain Motion in Adenylate Kinase Elucidated by NMR

order it is most simply given by (10–12):

\[ U(\Omega_{CM})/k_B T = -c_{20} D_{00}^2(\Omega_{CM}) \]  

(1)

where \( k_B \) is the Boltzmann constant, \( T \) is the temperature in K, \( c_{20} \) is the potential strength in units of \( k_B T \), and \( D_{00}^2 \) is the Wigner rotation matrix element. A conventional order parameter can be related to \( c_{20} \) as (10):

\[ S_{SRLS} = -<D_{00}^2(\Omega_{CM}(t))> \]  

(2)

where the brackets imply ensemble averaging using the probability distribution based on the potential function given in eq 1 (7).

The time-dependent part of the spin Hamiltonian for this two-body system was given previously (10, 27). The dynamic effects associated with the global and the local diffusion are incorporated into the spectral density through the diffusion operator (9, 10):

\[ \hat{\Gamma} = \hat{\Gamma}^{\text{global}}(\Omega_{LC}) + \hat{\Gamma}^{\text{local}}(\Omega_{LM}) + F^{\text{global}}(-\Omega_{CM}) + F^{\text{local}}(-\Omega_{CM}) \]  

(3)

where \( L \) denotes the fixed laboratory frame. The first two terms in this equation refer to the freely globally diffusing protein surroundings and locally diffusing N–H bond vector, respectively. The last two terms reflect the contributions to \( \hat{\Gamma} \) due to the potential \( U(\Omega_{CM}) \), which couples the global and local motions. \( \hat{\Gamma}^{\text{global}} \) and \( F^{\text{local}} \) are functions of the Euler angles \( \Omega_{CM} \) that transform the M frame into the C frame. This constitutes an effective two-body model for which a Smoluchowski equation representing the rotational diffusion of two interacting rotors is solved (8, 9). The solution features three eigenvalues (correlation times) for the local motion when \( S^2 = 0 \):

\[ (\tau_K)^{-1} = 6R_{1\perp}^2 + K^2(R_{1\parallel}^2 - R_{L\perp}^2) \]  

for \( K = 0; 1; 2 \)  

(4)

Each \( K \) value leads to its own spectral density component (28). For \( S^2 > 0 \) the \( j_{K=0}(\omega) \) term represents mixed modes between the global motion, \( R^C \), and the local motion component, \( R^L_{\perp} \). When \( R^L_{\perp} \geq R^C \) the \( j_{K=1}(\omega) \) and \( j_{K=2}(\omega) \) terms are dominated by \( R^L_{\parallel} \) and \( R^L_{\perp} \), given in eq 4. The “measurable” spectral density is then constructed out of the three \( j_K(\omega) \) components by incorporation of the orientation dependent functions that multiply the spin operators in the spin Hamiltonian (28).

Assuming that the \(^{15}\text{N CSA tensor} \) is axially symmetric and collinear with the dipolar N–H tensor, the spectral density for \(^{15}\text{N CSA} \) and \(^{15}\text{N-‘\( ^{1}\text{H} \) dipolar relaxation} \) in the coordinate frame of the local motion is given by (28, 29):

\[ J(\omega) = Aj_{K=0}(\omega) + Bj_{K=1}(\omega) + Cj_{K=2}(\omega) \]  

(5)

where \( A = (1.5\cos^2\beta_{MD} - 0.5)^2 \), \( B = 3\sin^2\beta_{MD}\cos^2\beta_{MD} \), \( C = 0.75\sin^2\beta_{MD} \), and \( \beta_{MD} \) is the “diffusion tilt” angle between the molecular diffusion axis \( Z_M \) and the N–H bond vector. In the present study, the SRLS parameters featured by \( J(\omega) \) include three diffusion rate constants: \( R^C \) (related to the global diffusion tensor), \( R^L_{\perp} \) and \( R^L_{\parallel} \) (related to the local diffusion tensor), one potential parameter \( c_{20} \), and the polar angle \( \beta_{MD} \) (diffusion tilt angle). Special cases include:

(i) isotropic fast local diffusion (local correlation time \( \tau_L \equiv \tau_1 = (6R_{1\perp}^2)^{-1} \approx \tau_L = (6R_{1\parallel}^2)^{-1} \)), implying \( \beta_{MD} = 0 \); then \( J(\omega) = j_{K=0}(\omega) \), and the NMR relaxation data can be fit with one (\( c_{20} \) if \( \tau_L \) is negligibly small) or two (\( c_{20} \) and \( \tau_1 \)) free parameters, in formal analogy with the original MF spectral density (4, 5), with \( \tau_L \) denoting the local motion correlation time; (ii) very anisotropic slow local motion (\( \tau_L \ll \tau_1 \ll \tau_t \) and \( \tau_L \sim 0 \)), denoted VALM below; then the last two terms in eq 5 are negligibly small compared to \( Aj_{K=0} \), provided \( \beta_{MD} \neq 54.7^\circ \), and NMR data can be reproduced with three free parameters (\( c_{20}, \tau_1, \beta_{MD} \)). The VALM spectral density is formally analogous (7, 14) to the reduced extended MF spectral density (6). If NMR data at more than one magnetic field are available, VALM can be extended by allowing \( \tau_L \equiv \tau_1 \) to be a free parameter. Then all the \( j_k(\omega) \) components contribute to \( J(\omega) \) in eq 5. For small \( \beta_{MD} \) angles, the SRLS spectral density is (given the above assumptions) formally analogous to the extended MF spectral density (30). The expression (1.5\cos^2\beta_{MD} - 0.5)^2 is formally analogous to \( S^2 \) (7, 14).

The CSA spectral density can be corrected for non-collinearity of the \(^{15}\text{N CSA} \) and \(^{15}\text{N-‘\( ^{1}\text{H} \) dipolar tensors} \), which are tilted at an angle \( \theta \) (33), by addition of \( \Delta J_{CSA} \) (7, 14). As discussed previously (7, 14), the angle \( \gamma_{MD} \) between the \( Y \) axis of the magnetic dipolar tensor and the \( x \) axis of the \( M \) frame was set at 90\(^\circ \). In this case, the perpendicular local motion occurs about an axis \( (\gamma_{MD}) \) lying in the peptide plane parallel to the \( N_C-\text{C}^\alpha \) bond, or the \( \text{C}^\alpha -\text{C}^\alpha \) axis.

After the spectral density function \( J(\omega) \) has been constructed out of its fundamental \( j_k(\omega) \) components using eq 5, the measurable \(^{15}\text{N relaxation quantities} \) \(^{15}\text{T}_1, ^{15}\text{T}_2, \) and \(^{15}\text{N-‘\( ^{1}\text{H} \) NOEs} \) are calculated as a function of \( \gamma_{MD} \), \( J(\omega_{\text{ON}}) \), \( J(\omega_{\text{ON} + \omega_{\text{ON}}} \), and \( J(\omega_{\text{ON} - \omega_{\text{ON}}} \), using standard expressions for NMR spin relaxation (3, 34).

The formal analogy between SRLS and MF spectral densities can be further clarified by giving the form of the expression for the SRLS \( j_k(\omega) \) functions. The solution of the two-body Smoluchowski equation consists of eigenmodes \( \lambda_1, \lambda_2 \), and weighing factors \( c_k \), such that \( j(\omega) = \sum_k c_k \lambda_k (1 + \omega^2 \tau_k^2) \). The eigenmodes \( \lambda_k \) represent pure or mixed dynamic modes, in accordance with the parameter range considered. For example, for \( \lambda_1^2 / \lambda_2 > 0.01 \) the global motion is given by the smallest eigenmode, with increasing weight as \( S^2 \) increases (Table 5 of ref 8). The local motion is given by a large number of mixed eigenmodes with two (for \( 0.2 < S^2 < 0.5 \)) or one (above \( S^2 = 0.5 \)) having weights comparable to that of the global motion eigenmode. Yet, the combined contribution of the (three or two) dominant eigenmodes to \( j(\omega) \) in the noted \( S^2 \) range is only about 80\%, with the rest contributed by a large number of large mixed eigenmodes with small individual weights. The SRLS function \( J(\omega) \) is constructed out of the \( j_k(\omega) \) components. The \( J(\omega) \) from the SRLS approach we use (as described above) and from MF have similar general forms and feature the same number of free parameters (more sophisticated forms of SRLS are available as needed) (7, 10). In this respect, they are “formally analogous” but not identical, because the MF \( J(\omega) \) are not precisely the same dynamic modes and associated weights, nor does MF distinguish between the different \( j_k(\omega) \) in eq 5. Identity is achieved only in the limiting cases where \( J(\omega) \) for SRLS yields the MF formulas. We found that the contribution of mixed modes is on the order of typical experimental errors when \( S^2(\text{SRLS}) > 0.9 \) and \( r(\text{SRLS}) <
Table 1: SRLS Models Used to Fit Experimental AKeco and AKeco*AP3A 15N NMR Relaxation Data Acquired at Two Magnetic Fields

<table>
<thead>
<tr>
<th>no</th>
<th>parameters*</th>
<th>SRLS model description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>c20</td>
<td>very fast internal motion (τ → 0)</td>
</tr>
<tr>
<td>2</td>
<td>c20 (S^2); τ(τ)</td>
<td>isotropic internal motion</td>
</tr>
<tr>
<td>3</td>
<td>c20 (S^2); R_ex</td>
<td>model 1 with exchange term</td>
</tr>
<tr>
<td>4</td>
<td>c20 (S^2); τ(τ); R</td>
<td>model 2 with exchange term</td>
</tr>
<tr>
<td>5</td>
<td>c20 (S^2); β_md (S^2); τ(τ)</td>
<td>very anisotropic internal motion (τ → 0)</td>
</tr>
<tr>
<td>6</td>
<td>c20 (S^2); β_md (S^2); τ(τ); R</td>
<td>anisotropic internal motion¹</td>
</tr>
<tr>
<td>7</td>
<td>c20 (S^2); β_md (S^2); τ(τ)</td>
<td>model 5 with exchange term</td>
</tr>
<tr>
<td>8</td>
<td>c20 (S^2); β_md (S^2); τ(τ)</td>
<td>model 6 with exchange term</td>
</tr>
</tbody>
</table>

*Analogous MF parameters are shown in parentheses. ¹For model 1, the τ → 0 assumption is practically equivalent to fixing τ at the lowest value for which the SRLS spectral densities could be calculated. ²For model 5, the τ → 0 assumption is equivalent to neglecting the jk=1(ω) and jk=2(ω) spectral density components. For models 6 and 8, where in practice τ ≪ τ, jk=1(ω), and jk=2(ω) components calculated for an isotropic local diffusion tensor with correlation time 1/6 R^2 (D) were used in eq 5.

40 ps. As indicated in ref 7, and further demonstrated in this study, the MF limits are not precisely attained over the parameter range relevant to folded proteins. The parameter most sensitive to departures from these limits is τ.

The theory outlined above has been implemented in a fitting scheme (7). The SRLS-based dynamic models employed to fit the two-field 15N relaxation data of AKeco are summarized in Table 1. In model 1, the local motion is so fast (τ → 0) that its effect on the spectral density is negligible. This assumption is equivalent in practice to fixing τ at the lowest value for which the SRLS spectral densities could be calculated. In model 2, it is assumed that the internal motion can be approximated as isotropic (τ_i = τ_j). Models 3 and 4 are derived from models 1 and 2, respectively, by addition of the free parameter, R_ex, to the transverse relaxation rate expressions to account for exchange processes on the microsecond to millisecond timescale. For models 1–4 β_md = 0, hence J(ω) = jk=1(ω) in eq 5. In model 5 (VALM), the local motion is assumed to be very anisotropic, i.e., τ_i ≪ τ_j ≡ τ and τ → 0. In this case, only τ_j enters the spectral density. The angle β_md is allowed to vary, affecting J(ω) through the coefficient A in eq 5. In model 6, the restriction τ → 0 is removed leaving τ_j as a free variable. The local motion (R^2) of an N–H bond restricted to the peptide plane is necessarily highly anisotropic (see below).

It was found that in this limit jk=1(ω) and jk=2(ω) in eq 5 are well approximated by their counterparts calculated for an isotropic local diffusion tensor with correlation time 1/6 R^2 (D). These approximations were used in models 6 and 8. Models 7 and 8 are derived from models 5 and 6, respectively, by addition of the free parameter R_ex.

MATERIALS AND METHODS

Sample Preparation. For preparation of uniformly 15N-labeled AKeco, Escherichia coli HB101 cells transformed with the pEAK91 plasmid were grown at 37 °C in Celltone-N medium (Martek Biosciences Corp., US) containing >98% 15N. The recombinant plasmid pEAK91 contained the intact gene coding for E. coli adenylate kinase (35). The previously described procedure for purification of AKeco (24) was improved by application of Blue-Sepharose affinity chromatography (35), followed by size-exclusion chromatography on a Sephacryl S-100 column (Pharmacia, Sweden). AKeco stock solution was prepared by thorough dialysis of the protein solution against 40 mM sodium phosphate buffer (pH 6.8) containing 10 mM sodium azide, followed by concentration on a Centricon-10 concentrator (Amicon, US). The concentration of the AKeco solution was determined based on the absorption coefficient A_277 = 0.5 mg/mL cm⁻¹ (36).

An appropriate amount of AP3A (Boehringer, Germany) inhibitor stock solution (pH 6.8) and 50% D₂O, prepared in the same buffer, were added to the concentrated AKeco solution to obtain fully saturated enzyme in the AKeco*AP3A sample. The ligand-free sample contained 1.75 mM 15N-labeled enzyme and 40 mM sodium phosphate buffer in 95% H₂O/5% D₂O. The inhibitor-bound sample contained 2 mM 15N-labeled enzyme, 2.5 mM AP3A, and 40 mM sodium phosphate buffer in 95% H₂O/5% D₂O. The protein samples were degassed and transferred to 5-mm NMR Shigemi cells. Protein monodispersion was ascertained previously (26).

NMR Spectroscopy. NMR experiments were carried out at 303 K on Bruker DMX-600 and DRX-800 spectrometers operating at 14.1 and 18.79 T, respectively, using 5-mm ¹H-¹³C-¹⁵N triple resonance inverse detection probes and B-VT-2000 and BTO-2000 temperature control units at 14.1 and 18.79 T, respectively. NMR data were analyzed on Silicon Graphics workstations using the software packages mmPipe and modelXY (37). The previously determined assignments of the AKeco ¹H-¹⁵N correlations (38), complemented and revised in our earlier study (26), were used. The assignments of the AKeco*AP3A ¹H-¹⁵N correlations were taken from ref 39.

Relaxation times T₁ and T₂, and NOE parameters were measured using established inversion recovery (40), spin–echo (41), and ¹N-¹H steady-state NOE (42) pulse sequences, as described in refs 43–45. A 0.5-s spin–echo period was used in the CPMG T₂ experiments. For NOE experiments, we used sequence 1B of ref 45, which features H₂O flip-back pulses to minimize saturation of water. Spectral widths were 1824.5 and 2432.6 Hz in the F₁ dimension, and 9615.4 and 12820.5 Hz in the F₂ dimension at 14.1 and 18.79 T, respectively. The ¹⁵N carrier was set at 117.5 ppm and was referenced indirectly to liquid NH₃ (46). 360 × 2048 complex points were acquired in the t₁ × t₂ dimensions for each time point.

The ¹⁵N T₁ and T₂ measurements were performed using a total of 40 and 64 transients per t₁ experiment, respectively. For the T₁ measurements of AKeco, nine time points were collected using parametric delays of 15, 127, 247, 367, 487, 647, 807, 1031, and 1287 ms at 14.1 T, and 15, 127, 327, 567, 807, 1047, 1367, 1767, and 2247 ms at 18.79 T. The experiment was repeated twice for time points 15, 487, and 1287 ms at 14.1 T, and 15, 807, and 2247 ms at 18.79 T. The delay between scans was set to 1.5 s at 14.1 T and 2 s at 18.79 T. For the T₁ measurements of AKeco*AP3A, eight time points were collected using parametric delays of 133.5, 245.5, 357.5, 485.5, 645.5, 805.5, 997.5, and 1285.5 ms at 14.1 T, and nine time points were collected using parametric delays of 15, 127, 327, 567, 807, 1047, 1287, 1607, and 2007 ms at 18.79 T. The experiment was repeated twice for each
time point at 14.1 T, and for 15, 807, and 2007 ms at 18.79 T. The delay between scans was set to 1.5 s at 14.1 T and 2.2 s at 18.79 T for both AKeCo forms. For the $T_2$ measurements of AKeCo, nine time points were collected using parametric delays of 8, 16, 24, 32, 48, 64, 80, 96, and 128 ms at 14.1 T, and eight time points were collected using parametric delays of 8, 16, 32, 48, 64, 80, 104, and 128 ms at 18.79 T. The experiment was repeated twice for time points 8, 64, and 128 ms. For the $T_2$ measurements of AKeCo*AP5A, nine time points were collected using parametric delays of 8, 16, 24, 32, 48, 64, 80, 96, and 128 ms at 14.1 T, and eight time points were collected using parametric delays of 8, 16, 32, 48, 64, 80, 104, and 128 ms at 18.79 T. The experiment was repeated twice for time points 8, 24, 64, and 128 ms at 14.1 T, and 8 and 48 ms at 18.79 T. The delay between scans was set to 1.6 s at 14.1 T and 1.9 s at 18.79 T for both AKeCo forms. The data were apodized with a cosine (cosine-bell) window function in $t_1$ ($t_2$). Duplicates were used to calculate average values of, and uncertainties in, the measured peak heights. Phenomenological $T_1$ and $T_2$ values and uncertainties were determined by nonlinear least-squares fitting of the experimental data to monoexponential equations (47).

The $^{15}$N-$^{1}$H NOE values were measured using pairs of spectra recorded in an interleaved mode with and without proton presaturation during the recycle delay. A total of 96 transients per $t_1$ experiment were recorded. The delay between scans was 3.8 and 6.6 s for AKeCo and 3.8, 6.6, or 12.1 s for AKeCo*AP5A at 14.1 T, and 5.4 s for AKeCo and 5.7 s for AKeCo*AP5A at 18.79 T. The data were processed as described above. The $^{15}$N-$^{1}$H NOE values were recorded in duplicate, and the replicates were used to determine uncertainties and mean values.

Data Analysis. The calculation of SRLS spectral densities is computationally intensive for $c_{20}$ values exceeding $\sim 10$ ($S^2$ exceeding $\sim 0.81$) and/or very fast internal motions. Therefore, in adapting SRLS to protein relaxation analysis we used precalculated2 two-dimensional grids of $j(0), j(\omega_H), j(\omega_H + \omega_N)$, and $j(\omega_H - \omega_N)$ to fit experimental $^{15}$N $T_1$, $T_2$, and $^{15}$N-$^{1}$H NOE data. (7) The $j_{k=0}, j_{k=1}$, and $j_{k=2}$ grids of spectral density values at the five frequencies were constructed under the assumption of isotropic global motion for sets of $c_{20}$ and $T_1$ (or $T_2$) values. An axial $^{15}$N chemical shielding tensor with $\sigma_{||} - \sigma_{\perp} = -170$ ppm, $r_{NH} = 1.02$ Å, and $\theta = -16^\circ$ (e.g., see ref 48) were used in these calculations. The $c_{20}$ grid dimension spanned the values between 0 ($S^2 = 0$) and 40 ($S^2 = 0.95$), and the $\tau$ dimension spanned the values between 0.0005$r_{\text{m}}$ and 1.4$r_{\text{m}}$. The ratio $\tau/r_{\text{m}}$ was interpolated to 0 as required. A two-dimensional polynomial interpolation using Neville’s algorithm (49) was employed for spectral density evaluation in the course of model fitting. The interpolation errors in both the $c_{20}$ and $\tau$ grid dimensions were estimated to be at least one order of magnitude smaller than the errors in the fitted microdynamic parameters. The fitting of experimental NMR data was based on Powell minimization (49) of a target function. The target function for spin $i$ was defined as the sum of the squared differences between experimental and calculated $T_1$, $T_2$, and $^{15}$N-$^{1}$H NOE values divided by the squared random errors:

$$
\chi^2_i = \sum_{j} \left[ \left( T_{1,i}^{\text{obs}} - T_{1,i}^{\text{calc}} \right)/\sigma_{T_{1,i}}^2 \right]^2 + \left[ \left( T_{2,i}^{\text{obs}} - T_{2,i}^{\text{calc}} \right)/\sigma_{T_{2,i}}^2 \right]^2 + \left[ \left( \text{NOE}_{i}^{\text{obs}} - \text{NOE}_{i}^{\text{calc}} \right)/\sigma_{\text{NOE}_{i}}^2 \right]^2
$$

where the sum runs over the magnetic fields used in acquiring the experimental data. The model selection scheme employed in the fitting program was based on $\chi^2$- and F-statistic testing and followed closely the schemes used for MF analysis, as described previously (7). Errors in microdynamic parameters were evaluated based on Monte Carlo simulations (50) using 100 randomly distributed synthetic data sets. Convergence was ascertained by obtaining practically identical results with a larger number of Monte Carlo simulations in representative calculations.

The fundamentals of the MF calculations were described earlier (51), and the details of their application to the AKeCo and AKeCo*AP5A data acquired at 14.1 T were given previously (26).

RESULTS AND DISCUSSION

The experimental $^{15}$N $T_1$, $T_2$, and $^{15}$N-$^{1}$H NOE data, acquired at 14.1 and 18.79 T for AKeCo are shown in Figure 2. It can be seen that the $^{15}$N-$^{1}$H NOE is the most sensitive parameter, with NOE values clearly reduced in the chain regions corresponding to domains AMPbd and LID. $T_1$ values are lower within AMPbd and within the LID chain segment extending from residue G122 to residue F137. The experimental $^{15}$N $T_1$, $T_2$, and $^{15}$N-$^{1}$H NOE data, acquired at 14.1 and 18.79 T for AKeCo*AP5A, are shown in Figure 3.
values acquired at both 14.1 and 18.79 T are reduced within
loop $\alpha_5\beta_3\alpha_5$ of domain AMPbd, loops $\alpha_5\beta_3$ and $\alpha_5\beta_2$ of
CORE, and residues Q160 and G214. The $T_1$ and $T_2$ profiles
display limited variability. The NOE and $T_2$ values are on
average smaller, and the $T_1$ values larger, for the ligand-
free enzyme, pointing qualitatively to a larger and more
flexible particle prevailing in solution in the absence of
inhibitor.

The combined data sets featuring 188 residues for AKeco and
187 residues for AKeco*AP5A were subjected to SRLS
analysis, with the experimental errors on average 2.5% for
AKeco and 3.5% for AKeco*AP5A. The $\chi^2$ probability
confidence level was set at 5% (in a few cases at 1%), and
the F-statistic probability confidence level at 20%. With these
criteria, the experimental data of 86% of the AKeco residues,
and 96% of the AKeco*AP5A residues, could be fit.

Global Diffusion. Domains AMPbd and LID of AKeco
ejecute in solution large-amplitude segmental motions (23,
24). On the NMR time scale, conformational averaging leads
to a well-defined average structure (26). A detailed discussion
of its global diffusion tensor, $R^G$, was presented previously
(14). It was concluded that isotropic diffusion with $\tau_m = 15.1 \pm 0.1$ ns constitutes an appropriate approximation, with
the provision that exchange contributions beyond Q160 may
also reflect geometric features associated with $R^C$ anisotropy
(26). The inhibitor-bound complex is quite rigid and similar
to the nearly spherical crystal structure (26, 52). Therefore,
its lends itself particularly well to global diffusion tensor
determination based on the quadratic approximation (53, 54).
This has been accomplished previously (26) to yield $\tau_m(app) = 11.6 \pm 0.1$ ns and a diffusion anisotropy $D_\perp/D_\parallel = 1.11$.
The quadratic approximation (53) makes possible calculating
(54) effective correlation times $\tau_m(i)$ for each residue $i$. Using
these $\tau_m(i)$ values led to practically the same results as using
$\tau_m = 11.6 \pm 0.1$ ns, with the same provision in regards with $R^G$ terms beyond Q160 as mentioned above for AKeco. On
the basis of these considerations, $\tau_m = 11.6$ ns was used in
all further calculations.

Local Motion. The combined two-field data sets of AKeco
and AKeco*AP5A were subjected to SRLS fitting (7) using
the eight SRLS models (Table 1), which can be classified into
the simplified models 1–4 and the somewhat more
general models 5–8. In the first model category, it was
assumed that $R^L$ is in the extreme motional narrowing limit
(models 1 and 3) or isotropic (models 2 and 4). The latter
simplification is justified when the time scale separation
between $R^L$ and $R^C$ is large, as in this case $j_{k(\omega)}$ is
dominated by the global diffusion, and determining $R^G$ with
reasonable accuracy is difficult. The model 5–8 category
includes general cases of weaker coupling potentials and
smaller time scale separations, when the tensor $R^L$ must be
at least axially symmetric. Since $R^L \gg R^C$ for models 1–4
and $R^L \ll R^C$ for models 5–8, we denote the former
parameter range as the “ps regime”, and the latter parameter
range as the “ns regime”. With AKeco the majority of the
CORE residues were associated with the ps regime and fit with
models 2 and 4. The AMPbd and LID residues were
associated with the ns regime and fit with models 6 and 8.
With AKeco*AP5A loop $\alpha_5\beta_3$, and representative residues of
loops $\beta_3/\alpha_5$, $\alpha_5/\beta_3$, and $\beta_3/\alpha_5$, were associated with
the ns regime and fit mostly with model 6. The rest of the
backbone was associated with the ps regime and fit with
models 1–4, pointing out outstanding rigidity.

The best-fit microdynamic parameters obtained with the
SRLS analysis are depicted in Figures 4 and 5. The squared
order parameters, $S^2$, are clustered into two distinct narrow
ranges. With AKeco $S^2 = 0.78–0.90$ in the ps regime and
0.18–0.49 in the ns regime, and with AKeco*AP5A $S^2 = 0.82–0.95$ in the ps regime and 0.15–0.38 in the ns regime
(Figure 4a). The local motion correlation times $\tau_L$ are shown
in Figure 4b. Note that with models 2 and 4 this parameter
represents the presumed isotropic correlation time $\tau \approx \tau_L$. This assumption appears to be justified by $\tau R_m = (0.3–8.7) \times 10^{-3}$ for AKeco and $(0.4–16.0) \times 10^{-3}$ for
AKeco*AP5A. With models 6 and 8, $\tau_\perp$ represents the parallel
component of the axial local diffusion tensor $R^L$; on the order
of 5 ps for AKeco and 11–31 ps for AKeco*AP5A. The
perpendicular component $\tau_\parallel$ of $R^L$, shown in Figure 4c,
says the range of 5.7–11.3 ns for AKeco and 5.6–9.6 ns (one outlier A11 with $\tau_\parallel = 10.7$ ns) for AKeco*AP5A.

The $R^G$ data appear in Figure 4d. The issue of experimental
confirmation of the best-fit $R^G$ terms was addressed previously
(14). On the basis of $T_{1n}$ experiments (55), the measurement of cross-correlated $^{15}$N CSA–$^{15}$N–$^1$H dipolar
relaxation rates $\eta_{ns}$ (56), numerical simulations and general
considerations (14), and the current AKeco*AP5A data, the
following results were obtained. Within AMPbd and LID
of AKeco $R^G$ lies largely between 2.3 and 4.4 s$^{-1}$ and is
likely to represent genuine conformational exchange (14).
It is reduced mostly below 2 s$^{-1}$ in the LID domain (two
outliers T155 with $R^G = 3.9$ s$^{-1}$, and R156 with $R^G = 6.1$
s$^{-1}$), and largely suppressed in the AMPbd domain upon
inhibitor binding, in accordance with domain motion being
discontinued. For both enzyme forms $R^G$ terms within CORE
below Q160 are likely to represent genuine exchange (14).
The AKeco*AP5A residues associated with outstandingly large $R_{ex}$ terms include I29, D84, T155, and R156.

The best-fit values of the coefficient $c_{20}$, which gauges directly the strength of the coupling potential (eq 1) associated with the order parameter $S_{SRLS}$ (eq 2), are shown in Figure 5a. With AKeco $c_{20} = 9.0-20.5 \, k_B T$ in the ps regime and $2.3-4.7 \, k_B T$ in the ns regime. With AKeco*AP5A $c_{20} = 11.4-40.0 \, k_B T$ in the ps regime, and $1.8-3.8 \, k_B T$ in the ns regime. The angle $\beta_{SD}$ between the local diffusion/ordering axis and the N–H bond assumes values of $14.7-21.0^\circ$ for AKeco and $11.6-20.0^\circ$ for AKeco*AP5A in the ns regime.

Validity Range of the MF Limit. The SRLS spectral density is expected to yield the MF spectral density as $\omega \rightarrow 0$. The range of $\omega$ values wherein SRLS and MF $J(\omega)$ can be considered to be practically identical is determined by the experimental error, $\omega$ and $S$. These ranges can be determined theoretically by simulating $T_1$, $T_2$, and NOE with SRLS and MF. We carried out such calculations using analogous microdynamic parameters, evaluated relative percentage errors, and considered convergence of SRLS $J(\omega)$ to the MF asymptote practically achieved if these data were comparable to the experimental errors. AKeco*AP5A turned out to be an exceptionally rigid molecular complex featuring a large number of residues fit with model 1, where $\omega = 0$, and model 2, where $\omega$ is small. This enabled assessing MF validity on the level of data fitting, besides theoretical simulations. The AKeco*AP5A data were subjected to MF fitting using the program DYNAMICS (57), and corresponding microdynamic parameters were compared for residues where both SRLS and MF selected model 1 or model 2. Relative percentage errors were evaluated, considering convergence of SRLS to MF practically achieved if these data were comparable to the Monte Carlo errors determined by the fitting process. Both simulation and data fitting indicated that SRLS $J(\omega)$ converges to MF $J(\omega)$ for $\omega = 0$. For finite values of $\omega$, we found with simulations that the relative percentage errors in $T_1$, $T_2$, and NOE remain below approximately 2% up to 7 (75) ps for $S^2 = 0.8 (0.95)$, for magnetic fields of...
and tilted out-of-plane at approximately 20° from the N—H bond, which is tilted out-of-plane at approximately 20° from the N—H bond, and the average ZM ordering (currently determined to be approximately $S^2 = 0.3$) higher than XM ordering (32). The rigorous approach is to add the rhombic term in eq 1, and carry out the SRLS fitting for an asymmetric ordering tensor. On the basis of data fitting Y_M lies within the peptide plane perpendicular to Z_M, which is tilted out-of-plane at approximately 20° from the N—H bond, and $\tau_{||} \ll \tau_L$. This is consistent with the N—H orientations motionally averaged by $\tau_{||}$ about Z_M, and the average Z_M axis reorienting about Y_M with correlation time $\tau_L$. The $\tau_L$ mode is on the ns time scale defined by the global diffusion correlation time, $\tau_0$. The angle $\beta_{MD}$ is on the order of 20°, in compliance with Y_M being aligned close to the $C_{i-1}^{\alpha} - C_{i}^{\alpha}$ axis (60). The angle H—N—C$^{\alpha}$ of 113° (ref 61) renders Y_M parallel to the N—C$^{\alpha}$ bond also geometrically feasible. The specific values of the various parameters converge to the physical picture of nanosecond peptide plane motion. With AKeco the conspicuous parameter clustering is suggestive of collective ns motions interpretable as domain motion. AKeco*AP$_3$A does not experience domain mobility and the ns motions may be of a loop-swinging type. Higher accuracy in model characterization will be achieved after implementing asymmetric ordering (14). The latter feature is also expected to have implications to the interpretation of order parameters in terms of fast asymmetric local motions (60).

Comparison between SRLS and MF Analyses. A detailed account of the results obtained with SRLS and MF for the inhibitor-bound enzyme is given in ref 14. The main results obtained for the ps regime CORE domain, and the ns regime AMPbd/LID domains, are summarized below for convenience. In the ps regime, MF $S^2$ is mostly higher than SRLS $S^2$, in excess of the experimental error. In some cases, they are practically the same. The MF $\tau_1$ value ranges between 5 and 50 ps, while its SRLS counterpart ranges mostly between 5 and 131 ps. Analysis of corresponding pairs indicated that MF underestimates local motion correlation times by factors of 3–6. In the ns regime, MF $S^2$ overestimates SRLS $S^2$ on average by 131%, representing the $S^2$ profile quite insensitive to the distinction between ps regime and ns regime dynamics. The MF profiles of $\tau_1$ and $\tau_s$ do single out AMPbd and LID qualitatively, but the quantitative differences are substantial, with MF $\tau_s$ underestimating SRLS $\tau_L$ on average by a factor of 11.

The best-fit parameters obtained with SRLS for the inhibitor-bound enzyme are shown in Figure 6 superimposed on the best-fit parameters obtained with MF. Most residues pertain to the ps regime, where the $S^2$ values obtained with SRLS and MF are comparable, with $<S^2> = 0.9$ (Figure 6).
The average MF $\tau_2$ value is 25 ps, and the average SRLS $\tau_1$ value is 125 ps. Analysis of corresponding pairs indicated that MF underestimates this parameter by factors of $4.5 \rightarrow 9$ (Figure 6b). SRLS identifies loops $\beta_1/\alpha_1$, $\alpha_2/\alpha_3$, $\alpha_5/\beta_4$, and $\beta_3/\alpha_7$ as flexible, by associating representative residues thereof with $\alpha$ regime dynamics. The pertinent parameters were determined as $<S^2> = 0.3$ (Figure 6a) and $<\tau_{1,2} = 7.6$ ns (Figure 6c). MF only identifies loops $\alpha_5/\beta_4$ and $\beta_3/\alpha_7$ as flexible, with $<S^2> = 0.73$ and $<\tau_{1,2} = 0.8$ ns. Thus, for slow local motions, MF overestimates $<S^2>$ by 143% and underestimates $<\tau_{1,2}$ by a factor of 9.5. When SRLS and MF identify $R_\alpha$ terms for the same residue, the magnitudes are mostly comparable (Figure 6d). SRLS identifies several additional $R_\alpha$ terms typically below 2 s$^{-1}$, except for the $R_\alpha$ contributions of residues T155 and R156.

It is concluded that the dynamic picture provided by SRLS is significantly more accurate and discriminating than the dynamic picture provided by MF. The well-defined meaning of the SRLS parameters, notably, the interpretation of $<\tau_{1,2}$ of AKeco as correlation time for domain motion (14), provides new physical insight. MF can mostly determine satisfactorily axial order parameters in the $\alpha$ regime.

**Relation between SRLS Dynamics and the Mechanics of E. coli AKeco Catalysis.** AKeco catalysis takes place through movements of the domains AMPbd and LID. With the ligand-free enzyme, ps regime dynamics is associated with the structural scaffold CORE (21), and ns dynamics with the segmentally mobile domains (23, 24) AMPbd and LID (14). The tightly clustered ns regime parameters concur to a dynamic model where $<\tau_{1,2}$ represents the correlation time for domain motion, found to be on the order of nanoseconds (14). To our knowledge, this is the first direct measurement of domain motion correlation time. This is very significant for understanding kinase catalysis. On the basis of the rationale outlined above for AKeco, the entire backbone of AKeco*AP5A is expected to feature ps regime dynamics. Therefore, the regions of interest are the ns regime loops $\beta_1/\alpha_1$, $\alpha_2/\alpha_3$, $\alpha_5/\beta_4$, and $\beta_3/\alpha_7$ of AKeco*AP5A, identified as flexible by SRLS. These are discussed below specifically.

The dynamic state of loops $\alpha_5/\beta_4$ and $\alpha_5/\beta_4$ can be related to the energetic counter-balancing of substrate binding concept set forth by Müller et al (17). On the basis of crystallographic B-factors, these authors found that in the AKeco-AP5A complex the active site is rigid, whereas loops $\alpha_5/\beta_4$ and $\alpha_5/\beta_4$ are flexible. Conversely, in the ligand-free enzyme the active site is flexible, whereas loops $\alpha_5/\beta_4$ and $\alpha_5/\beta_4$ are rigid. The “solidification” of loops $\alpha_5/\beta_4$ and $\alpha_5/\beta_4$ upon substrate release is hypothesized to serve as a “counterweight” balancing the substrate binding energy. This hypothesis was confirmed by a previous $^{15}$N NMR relaxation study (26). The present SRLS analysis indicates that loops $\alpha_5/\beta_4$ and $\alpha_5/\beta_4$ (except for residue 103) are rigid in AKeco, and representative residues of these loops are intrinsically flexible in AKeco*AP5A. Residues R156 and D158 of loop $\beta_3/\alpha_7$ are involved in binding to the AP5A phosphates 3–5. They form one of the two salt bridges that were found to change the conformation of the hinge connecting LID and CORE in the crystal state (21). The flexibility of these residues in the transition state, of which AKeco*AP5A is a mimic (21), may be related to the initiation of LID movement toward product release. Residue A11 of loop $\beta_3/\alpha_7$ pertains to the P-loop. This residue participates in a dense mesh of hydrogen bonds that connects the inhibitor with the enzyme. Its flexibility may be related to the site where transition state dissociation commences. Finally, the chain segment of AKeco*AP5A featuring the largest number of contiguous ns regime residues is loop $\alpha_5/\alpha_7$. In the ligand free enzyme, this chain segment is part of helix $\alpha_5$ (17). Upon inhibitor binding, it becomes a turn-type structure with hydrogen bonds between residues i + n and i, with n = 3, 4, 5 (21). Its flexibility, possibly of a loop-swinging type, may be related to the onset of AMPbd movement toward product release. This is similar to the role played by loop $\beta_3/\alpha_7$ in the context of LID motion. Finally, it is of interest to point out that all of the AKeco*AP5A loops featuring ns regime dynamics are located at the protein surface.

A comment on the ns regime residues of loops $\alpha_5/\alpha_7$ and $\alpha_5/\alpha_7$ of AKeco*AP5A are in order. All of these residues feature relatively low NOEs (Figure 3c). Yet, originally some were fit with models 2 or 4. As pointed out previously, these are false mimics that can be identified and avoided by selecting appropriate starting points in the fitting process (14). Also, some of these residues were fit with model 5, which we found to feature parameters of lower accuracy (62).

**CONCLUSIONS**

Ligand-free adenylate kinase from E. coli is a relatively large particle diffusing with $\tau_m = 15.1$ ns correlation time. Domain CORE features a rigid structure with rapid small-amplitude N–H bond vector fluctuations. The catalysis-related AMPbd and LID domains are engaged in nanosecond domain motion and substantial microsecond to millisecond conformational exchange. The AP5A-inhibitor bound form is outstandingly rigid and compact ($\tau_m = 11.6$ ns). The majority of its residues feature the conventional rapid rapidly fluctuating structure. The exceptions include loops $\beta_1/\alpha_1$, $\alpha_2/\alpha_3$, $\alpha_5/\beta_4$, and $\beta_3/\alpha_7$, which feature nanosecond long local motions. Loops $\alpha_5/\beta_4$ and $\alpha_5/\beta_4$ are associated with the “energetic counterweight balancing of substrate binding” hypothesis, which is borne out by the SRLS analysis. The other ns regime loops of AKeco*AP5A may be related to the onset of domain motion toward product release. In general, SRLS $^{15}$N relaxation is a powerful tool for elucidating intrinsic flexibility, notably, slow motions, in proteins. It is particularly promising in the context of multidomain enzymes, where domain motion is associated with catalysis.

**ACKNOWLEDGMENT**

E.M. gratefully acknowledges the hospitality of CSIT, FSU, Tallahassee, FL, during short visits promoting this work; we also thank CSIT for computational resources. We thank Dr. David Fushman (University of Maryland, College Park, MD) for sharing with us his program DYNAMICS.

**SUPPORTING INFORMATION AVAILABLE**

$^{15}$N $T_1$, $T_2$, and $^{15}$N-{$^1$H} NOE data of E. coli adenylate kinase acquired at 14.1 and 18.79 T at 303 K (Table S1). $^{15}$N $T_1$, $T_2$, and $^{15}$N-{$^1$H} NOE data of E. coli adenylate kinase in complex with the inhibitor AP5A acquired at 14.1 and 18.79 T at 303 K (Table S2). The results of SRLS fitting based on the combined AKeco data set, including estimated errors of the best-fit parameters (Table S3). The results of
SRLS fitting based on the combined AKece*AP3A data set, including estimated errors of the best-fit parameters (Table S4). The results of MF fitting based on the combined AKece*AP3A data set, including estimated errors of the best-fit parameters (Table S5).

REFERENCES

30. The analogies drawn between SRLS model 5 and reduced extended MF on one hand, and model 6 and extended MF on the other hand, are merely formal. For example, the K = 1 term of model 6 is associated with the local motion K = 2 term of extended MF (see below). Genuine SRLS/MF correspondence can be established in the mode-decoupled limit (R1 ≥ R2) based on a rigorous SRLS theory (29) validated in a perturbational approach for axial ordering (S5 nonzero), and further extended to asymmetric ordering (S5 and S2 = S′2 nonzero) (13). The spectral densities jk = jk + fL + fR (to be called LF below) are given by eqs 10 and B6 of ref 13. The term jR represents free local diffusion (jR = fR = K2(R5 - R1)), K = 0, 1, 2), whereas the sum j = jR + fR represents the SRLS contributions. If the local ordering tensor is assumed to be axially symmetric and the diffusion tilt angle βs is zero, only jR contributions to J(ω/LF). In this case, J(ω/LF) corresponds formally to the original MF formula (4, 5) with (S5)2 corresponding to the squared generalized MF order parameter and t0 to the effective MF local motion correlation time. For anisotropic local motion all of the components of jk contribute to J(ω/LF). If βMD is set equal to 90°, the K = 1 contribution of jR vanishes (the coefficient B in eq 5 of this paper is zero). In this case, J(ω/LF) corresponds formally to the extended MF formula (6) with t0 = r0 = r2, and (S5)2 and (S5)4 recast in terms of S2 and S5.
BI012132Q