Comparison of Backbone Dynamics of *apo*- and *holo*-Acyl-Coenzyme A Binding Protein Using ^{15}N Relaxation Measurements

Christian Rischel,‡ Jens Chr. Madsen,§ Kim Vilbour Andersen, and Flemming M. Poulsen*

Kemisk Afdeling, Carlsberg Laboratorium, Gamle Carlsberg Vej 10, DK-2500 Valby, Copenhagen, Denmark

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ABSTRACT: ^{15}N magnetic relaxation parameters T_1, T_2 and the nuclear Overhauser effect in the protein acyl-coenzyme A binding protein (ACBP) have been measured in the presence and absence of the ligand, palmitoyl-coenzyme A, in order to obtain information about local and global dynamical properties of the peptide backbone with and without the ligand bound in the binding site. The three-dimensional structures of acyl-coenzyme A binding protein are known for both states of the protein as determined from multidimensional heteronuclear NMR studies, and they have been shown to be essentially identical. However, the dynamics of the backbone is influenced by the presence of ligand in the binding site. The binding of ligand had significant and specific effects on the relaxation time T_1 for many of the ^{15}N in the peptide backbone, in particular those near residues with contacts to the ligand. Similarly, the nuclear Overhauser effect at ^{15}N near such residues increased. There were no significant changes in the T_2 relaxation. T_1 values showing a significant decrease and NOEs increasing in regions close to the binding site when the ligand was bound suggest two modes of action on the dynamics of the protein when the ligand is binding. The reduced T_1 indicates motion of lower amplitude in agreement with the structural constraints introduced by protein−ligand interactions. The increased NOEs may be a consequence of shorter time constants for dynamics of the atoms close to the binding site. The Lipari−Szabo model could not be satisfactorily applied to the entire set of experimental data. In particular, many of the measured NOE values exceeded the theoretical maximum predicted in this model.

With the rapid increase in the number of well-determined three-dimensional protein structures, it is becoming ever more important also to understand the physics and the chemistry that relate the geometrical outline of the protein structures and their binding sites with the function and action of the respective protein. Ligand binding and enzymatic processes are often associated with conformational changes in the structure, and these changes require flexibility of the system. Therefore, an interesting aspect of protein function is concerned with the dynamic properties of the atoms in the protein structure and the influence of ligand binding on these properties.

The complex between acyl-coenzyme A binding protein (ACBP)[1] and palmitoyl-coenzyme A seems an ideal system for a study of the relationship between protein flexibility and function. ACBP is a small four-helix bundle protein that binds long-chain acyl-coenzyme A esters. The protein is present in many eukaryotic systems ranging from yeast to mammals, and the amino acid sequence is highly conserved throughout the taxonomic system. The biological role of the protein is not fully understood; however, in relation to the chemical specificity of the protein−ligand interaction it has been suggested that the protein is a carrier protein or a support for pool formation of ligands. Other studies have suggested that the protein is a precursor for the formation of a neurotransmitter that it is involved in regulation of steroidogenesis and in the regulation of insulin release, respectively. For a review, see Knudsen et al. (1993).

The three-dimensional structures of both *apo*- and *holo*-ACBP have been determined by ^{1}H, ^{13}C, and ^{15}N NMR-spectroscopy (Andersen et al., 1991; Andersen & Poulsen, 1992, 1993; Kragelund et al., 1993). The four-helix protein binds the ligand palmitoyl-coenzyme A in a rather intricate way that facilitates a fully protected hydrophobic binding site for the palmitoyl part and binding of polar parts of the ligand to establish a hydrophilic surface of the protein−ligand complex. The structure of the protein is essentially not changed upon ligand binding, but a substantial number of ^{1}H and ^{15}N atoms have their chemical shifts significantly changed (Kragelund et al., 1993), showing that ligand binding has an impact on the protein not reflected in the structure. We have measured ^{15}N T_1 and T_2 relaxation times and ^{1}H−^{15}N NOEs of both systems in order to investigate the influence of ligand binding on the peptide backbone dynamics.

The investigation of protein backbone dynamics by ^{15}N relaxation measurements has recently been applied to several protein−ligand complexes. Akke et al. (1993) were able to demonstrate that binding of a calcium ion to calbindin reduced the mobility of the neighboring amino acid residues.

* To whom correspondence should be addressed.

‡ Also at the Niels Bohr Institute, Universitetsparken 5, DK-2100 Copenhagen East, Denmark.

§ Present address: Department of Molecular Biology, The Scripps Research Institute, 10666 N. Torrey Pines Road, La Jolla, CA 92037.

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[1] Abbreviations: ACBP, acyl-coenzyme A binding protein; HSQC, heteronuclear single-quantum correlation; NMR, nuclear magnetic resonance; CPMG, Carr−Purcell−Meiboom−Gill; NOE, nuclear Overhauser effect; ppm, parts per million; rf, radio frequency; TPPI, time-proportional phase incrementation; *apo*-ACBP, ACBP without ligand; *holo*-ACBP, ACBP with ligand. [The *apo* and *holo* prefixes are conventionally used to designate a protein without or with a functional group. Although it is not clear if this description applies to the ACBP−ligand system, we have followed previous publications (Kragelund et al., 1993; Knudsen et al., 1993) and used the convenient designations].

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However, a study of a phosphopeptide binding to the C-terminal SH2 domain of phospholipase $C_\gamma 1$ showed no definite changes (Farrow et al., 1994). Cheng et al. (1994) observed a drastic increase in signal intensities from a loop in FKBP-12 upon binding of the ligand FK506, accompanied by less flexibility in this loop region.

It is the aim of the present study to examine the influence of ligand binding to ACBP on the dynamics of the peptide backbone in order to gather information about the relevance of these properties for protein function and specificity.

METHODS

Experimental. Palmitoyl-coenzyme A and uniformly ^{15}N-labeled ACBP were prepared as described by Kragelund et al. (1993).

All experiments were performed at 14.1 T on a Bruker AMX-600 spectrometer equipped with a triple resonance 5 mm probe. The data were recorded by HSQC-based pulse sequences (Bodenhausen & Ruben, 1980) using the States–TPPI scheme (Marion et al., 1989) to obtain pure line shapes and quadrature detection in t_1. Several different pulse sequences have been proposed to measure T_1 and T_2. Here we have used the pulse sequences suggested by Kay et al. (1992). T_1 and T_2 relaxation times were measured for *apo*- as well as for *holo*-ACBP using relaxation delays of 50, 250, 450, 600, 800, 1100, and 1500 ms for the T_1 experiments and periods of 8, 24, 48, 64, 96, 128, 168, 240, and 320 ms for the T_2 experiments. The CPMG spin-echo period was 1.0 ms. For *apo*-ACBP, T_2 was also measured using a shorter CPMG spin–echo period of 0.2 ms to check for exchange contributions to T_2 (Orekhov et al., 1994). Each spectrum consisted of 1024 by 192 complex points with 16 scans per t_1 value for the T_2 experiments with spin–echo period 0.2 ms and 32 scans per t_1 value for the other spectra. The H_2O signal was suppressed by a 900 ms presaturation pulse.

$^{15}N-^1H$ nuclear Overhauser effects were measured using the pulse sequence described by Kay et al. (1989) with and without the 3 s ^1H saturation, which consists of a train of high-power 120° pulses spaced by 20 ms. The spectra consisted of 1024 by 192 acquired complex points, with 16 scans per t_1 value. Water suppression was achieved by a presaturation pulse with a total duration of 100 ms applied during the last five 20 ms delays of the ^1H saturation pulse train.

For all spectra, the ^1H spectral width was 14.9 ppm and the ^{15}N spectral width was 32.9 ppm. The ^{15}N carrier was positioned at 114.8 ppm relative to the nitrogen resonance of NH_3. The concentration of both samples was 1.6 mM in 600 μL. Both samples had a pH of 6.3 and spectra were recorded at a temperature of 298 K.

Data Analysis. The NMR data were zero-filled to double length and Fourier transformed using the program MNMR (Pronto Software Development and Distribution, Copenhagen, Denmark). ^{15}N and $^1H^N$ resonance assignments have previously been published (Andersen & Poulsen, 1993; Kragelund et al. 1993). The spectra were analyzed using the program Pronto (Pronto Software Development and Distribution, Copenhagen, Denmark), and cross-peak integration was performed by fitting the data to a two-dimensional Lorentzian line shape.

The relaxation times were found by fitting the peak integrals to an exponential decay as a function of the delay time using a nonlinear fitting procedure (Press et al., 1988), assuming the same noise level at all delay times. Uncertainties of the relaxation times were computed from the fit, using the deviations from the fit as an estimate of the noise level (Press et al., 1988).

The NOE for each $^{15}N-^1H^N$ was calculated by dividing the integral of the peak in the spectrum with ^1H saturation by the integral of the peak in the spectrum without ^1H saturation. All NOE experiments were performed three times to obtain an estimate of the uncertainty.

Several of the experiments have been repeated with two different freshly prepared samples. This is because we have previously experienced problems with sample decay of the ACBP–ligand complex during measurements. The variation between samples was less than 3% for most of the measured relaxation values.

Relaxation Theory. The relaxation of the ^{15}N nuclei is primarily caused by dipolar coupling with the H^N proton, chemical shift anisotropy, and chemical exchange processes. The NOE and T_1 are affected by the first two, whereas T_2 may also be affected by exchange. Due to the anisotropic nature of the dipolar coupling and the chemical shift, motions of the $^{15}N-^1H^N$ vector affect the relaxation parameters. Considering only these two relaxation mechanisms, the relaxation parameters can be expressed using the values of the spectral density function J evaluated at the frequencies 0, ω_N, $\omega_H + \omega_N$, ω_H, and $\omega_H - \omega_N$ (Abragam, 1961):

$$\frac{1}{T_1} = \frac{d^2}{4}[3J(\omega_N) + J(\omega_H - \omega_N) + 6J(\omega_H + \omega_N)] + c^2 J(\omega_N) \quad (1)$$

$$\frac{1}{T_2} = \frac{d^2}{8}[4J(0) + 3J(\omega_N) + J(\omega_H - \omega_N) + 6J(\omega_H) + 6J(\omega_H + \omega_N)] + \frac{c^2}{6}[4J(0) + 3J(\omega_N)] \quad (2)$$

$$NOE = 1 + \frac{\gamma_H}{\gamma_N} T_1 \frac{d^2}{4}[6J(\omega_H + \omega_N) - J(\omega_H - \omega_N)] \quad (3)$$

in which $d^2 = (\mu_0 h/8\pi^2)^2 \gamma_H^2 \gamma_N^2 (1/r_{HN}^3)^2$ and $c^2 = \omega_N^2(\sigma_\parallel - \sigma_\perp)^2/3$. ω_H and ω_N are the Larmor frequencies of the ^1H and ^{15}N nuclei, μ_0 is the permeability of free space, h is Planck's constant, γ_H and γ_N are the gyromagnetic ratios of the H and N nuclei, r_{HN} is the internuclear $^1H-^{15}N$ distance, and $\sigma_\parallel$ and $\sigma_\perp$ are the parallel and perpendicular components of the ^{15}N chemical shift tensor.

A qualitative impression of the relation between the dynamics of the $^1H-^{15}N$ vector and the relaxation parameters can be obtained directly from the expressions (1–3) above. $J(\omega)$ is twice the cosine-transform of the correlation function $C(t) = {}^1/_5\langle P_2(\mu_{HN}(0)\cdot\mu_{HN}(t))\rangle$, where P_2 is the second Legendre polynomial and μ_{HN} is a unit vector in the direction of the $^1H-^{15}N$ vector (Lipari & Szabo, 1982). Thus, $J(\omega)$ is mainly sensitive to the behavior of $C(t)$ on time scales of order $1/\omega$. A measurement of J at all five frequencies for a 70 residue protein (Peng & Wagner, 1992) showed that $J(0)$ and $J(\omega_N)$ are considerably larger than $J(\omega_H + \omega_N)$, $J(\omega_H)$, and $J(\omega_H - \omega_N)$. This means that for proteins of this size,

T_1 and T_2 are mainly determined by $J(0)$ and $J(\omega_N)$, whereas the NOE is also sensitive to the values of $J(\omega_H + \omega_N)$ and $J(\omega_H - \omega_N)$. Since $\omega_H \approx 10\omega_N$, we therefore qualitatively expect T_1 and T_2 to be most sensitive to motions of time scales around $1/\omega_N$ and the NOE to be sensitive to motions on the faster time scales around $1/\omega_H$. However, $C(t)$ is subject to the constraint $C(0) = {}^1/_5$. If $C(t)$ is monotonically decreasing, this means that an initial rapid drop in $C(t)$ due to fast fluctuations of high amplitude must also be reflected in a lower value of $C(t)$ at longer times. In this way, the *amplitude* of fluctuations much faster than $1/\omega_N$ will also affect T_1 and T_2, as a higher amplitude gives lower values of $J(0)$ and $J(\omega_N)$ and hence longer values of T_1 and T_2.

Rather than performing all the measurements necessary to determine the spectral density at the five frequencies, one may describe J by a formula with a reduced number of parameters and determine these parameters from measurements of T_1, T_2 and NOE. Originally Bloembergen et al. (1948) proposed J to be

$$J(\omega) = \frac{2}{5}\frac{\tau_c}{1 + \omega^2\tau_c^2} \tag{4}$$

with a single free parameter, the correlation time τ_c. However, this gives an unsatisfactory description of the data in protein NMR experiments, and the more elaborate form

$$J(\omega) = \frac{2}{5}\left(\frac{S^2\tau_c}{1 + \omega^2\tau_c^2} + \frac{(1 - S^2)\tau}{1 + \omega^2\tau^2}\right) \tag{5}$$

was proposed by Lipari and Szabo (1982). This so-called model-free approach describes the dynamics of the $^{15}N-{}^1H^N$ vector by an overall tumbling of the protein with correlation time τ_c and a faster, restricted (local) motion characterized by a correlation time τ and an order parameter S^2. For a $^{15}N-{}^1H^N$ vector completely fixed within the protein, $S^2 = 1$; for a completely free vector, $S^2 = 0$. Within this model, Kay et al. (1989) showed that the NOE is much more sensitive to the value of the local correlation time τ than T_1 and T_2, in agreement with the qualitative result found by considering the fundamental expressions (1−3) above.

To account for data that could not be described by the Lipari−Szabo model, Clore et al. (1990) suggested that two time scales of local motion were present and proposed the following form of J:

$$J(\omega) = \frac{2}{5}\left(\frac{S^2\tau_c}{1 + \omega^2\tau_c^2} + \frac{(S_f^2 - S^2)\tau}{1 + \omega^2\tau^2}\right) \tag{6}$$

where the order parameters of the fast and slow local motions are called S_f^2 and S_s^2, S^2 is defined as $S_f^2S_s^2$, and τ is the correlation time of the slow local motion. The time scale of the fast local motion is assumed to be too fast to be detected.

For the spectral density functions of Lipari and Szabo (1982) and of Clore et al. (1990) the overall correlation time τ_c is the same for all residues. For $\tau = 0$ the second term in both forms of J vanishes, and the ratio T_1/T_2 only depends on τ_c. The determination of τ_c using the average of this ratio and the simplified model $\tau = 0$ (Kay et al., 1989) is often applied.

T_2 values much lower than the expected value cannot be explained by dipolar relaxation or chemical shift anisotropy mechanisms, and they are usually ascribed to exchange broadening, which is caused by exchange of the system between two or more states with different ^{15}N chemical shifts (Ernst et al., 1987). Without information about the chemical shift difference, the exchange contribution is formally expressed by adding a free constant R_{ex} to the transverse relaxation rate

$$\frac{1}{T_2^{exp}} = \frac{1}{T_2^{theo}} + R_{ex} \tag{7}$$

where T_2^{theo} is the value predicted by the theory outlined above.

Overall tumbling rates and individual S^2 and τ for each atom as well as standard deviations of these quantities were calculated using a Monte Carlo procedure (Press et al., 1988; Palmer et al., 1991). The first step in the procedure is the generation of a number of data sets by adding random errors to the measured parameters. The errors are normally distributed with the measured standard deviation. We used 500 such data sets for each calculation. For each set the optimal parameters in the model is found by minimization of the sum of the squared deviations between the experimental values (including random error) and the values predicted by the model. The deviations are weighted by the experimental standard deviation, and minimization is performed by the Levenberg−Marquardt algorithm (Press et al., 1988). From the sets of optimized model parameters, an average and a standard deviation for each parameter can then be derived. We have calculated overall tumbling rates using the average values of the ratio T_1/T_2 for each of the backbone nitrogens. Points that deviated more than twice the standard deviation from the average were excluded by an iterative procedure. For each atom, S^2 and τ from the Lipari−Szabo model with the calculated (fixed) τ_c were then calculated.

RESULTS AND DISCUSSION

The measured ^{15}N relaxation parameters T_1 and T_2 as well as $^{15}N-{}^1H$ NOE have been measured for *apo*- and *holo*-ACBP, respectively, and are shown in Figures 1 and 2. For the NOEs we note that there is noticeable minimum of the NOEs for residues in the C-terminal residues of helices A1, A2, and A4 in both the *apo*- and the *holo*-forms of the protein. Similarly for the T_1s there is a noticeable and consistent increase in the measured values for the residues in the C-terminal of the helices in both systems combined with a clear trend that residues in the centers of the helices have smaller values of T_1 with sequence dependent minima. In the loop between residues 40 and 49 there is a well-defined minimum in the NOEs for both systems. For the measured T_2s in both forms there are no persistent sequence-dependent variation. The local minima for the T_2s seen at residues Ile27 and His30 are clearly residue and sequence position dependent variations.

The dynamic properties that are fundamental for the relaxation and NOE parameters can be described by a set of new parameters that are more directly related to the description of motion. Typically the parametrization according to Lipari and Szabo (1982) and extensions of this model (Clore

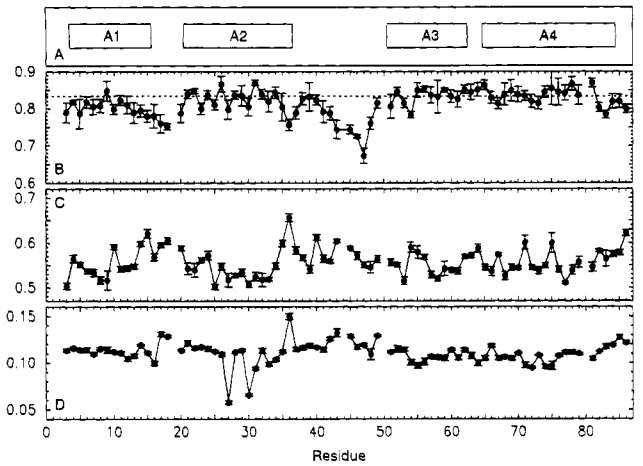

FIGURE 1: Measured ^{15}N relaxation parameters of ACBP. (A) α-helices of the solution structure (Andersen & Poulsen, 1993). (B) $^{1}H-^{15}N$ NOEs. The dashed line shows the theoretical maximum 0.834 of the Lipari−Szabo model. (C) T_1. (D) T_2. The ordinate axes in panels C and D are in units of seconds. For the *apo*-protein, the cross peaks from Ser1 and Gln2 have not been assigned, and the cross peaks from Lys50 and Leu80 could not be unambiguously assigned and measured due to overlaps.

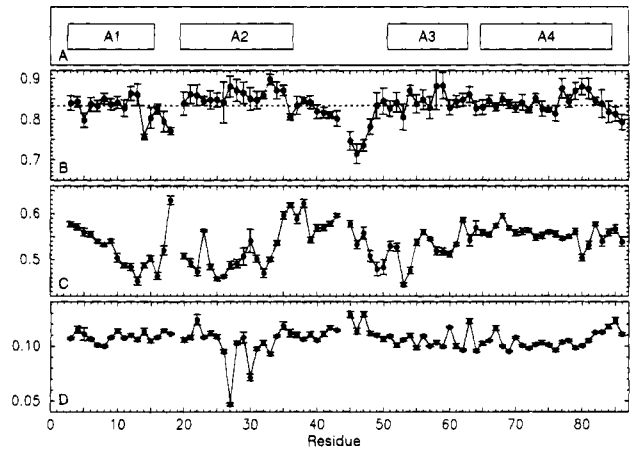

FIGURE 2: Measured ^{15}N relaxation parameters of the complex between ACBP and palmitoyl coenzyme A. (A) α-Helices of the solution structure (Kragelund et al., 1993). (B) $^{1}H-^{15}N$ NOEs. The dashed line shows the theoretical maximum 0.834 of the Lipari−Szabo model. (C) T_1. (D) T_2. The ordinate axes in panels C and D are in units of seconds. For the *holo*-protein, the cross peaks from Ser1 and Gln2 have not been assigned.

et al., 1990) have been widely employed. From the averages of the T_1/T_2 ratios, overall tumbling times of 6.26 ± 0.06 ns for *apo*-ACBP and 6.35 ± 0.08 ns for *holo*-ACBP can be calculated. Subsequent analysis of the relaxation and NOE data shows, however, that these parametrization models are not appropriate for analysis of the data. First, this is because the NOEs measured for a considerable number of residues are larger than the maximum value of 0.834 allowed in both parametrization schemes. Second, for many residues the measured T_1 and T_2 values are not concurrent, suggesting either that there is an additional exchange contribution to the measured T_2 relaxation or that the spectral density function is more complex than anticipated in the Lipari−Szabo model.

The use of the various parametrizations (4−6) of the spectral density functions described above provides convenient ways of rationalizing the relaxation data. However, they also imply additional restraints on the possible values of the relaxation parameters, constraints that do not follow from the fundamental formulas (1−3). One such constraint common to the mentioned forms of J is that the theoretical maximum value for the NOE is 0.834 at 60 MHz ^{15}N frequency, obtained with $S_2 = 1$, $\tau = 0$, and $\tau_c \gg 1/\omega_N$. This value is shown with dashed lines in the NOE plots in Figures 1 and 2. It is clear that both systems have many ^{15}N nuclei with NOE values significantly higher than the theoretical maximum 0.834. With τ_c fixed at 6.3 ns in the Lipari−Szabo model the even more restrictive maximum value of 0.807 is obtained. For *apo*-ACBP, 51 atoms have NOEs higher than 0.807 and 30 of those have NOEs higher than 0.834. For *holo*-ACBP, the numbers are 69 and 49, respectively.

One explanation of the high NOE values could be an experimental imperfection, e.g., insufficient proton saturation. However, we have performed the same experiment on other proteins, obtaining very few values violating the maximum (K. V. Andersen et al., unpublished results). It has been reported that fast exchange of the amide protons with water protons saturated by gated decoupling can give rise to artifacts in measured NOE values (Smith et al., 1987; Kay et al., 1989). However, in the present case the large NOEs were not associated with the regions of rapid hydrogen exchange (B. B. Kragelund, J. Knudsen, and F. M. Poulsen, unpublished results) which makes this explanation improbable.

The NOE values measured in other studies show some variation, although most experiments give values well below the theoretical maximum. The measurements of Redfield et al. (1992) on human interleukin-4 provide an example of average NOE values close to those of the present work (when the different spectrometer frequencies are taken into account). Here we have chosen the approach of Kay et al. (1989) to use the NOE values only in a qualitative way. As discussed above the NOE is the parameter most sensitive to the fast local motions, with a low value of the NOE indicating slow local motion (a high value of τ).

Excluding the NOE values, we have for each atom determined the two parameters S^2 and τ of the Lipari−Szabo model by fitting to the two experimental parameters T_1 and T_2. The analysis reveals, however, that the T_1 and T_2 data are incompatible with this model, as values of S^2 larger than 1 are obtained for many residues. This incompatibility may arise from too short experimental T_2 values due to exchange contributions of a few inverse seconds to the measured T_2. This effect has been observed in many proteins. Orekhov et al. (1994) have shown that using a shorter CPMG spin−echo period can reduce the exchange contribution to the measured T_2 if the exchange is not faster than the spin-echo period. T_2 measurements for *apo*-ACBP with a CPMG spin−echo period of 0.2 ms showed only an effect on the two residues Ile27 and His30 (the data are plotted in the supplementary material). For the rest of the residues this result shows that exchange contributions to T_2 on a time scale slower than 1 ms are not important. Exchange processes on a faster time scale may still be present, though.

Clore et al. (1990) proposed that a slower and a faster motion both in the subnanosecond range may be present in cases where the Lipari−Szabo formalism fails. However, the application of this parametrization was not possible for ACBP, neither free nor with ligand bound, with the NOEs being too large to enter the analysis, as T_1 and T_2 and the

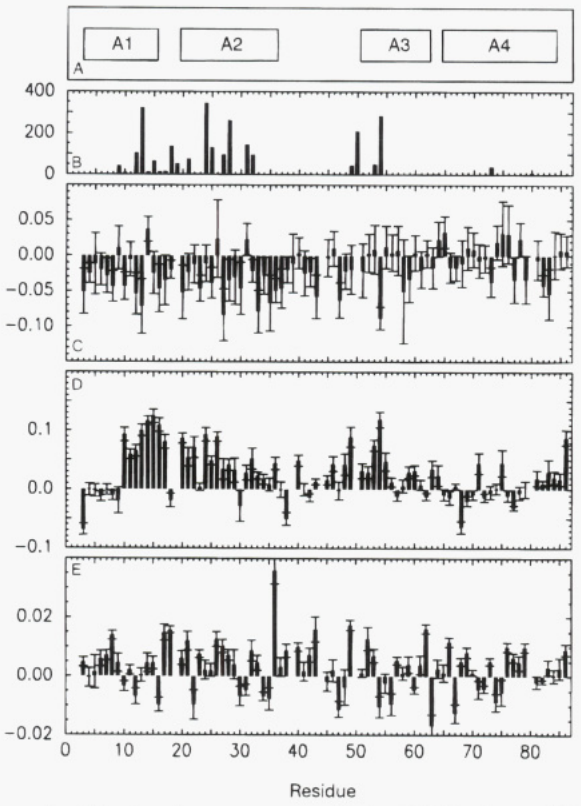

FIGURE 3: ^{15}N relaxation parameters of *holo*-ACBP subtracted from those of *apo*-ACBP. (A) α-helices of ACBP (Andersen & Poulsen, 1993). (B) Number of protein−ligand atom pairs with a distance of less than 5 Å counted for each residue in the protein. (C) $^{1}H-^{15}N$ NOE difference. (D) T_1 difference. (E) T_2 difference. The ordinate axes in panels D and E are in seconds.

NOEs are all three required to perform a calculation of the two order parameters and one correlation time. Furthermore, this model predicts higher values of T_1 and T_2 at a particular value of τ_c, rather than the low values of these parameters we observe for ACBP.

The ACBP system can clearly not be analyzed by the common types of parametrizations. However, a qualitative interpretation of the relaxation and NOE measurements can still be inferred directly from the fundamental forms (1−3).

The relaxation parameters undergo rather different changes upon ligand binding. The T_1 values undergo a general decrease in positions clearly correlated with the positions in which the ligand binds, but in agreement with the observations above the changes in T_2 values seem virtually uncorrelated with ligand binding and secondary structure. The NOE values generally become higher in the places where the ligand is bound, whereas there seem to be no systematic difference in the rest of the protein.

Figure 4 shows a stereo rendering of the ACBP−palmitoyl coenzyme A complex, with the ^{15}N atoms for which a T_1 difference has been measured shown as solid spheres. The bluish atoms are those that show a marked decrease in T_1 upon ligand binding, indicating a substantially lower local flexibility. It is seen that these atoms are predominantly those close to the binding site, as can also be seen in Figure 3. It is interesting to notice, that whereas Akke et al. (1993) and Cheng et al. (1994) only observed increased rigidity in the loops adjacent to the ligand, our data show that the dynamics of parts of α-helices can also be significantly altered by ligand binding. This can be seen particularly clearly in helix 1. Residues 4−9 experience no change of T_1 within the experimental error, but residues 10−16 all show a substantial decrease in T_1. Such a decrease is also seen in the beginning of helix 3, which is close to the fatty acid chain of the ligand, but not in the rest of the helix. These data indicate that an α-helix may well be considered as a rather flexible structural feature, since the fixing of one end does not affect the dynamics in the other end.

The changes in NOE values shown in Figure 3 have a larger uncertainty than the changes in T_1, and therefore the correlations between NOE changes and ligand binding are less clear. However, there is a marked tendency that helices 1 and 2 and the beginning of the long middle loop show an increase in NOE. The most significant change in the rest of the protein is the increase for Lys54 that has many contacts to the ligand, and apart from this little or no systematic pattern is seen. According to the qualitative interpretation

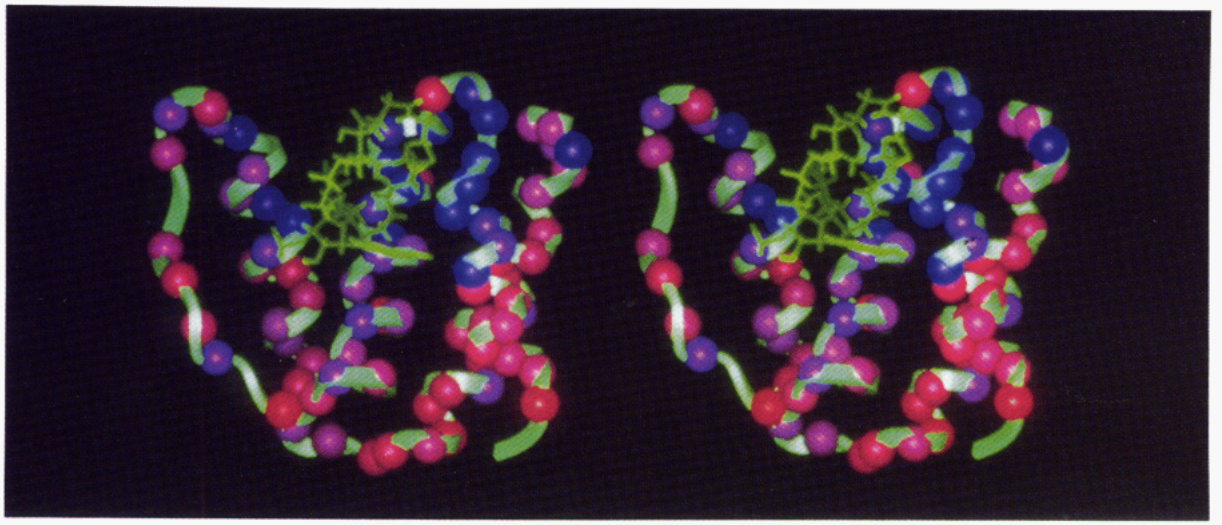

FIGURE 4: Difference in T_1 between *apo*- and *holo*-ACBP. The stereo drawing shows the lowest energy structure of Kragelund et al. (1993) with the ^{15}N nuclei for which a T_1 difference has been measured rendered as large spheres. The spheres have been colored according to the change in T_1 shown in Figure 3D, with continous change in color from red ($\Delta T_1 = -0.05$ s) to blue ($\Delta T_1 = 0.10$). The ligand is drawn with thick lines.

outlined above, the change toward a higher NOE indicates that the local dynamics occur on a faster time scale.

CONCLUSION

The measurement of ^{15}N relaxation parameters for *apo*- and *holo*-ACBP reveals that it is not always possible to rationalize such data in terms of the Lipari−Szabo model (Lipari & Szabo, 1982) or the extension of Clore et al. (1990), since these models impose new constraints on the theoretically allowed values of the parameters. A *qualitative* interpretation of the measurements can be inferred from the fundamental expressions, though.

The difference between the two systems turns up very clearly in the comparison of measured T_1 values, whereas the T_2 values are insensitive to the changes. The changes in NOE values are clear but less specific than the changes in T_1, possibly due to the larger uncertainty of the NOE changes. The difference in T_1 is strongly correlated with ligand binding, with residues close to the ligand experiencing a significant drop in T_1 upon ligand binding, and a simultaneous raise in NOE. These changes indicate fluctuations of lower amplitude and of shorter correlation time, thus demonstrating a decrease in flexibility of the residues in the binding site upon ligand binding. These findings are in good agreement with previous studies (Akke et al., 1993; Cheng et al., 1994), although the present data show changes in a much larger number of residues. Remarkably, the changes display little correlation with the secondary structure of ACBP.

A few residues have relaxation parameters that significantly deviate from the average. In *apo*- as well as in *holo*-ACBP, Ile27 and His30 have large exchange contributions to T_2 arising from motions on the millisecond to second time scale. Both systems have a few ^{15}N nuclei with values of T_1 and/or T_2 much higher than the average, which indicates a large amplitude of local fluctuations. At present, a satisfactory explanation of these large, strictly local variations cannot be given.

ACKNOWLEDGMENT

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SUPPLEMENTARY MATERIAL AVAILABLE

Figure S1 showing T_2 measurements for *apo*-ACBP with CPMG spin−echo periods of 0.2 and 1.0 ms (1 page). Ordering information is given on any current masthead page.

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