Analysis of the Backbone Dynamics of Interleukin-8 by $^{15}$N Relaxation Measurements

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The backbone dynamics of the cytokine interleukin-8, a symmetric homodimer of overall molecular mass 16 kDa, has been investigated at pH 5.2 by means of $^{15}$N relaxation measurements using heteronuclear two-dimensional inverse detected $^{1}H$-$^{15}$N spectroscopy. $^{15}$N T1, T2 and NOE data were obtained for 66 out of a total of 67 backbone amide groups. The overall correlation time is 9.10 (± 0.05) ns at 26°C. All residues exhibit very rapid motions on a time-scale of ≤20 ps. These very rapid motions alone can account for the $^{15}$N relaxation behaviour of 30 residues. The $^{15}$N relaxation data for another 21 residues can only be accounted for by the inclusion of an additional internal motion on a time-scale ranging from 0.5 to 3.5 ns. These residues are clustered at the N and C terminals, and in the loop regions connecting elements of secondary structures. Finally, the $^{15}$N relaxation data for another 15 residues could only be accounted for by the presence of chemical exchange on a time-scale ranging from ~170 ns to ~235 ns. In addition, the inclusion of chemical exchange improved the fit to the experimental data for 10 of the 30 residues whose $^{15}$N relaxation behaviour could be accounted for by very fast motions alone. The residues exhibiting chemical exchange line broadening cluster at the interface of the long C-terminal α-helix and the underlying β-sheet. It is suggested that this clustering is indicative of concerted rather than independent motions in regions of secondary structure, with motion at any one residue being propagated to neighbouring residues in van der Waals contact.

Keywords: interleukin-8; protein dynamics; heteronuclear relaxation

Protein mobility plays a key role in protein-ligand interactions and protein-protein recognition (Ringe & Petsko, 1985). One method of studying protein motion involves the analysis of nuclear magnetic resonance (NMR) relaxation processes (Lipari & Szabo, 1983). With the advent of inverse detection methods, it has now become possible to analyse the backbone dynamics of proteins on a residue by residue basis by means of $^{15}$N relaxation measurements (Kay et al., 1989; Clore et al., 1990a,b; Barbato et al., 1992; Kördel et al., 1992; Powers et al., 1992; Stone et al., 1992). In this communication we present an analysis of the backbone dynamics of the cytokine interleukin-8 (IL-8), a protein whose structure we have previously solved by both NMR and X-ray crystallography (Clore et al., 1990a,b; Baldwin et al., 1991; Clore & Gronenborn, 1991a,b, 1992).

IL-8 is a 16 kDa homodimer, each subunit comprising 72 residues, released from several cell types in response to an inflammatory stimulus and is associated with the selective capacity to attract neutrophils, basophils and T cells (Matsuushima & Oppenheim, 1988). The overall structure of IL-8 consists of two anti-parallel α-helices, separated by about 15 Å (1 Å = 0.1 nm), lying on top of a six-stranded anti-parallel β-sheet, with a topology (Clore et al., 1990a,b) reminiscent of that of the α1/α2 domains of the HLA class I histocompatibility antigen (Bjorkman et al., 1987). Recent studies employing synthetic analogues (Clark-Lewis et al., 1991), mutagenesis (Hebert et al., 1991) and molecular modeling (Clore & Gronenborn, 1991a,b, 1992; Gronenborn & Clore, 1991) have suggested that the N terminus, the loop from residues 31 to 36 and possibly the cleft between the two helices are involved in receptor binding. The analysis of the dynamic behaviour of free IL-8 may therefore yield insights into understanding its binding to and activation of its cell surface receptors. In addition, IL-8 provides the first example of a $^{15}$N relaxation study on a dimer.
Figure 1. A 600 MHz $^{15}$N-$^1$H NOE correlation spectrum of IL-8. The negative NOE peaks for Lys 3 and Glu 4 are shown as filled circles. The NOE peaks for Ala 2 and Leu 5 are near zero and hence cannot be observed at the contour level plotted; their location, however, is indicated. Recombinant human IL-8 was expressed in Escherichia coli as described (Furuta et al., 1989). Uniform ($>95\%$) $^{15}$N labelling was achieved by growing the bacteria in minimal medium containing $^{15}$NH$_4$Cl as the sole nitrogen source. The protein was purified by inclusion bodies as follows: after breaking the cells, the lysate was centrifuged and the pellet was washed with 1% (v/v) Triton X100, 0.75 M-urea and resuspended in 6 M-guanidine. IL-8 was refolded by sequential dialysis against 50 vol. of 3 M-urea, 2 M-guanidine, and finally 20 mM NaHPO$_4$ (pH 6.5). The precipitates were collected and redissolved in guanidine for greater recoveries. The refolded protein was purified on a Mono-S FPLC (Pharmacia) column, followed by high pressure liquid chromatography on a C4 column. The sample for NMR contained ~15 mg-IL-8 in 90% H$_2$O/10% $^2$H$_2$O (pH 5.2). All NMR data were collected at 268°C on a Bruker AM800 spectrometer operating in reverse mode. The $^{15}$N assignments were made on the basis of the previously assigned $^1$H resonances (Clore et al., 1989) and three-dimensional $^{15}$N-separated Hartmann-Hahn (Marion et al., 1989) and ROESY (Clore et al., 1990) spectra. The $^{15}$N relaxation data were measured using pulse sequences described previously (Kay et al., 1989a; Clore et al., 1990a) appropriately modified in the case of the $^{15}$N T$_1$ and T$_2$ experiments to eliminate cross-relaxation between dipolar and chemical shift anisotropy relaxation mechanisms (Boyd et al., 1990; Kay et al., 1992; Palmer et al., 1992). The $^{15}$N T$_1$ and T$_2$ experiments were recorded with a sweep width of 5000 Hz in the $^1$H F$_2$ dimension, with the carrier set to the centre of the amide NH region and the water resonance on the right-hand edge of the spectrum, using low power DANCE-style off resonance irradiation (Kay et al., 1989b) to suppress the water resonance. The $^{15}$N-$^1$H NOE experiments were recorded with a sweep width of 10,000 Hz in the $^1$H F$_2$ dimension using conventional low power irradiation to suppress the water resonance located in the centre of the spectrum. A total of 256 $t_1$ increments, each of 1024 data points for the $^{15}$N T$_1$ and T$_2$ experiments, and 2048 data points for the $^1$H-$^{15}$N NOE experiments, were recorded, giving a total acquisition time in $t_1$ of 404 ms and a spectral width of 1582 Hz in the $^{15}$N F$_2$ dimension. 32 transients/insertion were recorded for the $^{15}$N T$_1$ and T$_2$ experiments and 160 transients/insertion for the $^1$H-$^{15}$N NOE experiments. The recycle time was 1.2 s for the $^{15}$N T$_1$ and T$_2$ experiments and 5 s for the $^1$H-$^{15}$N NOE experiments. $^{15}$N T$_2$ data were obtained for 9 $t$ delays of 12.1, 29.2, 56.3, 84.8, 165.6, 327.2, 529.2, 731.2 and 1054.3 ms, and $^{15}$N T$_1$ data for 7 $T$ delays of 144, 287, 575, 1149, 1724, 2299 and 2373 ms. All NMR data were processed using NMR2 software (New Methods Research, Syracuse, NY, U.S.A.) and analysed with the programs PPPP and CAPP (Garrett et al., 1991) running on a Sun Sparc workstation. For the F$_2$ dimension, a linear baseline correction was applied for the $^{15}$N T$_1$ and T$_2$ data and a polynomial baseline correction of order 5 for the NOE data. The decays of cross-peak intensities with time in the $^{15}$N T$_1$ and T$_2$ experiments is exponential within experimental error, and the decays were fit to a single exponential by non-linear least-squares Powell minimization using the program IACSIMILE (Chance et al., 1979; Clore, 1983). The errors in the T$_1$ and T$_2$ values were obtained from conventional analysis of the variance-covariance matrix generated by the non-linear least-squares optimization routine (Chance et al., 1979; Clore, 1983). Out of the 66 amide groups for which T$_1$ and T$_2$ values were obtained, 63 T$_1$ values had errors $\leq 5\%$, 2 T$_1$ values (Thr12 and Ile40) had errors between 5 to 10\%, and 1 T$_1$ value (Ser14) had an error of 13\%; 54 T$_2$ values had an error of $\leq 5\%$, 11 T$_2$ values (Ala2, Tyr13, Ser14, Lys15, His18, Val27, Gly31, Cys34, Glu38, Ile39 and Ile40) had errors of 5 to 10\%, and 1 T$_2$ value had an error of $\sim 12\%$ (Ser30). The error in the experimental measurement of the $^{15}$N-$^1$H NOE was of the order of 2-6\%. It should be noted that the errors also include systematic error in the measurement of the $^{15}$N-$^1$H NOE arising from chemical exchange with water; for a $T_2$ of $\sim 2.8$ s for water and a recycle
The $^{15}$N relaxation data were measured using pulse sequences described previously (Kay et al., 1980a; Clore et al., 1990a) taking care to eliminate cross-correlation between dipolar and chemical shift anisotropy relaxation mechanisms (Boyd et al., 1989; Kay et al., 1982; Palmer et al., 1982). $^{15}$N T$_1$ and T$_2$ data were obtained for all backbone amide nitrogen atoms with the exception of Gin20 (for which the cross-peak was too weak to obtain reliable relaxation times), and $^{13}$N NOE data were obtained for all backbone amide groups. The $^{13}$H-$^{15}$N NOE correlation spectrum is shown in Figure 1, and the $^{15}$N T$_1$, T$_2$ and NOE values are plotted as a function of residue number in Figure 2A to C.

The overall rotational correlation time, $\tau_\phi$, was calculated from the T$_1$/T$_2$ ratios, excluding those residues with a T$_1$/T$_2$ ratio outside one standard deviation of the mean, as described by Clore et al. (1990a). The mean T$_1$/T$_2$ ratio for the amide nitrogen atoms of IL-8 was $8.52(\pm 1.62)$ with a maximum of 10.85 for His18 and a minimum of 3.05 for Glu4. Ala2, Lys3, Glu4, Leu5, Arg6 and Ser72 have T$_1$/T$_2$ ratios of < 0.60, implying a significant degree of internal motion. His18, Asp52, Val61 and Val62 have T$_1$/T$_2$ ratios of > 10.14, indicative of chemical exchange line broadening. The average T$_1$/T$_2$ of the remaining 56 residues is 8.59(\pm 5.54), and the calculated $\tau_\phi$ obtained by fitting the T$_1$/T$_2$ ratios of these residues simultaneously is 9.10(\pm 0.05) ns.

Of the 51 residues which did not require any contribution from chemical exchange to account for their $^{15}$N relaxation data, the T$_1$ and T$_2$ data of 30 residues, all with NOE values > 0.7, could be adequately fitted (i.e. within $\pm 1$ s.d.) by the simplified spectral density function of Lipari & Szabo (1982):

$$ J(\omega) = S^2 \tau_\phi (1 + \omega^2 \tau_\phi^2) $$

(1)

In which terms containing the internal correlation time $\tau_\phi$ are neglected (i.e. $\tau_\phi$ is $\leq$ 20 ps and hence makes a negligible contribution to T$_1$ and T$_2$). The order parameter $S^2$ for these 30 residues is $\geq 0.8$, indicating restricted internal motion. The remaining 21 residues in this group which had NOE values $\leq 0.7$ or their $^{15}$N T$_1$ and T$_2$ data could not be accounted for by equation (1) (i.e. the calculated T$_1$ and/or T$_2$ values lie outside $\pm 1$ s.d. of the corresponding experimental values). The $^{15}$N T$_1$, T$_2$ and NOE relaxation data for these 21 residues could not be fitted by the complete Lipari & Szabo (1982) model free spectral density function:

$$ J(\omega) = S^2 \tau_\phi (1 + \omega^2 \tau_\phi^2) + (1 - S^2) \tau_\phi (1 + \omega^2 \tau_\phi^2) $$

(2)

where $\tau_\phi = \tau_\phi \tau_\rho / (\tau_\phi + \tau_\rho)$. Specifically, equation (2) predicted significantly smaller NOE values than observed (see Clore et al., 1990a,b). As in previous examples (Clore et al., 1990a,b; Powers et al., 1992; Barbato et al., 1992), the $^{15}$N relaxation data for these residues could only be accounted for by the extension of the Lipari & Szabo (1982) model described by Clore et al. (1990b) in which the internal motions inside (fast) and outside (slow) the extreme narrowing limit are distinct with rates for the two regions differing by at least an order of magnitude (i.e. $\tau_\phi < 20$ ps, 200 ps $< \tau_\rho < \tau_\phi$). This is described by the spectral density function:

$$ J(\omega) = S^2 \tau_\phi (1 + \omega^2 \tau_\phi^2) + S^2 \tau_\phi (1 - S^2) \tau_\rho (1 + \omega^2 \tau_\rho^2) $$

(3)

where $S^2_\phi$ and $S^2_\rho$ are the order parameters for the fast and slow motions, $\tau_\phi$ is the internal correlation time for the slow motions (with $\tau_\rho = \tau_\rho \tau_\phi / (\tau_\rho + \tau_\phi)$), $\tau_\rho$ is the internal correlation time for the fast motions, is assumed to be $\leq 20$ ps so that terms containing $\tau_\rho$ are neglected. The values obtained for $\tau_\phi$ and $S^2_\phi$ range from 0.5 to 3.5 ns and from 0.2 to 0.9, respectively, while $S^2_\rho$ is $> 0.8$.

The relaxation data for the other 15 residues could only be accounted for by including an exchange line broadening term $\Delta \omega$ for T$_2$:

$$ 1/T_2(\text{obs}) = 1/T_2 \pm \Delta \omega $$

(4)

The lifetime for the chemical exchange processes can be estimated to lie between 170 ns and 2.25 ms (Powers et al., 1992). The $^{15}$N relaxation data for all but two of these residues could then be accounted for by equation (1). The $^{15}$N relaxation data for Thr37 and Arg68, however, could only be accounted for by equation (2) and chemical exchange. The internal correlation times $\tau_\rho$ for these two residues (51 and 68 ps, respectively) indicate that in addition to a slow chemical exchange process, their NH vectors undergo some motions that are slower than the ubiquitous fast thermal motions described by equation (1), but still located in the extreme narrowing limit. In addition, there were another ten residues whose $^{15}$N relaxation data could be fitted slightly better to equation (1) plus chemical exchange than to equation (1) alone.

The results of the analysis of the relaxation data are shown in Figure 2 and the location in the three-dimensional structure of IL-8 of the residues exhibiting the various types of relaxation behaviour is depicted in Figure 3.

Excluding the residues 2 to 6 and 72 at the N and C termini, respectively, the average value of the overall order parameter $S^2$ is 0.87(\pm 0.06) with a time of 3 s, this effect may result in a systematic increase of at most 20%, in the absolute value of the $^{15}$N-$^1$H NOE (Clore et al., 1990a) and probably accounts for the observation that in a few cases the measured $^{15}$N-$^1$H NOE value was greater than the theoretical maximum of 0.82. In such cases, a value of 0.82(\pm 0.1) was assumed for fitting the data to various spectral density functions. (It should be noted that the 5 unlabelled cross-peaks between $^3$H-$^{15}$N = 126 to 128 parts per million probably represent folded peaks arising from interactions via 2-bond $J$ couplings between the nitrogen atoms and non-exchangeable protons of the imidazole ring of the 2 histidine residues; they were not observed in the regular 2-dimensional $^{13}$C-$^{15}$N Overhauser enhancement correlation spectrum, in the $^{15}$N T$_1$, T$_2$ experiments, or in the 3-dimensional $^{15}$N-separated HOHAHA and ROESY spectra.)
Fig. 2.
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range spanning from 0.70 to 0.99, indicating that IL8 is a fairly rigid molecule. The average value for \(S^2\) (which is equal to \(S^2\) in the case of those residues whose relaxation data can be fitted to eqn (1)) is 0.88 (±0.05), which is equivalent to free diffusion within an axial-symmetric cone of semiangle \(\theta_{2g} = 17.4(±4.3)^\circ\). All these values are comparable to those found in previous studies (Cloro et al., 1990a).

Residues whose relaxation behaviour can be accounted for by the simplified Lipari & Szabo (1982) correlation function (eqn (1)) alone are, in general, located in regions of hydrogen bonded structure (e.g. the single \(3_{10}\) helical turn from residues 19 to 22, which is also stabilized by a hydrogen bond from the backbone amide group of Lys20 to the N-terminal of His18, the turn comprising residues 30 to 33, which connects \(\beta\)-strands 1 and 2 and is stabilized by 2 hydrogen bonds, residues 47 to 50 located in \(\beta\)-strand 3 in the vicinity of the disulphide bond between Cys89 and Cys50, and residues 59, 60, 69 and 70 in the C-terminal helix). The residues at the N and C termini exhibit a high degree of mobility with the overall order parameter \(S^2\) ranging from 0.2 to 0.6. These residues all require the presence of two internal motions (i.e. eqn (3)). Interpreting the order parameter \(S^2\) for the slow motions in terms of a two-state jump model on the nanosecond time-scale (Cloro et al., 1990a) yields an angle \(\phi_r\) between the NH vectors in the two states ranging from 48° to 83°. A number of other residues, located either at the N terminus (residue 7), the C terminus (residue 71), in loop regions (residues 10 to 12 and 14), in turns connecting \(\beta\)-strands (residues 31 to 36 between \(\beta\)-strands 2 and 3, and residues 42 and 44 to 46 between \(\beta\)-strands 3 and 4) or in the turn connecting \(\beta\)-strand 4 to the C-terminal helix (residues 54 to 55), reveal the presence of slow internal motions on a time-scale ranging from 1 to 3.5 ns and display lower than average overall order parameters \(S^2\). The lack of secondary structure allows these residues to move more independently since they are located on the surface of the molecule and are not restricted by packing within the hydrophobic core.

It is interesting to note that in the long series of N-terminal residues whose relaxation data is described by equation (3), a break occurs at residues 8 and 9. The internal motions in this short stretch are, in all likelihood, restricted by the interaction between the backbone amide proton of Gln8 and the amidoxyl ring of His33 (Cloro et al., 1990c) and by the disulphide bridge between Cys9 and Cys50 (located in the middle of \(\beta\)-strand 4). This contrasts with the situation at the second disulphide bond formed by Cys7 and Cys34, where both residues exhibit the additional slow internal motion, presumably due to the fact that Cys34 is located in a loop region.

A comparison of the crystallographic atomic root-mean-square (r.m.s.) displacements for the backbone nitrogen atoms derived from the B-factor values (Fig. 2F), the root-mean-square difference (r.m.s.d.) of the calculated NMR structures about their mean co-ordinate positions (Fig. 2G), and the overall order parameter \(S^2\) (Fig. 2H) indicates some degree of correlation between these three parameters. This is also depicted by the correlation plot and regression analysis shown in Figure 4. Not surprisingly, the correlation between \(S^2\) and the NMR r.m.s.d. (correlation coefficient = −0.86) is significantly better than that between \(S^2\) and the X-ray atomic r.m.s. displacements (correlation coefficient = −0.90). This is mainly due to the fact that, although the mobility of residues 4 to 6 in the X-ray structure is higher than average, they are still relatively well ordered on account of a salt bridge.

![Figure 2. Plots as a function of residue of: A, \(^{15}\)N T1; B, \(^{15}\)N T2 (displayed as linewidth = 1/(\(\pi T_2\)); C, \(^{15}\)N-\(^{1}\)H NOE values measured at 600 MHz; D, values calculated from the data of \(T_2\) for residues fit to eqn (2); E, the exchange broadness for residues fit to eqns (1) and (4) (filled circles, or open circles for those residues that can also be fit to eqn (1) alone) and for residues fit to eqns (2) and (4) (open squares); H, the overall order parameter \(S^2\) (filled circles for residues fitting eqns (1) or (2) with or without exchange line broadening; filled triangles for residues fitting eqn (3)); I, the fast \(S^2\) (circles) and slow \(S^2\) (triangles) motion order parameters. In J, the values of the \(\theta_{2g}\) (circles) and \(\phi_r\) (triangles) angles given by \(S^2 = (0.5 \cos \theta_{2g} + 0.5 \cos \phi_r)^2\) and \(\theta_{2g} = (1 + 3 \cos^2 \phi_r) / 4\), respectively, are displayed for residues whose relaxation behaviour is described by eqn (3); in this interpretation of the motions described by the spectral density function eqn (3), the slower motion is represented by a jump between 2 states, \(i\) and \(j\), while the faster motion is represented as free diffusion within 2 axially symmetric cones centred about the 2 states, \(i\) and \(j\); \(\theta_{2g}\) is the semi-angle of the cone, while \(\phi_r\) is the angle between the NH vectors in the 2 states, \(i\) and \(j\) (Cloro et al., 1990a). Also shown are \(F_0\), the crystallographic atomic displacements, (\(r^2\))^1/2; for the backbone nitrogen atoms calculated from the Debye-Waller B-factor values (\(r^2\))^1/2 = (3\(B/8\pi^2\))^1/2 (Baldwin et al., 1991); and G, the average r.m.s. difference of the backbone nitrogen atoms of the 30 individual calculated NMR structures about the mean co-ordinate positions (Cloro et al., 1990c). The FA/SHMIL program (Chance et al., 1979; Cloro, 1983) was used to analyse the relaxation data as described previously (Cloro et al., 1990b). The fitting procedure takes into account the experimental errors in the \(^{15}\)N relaxation data, and the errors in the values of the optimized parameters are obtained from analysis of the variance-covariance matrix. In fitting the experimental relaxation data to various functions, a fit to a given function is considered inadequate when 1 or more of the calculated relaxation parameters (i.e. \(T_2\), \(T_{12}\) or NOE) lies outside ±1 s.d. of the corresponding experimental value. The standard deviations of the experimental \(T_2\) and \(T_{12}\) data and of the calculated motional parameters are indicated by vertical bars. It should be noted that the errors for the calculated order parameters (\(S^2\), \(S^2\) and \(S^2\)), which range from 0.1 to 2%, with the majority being less than 0.5%, are smaller than the symbols. (The same is also true for some of the calculated values of \(S^2\)). A schematic representation of the secondary structure is shown at the bottom of the Figure.)
between the carboxylate group of Glu4 of one subunit and the NH$_3^+$ of Lys23 of the other subunit (Clore & Gronenborn, 1991a). In contrast, residues 4 to 6 are disordered in the solution NMR structures, consistent with the absence of non-sequential H-1H NOEs (Clore et al., 1989, 1990a; Clore & Gronenborn, 1991a) and the very low overall order parameters.

It should be emphasized, however, that the observed correlation between the overall order parameter $S^2$ and the r.m.s.d. of the calculated NMR structures is an indirect one, as the latter is directly related to the number and density of the $^1$H-$^3$H NOE derived interproton distance restraints. Clearly, the density of short (<5 Å) interproton distance contacts in exposed regions of the protein, such as loops, will be reduced, resulting in a higher NMR r.m.s.d. Consequently, even if such a loop region were rigid, for example due to stabilization by a salt bridge, it is likely that its NMR r.m.s.d. would be high. Moreover, it should be borne in mind that the NMR r.m.s.d. is also related to the degree of refinement, and thus any inference of mobility should be restricted to high resolution fourth generation NMR structures in which over 90% of the structurally useful NOEs have been assigned (Clore & Gronenborn, 1991b).

Residues that exhibit exchange line broadening cluster in regions of secondary structure, especially β-strands 2 and 3, the long C-terminal α-helix and, in particular, those residues that make contact between the helix and the underlying β-sheet (Figs 2E and 3). Line broadening arises from the existence of at least two distinct species with different chemical shifts. Since these residues have mutually stabilizing hydrogen bonds and buried hydrophobic side-chains, the grouping suggests a concerted movement of the residues between two or more conformations.

Many of the residues whose $^{15}$N relaxation data can be accounted for by the simplified Lipari &
Szabo (1982) correlation function (eqn (1)), together with chemical exchange (eqn (4)), are in van der Waals contact (Fig. 3). In β-strands 2 and 3, the backbone amide and carbonyl groups of Val41 and Leu49 are hydrogen-bonded and, in addition, there are contacts between the side-chains of Val41 and Leu51 and between Leu49 and Leu43. The side-chain of Val58 in the long C-terminal z-helix contacts the side-chain of Leu51 and the backbone amide group of Asp52 in β-strand 3. Contacts between residues in β-strand 1 and the overlying helix include Leu25 to Phe55, Val27 to Phe65 (of the other subunit), and Gln29 to Phe65 and Arg68. In addition, the side-chain of Leu25 in β-strand 1 contacts the side-chain of Val41 in β-strand 2. Many of these contacts involve the burying of hydrophobic side-chains. It is interesting to note that the only residue in β-strand 1 that does not exhibit exchange line broadening is Arg26. This residue is located at the C2 symmetry axis of the dimer, and therefore at the pivot point of any potential motion of the dimers relative to each other with respect to this axis. This could arise, for example, from a change in the angle between the long axes of the two central strands (i.e. strands 1 and 1') of the β-sheet at the dimer interface. Consequently, it is likely to experience only very small (if any) 15N chemical shift differences between different conformational states. That this type of motion may, indeed, occur is evidenced by the fact that the difference in the quaternary arrangement of the subunits between the solution and crystal structures can be directly attributed to a rigid body rotation of the two subunits about the C2 symmetry axis as a result of a change in the angle between the two central strands from approximately 108° in the solution structure to approximately 179° in the crystal structure (Clore & Gronenborn, 1991a).

Although both Lys15 and His18 exhibit exchange line broadening, they are not located in secondary structure elements. However, the adjacent residues, P016 and Pro19, respectively, are in van der Waals contact with Trp57 and Phe65 in the long helix, both of which exhibit exchange line broadening. Motions of these nearby aromatic side-chains may cause changes in the 15N chemical shifts of these residues. Other residues that exhibit exchange line broadening and are in contact with Pro19 include the side-chains of Val61 and Lys64 in the long helix.

Finally, there are two residues, Thr37 and Arg68, whose 15N relaxation behaviour can only be accounted for by both chemical exchange and a significant contribution from internal motion (i.e. eqns (2) and (4)). While both these residues are in close proximity to the residues exhibiting exchange line broadening, they are located at the boundaries of secondary structure elements, namely at the beginning of β-strand 2 (Thr37) and at the end of the C-terminal helix (Arg68). Hence, it is perhaps not surprising that, in addition to chemical exchange, these two residues may participate in some of the same type of motions that the more mobile residues display.

The pictorial description of the 15N relaxation data of IL-8 presented in Figure 3 makes it strikingly obvious that the ordered secondary structure elements exhibit a qualitative difference in dynamic behaviour from the more irregular loops and turns. Thus, whereas the residues located in the latter can move more freely and independently, a number of residues in the β-sheet and α-helices undergo motions on a time-scale longer than the rotational correlation time. The latter are in all likelihood concerted in nature since (1) the residues involved form a three-dimensional cluster, (2) both the six-stranded β-sheet and the two overlying helices in IL-8 are mutually stabilized by hydrophobic interactions at their interface (Clore et al., 1990a), and (3) structural changes in proteins tend to be highly cooperative. It therefore seems probable that any motion in one structural unit will be propagated to the other via interacting residues, such that compensatory conformational changes will ensure optimal arrangements of these elements in terms of interaction energies. Such motions can involve concerted intra-domain local motions as well as rigid body motions of the two subunits relative to each other.

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References


Clore, G. M. & Gronenborn, A. M. (1991a). Comparison of
the solution nuclear magnetic resonance and crystal structures of interleukin-8. *J. Mol. Biol.* 217, 611–620.


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