Transient Structure Formation in Unfolded Acyl-coenzyme A-binding Protein Observed by Site-directed Spin Labelling

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Paramagnetic relaxation has been used to monitor the formation of structure in the folding peptide chain of guanidinium chloride-denatured acyl-coenzyme A-binding protein. The spin label (1-oxyl-2,2,5,5-tetramethyl-3-pyrroline-3-methyl)methanesulfonate (MTSL) was covalently bound to a single cysteine residue introduced into five different positions in the amino acid sequence. It was shown that the formation of structure in the folding peptide chain at conditions where 95% of the sample is unfolded brings the relaxation probe close to a wide range of residues in the peptide chain, which are not affected in the native folded structure. It is suggested that the experiment is recording the formation of many discrete and transient structures in the polypeptide chain in the preface of protein folding. Analysis of secondary chemical shifts shows a high propensity for $\alpha$-helix formation in the C-terminal part of the polypeptide chain, which forms an $\alpha$-helix in the native structure and a high propensity for turn formation in two regions of the polypeptide that form turns in the native structure. The results contribute to the idea that native-like structural elements form transiently in the unfolded state, and that these may be of importance to the initiation of protein folding.

Keywords: protein folding; unfolded state; spin labelling; NMR; paramagnetic relaxation enhancement

Introduction

Understanding the folding of polypeptide chains into biologically active proteins is a great challenge in structural biology. The identification of proteins that acquire a folded structure only in the event of binding a molecular target has demonstrated that understanding protein folding is important for the comprehension of cellular function and gene regulation. Structural description of the unfolded state may add to the understanding of the driving forces in the folding process. In the unfolded state of a protein, the polypeptide chain is expanded towards a random coil; however, in most cases, a fully random chain is obtained only under very strong denaturing conditions. In the case of staphylococcal nuclease, residual structure was observed in as much as 8 M urea. Residual structures have been observed in the unfolded states of many other proteins. Structural elements such as native-like turns and peptide segments with a high helix-forming propensity in these unfolded states have been described. The similarity to the native structure indicates that these fluctuating structures in the unfolded state may be of importance to the protein folding process. Indeed, the folding properties of the B-domain of protein A could be predicted from diffusion–collision theory with preformed structure in the unfolded state. Likewise, stabilising $\alpha$-helices in the unfolded state of acylphosphatase by addition of trifluoro-acetic acid (TFA) has been shown to accelerate the folding process of this protein significantly. For $\alpha$-lactalbumin, lysozyme, RNase A, apomysoglobin and carbonic anhydrase, trapped water molecules in the unfolded state have been observed by $^{17}$O magnetic relaxation dispersion.

Abbreviations used: ACBP, acyl-coenzyme A-binding protein; GuHCl, guanidinium chloride; HSQC, heteronuclear single quantum coherence; MTSL, (1-oxyl-2,2,5,5-tetramethyl-3-pyrroline-3-methyl)methanesulfonate; PRE, paramagnetic relaxation enhancement. E-mail address of the corresponding author: fmp@apk.molbio.ku.dk

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Here, acyl-coenzyme A-binding protein (ACBP) is used as the system to study structure formation in the unfolded state. From mutation studies, it is suggested that the formation of a native-like structure including eight conserved hydrophobic residues in the N-terminal and C-terminal helices is rate-limiting in the folding of ACBP. Recently, it was found that for certain residues, partial protection against hydrogen exchange precedes the rate-limiting step in the folding of the protein. Especially in the C-terminal helix, many residues seem to engage in transient hydrogen bonds, indicating that very local structure in this part of the protein forms prior to the global folding. These findings encouraged a description of the unfolded state of ACBP. Here we have used site-directed spin-labelling and paramagnetic relaxation enhancement (PRE) to probe structures in the unfolded state of ACBP. As the effect of PRE by an unpaired electron is observable on protons at distances up to 20 Å, long-range structural information can be obtained in the dynamic ensemble of highly flexible structures resembling the unfolded state. Five Cys mutants were constructed and the introduced thiol groups were modified with the paramagnetic spin label MTSL (Figure 1).

**Table 1.** Stability of reduced spin-labelled mutants of ACBP at 298 K in 20 mM sodium acetate, pH 5.3

<table>
<thead>
<tr>
<th>Mutant</th>
<th>$[\text{GuHCl}]_{50%}$ (M)</th>
<th>$m$-value (kJ mol$^{-1}$M$^{-1}$)</th>
<th>$\Delta G$ (kJ mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>1.70 ± 0.01</td>
<td>14.7 ± 0.1</td>
<td>25.0 ± 0.2</td>
</tr>
<tr>
<td>T17C-MTSL</td>
<td>1.66 ± 0.01</td>
<td>13.9 ± 0.8</td>
<td>23.1 ± 1.3</td>
</tr>
<tr>
<td>V36C-MTSL</td>
<td>1.57 ± 0.01</td>
<td>14.2 ± 0.4</td>
<td>19.5 ± 0.6</td>
</tr>
<tr>
<td>M46C-MTSL</td>
<td>1.63 ± 0.01</td>
<td>12.6 ± 0.4</td>
<td>20.5 ± 0.7</td>
</tr>
<tr>
<td>S65C-MTSL</td>
<td>1.22 ± 0.02</td>
<td>15.5 ± 1.2</td>
<td>18.9 ± 1.5</td>
</tr>
<tr>
<td>I86C-MTSL</td>
<td>1.46 ± 0.01</td>
<td>13.4 ± 0.4</td>
<td>19.6 ± 0.6</td>
</tr>
</tbody>
</table>

**Results**

Spin labelling

In order to ensure efficient labelling, the ACBP Cys variants T17C, V36C, M46C, S65C, and I86C were labelled specifically with a threefold excess of the thiol-specific spin label MTSL at pH 8.0 for 16 hours in the dark at room temperature; almost
Complete labelling of the target protein was achieved. After purification by reverse-phase HPLC, a homogeneous preparation was obtained, as judged by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS). Introduction of the spin label destabilises the protein slightly in GuHCl stability experiments (Table 1).

The conditions for the subsequent study of the unfolded state at a low concentration of GuHCl were chosen such that approximately 95% of the protein population was unfolded. V36C-MTSL, S65C-MTSL, and I86C-MTSL were studied at 1.9 M GuHCl, while T17C-MTSL and M46C-MTSL were studied at 2.2 M GuHCl to compensate for the differences in stability among the mutant proteins. For comparison, wild-type ACBP is 95% unfolded at 2.2 M GuHCl.

Residual helical structure in unfolded ACBP

From a set of one HSQC and six triple resonance NMR spectra, $^{13}$C, $^{15}$N, and $^2$H backbone chemical shifts for all residues except Asp56 and Ala72 in spin-labelled ACBP,I86C-MTSL were assigned under denaturing conditions of 1.9 M GuHCl, where two sets of easily distinguishable cross-peaks were observed. A low-intensity set resulting from the native structure, and a high-intensity set of peaks, which clearly represents the unfolded state of the protein, as HSQC spectra at 0 M, 1.9 M, and 4 M GuHCl show that most of the dispersion in the $^2$H dimension of the spectrum of the native state is lost at 1.9 M GuHCl (Figure 2).

The differences observed between the spectra at 1.9 M GuHCl and 4 M GuHCl can be attributed to the increased concentration of salt. From a set of HSQC spectra at 1.9 M, 2.25 M, 2.6 M, and 4 M GuHCl, a linear or near-linear relationship between chemical shifts for all $^2$H and $^{15}$N, and the concentration of GuHCl was observed. No cooperative transition in the chemical shift changes is thus apparent. The change in $^1$H chemical shifts when the concentration of GuHCl is raised from 1.9 M to 4 M is less than 0.2 ppm, and is uniform along the protein sequence. On the other hand, $^{15}$N chemical shift changes are not uniform, and vary from 0.1 ppm to 1.4 ppm. The largest changes in $^{15}$N chemical shifts are in the regions 70–86 and 24–30 (Figure 3). This finding suggests that the parts of the polypeptide comprising helix 2 in the native structure, and especially the part comprising helix 4, have some residual structure in the unfolded state at 1.9 M GuHCl.

The chemical shifts of backbone $^{13}$C and CO, and to a smaller extent, $^{13}$C are dependent on secondary structure. These shifts show for ACBP, I86C-MTSL in 1.9 M GuHCl small but distinct deviations from random coil shifts (Figure 4). In the case of $^{13}$C all chemical shifts are found to be around 0.4 ppm higher than random coil values, except for residues 66-82, which are shifted more than 1 ppm. The $^{13}$C chemical shifts show no significant deviation from random coil chemical shift values. The CO chemical shifts show a continuous stretch of residues from 64 to 84 (the gap is due to missing assignments) with positive secondary chemical shifts. Together the $^{13}$C and CO chemical shift deviations indicate that the C-terminal part of the polypeptide has some residual helical structure in the unfolded state at 1.9 M GuHCl and that the propensity is two- to threefold higher in this part of the polypeptide than in the rest of the polypeptide.
This is in agreement with the larger change in $^{15}$N chemical shift upon increased concentration of GuHCl (Figure 3). The Cα secondary chemical shifts and $^{15}$N chemical shift changes upon increased concentration of GuHCl suggest the existence of residual structure around residues 24–30, but to a smaller extent than in the C-terminal region.

In the HSQC spectra at 1.9 M GuHCl, cross-peaks from residues 71 to 84 are broadened relative to cross-peaks from residues located in other parts on the protein. Such broadening may result from exchange between two (or more) conformations or it may result from altered relaxation properties. Whether the origin of the broadening is related to exchange or relaxation, the observation supports the existence of residual structure in the C-terminal part of the protein.

The assignment of the HSQC spectra of the other four MTSL-labelled mutants was achieved by comparison with the HSQC spectrum of I86C-MTSL. The assignments were transferred readily from the I86C-MTSL spectrum. Only minor changes were observed between the HSQC of all mutants, indicating that no significant structure or structural change is introduced by the presence of the spin label.

Paramagnetic relaxation enhancement in spin-labelled ACBP

The effect of the MTSL spin label on the transversal relaxation rate of backbone amide protons was measured as the decrease in the intensity (height) of HSQC cross-peaks of the paramagnetic sample compared with the intensity of HSQC cross-peaks in the reduced diamagnetic sample. Samples of spin-labelled protein under both native and mildly denaturing conditions (95% unfolded) were studied at pH 5.3. In the native state of ACBP, due to the structure, resonance intensities from nuclei close to the spin label are affected in a distance-dependent manner (Figure 5). Comparing the PRE profiles of the native structure with the published NMR-structure (Figure 1) shows that the effect of the spin labels can be observed up to 20 Å from the paramagnetic centre. From the effects of the spin labels, even the face of a helix pointing towards the spin label can be identified. In the bottom right panel of Figure 5 showing the PRE profile for I86C-MTSL in the native state, this effect is clearly seen on residues 24, 27, 31, and 34, which are on the side of the helix facing the spin label, and which are affected by the spin label more strongly than the neighbouring residues.

Figure 5. PRE of backbone amide protons in spin-labelled ACBP under native and mildly denaturing conditions (95% unfolded) at pH 5.3 and 298 K. Five Cys-substituted and spin-labelled variants of ACBP, T17C-MTSL, V36C-MTSL, M46C-MTSL, S65C-MTSL, and I86C-MTSL were analysed. The HSQC spectra of the protein sample before (paramagnetic) and after (diamagnetic) reduction of the spin label with ascorbate were recorded and the intensity ratio of the $^1$H$^-$N cross-peaks determined. Arrows indicate the position of the modified amino acid residue. Blank spaces in the diagrams are either due to proline residues (position 19 and 44) or the inability to measure the peak height as a consequence of overlapping or non-assigned residues. Dotted lines in the right-hand panels are simulated effects of the spin labels in a set of 96 random unstructured polypeptides.
From Figure 5, it is seen that effects of the spin label in the unfolded form can be observed far beyond the nuclei in the covalent vicinity of the modification. The widespread effects suggest that the spin label makes contact with a large part of the unfolded peptide chain. For instance, for the I86C-MTSL, a comparison of the effect of the spin label in the absence of GuHCl and in the presence of 1.9 M GuHCl shows this very clearly. There are significant effects on residues 21–75 under denaturing conditions, which are not seen in the folded protein, and effects in the folded protein on residues 5–20 are absent under denaturing conditions. Likewise, for the other mutant proteins, it was observed that around the sites of the spin labels, increased relaxation extended much further along the sequence under denaturing conditions compared with the native conditions, where sharper limits for the effects of the spin label are observed. Also, a spin label at site A affects the sites modified in the other variants in the same way that spin labels at these sites affect site A. This complementarity in the PRE effects together with the similar HSQC spectra for all five spin-labelled variants indicates that the observed effects are not induced by the modifications. Apart from these effects, we note that the N terminus seems to be the part of the molecule affected least by the paramagnetic centre. Even in T17C-MTSL, the relaxation enhancement along the sequence towards the N terminus evidently levels off almost as simulated for a random coil (Figure 5), suggesting that the N-terminal part of the polypeptide is unstructured and makes only few contacts with the rest of the molecule in the unfolded state.

**Titration of the unfolded structure**

To determine the effect of GuHCl on the unfolded structure, both the oxidised and reduced form of I86C-MTSL were studied by NMR and fluorescence in 0–5 M GuHCl. Whereas the oxidised and reduced forms of I86C-MTSL give identical results when measured by fluorescence, distinct differences are observed by NMR (Figure 6). In the NMR experiment, the reduced sample, where the spin label is inactivated, shows a single cooperative transition, which can be fit to a two-state unfolding transition. The same m-value ($14.3(\pm0.8)$ kJ mol$^{-1}$ M$^{-1}$) and mid-point ($1.56(\pm0.02)$ M) of the transition were found as when using intrinsic tryptophan fluorescence (Table 1). In the NMR data on the oxidised samples, the peak intensity emerge much more slowly than in the reduced sample (Figure 6(b)). The fluorescence experiments show that the effect seen in the NMR experiments is not due to differences in the stability of the oxidised and reduced form of the spin-labelled protein. Rather, the effect is assigned to residual structure in the unfolded state ensemble. The recurrence of the Trp side-chain peak intensity in the oxidised sample with increasing concentration of GuHCl can be caused by a single cooperative transition between two thermodynamically distinct states, or by a gradual non-cooperative loss of hydrophobic interactions stabilising the structures in the unfolded state, which would lead to an ensemble of less compact structures. Attempts to fit a cooperative transition to the increasing Trp signals failed, which is in agreement with the absence of cooperativity in the $^{15}$N chemical shifts upon increased concentration of denaturant. The expected small amount of buried hydrophobic surface would, however, result in a low cooperative transition, hampering the analysis of equilibrium data.

**Discussion**

**Structure of the unfolded state of ACBP under mildly denaturing conditions**

The effects of the spin labels can be interpreted as either an ensemble of unfolded structures, where each structure is satisfying the experimental data, or as an ensemble of unfolded structures, where as an average the ensemble satisfy the experimental data. Gillespie & Shortle5 presented structures of an intrinsically unfolded fragment of ACBP, I86C-MTSL at pH 5.3 and 298 K. (a) Equilibrium stability measured by intrinsic Trp fluorescence on samples with both oxidised (%) and reduced (■) spin label. The Trp residues were exited at 280 nm and the emission at 356 nm was measured. The measured fluorescence intensity was fit to a two-state transition and normalised to give the unfolded fraction. (b) The height of the peak from Trp55 and Trp58 in unfolded ACBP was measured from a series of 1D $^1$H NMR spectra. Intensities were measured before (%) and after (■) reduction with ascorbate. The data from the reduced spin label samples were fit to a two-state unfolding transition (continuous line).
staphylococcal nuclease based on restraints from PRE effects, whereas Mok et al. used long-range nuclear Overhauser effect (NOE) restraints for the structure determination of partially deuterated drk SH3. These structures were calculated to satisfy all the restraints. As discussed by those authors, the $r^{-6}$ distance-dependence of the NOE and PRE effects is likely to result in structures that are too compact. For the drk SH3 domain, Choy & Forman-Kay demonstrated that, from a large number of structures, it was possible to identify clusters of structures satisfying the experimental data not individually but as an ensemble. The structures were much less compact than the structures of their initial structure calculation and in better agreement with other data on the unfolded state. This result suggests that the polypeptides in the unfolded state of a protein generally are less structured than the structures of their initial calculation and in better agreement with other data on the unfolded state.

Figure 7. Secondary structure propensity and hydrophobicity of ACBP. (a) α-Helix propensity at pH 5.3 and 1 M ionic strength calculated by AGADIR. (b) β-Turn propensity calculated from the scale of Levitt with a window size of seven amino acid residues. (c) Hydrophobicity of ACBP calculated from the hydrophaticity scale of Kyte & Doolittle with a window size of seven amino acid residues.

Whether the interactions observed in the spin-label experiments represent a distinct thermodynamic state with a cooperative transition to the unfolded protein cannot be determined from the current data. In cytochrome $c$, a barrier-limited
transition between an extended and a compact unfolded state was observed.20 However, the existence of fluctuating secondary structure and transient loop formation do not necessarily lead to the formation of a compact unfolded state. In unfolded protein L, both local and long-range effects of introduced spin labels were observed.6 A study of the folding process by small-angle X-ray-scattering did not reveal any burst decrease in the radius of gyration. This indicates that the collapse of the polypeptide chain occurs simultaneously with the formation of the rate-limiting event in the folding reaction.21

Folding of ACBP is initiated by a search for productive interactions

The present work has demonstrated that transient long-range interactions and segments with a propensity for helix and turn formation are present in the unfolded state of ACBP under conditions near the folding transition, where the sample is 95% unfolded. Under these conditions, ACBP can still fold and form a native structure. This suggests that the interactions that lead to folding under these conditions are similar to those under non-denaturing conditions. Similarly, the formation of transient long-range and short-range interactions seen under conditions near the folding transition in this work may as well take place under non-denaturing conditions. In a recent study, ACBP was shown to form transient structures at the folding transition at low pH. Two populations of the unfolded state were present, one that had the potential to fold, and one that did not.22 Accordingly, the transient structure formation as seen in this work may result both in folding productive and folding unproductive structures. In other words, the transient structure formation may be the early stages in protein folding where the polypeptide chain is in a search for folding productive interactions.

Previous work12,13,22 has suggested that helix 4 in particular is involved in the early folding events of ACBP. The present results thus suggest that the structures found in the unfolded state near the folding transition resemble structure formed during the initial folding events. Similar considerations have been made for the α-spectrin SH3 domain.23 A comparison of structure observed in the denatured state ensemble with the folding transition state depicted by protein engineering24 showed that the residues comprising the distal β-hairpin formed in the transition state are partly structured in the denatured state ensemble. Together with the present work, this suggests that parts of the polypeptide chain with a high propensity for secondary structure formation may be important for initiation of the folding reaction.

Recent high-resolution kinetic experiments on dansyl-labelled ACBP showed that during folding an intermediate formed with a time constant of 80 μs.25 This intermediate is only 1% populated at 1.9 M GuHCl and cannot account for the structure in the unfolded state observed in the present study. The intermediate rather represents a collapsed structure in which about 30% of the hydrophobic surface has been buried and where fast formation of transient hydrogen bonds and fluctuating helical structure occurs, primarily in the C-terminal part.13 This intermediate may represent a consolidation of the structures observed here in the unfolded state. From the conformational restricted intermediate state, formation of native-like interactions between helix 1 and helix 4, the N and C-terminal helices, is the rate-limiting step before the native state of ACBP is reached.12 A representation of events in the folding process of ACBP is shown in Figure 8.

Materials and Methods

The Cys mutants of ACBP were constructed by site-directed mutagenesis, ligated into the pET-3a vector and expressed in Escherichia coli strain BL21(DE3)/pLyS3. Cultures for expression of isotope-labelled protein were grown in M9 minimal medium with [15N](NH₄)₂SO₄ as

![Figure 8. Folding sequence of ACBP. From an ensemble of unfolded structures (U), where transient structures form the intermediate (I) is reached. I is a loosely packed ensemble of structures with fluctuating helical structure primarily in the C-terminal (blue) part of the polypeptide. In the rate-limiting structure (‡), helices in the N-terminal (yellow) and the C-terminal (blue) part form native-like interactions before the native state (N) is reached. MOLSCRIPT26 was used for the preparation of this Figure.](image)
sole nitrogen source. For \(^{13}\)C,\(^{15}\)N-labelled protein, \(^{13}\)C\(^{15}\)N-glucose was used as carbon source.

The cysteine residues of the purified mutants were modified with (1-oxyl-2,2,5,5-tetramethyl-3-pyrroline-3-methyl)methanesulfonate (MTSL, Toronto Research Chemicals Inc., Canada). The protein was dissolved in 0.1 M Tris–HCl (pH 8.0) at 1 mg ml\(^{-1}\), flushed with Ar, and a 3 M excess of MTSL from a 40 mM stock in acetonitrile was added. The reaction was left overnight in the dark at room temperature and then the protein was purified by HPLC. The homogeneity of the purified spin-labelled protein was verified by MALDI-TOF MS.

Equilibrium unfolding measurements were performed by preparing 4 \(\mu\)M samples of the spin labelled ACBP mutants in 20 mM sodium acetate (pH 5.3) with increasing concentrations of GuHCl. For measurements on reduced samples, a 2.5-fold excess of ascorbate was added to the stock of spin-labelled protein prior to the preparation of the samples. The samples were excited at 280 nm and fluorescence at 356 nm was measured using a Perkin–Elmer LS50B luminescence spectrometer. The data were fit to the equation for a two-state transition as described by Jackson & Fersht. The mid-point of the unfolding transition \([\text{GuHCl}]_{\text{u}} = \Delta G/m\) is reported in Table 1.

NMR samples were prepared by dissolving freeze-dried, spin-labelled protein in 20 mM sodium acetate (pH 5.3), 10\% \(^{2}H_{2}O\) with the appropriate concentration of GuHCl. Protein concentrations were between 0.5 mM and 0.7 mM in a volume of 650 \(\mu\)l. \(^{1}H,^{15}N\)-HSQC NMR spectra were recorded on a Varian Unity Inova 750 instrument at 298 K. Spectra were recorded on each sample both before and after reduction of the spin label with a 2.5-fold excess of ascorbate added from a 0.5 M stock.

Assignment of backbone chemical shifts was performed on a 1.5 mM (\(^{13}\)C,\(^{15}\)N)-labelled ACBP, 186C modified with MTSL and subsequently reduced with ascorbate. A \(^{1}H,^{15}N\)-HSQC spectrum and the following triple resonance spectra were recorded, HNCO, HN(CA)CO, HN(CO)CA, HNCA, HNCACB, CBCA(CO)NH (all pulse programs from Varian ProteinPack) at 298 K on a Varian Unity Inova 750 instrument. NMR data were processed by nmrPipe and analysed using Pronto3D.

In order to compare the PRE measured under denaturing conditions with the expected effects in an ensemble of random unstructured polypeptide chains, XPLOR was used to generate 96 structures by unconstrained molecular dynamics at 10,000 K, starting from a fully extended polypeptide chain. For each structure, the distance from every backbone HN to each of the five spin-labelled sites was measured and the paramagnetic contribution to the transversal relaxation rate, \(R_{\text{ep}}\), calculated from:\(^{29}\)

\[
R_{\text{ep}} = \frac{K}{\tau_{c}} \left( 4\tau_{c} + \frac{3\tau_{c}}{1 + \omega_{1/2}^{2}} \right)
\]

where \(\tau_{c}\) is the HN–spin label distance. \(K\) is 1.23\(\times\)10\(^{-32}\) cm\(^{2}\) s\(^{-2}\) for the interaction between a single electron and a proton. \(\omega_{1/2}\) is the Larmor frequency of a proton (750 MHz). \(\tau_{c}\) is the correlation time for the electron-nuclear dipole–dipole interaction, which was set to 4 ns.\(^{3}\)

Assuming that the transversal relaxation rate in the diamagnetic sample, \(R_{\text{ep}}^{\text{red}}\), is 12.6 s\(^{-1}\) (4 Hz line-width), the ratio between the intensity of a peak in the paramagnetic sample, \(I_{\text{ox}}\), and the diamagnetic sample, \(I_{\text{red}}\), can be calculated from:\(^{30}\)

\[
\frac{I_{\text{ox}}}{I_{\text{red}}} = \frac{R_{\text{ep}}^{\text{red}} \exp(-R_{\text{ep}} t)}{R_{\text{ep}}^{\text{red}} + R_{\text{ep}}^{\text{ox}}}
\]

where \(t\) is the duration of the INEPT delays (~10 ms) in the HSQC pulse sequence where the proton magnetisation will be in the transversal plane. \(I_{\text{ox}}/I_{\text{red}}\) for all 96 structures were averaged and are plotted in Figure 5.

Data bank accession number

The chemical shift assignments have been deposited at the BioMagResBank, accession number 5351.

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References


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