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Hydration of the partially folded peptide RN-24 studied by multidimensional NMR

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Summary

Peptide–water interactions of a ribonuclease C-peptide analogue, RN-24 (Suc-AETAAAKFLRAHA-NH_2), which exhibits significant helicity, have been studied in solution using homonuclear 2D and 3D NMR cross-relaxation experiments. Dipolar peptide proton–water proton interactions are indicated by a large number of NOESY-type cross peaks at the H_2O resonance frequency, most of them with opposite sign relative to the diagonal. Some cross peaks arise from intrapeptide cross relaxation to labile protons of histidine, threonine, lysine and arginine side chains. The observed peptide–water interactions are rather uniformly distributed, involving peptide backbone and side chains equally. The data are consistent with rapid fluctuations of the conformational ensemble and the absence of peptide regions that are highly shielded from bulk solvent, even in a peptide that exhibits high propensities for formation of helical secondary structure.

Introduction

One approach to elucidate the earliest events in protein folding is based on the study of the propensities of polypeptide fragments to adopt folded conformations in aqueous solution (Wright et al., 1988). Early folding events, which are difficult to detect in intact proteins because of the rapid and highly cooperative nature of the folding process, can be modeled using short peptide fragments of proteins due to the absence of long-range interactions which drive the cooperative folding process. In recent years, a number of peptide fragments of proteins have been investigated by NMR and circular dichroism and have been shown to have conformational preferences for secondary structure (Dyson et al., 1988a,b; Goodman and Kim, 1989; Peña et al., 1989; Bruch et al., 1991; Jiménez et al., 1993; Waltho et al., 1993). Although it is clear from sequence, pH and temperature dependence studies (e.g. Dyson et al., 1988b; Waltho et al., 1993) that intrapeptide interactions are important in stabilizing ordered structure, the role of the water solvent in these systems is not well understood. A number of theoretical studies using molecular dynamics simulations with explicit inclusion of solvent molecules (Di Capua et al., 1990; Soman et al., 1991,1993; Tirado-Rives and Jorgensen, 1991; Daggett and Levitt, 1992; Brooks and Case, 1993) indicate that water molecules tend to compete with intrapeptide hydrogen bonds and destabilize secondary structure.

NMR methods have been developed recently to investigate hydration of biological macromolecules (Otting and Wüthrich, 1989; Otting et al., 1991a,b) via NOESY- and ROESY-type dipolar cross-relaxation processes between water protons and polypeptide protons. Until now, the method has been applied to study hydration of several biomolecules, including the protein BPTI and the highly flexible 'random coil' peptide oxytocin (Otting et al., 1991b). The behavior of BPTI should be representative for a globular protein: NOEs are seen to both the slowly exchanging interior water molecules (with residence times $\tau > 10$ ns) and to fast exchanging surface water molecules (with correlation times $\tau < 1$ ns for the corresponding dipolar correlation function). For most protons in the interior of the protein, no contacts with either bulk or bound water can be detected. By contrast, hydration of oxytocin covers the entire molecule, a fact which has been attributed to its random coil character (Otting et al., 1991b).

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In this paper we present the results of an investigation of the hydration of a ribonuclease C-peptide analogue, RN-24, with sequence Suc-AETAAAKFLRAHA-NH_2. This analogue is not fully random but has a high population, approximately 60% by CD spectroscopy (Osterhout et al., 1989), of helical conformers in aqueous solution. Previous NMR investigations showed that RN-24 populates helical conformations predominantly in the center of the sequence, and that there is also a high population of a conformation in which the backbone is largely extended at the N-terminus, allowing formation of a salt bridge between Glu^2 and Arg^{10} (Rico et al., 1984; Osterhout et al., 1991). The peptide also samples partially or fully unfolded states.

Materials and Methods

Peptides were synthesized as described previously (Dyson et al., 1988a; Osterhout et al., 1989). An NMR sample of RN-24 (8 mM, pH 5.2) was prepared in 90% H_2O/10% D_2O. The probe temperature for all 2D and 3D NMR experiments was set to 278 K.

NMR spectra were acquired on a Bruker AMX-500 spectrometer. 2D NOESY experiments were collected using a jump-and-return pulse sequence (Fig. 1A) or a slightly modified version of the pulse sequence proposed by Otting et al. (1991a). 2D ROESY, 3D NOESY-TOCSY and 3D ROESY-TOCSY experiments were collected with the pulse sequences of Otting et al. (1991a) with small modifications: a CW ROESY spin-lock was used during the ROE mixing time (Figs. 1B,D) instead of a series of $\pi/6$ pulses, and a DIPSI-2 (Shaka et al., 1988) instead of an MLEV-17 sequence was used during the TOCSY mixing times (Figs. 1C,D). In the 3D ROESY-TOCSY sequence, two additional 90° pulses flank the ROE mixing period (Fig. 1D) (Griesinger and Ernst, 1987). Parameters for the various spin-lock pulses and delays are included in the figure caption.

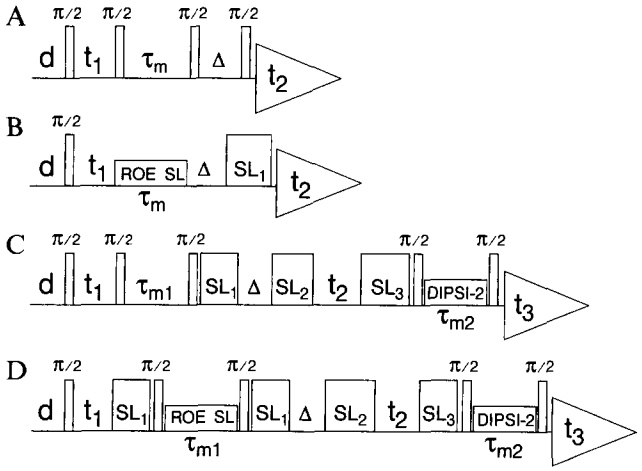

Fig. 1. Pulse sequences used in the RN-24 hydration experiments for (A) 2D NOESY, (B) 2D ROESY, (C) 3D NOESY-TOCSY and (D) 3D ROESY-TOCSY spectra collection with phase cycles analogous to those described by Otting et al. (1991a). Typical parameters are: d = 1.8 – 2.0 s for the recycling delay, Δ = 180 μs for the defocusing delay; mixing times: NOESY τ_m = 250 ms, ROESY τ_m = 200 and 190 ms (spin-lock applied for 150 ms) for 2D and 3D spectra, respectively, TOCSY τ_m = 35 ms. The spin-lock pulses were SL_1 = 1.2 ms for 2D ROESY spectra, SL_1 = 0.5 ms, SL_2 = 1.0 ms, SL_3 = 2.0 ms for 3D NOESY-TOCSY spectra and SL_1 = 0.9 ms, SL_2 = 2.0 ms, SL_3 = 1.0 ms for 3D ROESY-TOCSY spectra. The pulse heights symbolize the applied rf power. The rf field strength γB_1 for the ROESY mixing period was set to about 4 kHz.

Quadrature detection in ω_1 (and ω_2 for 3D spectra) was achieved using TPPI in all pulse sequences, with the carrier placed at the water resonance. In order to avoid frequency-dependent phase corrections, the first t_1 delay (and t_2 delay for 3D spectra) was set to $\Delta t - 4\tau_{90}/\pi$, where Δt is the time increment of the corresponding time domain (Marion and Bax, 1989). During processing, the FIDs were shifted to the right by one point and the first point was set to zero or linearly back-predicted. For 2D spectra, 512 t_1 data points with 32 scans each were collected and $200(t_1) \times 100(t_2) \times 1024(t_3)$ data points with eight scans per t_1 point were collected for 3D spectra.

Data were processed on an IRIS 4D workstation using the FELIX software provided by Dr. Dennis Hare. Typically, $1024(\omega_1) \times 4096(\omega_2)$ and $256(\omega_1) \times 256(\omega_2) \times 1024(\omega_3)$ real matrices were generated for 2D and 3D data sets, respectively. The residual water signal was removed from the spectra by applying a sine-bell low-pass filter on the time domain data along the direct dimension (Marion et al., 1989). The first one to four points for FIDs in all dimensions were linearly back-predicted and zero-filling was applied in all dimensions. For 2D spectra, cosine-bell or skewed sine-bell window functions were used along t_1 and t_2; for 3D spectra, cosine-bell window functions were used along all dimensions. Linear or higher order polynomial baseline correction was applied after Fourier transformation along the acquisition dimension.

Results and Discussion

Figure 2 shows the 1D and 2D cross sections at the ω_1 frequency of the water signal from a homonuclear ^1H 3D NOESY-TOCSY and a 2D ROESY spectrum of RN-24. These data are consistent with the corresponding homonuclear ^1H 3D ROESY-TOCSY and 2D NOESY experiments (spectra not shown). Homonuclear 3D experiments provide increased spectral resolution and additional intra-residue TOCSY connectivities for spin system identification in cases of resonance overlap. Cross peaks in the homonuclear TOCSY-NOESY spectra (Figs. 2C,D) correspond to magnetization transfer following the path

$$H_w(\omega_1) \xrightarrow{\text{NOE}} H_A(\omega_2) \xrightarrow{\text{TOCSY}} H_B(\omega_3) \qquad (1)$$

where H_w are the water protons, and H_A, H_B are two peptide protons belonging to the same residue.

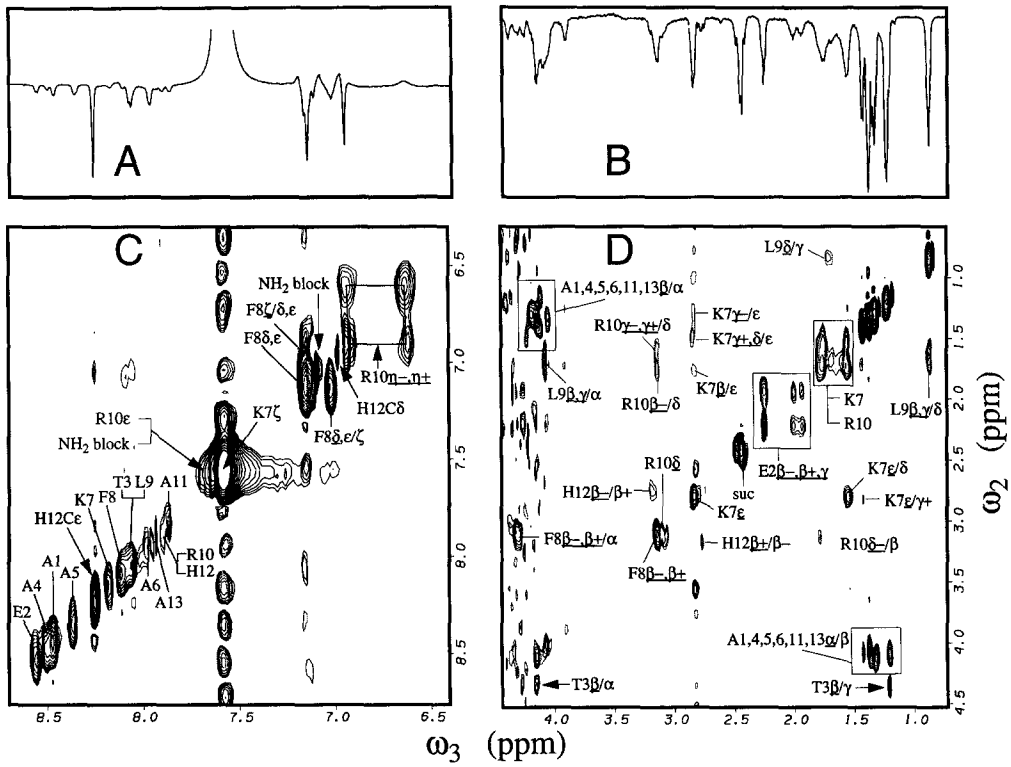

Fig. 2. Proton NMR spectra showing NOEs and ROEs between RN-24 protons and water protons. (A,B) Cross sections of a 2D ROESY; (C,D) cross sections of a 3D NOESY-TOCSY, both corresponding to the water resonance of RN-24 at $\omega_1 = \omega_{H_2O}$. In C and D, cross peaks marked with an arrow (except for baseline noise) denote positive cross-peak signs. Protons which evolve during t_2 (protons H_A in Eq. 1) are underlined; protons involved only in TOCSY but not in NOESY transfers are not underlined. In C, resolved backbone and side-chain amide and aromatic protons are labeled using the one-letter amino acid residue code; 'NH_2 block' refers to the C-terminal NH_2 proton pair of RN-24. Some cross peaks involving backbone α- and side-chain protons are labeled in D. Data acquisition parameters are described in Fig. 1. While short correlation times lead to negative cross peaks ($\Gamma_{NOE} > 0$, $\Gamma_{ROE} > 0$), long residence times cause $\Gamma_{NOE} < 0$, $\Gamma_{ROE} > 0$; chemical exchange peaks have a positive sign in both NOESY and ROESY spectra ($\Gamma_{ex} < 0$). The spectra are plotted after inverting the low-field region (6–9 ppm) to improve readability. The spectra were collected at 500 MHz proton frequency and at a temperature of 278 K.

Most cross peaks in the 3D NOESY-TOCSY and 2D ROESY spectra (Fig. 2) exhibit negative signs (i.e., the corresponding NOESY and ROESY cross-relaxation rate constants, Γ_{NOE} and Γ_{ROE}, are both positive), indicating that the corresponding water–peptide dipolar interactions are dominantly modulated on time scales τ that are fast compared to ω_0^{-1} ($\tau < 350$ ps). These peaks involve all backbone and nearly all side-chain protons. However, in some cases peaks do overlap or peak intensities are attenuated due to the application of a low-pass filter (see Materials and Methods). In Figs. 2C,D, all positive NOE cross peaks ($\Gamma_{NOE} < 0$, marked with an arrow) involve either a labile proton or a proton in close spatial proximity to a labile proton. In the first case direct chemical exchange between H_2O and the labile proton occurs, which is manifested by positive cross peaks ($\Gamma_{ex} < 0$) in both the NOESY-TOCSY and the ROESY spectra. Such peaks are observed for the labile side-chain N^{ζ} and N^{η} protons of Lys^7 and Arg^{10}, respectively (Fig. 2), and reflect direct exchange with the solvent. In the other case, positive cross peaks in the NOESY-TOCSY spectrum ($\Gamma_{NOE} < 0$) correspond to negative peaks in the ROESY spectrum ($\Gamma_{ROE} > 0$), as expected for an intrapeptide cross-relaxation mechanism. Therefore, these cross peaks visualize cross relaxation to the labile proton, which is in fast exchange with H_2O (10 ns $< \tau <$ 1 ms), rather than directly to H_2O (Liepinsh et al., 1993). Examples are the side chain of Thr^3, which cross relaxes with the labile hydroxyl proton, and the ring C^{δ} and C^{ε} protons of His^{12}, which reflect cross relaxation with a labile ring NH proton (Fig. 2). Additional direct cross relaxation between the water and Thr^3 and His^{12} residues might also be present, but is obscured by the stronger cross-relaxation process to the labile proton resonating at the water frequency. Generally, such NOE effects to labile protons can hinder direct quantitation of true hydration cross peaks. No cross-relaxation effects involving the labile proton of a (possibly) protonated carboxylic acid group of Glu^2 are observed, in agreement with earlier studies (Liepinsh et al., 1993).

From the negative NOEs in Fig. 2 it follows (except for overlapping peaks) that the whole backbone of RN-24 interacts with highly mobile water molecules. Overlaps of NH resonances involve Leu^9 and Thr^3, as well as Arg^{10} and His^{12}. However, peak amplitude differences, when compared to the rest of the backbone cross peaks, are observable for various H_2O NOE cross peaks: Ala^6 shows

a strong ROESY and a weak NOESY peak, which may be associated with a time-scale effect where the corresponding effective correlation time approaches 350 ps. For the C-terminal NH protons of Ala^{11} and Ala^{13}, both NOESY and ROESY cross peaks are weak, which is characteristic for short correlation times.

NOEs between H_2O and backbone α-protons can best be assessed via true 3D cross peaks, as shown in Fig. 2D. Moreover, negative NOEs between H_2O and side-chain protons are shown in Figs. 2C and D. Cross relaxation between H_2O and the peptide protons which are underlined gives rise to the corresponding cross peak. It appears that side-chain hydration occurs in all amino acids of RN-24, irrespective of their hydrophobicity. An example is Leu^9, for which all β-, γ- and methyl protons show NOEs to H_2O (Fig. 2D). This is not unexpected, since in a small peptide like RN-24 hydrophobic side chains cannot be buried effectively by other amino acids. We cannot identify peptide protons that are shielded from water and do not cross relax with H_2O. The data imply that hydration is occurring at all sites along the peptide backbone and the side chains, irrespective of the sequence position and hydrophobic character of the amino acids.

Uniform fast-time-scale hydration has been found for oxytocin and this has been attributed to its flexibly disordered behavior (Otting et al., 1991b). In the current context similar hydration characteristics are observed for RN-24, even though it adopts significant populations of secondary structure. Although the various fast interchanging conformational substates, such as folded, partially folded and unfolded states, are expected to have variable hydration properties, the experimental data reflect the ensemble average and do not allow unambiguous determination of the solvation of individual conformational substates; the spectra reflect primarily the 'background' hydration originating from the manifold of unfolded or partially unfolded substates. Molecular dynamics simulations on RN-24 indicate that, even within this largely helical peptide, transient deviations from ideal helical geometry frequently occur that are often associated with formation of water–peptide hydrogen bonds (Soman et al., 1993).

The current study provides experimental evidence that both the backbone and side chains of the peptide fragment RN-24 are on average fairly uniformly surrounded by highly mobile water molecules which might facilitate interconversions between conformational substates. The secondary structure in RN-24 is only marginally stable. It is expected that during protein folding, substantial desolvation of backbone and side chains will accompany the progressive stabilization of local elements of structure. Indeed, differential hydration of side chains has recently been observed (Yao et al., 1994) for a peptide that forms an unusually highly populated type VI turn conformation which is stabilized by local hydrophobic interactions. Furthermore, we have recently observed differential backbone hydration in a peptide that forms a stable coiled coil conformation (A. Karimi and P.E. Wright, unpublished observations).

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