Time-resolved isotope-edited IR spectroscopy – Detailed insight into the fast folding dynamics of α-helical peptides

M Volk, A Pozo Ramajo

Department of Chemistry and Surface Science Research Centre, University of Liverpool, Liverpool, L69 3BX, UK

S A Petty, A Starzyk, S M Decatur

Department of Chemistry, Mount Holyoke College, South Hadley, Massachusetts, 01075, USA

Main contact email address: *m.volk@liv.ac.uk*

Introduction

The question of how a protein folds to its unique three-dimensional structure, which is the basis of the biological function of the protein, is one of the most challenging questions currently investigated in biological research. *Dynamic* aspects of this folding process are important for elucidating the mechanism by which protein folding proceeds, but are also of high relevance in the context of predicting the final structure of engineered peptides. Natural proteins as well as designed peptides will only adopt the predicted lowest free-energy functional structure on a reasonable time scale, if there is an efficient folding pathway for the polypeptide which avoids the accumulation of metastable non-functional folds.

The first phase of protein folding is generally accepted to be the formation of secondary structures, such as α-helices, β-sheets or turns, which then may act as nucleation sites for the formation of the full structure. Thus, the investigation of the dynamics of secondary structure formation is of utmost importance for protein folding, and has received increased attention in recent years. In particular, the folding of α-helical peptides has been reported to occur on the 100 ns-time scale, mostly from experiments using fast laser-induced temperature jumps[1,2]. The rise in temperature disturbs the helix-coil equilibrium of the peptide, and the resulting helix-coil relaxation can be observed by a suitable technique, such as nanosecond time-resolved IR-spectroscopy of the amide I-band near 1650 cm^{-1}, which is highly sensitive to secondary structure[3].

Standard IR spectroscopy is limited to the observation of the *overall* helical content of such peptides, as it does not allow the distinction between different secondary structures at residue level. However, IR spectroscopy can be made site-specific by isotopic labeling of specific backbone carbonyls with ^{13}C, which shifts the amide I frequency by approximately 40 cm^{-1}. This method has been used previously for observing helix-coil stability of α-helical peptides at residue level[4].

Here, we report results of dynamic measurements on a series of α-helical model peptides. The helix folding dynamics of different peptide sections were observed separately, using isotopic labeling and IR detection in the amide I band. The results show that the α-helix folds more rapidly at the helical C-terminus than at the N-terminus.

Experimental

The following labeled peptides were investigated (underlined residues are ^{13}C-labeled):

4A	AAAAKAAAAKAAAAKAAAAY-NH_2
4AL1	<u>AAAA</u>KAAAAKAAAAKAAAAY-NH_2
4AL2	AAAAK<u>AAAA</u>KAAAAKAAAAY-NH_2
4AL3	AAAAKAAAAK<u>AAAA</u>KAAAAY-NH_2
4AL4	AAAAKAAAAKAAAAK<u>AAAA</u>Y-NH_2

Synthesis, CD spectra and FTIR analysis of these peptides have been described previously[4]. No significant absorbance was found at 1600 cm^{-1} for the unlabeled peptide 4A, but FTIR spectra of the labeled peptides show a band near 1600 cm^{-1}, due to the labeled residues in helical conformation. The size of this absorbance band correlates well with the predicted helix content of the labeled sections, which is significantly lower for 4AL1 and 4AL4 than for 4AL2 and 4AL3, and thus did confirm the predicted fraying of the helix at both the N- and C-terminus.

Peptides were dissolved at a concentration of 20 mg/ml in 0.1 % phosphoric acid D_2O buffer and placed in a temperature-controlled IR cell (50 μm spacer). A Nd:YAG/dye laser system with IR difference frequency generation (CLF laser loan pool system NSL4) provided 3 mJ pump pulses of 7 ns pulse width for direct excitation of the overtone band of D_2O at 1970 nm, yielding temperature jumps of 5°C. The size of the temperature jumps was determined from the induced absorbance change of D_2O at 1570 cm^{-1}, where no peptide absorbance is found, calibrated by temperature-dependent FTIR spectra. Time-resolved IR absorbance changes at 1601 cm^{-1}, corresponding to the amide I frequency of the ^{13}C-labeled residues in helical conformation were monitored as described previously[2], using a tunable IR-laser diode and a 50 MHz IR-detector (overall signal rise time: 14 ns).

Results

Absorbance changes at 1601 cm^{-1}, induced by temperature jumps from 11°C to 16°C, are shown in Figure 1. For all peptides, we observed an instantaneous absorbance bleach, which is largely due to an absorbance decrease of D_2O at 1601 cm^{-1} with increasing temperature. A smaller part of the instantaneous bleach arises from a temperature-induced shift of the helical amide I frequency, as reported before[1,2]. For the unlabeled peptide 4A, no further absorbance changes were observed at 1601 cm^{-1} on the nanosecond time scale, (Figure 1), although the absorbance at 1632 cm^{-1} decreases upon helix melting on the 100 ns-time scale, as expected (data not shown). For the labeled peptides, the absorbance at 1601 cm^{-1} decreases on the 100 ns-time scale due to the melting of α-helical labeled peptide sections. As expected, the amplitude of these absorbance changes is smaller for the peptides labeled at the peptide ends than for those labeled in the center, due to the lower helix content of the peptide ends. The segmental helix-coil relaxation times, obtained from fits of data for temperature jumps from 4°C to 9°C and 11°C to 16°C, are given in Figure 2.

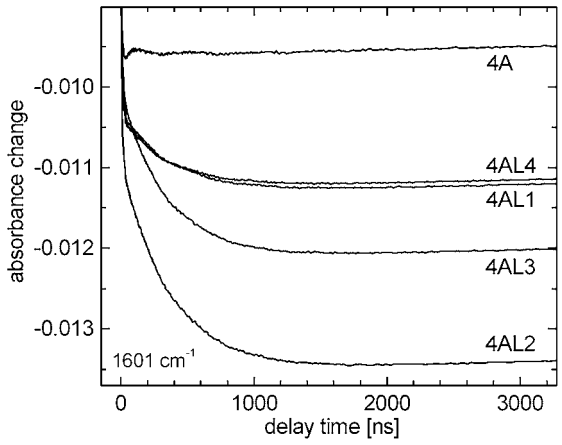

Figure 1. Time-dependent absorbance changes at 1601 cm^{-1} for the unlabeled peptide 4A and the labeled peptides 4AL1, 4AL2, 4AL3 and 4AL4, after temperature jumps from 11°C to 16°C.

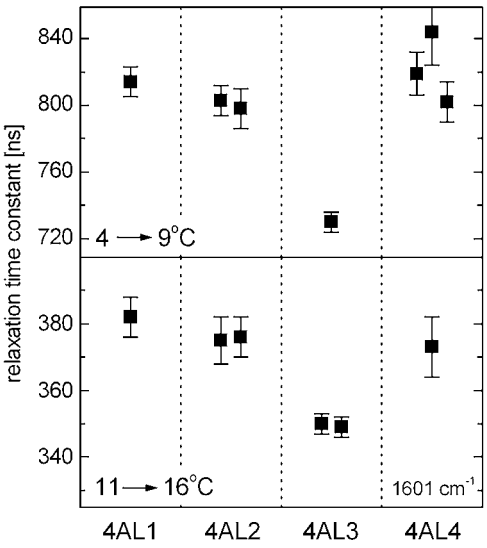

Figure 2. Segmental helix-coil relaxation time constants measured on the isotopically labeled peptides after temperature jumps from 4°C to 9°C and 11°C to 16°C, respectively.

Figure 2 shows that the third peptide section has a significantly faster helix-coil relaxation than the other sections. Most importantly, the helix-coil relaxation of the third section is faster than that of the second section, although the two sections are identical in amino acid content and sequence, have the same neighboring residues and have a highly similar helix content[4].

Discussion

As described below, we ascribe the observed difference between the helix-coil relaxation times of the second and third peptide sections to different dynamics of the C- and N-terminal helix ends. Previous dynamic measurements on α-helical peptides isotopically labeled in different sections[5] were not able to show this effect, since the different sections were highly inhomogeneous due to the presence of capping groups. Similarly, the dynamics of the first and fourth sections in the peptides investigated here may be affected by end or capping effects, and thus will not be discussed any further.

Alanine-based peptides form only marginally stable α-helices and are in a dynamic equilibrium between structures containing disordered *and* helical peptide segments of continuously changing length. It has been shown previously that most of the helical structures extend over the center of the peptide, whereas the ends are frayed[4]. With an overall helicity of 50-60% in the peptides investigated here, there is a significant probability for helices to start in the second section and/or end in the third one, as shown schematically in Figure 3. Upon increasing the temperature, helical structures decrease in length by "unravelling" from their ends. Thus, the segmental helix-coil relaxation times observed for the second and third peptide sections are expected to be dominated by the helix-coil relaxation of the α-helical N- and C-terminal ends, respectively, and our results show that the helix-coil dynamics of the C-terminal helix end is faster than that of the N-terminal end.

The two ends of an α-helix are not structurally equivalent. In particular, the backbone carbonyls of the last three residues at the C-terminal end are not hydrogen bonded. This leads to reduced steric restrictions and a wider conformational distribution of the final helical residues[6]. Also, these carbonyls are less protected from solvent access by side chains than those at the helical N-terminus. We propose that the faster helix-coil dynamics at the C-terminal helix end observed here result from these effects which may lead to a lower activation barrier for intrapeptide hydrogen bond formation.

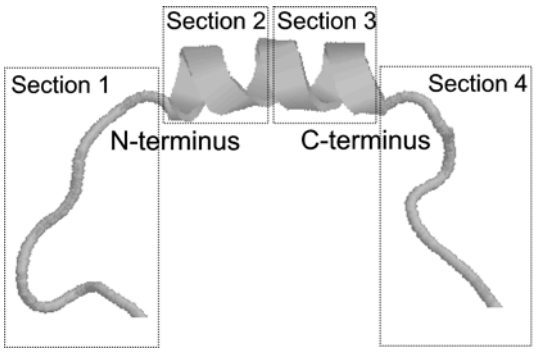

Figure 3. Snapshot of the distribution of helical structure over the different sections of a short α-helical peptide (schematic).

Faster helix folding and unfolding at the C-terminal end has previously been predicted by Molecular Dynamics simulations[7,8], but the results reported here are the first experimental observations of this effect. We conclude that helix propagation is direction dependent, i.e. an α-helix, once nucleated, grows more rapidly towards the peptide's C-terminus than towards the N-terminus. This has important implications for describing the first steps of protein folding and will need to be taken into account when trying to simulate this process.

Conclusion

We have shown that time-resolved IR spectroscopy in combination with isotopic labeling can be used to obtain helix-coil dynamics of individual peptide sections. The results show that the folding of an α-helix proceeds more rapidly at the helical C-terminus than at the N-terminus, presumably because of reduced steric restrictions at this helix end due to the non-hydrogen bonded carbonyl groups.

Acknowledgements

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