Coupled Vibrations in Peptides and Proteins: Structural Information Using 2D-IR Spectroscopy

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Summary

Predicting the folded structure of a protein requires a detailed understanding of the fundamental forces that drive protein folding, and obtaining this understanding is an ongoing challenge involving several scientific disciplines. In this thesis we provide experimental evidence of how detecting the coupling between vibrations using two-dimensional infrared (2D-IR) spectroscopy can give access to structural information at the molecular level of proteins and peptides. The information that we obtain can be used to determine general principles that lie at the basis of the stabilization of the folded structure of proteins and peptides. The size of the systems that we study ranges from a dimer formed by a few atoms to proteins with several hundreds of atoms. We investigate two types of vibrations: those involved in salt bridges, and backbone amide I vibrations. The first three chapters of this thesis are introductory. In Chapter 1 we introduce the basic structural features of proteins, putting emphasis on secondary structure and tertiary contacts. We also introduce vibrational spectroscopy, and 2D-IR spectroscopy in particular, as a powerful tool to investigate these structural features. In Chapter 2 we provide a description of 2D-IR spectroscopy in the frequency-domain configuration, using a narrow-band pump broad-band probe setup, and discuss the essential elements of a typical 2D-IR spectrum of two coupled oscillators. Chapter 3 contains a brief summary of non-linear response theory, followed by a description of the vibrational-exciton model for two and three coupled anharmonic oscillators. This formalism allows us to retrieve structural information from the non-linear response of a system upon interaction with resonant light fields. The following six chapters of this thesis describe the outcome of our research. In Chapter 4 we present the characterization of the 2D-IR response of salt bridges in solution. Salt bridges are important interactions for stabilizing molecular structure, but up to now it has been difficult to study their geometry in solution. We investigate a salt bridge between guanidinium (Gdm^+) and acetate (Ac^-). We find that as a result of salt bridge formation the vibrational modes of Gdm^+ and Ac^- become coupled, which is manifested as cross peaks between these modes in the 2D-IR spectrum. From a fit to the 2D-IR spectra using the vibrational-exciton model, we determine that the salt-bridge geometry is planar. We also investigate a salt bridge between methylguanidinium ($MeGdm^+$) and Ac^-, and the 2D-IR spectra of this system enables us to detect two possible bidentate geometries of this salt bridge. In Chapter 5 we investigate the 2D-IR response of salt bridges between arginine (Arg^+) and carboxylate (Glu^-) in peptides in solution. We inves-

tigate three peptides whose structure is stabilized by such salt bridges: a β-turn, an α-helical peptide, and a coiled coil. We find changes in the 2D-IR response of these salt bridges as compared to the isolated salt bridges, most likely due to conformational restrictions. We find evidence for a bidentate salt bridge in the β-turn and a monodentate one in the α-helical peptide, whereas the coiled coil shows signatures of both types of salt bridge. Chapter 6 contains a study of the amide I vibrations of two tripeptides. We investigate how their conformation is influenced by the presence or absence of charge on the C-terminus and side groups, and we analyze the measurements using the vibrational-exciton model. With these results we obtain the dihedral angles (ϕ, ψ) of the central C_α atom, and find that these angles are insensitive to the charges of the end and side groups of the tripeptides. Chapter 7 contains a study of the chemical-denaturation mechanism of Gdm^+, a widely used denaturant. We study the effect of Gdm^+ on α-helices and β-sheets by investigating the guanidinium-induced denaturation of two well-known proteins, lysozyme and α-chymotrypsin. We find that upon Gdm^+-denaturation, the β-sheet protein shows a complete loss of β-sheet structure, whereas the α-helical protein maintains most of its secondary structure. These results suggest that Gdm^+ disrupts β-sheets much more efficiently than α-helices, possibly because in the former hydrophobic interactions are more important. In Chapter 8 we study the denaturation mechanism of guanidinium further by probing changes in the secondary structure of a designed mini-protein, a zinc-finger mutant, which has structural properties that differ from most natural proteins. Our results indicate that the α-helical segment is stabilized by hydrophobic interactions, and that it unfolds when the hydrophobic core is disrupted. We find residual β-sheet structure, which suggests that this motif is not stabilized by hydrophobicity, and that it is not significantly destabilized by the absence of a hydrophobic core. In Chapter 9 we introduce the topic of amyloid-fibril formation, and show how the appearance of fibrils in lysozyme can be induced with a 'temperature-shock'. Nucleation of fibrils probably occurs after the hydrophobic core of the protein is exposed due to thermal denaturation. We show that 2D-IR spectroscopy is a good probe to follow protein fibril formation.