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The protein irreversible denaturation studied by means of the bending vibrational mode

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HIGHLIGHTS

- We study the microscopic mechanisms at the base of the protein irreversible unfolding.
- We follow the thermal evolution of the Amide I and II vibrational modes.
- The driving force is the coupling between hydration water and protein.
- After the denaturation the contribution from alpha-helices decreases.
- After the denaturation the contribution from beta-sheets increases.

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ABSTRACT

We study by means of the infrared bending vibrational mode the microscopic mechanisms that are at the base of protein irreversible denaturation. In particular, we follow the thermal evolution of the Amide I and II vibrational modes of lysozyme residuals from ambient temperature toward the temperature of irreversible unfolding. Our results indicate that the thermal changes of the coupling, by means of the hydrogen bond, between hydration water molecules and the different chemical groups of the protein are the main microscopic mechanisms underlying the unfolding process.

1. Introduction

The formation and stability of the chemical structure of proteins depends on the occurrence of promiscuous non-covalent interactions, which can form between any suitable pair of residues. This leads to the possibility of forming transient intermediate structures, through "non-native" interactions or frustration [1], that can be interpreted as roughness on the folding energy landscape [2].

Proteins are very complex systems characterized by a number of amino acid residuals whose flexible chain folds in a precise way to form the protein native state. The folding process depends on many variables such as temperature, pressure and pH. Furthermore, the existence of non-native interactions can retard or disrupt the protein search for its native state, but it may also direct the beginning of the folding process [3]. In terms of frustration, the native states of proteins are considered

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to be minimally frustrated, so more stable [4]. If the kinetics of the folding process presents any anomalies (misfolding), some conformational variations, such as from α-helix or random coil to β-sheet, may be induced. Usually this kind of variations cause protein aggregation with subsequent formation of insoluble fibrils [5] and the occurrence of some neurodegenerative illnesses such as Alzheimer's and Parkinson's diseases.

It is well known that the biological activity of proteins is governed by their structural and dynamical properties and by the interaction with the solvent [6,7]. In particular, water is the solvent of life par excellence and at least a single layer of water molecules surrounding the protein surface is needed to trigger its biological activity [8,9]. The uniqueness of water is essentially due to its strong ability to form "stable" hydrogen bonds (HB). In biological systems water, by hydrogen bonding different amino acid residuals, acts as a glue contributing to the structural conformation of the system [10].

One of the most used experimental techniques to study protein structure and ligand–protein interaction is InfraRed spectroscopy (IR) [11–13]. Although X-ray diffraction, Circular Dichroism and Nuclear Magnetic Resonance (NMR) spectroscopy have an higher resolution, IR spectra can be obtained from samples in physical states not amenable to the high-resolution methods and provide useful structural information. In the IR spectrum of proteins, the most intense band is that of Amide I (1600–1700 cm^{-1}) which is mostly a carbonyl (C=O) stretching [12,14]. In details, the Amide I band of proteins can provide useful structural information because it represents several overlapping bands of different structural elements, such as α-helices, β-sheets, turns, and irregular structures. For example, β-sheets components are centered at about 1620 cm^{-1} and at about 1685 cm^{-1}, α-helix at about 1654 cm^{-1} and random coil at about 1672 cm^{-1} [12].

Other Amide bands fall in the same IR spectral region of Amide I, namely Amide II and Amide III extending respectively from 1480 to 1580 cm^{-1} and from 1300 to 1450 cm^{-1}. The Amide II mode is essentially the combination of the N–H in plane bending and of the C–N stretching, while Amide III consists of more complex vibrational modes [14,15]. The different Amide contributions to the IR bending region are reported in Fig. 1. In the figure, the Amide I and II vibrational modes are also represented on a peptide fragment.

We are mainly interested in the thermal evolution of the solvent accessible regions of the protein backbone during the denaturation process. For this reason, we will focus mainly on the Amide II band that is more influenced by the interaction with the solvent. Besides, Amide I and III bands are very complex because they consist of different overlapping components. The shape of the Amide IR bands depends on the coupling among neighboring amide groups and intramolecular hydrogen bonding.

It is noteworthy to stress that interaction leads to coupling, meaning that when two oscillators interact with each other, the vibration of one oscillator exerts an oscillating force on the vibration of the other. Indeed, when two oscillators contribute together to one or more normal modes of the system of oscillators, we can say that they are coupled. When a hydrogen bond is formed, the bending vibration of water molecules couples with the N–H bending and with the C=O stretching of protein residuals. The C=O stretching is in turn coupled with the C–N stretching mode. So by looking at these modes we can probe the interaction of hydration water with the hydrophilic moieties of the protein surface. These concepts are crucial for our analysis which is in fact aimed to study the effect of the coupling between hydration water and amide groups of proteins in driving their physical and chemical properties responsible for the biological activity.

The focal point of our work is to shed light on the microscopic mechanisms that intervene toward the protein irreversible unfolding when the solvent accessibility changes dramatically and the native state of the protein is completely corrupted.

2. Materials and methods

Hen egg white lysozyme is a small globular protein (14.4 kDa) constituted by 129 amino acid residuals. It is present within animal tissues and is usually used as anti-inflammatory and antibacterial. Lysozyme samples were obtained from Fluka (L7651 three times crystallized, dialyzed, and lyophilized) and used without further purification. Samples were dried, hydrated isopiestically, and controlled by means of a precise procedure [16] in order to reach a hydration level, h (grams of water per gram of dry protein) equals to 0.3. This value corresponds to the coverage of the protein surface by the first hydration shell or layer.

Fourier Transform InfraRed (FTIR) absorption measurements were performed at ambient pressure in the OH bending spectral region, in the temperature range $290 \leq T(K) \leq 360$. We used a Bomem DA8 Fourier transform spectrometer, operating with a Globar source, in combination with a KBr beamsplitter and a DTGS/KBr detector. To avoid saturation effects, we used the attenuated total reflection (ATR) geometry, generally insensitive to sample thickness. The spectra of interest were recorded with the resolution of 4 cm^{-1}, automatically adding 200 repetitive scans in order to obtain a good signal-to-noise ratio and highly reproducible spectra; then they are normalized by taking into account the effective number of absorbers [17]. The analysis of the measured IR spectra is performed by means of a peak deconvolution by considering the contributions from water and from the different amide components. For what concerns the water OH bending mode, which is much more less intense than the water OH stretching mode at higher frequency, we distinguish two different contributions discriminating between water molecules involved in the HB network (NW water contribution) and not involved in the HB network (NN water contribution). In Fig. 2 the OH bending of bulk water at ambient temperature is reported to highlight the existence of these two contributions.

In order to fit the Amide I region we use five Gaussian functions centered at: 1685 cm^{-1} (β-sheet); 1672 cm^{-1} (random coil); 1654 cm^{-1} (α-helix); 1640 cm^{-1} (NN water component) and 1620 cm^{-1} (β-sheet). The water component (NW) coming from water molecules hydrogen bonded in their characteristic network is centered at about 1560 cm^{-1} and is broader with