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Temperature- and pressure-induced unfolding of ubiquitin

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Abstract

Temperature- and pressure-induced denaturation of the protein ubiquitin was investigated using Fourier-transform infrared (FTIR) spectroscopy. On the basis of IR spectral parameters, different states are distinguished and a tentative stability diagram of the protein is presented. Features of the secondary structure remain distinct at high pressure and part of the protein restructures. Contrary to the case for temperature denaturation, the secondary structure changes during compression and decompression are fully reversible and no aggregation occurs.

1. Introduction

Most studies dealing with protein denaturation have been carried out at atmospheric pressure using temperature, pH, or chemical denaturants as experimental variables [1]. Besides temperature and chemical potential, pressure represents a further important thermodynamic variable and the volume changes obtained, which are associated with the observed transitions, correspond to a fundamental physical parameter of the protein–solvent system. A more complete description of the pressure-denatured states of proteins, therefore, should be quite useful to understanding of protein folding phenomena. Different modes of denaturation, including pressure, may correlate differently with the roughness of the energy scale and slope of the folding funnel [2]. A number of reviews on effects of pressure on proteins are available now [3–7]. In addition to being an important thermodynamic variable, pressure can also be used as a valuable means of triggering and investigating the kinetics of the unfolding/refolding transition of proteins [7, 8].

Here we used high pressure Fourier-transform infrared (FTIR) spectroscopy to study the reversible pressure-induced unfolding and denaturation of ubiquitin and we compare the results with those obtained for the temperature-induced transition. Ubiquitin is a single-domain protein without disulphide bonds with 76 amino acids and a molecular weight of 8.5 kDa [9]. Its crystalline state contains about 16% α-helices, 37% β-sheets, and 37% other structures [10]. Four of the five β-strands form a β-sheet plane with a α-helix arranged above.

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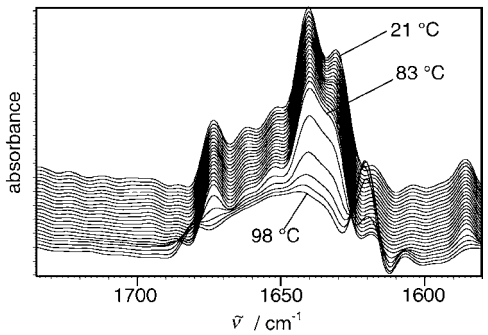

Figure 1. Deconvoluted FTIR spectra of ubiquitin as a function of temperature.

2. Materials and methods

Ubiquitin was purchased from Sigma Chemical Co. The protein was dissolved at a concentration of 5% w/w in a 10 mM TRIS-buffer in 99.9% D_2O at pH 7 for the high pressure experiments. For the temperature-dependent measurements we used a 10 mM phosphate buffer. The pH value of the solution was adjusted to pH 7. Before the measurements, the solution was heated to 55 °C for 1 h to allow for deuterium exchange of the labile amide protons of ubiquitin. FTIR spectroscopy was used to determine the secondary structure elements. Details of the experimental set-up are described elsewhere [8]. Fourier self-deconvolution of the IR spectra was performed with a resolution enhancement factor of 2.4 and a bandwidth of 12 cm^{-1}. The fractional intensities of the secondary structure elements were calculated from a band-fitting procedure assuming a Gaussian–Lorentzian line-shape function [11, 12].

3. Results

Figure 1 shows the deconvoluted IR spectra of ubiquitin between 20 and 100 °C at pH 7.0 and ambient pressure after H/D exchange at 55 °C. Up to about 83 °C, the band maximum increases slightly and then it shifts drastically to higher wavenumbers. At 83 °C, two new bands appear, around 1620 and 1684 cm^{-1}, respectively, which are characteristic of the formation of intermolecular β-sheet structures and are thus indicative of the unfolding and aggregation of the protein at that temperature. The evolution of secondary structures with temperature is illustrated in figure 2. Within the experimental error, the fractional intensities of the intramolecular β-sheets ($\sim$33%), α-helices ($\sim$12%) and turns ($\sim$15%) remain constant, and are therefore not included in the plot. The temperature-induced increase of band intensities of intermolecular β-sheets is clearly visible above 83 °C, which is accompanied by a corresponding decrease in band intensities of disordered (non-periodic) structures.

The pressure dependence of the deconvoluted FTIR spectra of ubiquitin at pH 7.0 and 21 °C are shown in figure 3. With increasing pressure, the amide I′ band maximum decreases from 1638.7 cm^{-1} at 1.4 kbar to 1637.6 cm^{-1} at 5.4 kbar, which is expected to occur for hydrogen-bonded carbonyl groups in the elastic regime [13]. Above 5.4 kbar the band maximum shifts to larger wavenumbers, indicating that the pressure-induced unfolding of the protein takes place. The changes of the fractional intensities of the different amide I′ subbands are presented in figure 4. Within the experimental error, the fractional intensity of intramolecular β-sheets is constant, and is therefore not included in the plot. The pressure-induced denaturation as judged by changes in the fractional band intensities also begins at 5.4 kbar and the transition is complete at 6.4 kbar. At the transition, the fractional intensities of α-helices and turns increase whereas the population of disordered (non-periodic) structures decreases. Assuming that the

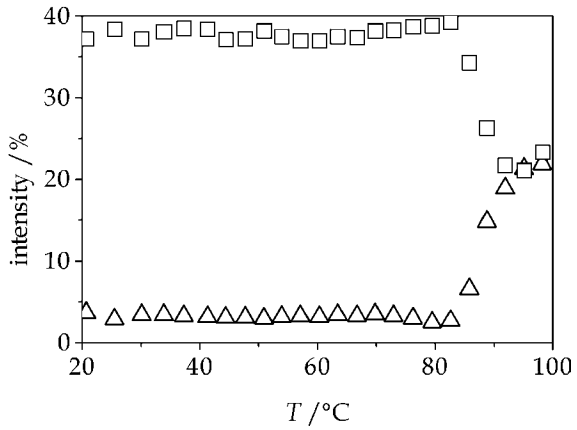

Figure 2. Fractional intensities of secondary structure elements (□: disordered; △: intermolecular β-sheets) of ubiquitin as a function of temperature.

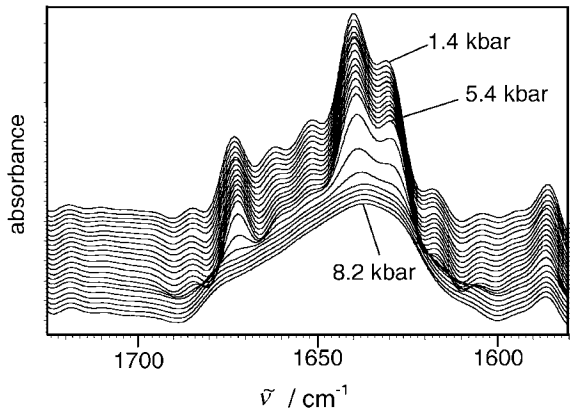

Figure 3. Deconvoluted FTIR spectra of ubiquitin at different pressures for $T = 21\,^{\circ}\mathrm{C}$.

changes taking place can be assigned to a simple two-state unfolding process, the volume change of unfolding can be calculated to be $\Delta V^0 = -50 \pm 20$ ml mol^{-1}.

Corresponding measurements of the pressure-induced unfolding of ubiquitin at different temperatures result in a p, T stability diagram, which is shown in figure 5. At 31 °C, the point of maximum pressure stability is reached.

4. Discussion and conclusions

The FTIR measurements indicate that ubiquitin unfolds and aggregates above 83 °C. The protein unfolds at pressures above about 5 kbar in the temperature range from 7 to 50 °C. Interestingly, the pressure-denatured state contains more α-helical and turn structures than the native state. The increase of these structure elements is accompanied by a decrease of disordered structures, which must arise from an overall volume decrease of the system. Thus no complete unfolding forming essentially disordered structures, but instead a partial rearrangement of the secondary structure elements is observed at high pressure for this particular protein.

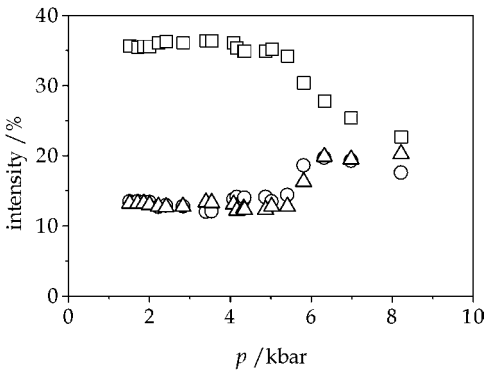

Figure 4. Fractional intensities of secondary structure elements (□: disordered; ○: α-helical; △: turns) of ubiquitin at different pressures for $T = 21\,^{\circ}\mathrm{C}$.

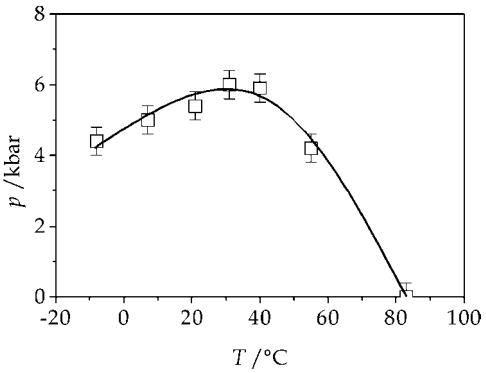

Figure 5. p, T stability diagram of ubiquitin.

Interestingly, also in kinetic studies a stable partially structured state, the A-state, was observed for ubiquitin under special solvent conditions [14]. In the A-state, a partially structured α-helix covers the hydrophobic face of the β-sheets, and this α-helix seems to be considerably more flexible than that in the native state. It has been speculated that the A-state could plausibly lie on the folding pathway, with the A-to-native transition being a late step in the folding process. Pressure has indeed been shown in a series of studies now to be able to stabilize conformational substates which are observed in kinetic folding studies under ambient pressure conditions [7, 15]. This could as well be the case in this particular situation. To date, only a few other proteins are known which form ordered structures under high pressure. In the model protein (L-)polylysine pressure induces a transformation from an unordered to a α-helical form [16]. In BPTI, application of pressure transforms α-helical and disordered structures into a β-sheet [13].

References

[1] Fersht A 1999 *Structure and Mechanism in Protein Science* (New York: Freeman)
[2] Bryngelson J D *et al* 1995 *Proteins: Struct. Funct. Genet.* **21** 167
[3] Weber G and Drickamer H 1983 *Q. Rev. Biophys.* **16** 89
[4] Heremans K and Smeller L 1998 *Biochim. Biophys. Acta* **1386** 353
[5] Silva J L and Weber G 1993 *Annu. Rev. Phys. Chem.* **44** 89
[6] Gross M and Jaenicke R 1994 *Eur. J. Biochem.* **221** 617
[7] Winter R and Jonas J (ed) 1999 *High Pressure Molecular Science (NATO ASI Series E, vol 358)* (Dordrecht: Kluwer Academic)
[8] Panick G *et al* 1998 *J. Mol. Biol.* **275** 389
[9] Rechsteiner M 1984 *Ubiquitin* (New York: Plenum)
[10] Vijay-Kumar S *et al* 1985 *Biochemistry* **82** 3582
[11] Byler D M and Susi H 1986 *Biopolymers* **25** 469
[12] Prestrelski S J *et al* 1991 *Biochemistry* **30** 133
[13] Goossens K *et al* 1996 *Eur. J. Biochem.* **236** 254
[14] Harding M M *et al* 1991 *Biochemistry* **30** 3120
[15] Jonas J *et al* 1998 *Biophys. J.* **75** 445
[16] Wong P T T *et al* 1990 *Biochemistry* **29** 254