Pressure–Temperature Induced Unfolding and Aggregation of Proteins

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Abstract. The effect of pressure and temperature on protein unfolding and aggregation as detected by infrared spectroscopy is discussed with a number of examples. At 45–50 °C the spectra of pressure denatured myoglobin and lipoxygenase show bands, but only after pressure release, that are typical for temperature induced protein aggregation.

1 Introduction

Proteins can be unfolded by high hydrostatic pressure as well as by low or high temperature. This gives rise to an elliptic phase diagram in the temperature-pressure plane which describes the conditions under which the protein is in the native or the unfolded state at a given temperature and pressure with a specified solution composition. For the interpretation of the elliptic shape of the phase boundaries we favour a thermodynamic model that assumes that the changes in entropy, ΔS, and in volume, ΔV, of the unfolding are temperature and pressure dependent. Within this physical model, the diagram gives also unique information on the difference in heat capacity, ΔC_p, compressibility, Δβ, and thermal expansion, Δα, between the unfolded and the folded state of the protein. The latter quantities can be related to the differences in dynamics of the protein conformations. Direct information on these quantities is difficult to obtain otherwise [1].

In many, if not almost all, cases the diagrams are obtained from kinetic rather than from equilibrium data. The first diagram that was obtained by Suzuki [2] on the denaturation of ovalbumin and hemoglobin was of such a kind. Many kinetic diagrams have been published since then. The inactivation kinetics of microorganisms gives similar diagrams strongly suggesting that the inactivation of proteins and/or enzymes is the main factor controlling these events [3, 4]. Similar diagrams are observed for the survival conditions of deep sea bacteria. Yayanos has proposed calling these pTk diagrams [5]. In all these instances it is still possible to interpret the data in terms of a thermodynamic model as is done in the formulation of rate processes. The denaturation process is usually interpreted in terms of a preequilibrium between the native (N) and the unfolded (U) state followed by an irreversible step which leads to the inactivated enzyme or the aggregated protein (A):
The negative activation energies that are obtained for the denaturation of proteins at high pressure [2] strongly supports the model that the kinetic data largely reflect the thermodynamics of the unfolding of the protein from the N to the U state.

The pressure- and temperature-induced conformational transitions and unfolding can be studied with a number of spectroscopic techniques. Although NMR spectroscopy is expected to give potentially the most details on the mechanism of unfolding [6], Fourier transform infrared (FTIR) spectroscopy in combination with the diamond anvil cell makes it possible to follow the changes in the secondary structure. For the temperature unfolded proteins an extensive intermolecular hydrogen bond network develops which is absent in the pressure unfolded proteins. This leads us to use pressure as a rather unique tool to unravel the mechanism of protein aggregation.

2 Pressure–Assisted Cold Denaturation

Cold denaturation of proteins is a natural consequence of the elliptic phase diagrams of protein stability [1]. Pressure assisted cold denaturation of ribonuclease was first observed with NMR techniques by Jonas [6]. The approach makes use of the fact that the freezing point of ice I is about -20 °C at 200 MPa. When the infrared spectra of the cold, heat and pressure denaturated horse heart metmyoglobin are compared at pH 4, interesting differences are noted [7]. The spectrum of the heat denatured protein shows the typical bands of the intermolecular hydrogen bonded interactions visible in many proteins. The spectrum of the pressure denatured protein and the pressure assisted cold denatured protein are very similar but not identical. In both instances there is no indication of intermolecular hydrogen bonding. The helical content of the protein is strongly reduced in favour of the formation of sheet structures. The effect of pressure on the temperature induced aggregation of this protein is discussed in Sect. 4 [8].

3 Hydrogen/Deuterium Exchange and Enzyme Inactivation

When a protein is dissolved in D₂O, the protons exchange for deuterons. The solvent exposed protons exchange rather quickly but the internal ones do so very slowly unless the protein unfolds. The rate of exchange can be measured by either NMR or infrared spectroscopy. Again the observed rate contains the equilibrium of the unfolding and it should be possible to obtain \( pTk \) diagrams for the H/D exchange. This has been done in detail for lipoxygenase and there is a good correlation with the kinetics of inactivation of the enzyme activity [9].

Less systematic investigations have been performed on the correlation between the effect of temperature and pressure on β-galactosidases from different biological sources. The experiments essentially confirm the idea that the stability of enzymes and proteins can be correlated with the rate of H/D exchange [10].