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Calorimetric observation of lysozyme degradation at elevated temperature in water and DMSO-water mixtures

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ABSTRACT

The reversibility of protein denaturation is an essential factor for biotechnology. Previous differential scanning calorimetry (DSC) studies demonstrated the development of the low-temperature shoulder on the calorimetric denaturation peak of lysozyme in successive heating-cooling cycles, which implies irreversible denaturation. However, this effect was not thoroughly investigated. In the present work, we have quantitatively studied the effect of incubation at the elevated temperature on the state of lysozyme in water and mixtures of water with dimethyl sulfoxide (DMSO) using DSC. The changes to the state of the lysozyme molecule indicated by DSC thermograms were also evaluated by circular dichroism (CD), Fourier transform infrared spectroscopy (FTIR) and dynamic light scattering (DLS) techniques. It is noted that with the increase of duration or temperature of incubation, the low-temperature peak on DSC thermograms grows, while the height of the unfolding peak of native structure decreases. The increase in the height of the low-temperature peak in DSC scans correlates with the development of the sideband associated with the absorption of the carboxyl group in the infrared spectra. This result suggests that the low-temperature endothermic peak corresponds to the unfolding of the deamidated protein. At the same time, DLS measurements indicate absence of aggregation, while FTIR and CD data demonstrate that deamidated protein maintains a native-like structure. The evaluation of the DSC thermograms allowed to determine the rates and activation energy of the degradation of protein molecules at the elevated temperatures. The addition of DMSO slows down the protein degradation but has little effect on the apparent activation energy of the process.

1. Introduction

The reversibility of thermal denaturation is an important factor for the production and applications of proteins. According to the thermodynamic dogma of Anfinssen [1], when the protein is reintroduced into the native environment from the denaturating conditions, it will spontaneously return to the native state. However, aggregation or chemical degradation of protein molecules in the denaturing medium may prevent the protein from returning into the native state [2–5]. Such processes are commonly described in terms of the two-stage Lumry-Eyring model of irreversible protein denaturation [6–8]. It is important to monitor the reversibility of the denaturation to optimize the biotechnological processes involving proteins.

Differential scanning calorimetry (DSC) is an often-used tool for monitoring protein thermal denaturation [7,9–13]. DSC can be used to test the reversibility of the denaturation by repeating heating and cooling scans [14]. In the case of fully reversible denaturation, the heat effect of the process must be equal in the first and subsequent heating scans. On the other hand, DSC does not provide direct information on the protein structure; thus, DSC should be combined with such methods as Infrared (IR) or Circular dichroism (CD) spectroscopy to get information both on the thermochemical and structural changes upon denaturation.

The reversibility of thermal denaturation of lysozyme, one of the most commonly studied model proteins, which has applications in the food and medical industries [15–18], was investigated in several works. While it was found that the thermal denaturation of lysozyme is highly-reversible [19], some authors [20–22] noted particular changes of DSC thermograms after repeating heating and cooling cycles, with additional low-temperature peak appearing in the scans. In the works [22–24] irreversible denaturation of lysozyme was attributed to aggregation; however, the phenomenon of the additional calorimetric peak

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