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Fast scanning calorimetry of lysozyme unfolding at scanning rates from 5 K/min to 500,000 K/min

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ABSTRACT

Background: Protein denaturation is often studied using differential scanning calorimetry (DSC). However, conventional instruments are limited in the temperature scanning rate available. Fast scanning calorimetry (FSC) provides an ability to study processes at much higher rates while using extremely small sample masses [ng]. This makes it a very interesting technique for protein investigation.
Methods: A combination of conventional DSC and fast scanning calorimeters was used to study denaturation of lysozyme dissolved in glycerol. Glycerol was chosen as a solvent to prevent evaporation from the micro-sized samples of the fast scanning calorimeter.
Results: The lysozyme denaturation temperatures in the range of scanning rates from 5 K/min to ca. 500,000 K/min follow the Arrhenius law. The experimental results for FSC and conventional DSC fall into two distinct clusters in a Kissinger plot, which are well approximated by two parallel straight lines.
Conclusions: The transition temperatures for the unfolding process measured on fast scanning calorimetry sensor are significantly lower than what could be expected from the results of conventional DSC using extrapolation to high scanning rates. Evidence for the influence of the relative surface area on the unfolding temperature was found.
General significance: For the first time, fast scanning calorimetry was employed to study protein denaturation with a range of temperature scanning rates of 5 orders of magnitude. Decreased thermal stability of the micro-sized samples on the fast scanning calorimeter raise caution over using bulk solution thermal stability data of proteins for applications where micro-sized dispersed protein solutions are used, e.g., spray drying.

1. Introduction

The unfolding of protein native structure caused by heating is an endothermic process which is commonly studied by Differential Scanning Calorimetry (DSC). Information typically achieved from DSC studies includes the temperature of unfolding (commonly attributed to the maximum of the calorimetric peak) as well as the unfolding enthalpy. More detailed DSC approaches were developed allowing some insight into the reversibility and mechanism of the transition [1, 2], which include recording DSC thermograms at several different scanning rates. Theoretical provisions of this approach were described [3, 4]. Different kinetic schemes of the denaturation give rise to the distinct scan rate dependences of the DSC curves. Fig. 1 A demonstrates a set of thermograms calculated for an irreversible one step mechanism (N → D, N represents native protein conformation, D – denatured protein); Fig. 1 B shows a set of thermograms for a reversible mechanism (Fig. 1 B, N ⇌ D); Fig. 1C shows a set of thermograms for a two-stage denaturation process (N ⇌ U → D, U represents an unfolded protein conformation, D – final irreversibly denatured conformation). The numbers on the picture indicate the ratio between temperature scanning rate and kinetic rate constant of the formation of the denatured protein (D) at a particular reference temperature (see Supplementary material).

As can be seen from the Fig.1A–C, the range of scan rate variation must be quite significant (4–5 orders of magnitude) to discriminate between different kinetic schemes. On the other hand, commonly available DSC instruments with sufficient sensitivity for protein unfolding measurements only allow for limited variation of the scanning rate (typically one order of magnitude, two orders of magnitude at maximum).

Fast scanning calorimetry (FSC) is a method which allows for heating and cooling at rates of up to MK/s. Applications of FSC in research of biomolecules were developed in recent years [5–10].The required sample size for FSC is very small, around 5–100 ng which is a great advantage for the field of protein research where samples are

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