

Thermal denaturation of Lysozyme

Introduction

In biochemical, biophysical or pharmaceutical research, proteins are an important subject in the development of new drugs or treatments.

The stability parameters of these proteins are necessary for all these developments. It is therefore necessary to know, for example, the denaturation temperatures of the proteins studied, as well as the energy involved in these denaturations (denaturation enthalpy and temperature).

The technology most commonly used to access these thermodynamic parameters is differential scanning calorimetry (DSC). One of the major problems with this technology is the large quantity of protein required to obtain usable results: up to 1 ml per experiment, for proteins that can sometimes be very expensive to produce.

In this context, Calneos has developed the Ultimate DSC, which allows the use of less than 100 μ L of sample in extractable crucibles. One of the advantages is the drastic reduction in the amount of sample required to obtain usable thermograms. Another advantage is the elimination of tedious and sometimes unreliable cleaning procedures.

Experimental protocol

100μL of several solutions of lysozyme in PHB buffer were placed in a 100μL crucible. Different concentrations were used, representing different masses of lysozyme analysed respectively. The reference crucible was filled with the same volume of PHB buffer.

The Ultimate DSC was programmed to perform a temperature ramp from 40°C to 95°C at 1°C/min. The thermograms obtained are shown on the right.

- Heat Flow (mW/µg)-98,4µg - Heat Flow (mW/µg)-90,6µg - Heat Flow (mW/µg)-90 - Heat Flow (mW/µg)-90 - Heat Flow (mW/µg)-20µg - Heat Flow (mW/µg)-2

Conclusion

The Ultimate DSC allows the

enthalpy and denaturation temperature of Lysozyme to be measured using 5 to 10 less sample than currently available instruments. Each thermogram was obtained in less than 60 minutes, allowing rapid analysis. The Ultimate DSC saves large amounts of sample and allows you to work faster.

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