Micro Reaction Calorimeter - µRC™

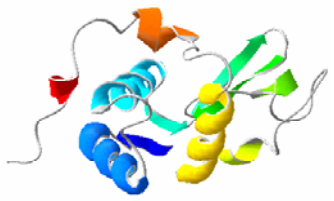
Technical Application Note 14

Scanning - Protein Denaturation

Introduction

In addition to Titration mode, the THT µRC Micro Reaction Calorimeter has facility to operate as a differential scanning calorimeter. As a preliminary example in this area the thermal denaturation of the 'model' protein Lysozyme has been studied.

Lysozyme occurs in tears, other exocrine secretions, and in very large amounts in chicken egg albumen. Its antibacterial action depends on the cleavage of the β (1→4)glycosidic linkage between alternating units of N-acetylmuramic acid and N-acetylglucosamine that form long-chain mucopolysaccharides in bacterial cell walls.



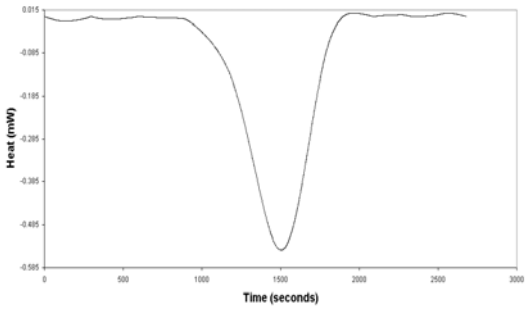
3D Structure of Lysozyme

Experimental

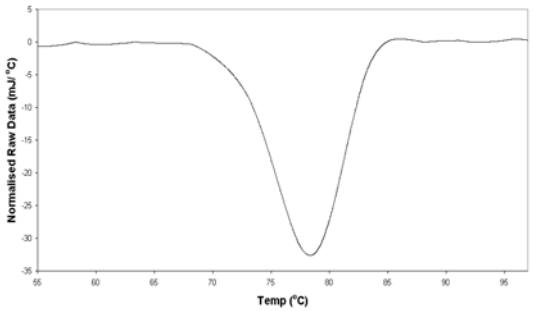
4mg/ml of lysozyme from chicken egg white (Sigma L-6876) was prepared in 20mM Sodium Acetate buffer, pH 5.2. 1.7ml of lysozyme was added to the removable HPLC-style glass vial sample holder and loaded into the µRC. A second vial was filled with buffer and used in the reference cell. The system was ramped at scan rate of 1°C/min.

Results

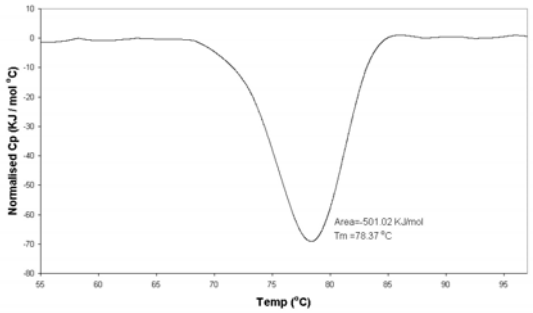
RAW DATA - BASELINE CORRECTED
Heating Scan 1°C / min Lysozyme 4mg/ml in 20mM Na Acetate Buffer, pH 5.2



NORMALISED RAW DATA
Heating Scan 1°C / min Lysozyme 4mg/ml in 20mM Na Acetate Buffer, pH 5.2

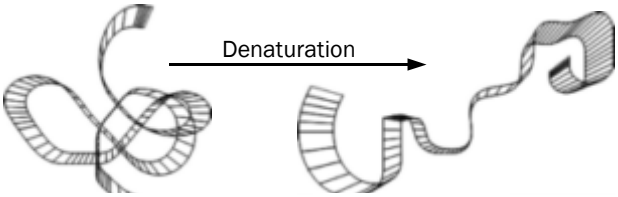


NORMALISED Cp
Heating Scan 1°C / min Lysozyme 4mg/ml in 20mM Na Acetate Buffer, pH 5.2



The µRC yields results for lysozyme of Tm = 78.4°C and ΔH = 501 KJ/mol

Discussion and Conclusions



Denaturation of Lysozyme

During heating the lysozyme undergoes a conformational change resulting in an endothermic transition. This is clearly observed in the µRC. Peptide bonds generally remain intact and the protein retains its original primary structure. Lysozyme is one of the few natural proteins that exhibits thermal reversibility. Upon cooling the denatured protein can return to its native state and resume its specific biological activity.

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