

Differential scanning calorimetric studies of a *Bacillus halodurans* alpha-amylase

Abstract:

The thermal unfolding of Amy 34, a recombinant alpha-amylase from *Bacillus halodurans*, has been investigated using differential scanning calorimetry (DSC). The denaturation of Amy 34 involves irreversible processes with an apparent denaturation temperature ($T(m)$) of 70.8 degrees C at pH 9.0, with four transitions, as determined using multiple Gaussian curves. The $T(m)$ increased by 5 degrees C in the presence of 100-fold molar excess of $CaCl_2$ while the aggregation of Amy 34 was observed in the presence of 1000-fold molar excess of $CaCl_2$. Increase in the calcium ion concentration from 1- to 5-fold molar excess resulted in an increase in calorimetric enthalpy ($\Delta H(cal)$), however, at higher concentrations of $CaCl_2$ (up to 100-fold), $\Delta H(cal)$ was found to decrease, accompanied by a decrease in entropy change (ΔS), while the $T(m)$ steadily increased. The presence of 100-fold excess of metal chelator, EDTA, resulted in a decrease in $T(m)$ by 10.4 degrees C. $T(m)$ was also decreased to 61.1 degrees C and 65.9 degrees C at pH 6.0 and pH 11.0, respectively