Denaturation and unfolding of Ribonuclease A with µDSC3 Evo

Introduction:

Ribonuclease (commonly abbreviated RNase) is a type of nuclease that catalyzes the degradation of RNA into smaller components. Bovine pancreatic RNase A is one of the classic model systems of protein science.

Fig. 1 - Representation of the three dimensional structure of Ribonuclease A – Source : http://en.wikipedia.org/wiki/Ribonuclease

$$N \underset{k_2}{\overset{k_1}{\longleftrightarrow}} U$$

Fig. 2 – Unfolding / refolding mechanism, where N and U are the native and unfolded enzymes. k1 and k2 are the unfolding and refolding rate constants

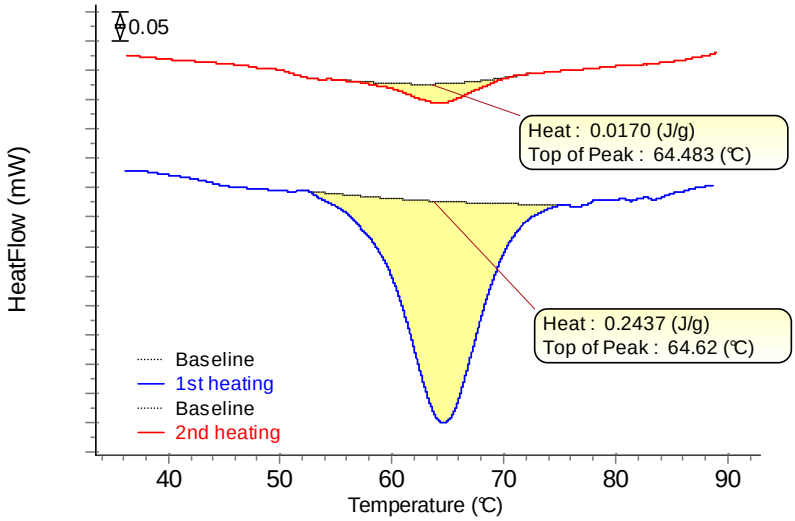

Fig. 3 – Denaturation of RNase A: superposition of first and second heating

Experimental conditions

The analyzed sample is a 5.10^{-4} molar aqueous solution of Ribonuclease A. About 750µL of sample were introduced in a µDSC3 Evo batch cell. For compensation, a buffer solution was introduced in a reference cell. Both were heated between 25°C and 90°C at 1 $K.min^{-1}$, cooled back down to 25°C at the same rate and then cycled again.

Conclusion

An endothermic peak linked with the denaturation of the enzyme is recorded during both heating phases, at a similar temperature. The peak obtained during the second heating is relevant of the partial reversibility of RNAse A unfolding process.

Amount of solution in sample cell (mg)	Amount of RNase in sample cell (mg)	Amount of buffer in reference cell (mg)
735.18	**5.04**	**743.95**

Tab. 1 – Used amounts of sample

Test	Heat (J/g solution)	Heat (kJ/mol RNAse*)	Heat (J/g RNAse)	Tm
1st heating	0.2437	487.4	35.58	64.62
2nd heating	0.0170	34.0	2.48	64.48

Tab. 2 – results of peak integrations - Molar mass of RNAse A is chosen equal to 13.7kDa

µDSC7 Evo
-45°C to 120°C

www.setaram.com
sales@setaram.com

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