Thermostability of a Plant Protein MoMo30 using a CFX Real-Time Thermocycler

Other

Morgan Coleman (mcoleman@msm.edu), Morehouse School of Medicine

Protein stability is an important aspect of characterizing a protein. Methods such as differential scanning calorimetry (DSC) can be cumbersome and not generally available in a core lab. Here we describe the use of the CFX Opus 96 Real-Time PCR system to determine the melt profile of a medical plant protein we call MoMo30.

Thermal stability of MoMo30 was determined using the GloMelt Thermal Shift Protein Stability Kit produced by BioTrend (Cat. No. 33021-T). This kit works on the principle that the GloMelt fluorescent dye binds preferentially to hydrophobic residues normally found on the interior of proteins. As proteins melt the residues are exposed and bind to the dye. Positive controls for this analysis included IgG and lysozyme. Nuclease-free water was used as a negative control. MoMo30 was placed in several different conditions such as water, 10% saponin, urea 8M, and 1% SDS. Analysis was performed in triplicate as described by the protocol included with the kit. Thermal stability was measured using the Bio-Rad CFX Opus 96 Real-Time PCR System. The melt curve protocol ran from 10°C to 100°C increasing 0.5°C every 30 seconds with a lid temperature of 50°C. The melt curve data was analyzed using the Bio-Rad CFX Maestro Software.

We found that even in the harshest conditions, MoMo30 did not unfold to generate a melt curve. The controls IgG and lysozyme generate melt curves as expected, with melt temperatures reaching approximately 73°C. Because MoMo30 did not unfold, even at the highest temperature setting, the melt curve appears as a straight line.

The findings of this work demonstrate that the GloMelt assay is a way to conveniently measure the thermostability of proteins using The CFX instrument.