Time-Dependence of Thermal Hysteresis Activity

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Thermal hysteresis (TH) activity in hyperactive AFPs is more than 10 times higher than it is in moderate AFPs at equimolar concentrations. Although the dependence of TH activity on AFP concentration has been extensively studied, the time-dependence of this process is still unclear. We used a custom-made nanoliter osmometer and a novel microfluidics systems in order to study the effect of time on TH activity of hyperactive AFPs from *Tenebrio molitor*, the common mealworm, (*Tm*AFP), and *Marinomonas primoryensis*, an Antarctic bacterium, (*Mp*AFP) and moderate AFPs (AFPIII from ocean-pout). We show that during the first minutes after the melting of an ice mass to a single crystal, its exposure time to *Tm*AFP is a crucial factor. TH activity could be increased 10-fold at the μM concentrations tested (1-40 μM) by delaying the onset of cooling. At higher *Tm*AFP concentrations, the effect of long exposure times is reduced. Accordingly, we found that the effect of long exposure times (12 h) at a very low *Tm*AFP concentration (1 μM) can increase the TH activity almost to the TH activity of a 10-fold higher AFP concentration. *Mp*AFP showed similar results to *Tm*AFP. However, AFPIII showed a different degree of time-dependence, as TH activity increased by only 50% after a relatively long exposure time (3 h). In microfluidic exchange experiments, *Tm*AFP prevented growth of an ice crystal after the removal of the protein solution. On the other hand, during the exchange of AFPIII protein solution, crystal growth usually commenced. These results are of significant importance in the study of the AFP mechanism, and reveal yet another difference between moderate and hyperactive AFPs.

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