Effects of Mono- and Di- Saccharide Osmolytes on the Stability and Folding Dynamics of src SH3

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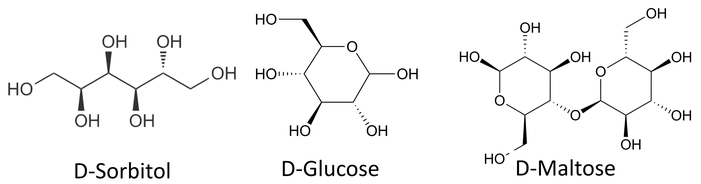
Abstract

Osmolytes are small molecules that stabilize protein's native structures in living systems. In order to better understand this process, we investigate how changes in specific structural properties of osmolytes affect folding dynamics and stability of proteins. Comparing the effects of osmolytes with varying quantities of cyclic glucose subunits, varying types of glycosidic linkages of cyclic glucose subunits, and a linear glucose analogue on the stability and folding dynamics of proteins may reveal the potential role of these structures on osmolyte-protein interactions. We characterize the effects of mono- and di- saccharide osmolytes on the stability and folding kinetics of the srcSH3 model protein using sorbitol, glucose, and maltose solutions with chemical denaturant using fluorimetry and stopped-flow spectroscopy.



Methods

Folding kinetics will be followed after the protein is perturbed from either its unfolded (U) or folded (F) states in solutions containing one osmolyte condition/experiment and GdmCl using a stopped-flow fluorimeter. Chevron plots can be fit to study folding kinetics of proteins (1).

Thermodynamics of the protein will be followed after the protein equilibrates at different [GdmCl] solutions containing one osmolyte condition/experiment using a fluorimeter. Denaturation Melts can be fit to study the thermodynamics of proteins (2).

Trials are completed using .1M Tris, .25M NaCl buffers, pH 7 at 298K. The third kinetic trace was used to fit k_{obs} for each [GdmCl] point to create the chevron plot. Reported average values ± standard error *denotes use of IgorPro software reported errors.

Results

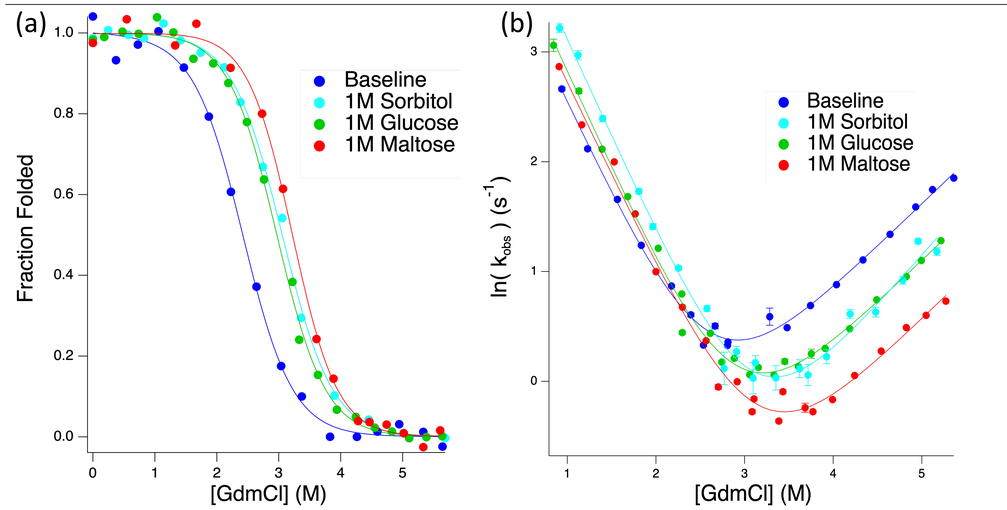


Figure 1: **Mono- and Di—Saccharide Osmolytes affect thermodynamic stability and kinetics of SH3.** Denaturation Melts calculated using normalized fluorometric ratio plotted vs [GdmCl] (a). Chevron calculated ln(k_{obs}) obtained via stopped-flow kinetic traces fits vs [GdmCl] (b).

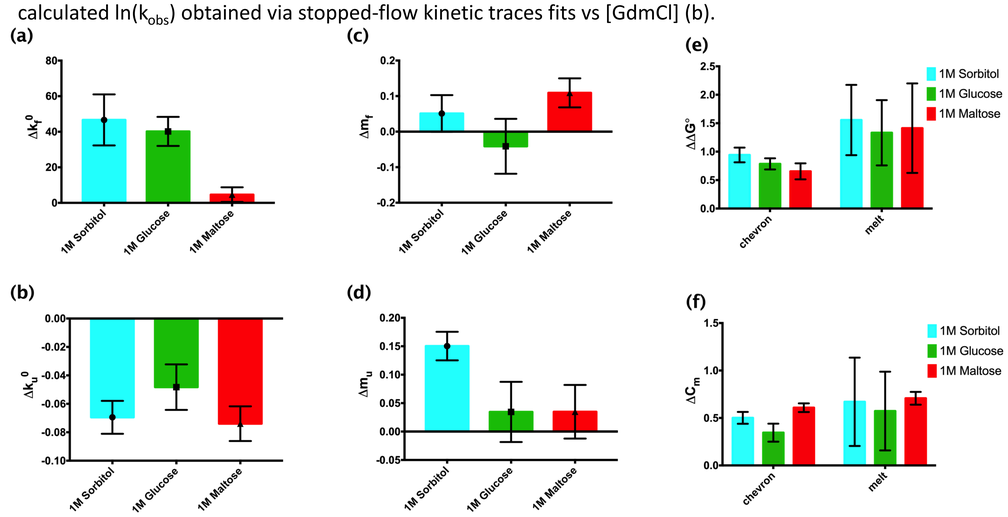


Figure 2: Mono- and Di—Saccharide osmolytes differentially affect thermodynamic stability and kinetics of SH3. Changes in kinetic mean fit coefficients from baseline to osmolyte condition are shown with error bars ± SEM (a-d). Change in mean values for thermodynamic parameters from baseline to osmolyte conditions are shown, including both chevron and melt derived values (e,f).

Table 1: Average Chevron Derived Fit Values

Condition	k_f^0 (s^{-1})	m_f (M^{-1})	k_u^0 (s^{-1})	m_u (M^{-1})	m (kcal mol^{-1} M^{-1})	ΔG° (kcal mol^{-1})	C_m (M)
Baseline	71.1 ± 1.40	-1.79 ± .04	.104 ± .011	.775 ± .024	1.52 ± .02	3.87 ± .05	2.55 ± .04
1M Sorbitol*	117.8 ± 14.3	-1.74 ± .03	.035 ± .003	.925 ± .008	1.58 ± .02	4.81 ± .12	3.05 ± .04
1M Glucose	111.3 ± 8.0	-1.83 ± .07	.056 ± .011	.810 ± .025	1.56 ± .03	4.53 ± .13	2.90 ± .08
1M Maltose	75.7 ± 3.9	-1.68 ± .01	.030 ± .005	.810 ± .041	1.47 ± .03	4.66 ± .08	3.16 ± .01

Table 2: Average Melt Derived Fit Values

Condition	m (kcal mol^{-1} M^{-1})	ΔG° (kcal mol^{-1})	C_m (M)
Baseline	1.33 ± .14	3.19 ± .36	2.40 ± .02
1M Sorbitol	1.55 ± .17	4.75 ± .50	3.07 ± .47
1M Glucose	1.52 ± .15	4.53 ± .45	2.97 ± .45
1M Maltose	1.46 ± .25	4.61 ± .70	3.10 ± .06

Tables 1 and 2: Average Chevron (1) and Melt (2) derived fit values. Averages of all trials are shown ± standard error of the mean. Melt derived m and ΔG° values reported via IgorPro Fitting software.

Discussion

- Sorbitol, Glucose and Maltose increase the C_m and ΔG° values, indicating that they increase the stability of src SH3.
- All sugars seem to have a greater affect on the unfolding kinetics of src SH3 than for refolding.
- Maltose has approximately twice the effect than both glucose and sorbitol, suggesting the increase in the glucose subunit has a direct impact on the stabilizing ability of the sugar.
- Unfolding m_u and folding m_f values do not change significantly for the sugars, indicating that they do not affect the structure of the transition state.
- All the sugars stabilize thermodynamic values (+ΔΔG°, +ΔC_m), and reduce unfolding rate constant k_u^0 in a seemingly size dependent manner.

Future Experimentation

Various osmolyte structural properties will be examined in future experimentation including: varying cyclic sugar osmolytes with differences in number of monosaccharide subunits, cyclic monosaccharide composition and glyosidic linkages

References

1. Greenfield, N. J. (2006). Analysis of the kinetics of folding of proteins and peptides using circular dichroism. *Nature protocols*, *1*(6), 2891.

2. Street, T. O., Courtemanche, N., & Barrick, D. (2008). Protein folding and stability using denaturants. *Methods in cell biology*, *84*, 295-325.