Communication: Folding of glycosylated proteins under confinement

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Conjugating flexible polymers (such as oligosaccharides) to proteins or confining a protein in a restricted volume often increases protein thermal stability. In this communication, we investigate the interplay between conjugation and confinement which is not trivial as the magnitude and the mechanism of stabilization are different in each instance. Using coarse-grained computational approach the folding biophysics is studied when the protein is placed in a sphere of variable radius and is conjugated to 0–6 mono- or penta-saccharides. We observe a synergistic effect on thermal stability when short oligosaccharides are attached and the modified protein is confined in a small cage. However, when large oligosaccharides are added, a conflict between confinement and glycosylation arises as the stabilizing effect of the cage is dramatically reduced and it is almost impossible to further stabilize the protein beyond the mild stabilization induced by the sugars. © 2011 American Institute of Physics. [doi:10.1063/1.3650700]
of the form $U = K[(c/2r)^3 - 2(c/2r)^2 + 1]$ \cite{14} in which $r$ is the distance between the $i$th residue and the ball, and $C$ is the distance between the ball shell, where $C$ was set to 4 Å and $K$ was set to 100. We examined how the size of the cage and the number of conjugated sugars alter protein stability. We also explored the effect of the length of the conjugated sugars on the cross talk between glycosylation and confinement and thus on the degree of the length of the conjugated sugars alter protein stability. We also explored the effect of the size of the cage and the number of conjugated sugars on the cross talk between glycosylation and confinement and thus on the degree of the length of the conjugated sugars alter protein stability.

Typically, confining proteins in small spaces results in greater protein stability as reflected by an increase in the folding temperature, $T_F$ (obtained from the peak of the specific heat curve and corresponding to the temperature at which protein stability is zero). While various proteins follow a similar scaling law for stabilization upon confinement, it is unclear whether this scaling law is valid for conjugated proteins. Proteins are linear polymers and the conjugation transforms them into branched polymers, which may restrict their compressibility. To examine the thermodynamics of branched proteins under confinement, we designed two sets of glycosylated variants of the SH3 domain with increasing numbers of glycans (between 1 and 6 oligosaccharide chains). One set of variants used up to six mono-saccharide GlcNAc units and the second set of variants incorporated up to six penta-oligosaccharide Man$_3$GlcNAc$_2$ units.

The bare effect of glycosylation on protein stability was examined by studying the folding thermodynamics in the bulk (i.e., a confined sphere with a radius of $\infty$). In the bulk, glycosylation with the monosaccharide increases $T_F$ by 0.6%–1.2% and glycosylation with the penta-saccharide increases $T_F$ by 0.5%–1.9% compared to the $T_F$ of the unmodified protein. This increase in $T_F$ translates into an increase in the population of the folded state from 50% to 62% for SH3-(GlcNAc)$_b$ and to 70% for SH3-(Man$_3$GlcNAc$_2$)$_b$.

To investigate the dependence of stability on confinement, we calculated the $T_F$ of the two sets of glycosylated variants in balls of different sizes (Fig. 1). Confining the SH3 variants conjugated to mono-saccharides in a small sphere (whose radius is similar to the $R_g$ of each variant in the bulk) results in significant stabilization, with $T_F$ increasing by about 10% (translates to a shift in the population of the folded state from 50% to 85%), regardless of the degree of glycosylation (Fig. 1(a)). The degree of stabilization upon confinement is considerably more limited for the variants with the larger oligosaccharides. For SH3 glycosylated with penta-saccharides, the stabilization by confinement decreases as the degree of glycosylation increases (Fig. 1(b)). For example, while the $T_F$ of the variant with six GlcNAc increased by 9.7% when it was placed in a sphere of radius $1.1R_g^U$, the $T_F$ of the variant with six Man$_3$GlcNAc$_2$ increased by only 3.6% when similarly confined, compared to unmodified SH3 in the bulk.

The dependence of the stability of mono-saccharide variants on the size of the ball is plotted in Figure 1(c) and is similar to that of the unmodified variants of SH3. The data are plotted on a logarithmic scale and fitted to a power-law function $\Delta T_F \propto (R/R_g^U)^{-\gamma}$. Takada et al.\cite{14} have shown that...
that for many small proteins (having different topologies), the dependence of stability on degree of confinement in a cylinder follows the same scaling exponent of $\gamma = 3.2$. In our simulations, we observed $\gamma = 2.9$ for the unmodified SH3 in accordance with the observations of Takada et al. A scaling exponent $\gamma = 2.5$ for SH3 was also found in simulations using a ball with a hard repulsive cavity (similar to the potential used here).\(^{20}\) In that work, however, the scaling exponent parameter differed significantly with the nature of the repulsive potential and with the topology of the protein.

The scaling exponent parameters for the six glycosylated variants of SH3 involving mono-saccharides are between 2.4 and 3.1 (Fig. 1(c)). This implies that conjugation of a small glycan does not change the nature of the protein although the excluded volume of a glycan ring is larger than that of an amino acid. When the larger conjugates were attached to the protein, it was observed (Fig. 1(d)) that the variant with few penta-saccharides follow the scaling law that was described for short glycans, but variants with a larger number of long glycans deviate significantly from that rule (e.g., SH3 with five or six Man$_3$GlcNAc$_2$ have $\gamma \approx 1$). This implies that when a significant part of the protein is glycosylated with bulky glycans (i.e., branched chains with a large excluded volume), the change in stability upon confinement is smaller. To understand the discrepancy between the effect of short and long glycans on folding stability upon confinement, we thoroughly analyzed the folding thermodynamics of four representative variants with low and high degrees of glycosylation (i.e., with a single or six conjugated sugar moieties of each type).

Figure 2 shows the enthalpic and entropic components of both the folded and unfolded states of the four glycosylated variants of SH3 as well as of the unmodified protein. For SH3 conjugated with mono-saccharides, the folded state is hardly affected by the conjugation or the confinement (Fig. 2(a)). The unfolded state, however, is strongly affected by the confinement as both the enthalpy and the entropy are reduced (Fig. 2(a)). Since the entropy of the unfolded state is reduced more than its enthalpy, there is an overall thermodynamic stabilization of the protein, as reflected by the increase in $T_F$ when folding takes place in small cavities. The relative effect of the degree of glycosylation on the enthalpy and entropy of the unfolded state is quite small, compared to the effect of confinement.

A stronger decrease in entropy than in enthalpy is observed also for the unfolded state of SH3 when it is conjugated with penta-saccharides (Fig. 2(b)). As for the unmodified protein or the protein with the short glycan, the reduction in entropy is not compensated by the reduction in enthalpy and so results in a higher $T_F$. However, in the case of the long glycan moieties, the decrease in entropy is smaller than that which occurs for the corresponding variant with the shorter glycan. The $T_F$ of the SH3 variant glycosylated with six penta-saccharides remains almost unaffected upon reducing the radius, $R$, of the confined space. The enthalpy and entropy of this variant decreases similarly as the sphere contracts (Fig. 2(b)) and, therefore, the free energy of the unfolded state in a small sphere is similar to that in the bulk and the overall thermal stability is not affected by the confinement.

To understand the dependence of the stabilizing effect of confinement on the length of the oligosaccharide chain, we followed two protein structural properties, namely, the average radius of gyration of the protein, ($R_g$), and the average number of native contacts, ($Q$), in the folded (F) and unfolded (U) states during folding reactions simulated in confined spaces of different sizes. The change in the ($R_g^U$) of four variants of SH3 upon confining in increasingly small spheres is nearly the same ($\sim$3.5 Å), indicating that the degree of compaction of the unfolded state is constant irrespective of the type or number of glycans attached to the protein (Fig. 3(a)). The changes in ($Q^U$), however, strongly depend on the type and number of attached glycans (Fig. 3(b)). When small glycans are attached, a substantial number of native contacts ($\sim$30 contacts) are formed on average in the unfolded state upon confinement. This is similar to the number formed in the unfolded state of the unmodified protein in a confined space. When a larger glycan is attached, however, a smaller
number of native contacts (~23 contacts) are formed upon confinement. The number of contacts in the unfolded state in a highly confined space is even more limited when six of the longer glycans are conjugated to the protein (⟨Q⟩ increases by only 13 contacts upon confinement).

The folded state is hardly affected by confinement or by the glycosylation. The values of ⟨Rg⟩ and ⟨Q⟩ for the unfolded state are almost identical for all variants and all confinement conditions (Fig. 3) and this is supported by the constant enthalpy and entropy of the folded state (Fig. 2). The increase in ⟨Q⟩ upon confining the unfolded state is compensated for by the decrease in its enthalpy. The decrease in the entropy of the unfolded state upon compaction (as reflected by its ⟨Rg⟩, Fig. 3) is larger than the decrease in its enthalpy, with the overall result being the observed increase in thermodynamic stability with increasing confinement.

Glycosylation in the bulk results in the loss of some native contacts in the unfolded state and this is accompanied by an expansion of the protein’s dimensions (i.e., larger ⟨Rg⟩). The stabilization upon glycosylation stems from the unwinding of some residual interactions in the unfolded state and is thus enthalpic in origin. Consequently, confining the protein in a small spherical cage can result in much larger stabilization, which is dominated by the reduced entropy of the unfolded state. The loss of entropy that follows restricting the allowed space is accompanied by a gain in residual structure (namely, a lower enthalpy for the unfolded state). Glycosylation, however, is characterized by a loss of residual structure in the unfolded state and an expansion of the unfolded state and the stabilization, which is typically ~5 fold smaller than the maximal stabilization that can be achieved by confinement, is enthalpic in origin. Confining proteins that are conjugated to short oligosaccharides results in a similar stabilization effect as achieved for the unmodified protein. A conflict between confinement and glycosylation arises when longer oligosaccharides are used. In particular, when the protein is highly glycosylated with bulky oligosaccharides, the opportunity for further compaction of the unfolded state and further increases in its residual structure is limited. In this case, the ability of confinement to enhance stability is negligible.

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12S. Q. Zhang and M. S. Cheung, Nano Lett. 7(11), 3438 (2007).