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Correction to Context-Dependent Effects of Asparagine Glycosylation on Pin WW Folding Kinetics and Thermodynamics

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In extracting folding and unfolding rate information from our apparent rate constant data, and in fitting the extracted folding rates as a function of temperature to Kramer's model, we inadvertently used the pre-jump equilibrium temperature instead of the post-jump temperature: a difference of 12 °C. The absolute folding and unfolding rate values for each WW variant in Tables 3 and 4 are different than reported in the original paper by < 2-fold for all but 2 cases that are slightly > 2-fold, but most of the folding and unfolding rate ratios (comparing the folding or unfolding rates of the glycosylated vs. non-glycosylated WW variants) are similar to the values presented in the original paper. The changes to the data do not change the conclusion of our paper that specific, evolved protein-glycan contacts must also play a role in mediating the beneficial energetic effects on protein folding that glycosylation can confer.

The corrected data appear in Tables 3 and 4 below. The corrected versions of Figures S44 to S55 (the data that Tables 3 and 4 summarize) are provided in the Supporting Information. The following sentences in the Results section text referring to our kinetic data (pp. 15364):

The modest stabilizing effect of the Asn to Asn-GlcNAc substitution at positions 20 and 30 appears to be a result, in part, of an increased folding rate. Glycosylation at position 20 (compare **20** with **20g**) increases the folding rate [?]1.1-fold (Table 3). Glycosylation at position 30 (compare **30** with **30g**) has a similar effect. These small folding rate increases agree with the predictions of the computational model and could be consistent with a small amount of denatured-state destabilization as a consequence of glycosylation. However, the decreased unfolding rate of **20g** relative to **20** (also predicted by the model) could indicate that glycosylation at position 20 actually stabilizes the folding transition state and native state simultaneously.

are changed to:

The modest stabilizing effect of the Asn to Asn-GlcNAc substitution at position 20 appears to be primarily due to an increased folding rate (**20g** folds 1.5 times faster than **20**). This folding rate increase agrees with the predictions of the computational model, and could be consistent with a small amount of denatured-state destabilization as a consequence of glycosylation. However, the unfolding rates of **20g** and **20** are indistinguishable, which disagrees with the predicted decrease in unfolding rate upon glycosylation. The stabilizing effect of glycosylation at position 30 appears to come primarily from a decrease in unfolding rate (**30g** unfolds 0.7 times as fast as non-glycosylated **Pin WW**), as predicted by the model. This decrease compensates for an unexpected decrease in folding rate: **30g** folds

Associated Content

Supporting Information. The corrected versions of Figures S44 to S55 are now incorporated into the SI. This material is available free of charge via the Internet at <http://pubs.acs.org>.

0.8 times as fast as **Pin WW** whereas the model predicted a large increase in folding rate. Results for **33** and **33g** are similarly inconsistent with the predictions of the model.

In addition, the following sentence in the Discussion section text about our kinetic data (pp. 15366):

For example, the observed increased stability and increased folding rate of **20g** relative to **20**, and of **30g** relative to **30**, could be the result of simultaneous transition state and native state stabilization (rather than destabilization of the denatured ensemble), reflecting the presence of favorable glycan-protein contacts at these positions.

is changed to:

For example, the observed increased stability of **20g** and **30g** relative to **20** and **30**, respectively, could be the result of favorable native-state GlcNAc-protein contacts at these positions.

Experimentally Measured and Computationally Predicted Folding Rates for Pin WW Variants Having Either Asn or Asn-GlcNAc at the Indicated Positions^a

Table 3

structural context	protein	residue at indicated position	measured folding rate ^b (10 ³ s ⁻¹)	(k _d [Asn-GlcNAc])/(k _d [Asn])	
				measured	predicted ^c
β-strand 1	14	Asn	<i>d</i>	<i>d</i>	2
	14g	Asn-GlcNAc	<i>d</i>		
loop 1	17	Asn	5.8 ± 0.2	0.80 ± 0.04	3.6
	17g	Asn-GlcNAc	4.7 ± 0.1		
	18	Asn	8.0 ± 0.2	0.71 ± 0.03	3.7
	18g	Asn-GlcNAc	5.6 ± 0.2		
	19	Asn	6.6 ± 0.2	0.77 ± 0.06	2.8
	19g	Asn-GlcNAc	5.1 ± 0.4		
	20	Asn	4.5 ± 0.2	<i>1.48 ± 0.09</i>	<i>2.4</i>
	20g	Asn-GlcNAc	6.6 ± 0.3		
β-strand 2	23	Asn	<i>d</i>	<i>d</i>	1.1
	23g	Asn-GlcNAc	<i>d</i>		
	PinWW	Asn	11.6 ± 0.7	<i>d</i>	0.9
	26g	Asn-GlcNAc	<i>d</i>		
loop 2	Pin WW	Asn	11.6 ± 0.7	0.81 ± 0.05	3.4
	30g	Asn-GlcNAc	9.4 ± 0.2		
β-strand 3	33	Asn	6.3 ± 0.7	<i>0.70 ± 0.16</i>	<i>0.8</i>
	33g	Asn-GlcNAc	4.4 ± 0.8		

^a Variants for which experimental observations agree with computational predictions are italicized.

^b Measured folding rates at 55 °C (328.15 K) were calculated based on relaxation data from laser temperature jump experiments on 100 μM solutions of each Pin WW domain variant in 20 mM sodium phosphate buffer (pH 7; see supporting information for details). Uncertainties represent the standard error in folding rates.

^c Predicted folding rate ratios at the indicated positions were calculated based on the all-atom native-topology model at the calculated melting temperature of the corresponding non-glycosylated Asn-containing Pin WW variant (in contrast with the measured folding rate ratios, which are all at 55 °C).

^dThe folding kinetics of peptides **14**, **14g**, **23**, **23g**, and **26g** could not be analyzed via laser temperature-jump experiments due to their low thermal stability.

Table 4

Experimentally Measured and Computationally Predicted Unfolding Rates for Pin WW Variants Having Either Asn or Asn-GlcNAc at the Indicated Positions^a

structural context	protein	residue at indicated position	measured unfolding rate ^b (10 ³ s ⁻¹)	(k _u [Asn-GlcNAc])/(k _u [Asn])	predicted ^c
β-strand 1	14	Asn	<i>d</i>	<i>d</i>	0.50
	14g	Asn-GlcNAc	<i>d</i>		
loop 1	17	Asn	6.1 ± 0.4	0.89 ± 0.08	0.58
	17g	Asn-GlcNAc	5.4 ± 0.3		
	18	Asn	6.9 ± 0.3	0.99 ± 0.09	0.56
	18g	Asn-GlcNAc	6.9 ± 0.5		
	19	Asn	5.4 ± 0.3	1.17 ± 0.12	0.33
	19g	Asn-GlcNAc	6.4 ± 0.6		
	20	Asn	5.9 ± 0.4	1.15 ± 0.11	0.45
	20g	Asn-GlcNAc	6.8 ± 0.4		
β-strand 2	23	Asn	<i>d</i>	<i>d</i>	0.77
	23g	Asn-GlcNAc	<i>d</i>		
	PinWW	Asn	8.0 ± 0.7	<i>d</i>	4.3
	26g	Asn-GlcNAc	<i>d</i>		
loop 2	Pin WW	Asn	8.0 ± 0.7	0.68 ± 0.08	0.70
	30g	Asn-GlcNAc	5.4 ± 0.4		
β-strand 3	33	Asn	42.5 ± 7.3	1.13 ± 0.39	2.4
	33g	Asn-GlcNAc	48.0 ± 14.5		

^a Variants for which experimental observations agree with computational predictions are italicized.

^b Measured unfolding rates at 55 °C (328.15 K) were calculated based on relaxation data from laser temperature jump experiments on 100 μM solutions of each Pin WW domain variant in 20 mM sodium phosphate buffer (pH 7; see supporting information for details). Uncertainties represent the standard error in folding rates.

^c Predicted unfolding rate ratios at the indicated positions were calculated based on the all-atom native-topology model at the calculated melting temperature of the corresponding non-glycosylated Asn-containing Pin WW variant (in contrast with the measured unfolding rate ratios, which are all at 55 °C).

^dThe unfolding kinetics of peptides **14**, **14g**, **23**, **23g**, and **26g** could not be analyzed via laser temperature-jump experiments due to their low thermal stability.