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

Joshua L. Price, Dalit Shental-Bechor, Apratim Dhar, Maurice J. Turner, Evan T. Powers, Martin Gruebele, Yaakov Levy,* and Jeffery W. Kelly*

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Supporting Information

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 prejump equilibrium temperature instead of the postjump 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 two 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 nonglycosylated 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 versions of Figures S44–S55, showing the data that are summarized in Tables 3 and 4, are provided in the Supporting Information.

Page 15364. The text in the Results section referring to our kinetic data (the first five sentences of paragraph 9) should be replaced with the following:

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

Table 3. Experimentally Measured and Computationally Predicted Folding Rates for Pin WW Variants Having Either Asn or Asn–GlcNAc at the Indicated Positions[a]

				(k_f[Asn–GlcNAc])/(k_f[Asn])	
structural context	protein	residue at indicated position	measured folding rate[b] (10^3 s^{-1})	measured	predicted[c]
β-strand 1	**14**	Asn	–[d]	–[d]	2
	14g	Asn–GlcNAc	–[d]		
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	*1.48 ± 0.09*	*2.4*
	20g	Asn–GlcNAc	6.6 ± 0.3		
β-strand 2	**23**	Asn	–[d]	–[d]	1.1
	23g	Asn–GlcNAc	–[d]		
	PinWW	Asn	11.6 ± 0.7	–[d]	0.9
	26g	Asn–GlcNAc	–[d]		
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	*0.70 ± 0.16*	*0.8*
	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 nonglycosylated Asn-containing Pin WW variant (in contrast with the measured folding rate ratios, which are all at 55 °C). [d]The 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.

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Table 4. Experimentally Measured and Computationally Predicted Unfolding Rates for Pin WW Variants Having Either Asn or Asn−GlcNAc at the Indicated Positions[a]

				$(k_u$[Asn−GlcNAc])/$(k_u$[Asn])	
structural context	protein	residue at indicated position	measured unfolding rate[b] (10^3 s^{-1})	measured	predicted[c]
β-strand 1	**14**	Asn	–[d]	–[d]	0.50
	14g	Asn-GlcNAc	–[d]		
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	–[d]	–[d]	0.77
	23g	Asn-GlcNAc	–[d]		
	PinWW	Asn	8.0 ± 0.7	–[d]	4.3
	26g	Asn-GlcNAc	–[d]		
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 nonglycosylated Asn-containing Pin WW variant (in contrast with the measured unfolding rate ratios, which are all at 55 °C). [d]The 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.

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 nonglycosylated **Pin WW**), as predicted by the model. This decrease compensates for an unexpected decrease in folding rate: **30g** folds 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.

Page 15366. In addition, the text in the Discussion section about our kinetic data (the second sentence of paragraph 5) should be replaced with the following:

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.

■ ASSOCIATED CONTENT

Supporting Information

Complete experimental methods, compound characterization, circular dichroism data, and laser temperature jump kinetic data, including the corrected versions of Figures S44−S55. This material is available free of charge via the Internet at http://pubs.acs.org.