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INCREASING PROTEIN STABILITY BY IMPROVING BETA-TURNS

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Abstract

Our goal was to gain a better understanding of how protein stability can be increased by improving β-turns. We studied 22 β-turns in nine proteins with 66 to 370 residues by replacing other residues with proline and glycine and measuring the stability. These two residues are statistically preferred in some β-turn positions. We studied: Cold shock protein B (CspB), Histidine-containing phosphocarrier protein (HPr), Ubiquitin, Ribonucleases Sa2, Sa3, T1, and HI, Tryptophan synthetase α-subunit (TSα), and Maltose binding protein (MBP). Of the fifteen single proline mutations, 11increased stability (Average = 0.8 ± 0.3; Range = 0.3 – 1.5 kcal/mol), and the stabilizing effect of double proline mutants was additive. Based on this and our previous work, we conclude that proteins can generally be stabilized by replacing non-proline residues with proline residues at the i + 1 position of Type I and II β-turns and at the i position in Type II β-turns. Other turn positions can sometimes be used if the φ angle is near −60° for the residue replaced. It is important that the side chain of the residue replaced is less than 50% buried. Identical substitutions in β-turns in related proteins give similar results. Proline substitutions increase stability mainly by decreasing the entropy of the denatured state. In contrast, the large, diverse group of proteins considered here had almost no residues in β-turns that could be replaced by Gly to increase protein stability. Improving β-turns by substituting Pro residues is a generally useful way of increasing protein stability.

Keywords

protein stability; beta-turn, proline; glycine, conformational entropy

Introduction

For both basic research and industrial applications, general methods are needed for increasing protein stability1,2. *In vitro* selection has been used successfully to increase protein stability3,4, but more often site-directed mutagenesis is used to optimize the various forces that contribute to protein stability 5–12. In a previous study, we showed that improving the β-turn sequences in RNase Sa was a good method for increasing the stability13. By incorporating five mutations to improve the β-turns in RNase Sa, we have increased the stability by 4.5 kcal/mol. With a total of eight mutations, including the β-turn mutations, we were able to increase the stability by 9.5 kcal/mol, and this increased the melting temperature by almost 30°C (Hailong Fu, unpublished observations). Here, we

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extend our previous studies to three other microbial RNases and to six unrelated proteins to gain a better understanding of how to increase protein stability by improving the β-turns.

β-turns are the smallest type of protein secondary structure, and they make up about 25% of the residues in proteins 14¯18. These turns usually contain four residues designated as i, i+1, i+2 and i+3, and may be grouped into six different classes depending on the φ and ψ angles for the two central residues 18. The content of each β-turn type is: I (34%), II (13%), VIII (9%), I ' (5%), II ' (3%), IV (37%) 18. Type VI β-turns have a cis Pro at position i+2 and are rare 18. If the turns do not meet the criteria for the first five turn types, they are classed as type IV. Statistical analyses have identified the occupancy frequency for amino acids at the four β-turn positions for each type of turn 17. Experimental and theoretical approaches to the study of β-turn was reviewed by Frieden et al.19 and the important roles of β-turns in protein folding and stability was recently reviewed by Marcelino and Gierasch 20.

The Matthews lab showed convincingly that proteins can be stabilized by mutations that decrease the entropy of unfolding 21. This was done both by replacing an Ala with a Pro and by replacing a Gly with an Ala 21. Later it became clear that proteins could also be stabilized by adding Gly residues at certain positions in β-turns 22¯25. In these cases and others 26,27, the stability increases result because steric strain in the folded protein is reduced. In one of the earliest studies, the Suzuki lab showed convincingly that adding Pro was an effective method for stabilizing proteins, especially when the Pro was introduced at second sites in β-turns or at N-caps of α-helices 28. More recent studies have shown that several factors determine whether a Pro substitution 29¯33or a Gly substitution 22¯24,34 will increase or decrease the stability of a protein. Detailed studies of individual β-turns have been especially informative in improving our understanding of the contribution of Pro and Gly to protein stability 25,34¯37.

In this paper, the strategy used to stabilize RNase Sa was applied to nine proteins ranging in size from 66 to 370 residues: (1) the cold shock protein from *Bacillus subtilis* (CspB-Bs), (2) the histidine-containing phosphocarrier protein from *Bacillus subtilis* (HPr-Bs), (3) yeast ubiquitin, (4) RNase Sa2, (5) RNase Sa3, (6) RNase T1, (7) RNase HI, (8) tryptophan synthatase α-subunit (TSα) and (9) maltose binding protein (MBP). These proteins all have well-defined x-ray structures, and their stabilities have also been extensively studied by either chemical or thermal denaturation 38¯45.

Materials and Methods

General

The reagents used for protein purification and the spectroscopic experiments were of the best grade available. Proteomics grade urea was used and purchased from Ameresco (Solon, OH).

Preparation of plasmids and proteins

The plasmids for RNase HI, ubiquitin, TSα, and CspB-Bs were kind gifts from Drs. Susan Marquesee, George Makhatadze, Bob Matthews, and Franz Schmid. The plasmid for MBP was purchased from New England BioLabs (Ipswich, MA). The plasmid for HPr-Bs has been described previously 45. The primers were obtained from Integrated DNA Technologies (www.idtdna.com). Site-directed muagenesis was performed using the QuikChange™ Site-Directed Mutagenesis Kit from Stragagene (www.stratagene.com). Mutant plasmids were sequenced at the Laboratory for Plant Genome Technology, Texas A&M University. All proteins were expressed and purified as described 38¯46.

Denaturation Experiments

Denaturation experiments were performed using an AVIV circular dichroism Spectrometer model 62 DS using methods described 38–45. For thermal denaturation curves, the buffers used were: HPr-Bs, 10 mM Sodium Phosphate, pH 7.0; RNase Sa2 and RNase T1, 30 mM MOPS, pH 7.0; RNase Sa3, 30 mM NaOAc, pH 5.0; CspB-Bs and MBP, 30 mM NaPi, pH 7.0; RNase HI, 30 mM NaOAc, pH 5.5 in 1M GuHCl; TSα, 30 mM NaPi, pH 7.0, with 0.1 mM EDTA and 10 μM DTT. The thermal denaturation of ubiquitin at pH >5 is not reversible; therefore, urea denaturation curves were determined for this protein at 25°C in 30 mM NaOAc, pH 5.0. The analysis of denaturation curves has been described 47,48.

Results

Design of mutations

The program PROMOTIF 16 was used to search the pdb files of the proteins of interest to identify β-turn sequences. The study was limited to type I, II and IV β-turns since they represent the majority of the different types of known β-turns. The statistical preferences of the turn residues from Guruprasad and Rajkumar 17 were used to identify non-proline and non-glycine residues in positions where Pro or Gly are preferred. We applied additional criteria for Pro or Gly replacement based on our previous study 13. We did not target residues that had charged or aromatic side chains, were involved in hydrogen bonding, or were near a disulfide bond. Pro substitutions were not made if the backbone amide proton participated in a hydrogen bond or if the residue had a bulky hydrophobic side chain. In total, 22 sites from the 9 proteins were selected for substitution (15 for Pro and 7 for Gly), and the characteristics of these sites are given in Table I. Their positions in the protein structures are shown in Figure 1. It is somewhat surprising that only 22 sites were suitable for substitution, considering that 8 sites were identified previously for RNase Sa which has only 96 residues 13. The number of sites varies in different proteins. For example, we did not find any suitable sites in thioredoxin and NTL9, although each protein has more than 8 turn sequences. No protein in our set had more than 3 acceptable sites. The most turn sequences, 13, were found in the 370 residue MBP, but only 3 sites were suitable for mutation. Perhaps modulating β-turn sequences was used in the evolution of the proteins to modify both the stability and kinetics of folding 20.

Characterizing wild-type proteins and variants

We did not expect single mutations on the surface to significantly alter the three dimensional structure of the proteins. Nevertheless, the structural properties of the wild-type proteins and their variants were characterized using circular dichroism spectroscopy. The far-UV CD spectra were very similar for all wild-type proteins and their variants (data not shown). In addition, for RNase Sa2, RNase Sa3 and RNase T1, all variants showed activity similar to the wild-type proteins (data not shown). These results suggest that the protein conformations are not changed significantly by the mutations made in this study.

The stabilities of the wild-type proteins and their variants were measured using thermal (Table II) or urea denaturation (Table III). The thermal denaturation of ubiquitin at pH > 5 is not reversible so urea denaturation was used to measure the stability. For all of the proteins, results for the wild-type proteins were in reasonable agreement with previous studies 38–45.

Discussion

In Table IV, the stability changes for each of the variant proteins are given along with parameters that help in interpreting the results. For the 15 mutations to Pro residues, 11 increased the stability by an average of 0.8 ± 0.3 kcal/mol, 3 left the stability unchanged,

and 1 decreased the stability by 0.7 kcal/mol. The large decrease in stability was for the T24P mutation of TSα. The Thr side chain in wild-type TSα is 62% buried and this position was probably not able to tolerate a Pro residue for steric reasons. This is probably also the reason that neither of the two Pro substitutions in MBP gave an increase in stability. This, along with our previous results 13, suggests that Pro substitutions should only be made at positions where the side chain of the residue to be substituted is less than 50% buried. These results suggest that adding Pro residues to the appropriate positions in β-turns is a generally useful way of increasing protein stability.

The results with Gly mutations were less promising than expected based on our previous study (13). Based on statistical preferences, the biggest increases in proteins stability are expected when non-Gly residues at the i+2 position of Type I' or Type II β-turns are replaced by Gly. The statistical preference is more than twice as large as any of the preferences shown in the last column in Table I. In the large, diverse group of proteins studied here, none of these substitutions were available. This was surprising because two were available in RNase Sa, 96 residues, which was used in the previous study, and both substitution with Gly led to a stability increase (13). In our previous study with RNase Sa, a Gly substitution, S42G, at the i+3 position in a Type I turn also led to a stability increase of 0.7 kcal/mol. Here, we found that the same substitution in RNase T1, S37G, also led to a stability increase of 0.4 kcal/mol. For the four mutations that increased the stability, the φ and ψ values for the residues in the wild-type protein were all in the L-α region of the Ramachandran plot: $0° < \varphi < 90°$ and $0° < \psi < 90°$. For the 6 other mutations to Gly reported here, the φ and ψ values were not in the L-α region of the Ramachandran plot, and no increases in stability were observed. This is expected. In general, replacing any other residue with Gly will lead to more flexibility in the unfolded state and a decrease in stability. In fact, protein stability can be increased by replacing carefully chosen Gly residues by residues with side chains 21. In addition, proteins can be stabilized by replacing Gly residues with positive φ angles with D-Ala residues26 or by replacing Gly residues at the C-cap position of α-helices with D-amino acids27. The non-Gly residues in the L-α region of the Ramachandran plot have unfavorable interaction between the β carbon of the residue and the peptide backbone and this leads to steric strain. Thus, an increase in stability can best be achieved by replacing non-Gly residues at the i + 2 position of either Type I' or II β-turns where the non-Gly residue has a positive φ angle, but few of these sites are available. A careful study on increasing protein stability by modifying Type I ' β-turns was recently published 49.

RNases Sa, Sa2, and Sa3 come from different strains of *Streptomyces aureofaciens*. RNase Sa2 is 56% and RNase Sa3 is 69% identical in sequence to RNase Sa. In Table V we compare results for mutations in the equivalent β-turns in the three proteins. The increases in stability are similar for the first two mutations, but for the third mutation a decrease in stability was observed for RNase Sa and increases for RNases Sa2 and Sa3. This results because an aromatic residue is replaced in the case of RNase Sa, but not for the other two proteins. Several lines of evidence suggest that aromatic residues stabilize β-turns 13.

As noted in the introduction, we have recently prepared a variant of RNase Sa with the T_m increased by almost 30° (Hailong Fu, unpublished observations). This required that we add an active site mutation to inactivate the enzyme in order to express the enzyme in *E. coli* 50. From our studies of the β-turns13 and Asp79 in RNase Sa 10, it is clear that evolution could have stabilized RNase Sa to a greater extent without a significant loss of enzyme activity. The microbial RNases are excreted from the cell for their function after synthesis 41. Perhaps they have not become more stable because they kill the cell if their stability becomes too great and they fold before secretion. Another microbial RNase, barnase, has a unique inhibitor, barstar, that is synthesized along with barnase in order to protect the cell51.

Barnase has no disulfide bonds, so it can fold in the reducing environment inside the cell, but the other microbial RNases apparently cannot unless their stability is increased.

Suzuki's idea that proteins could be stabilized by adding Pro was based on a comparison of mesophilic enzymes with their thermophilic counterparts 28. The comparison suggested that the increased number of Pro found in thermophilic enzymes might contribute to their enhanced stability. Extensive studies of the large enzyme oligo-1,6-glucosidase confirmed this idea. This is also observed with the small protein HPr. The thermophilic HPr from *Bacillus staerothermophilus* (HPr-Bst) has a Pro at position 56. The stability of both P56A and P56G (HPr-Bst) are 0.8 kcal/mol less than wild-type 52. Here we show that when Pro is added to position 56 in the mesophilic protein (A56P HPr-Bs), the stability is increased by 0.6 kcal/mol (Table IV). Note that this substitution is at the i position of a Type II turn. In RNases Sa2 and Sa3, this same substitution also leads to large increases in stability (Table IV).

Table IV shows the predicted increases in stability if the mutation only affects the conformational entropy of the denatured state (Column 5). These predicted values are based on the allowed areas in a Ramachandran plot estimated using the van der Waals contact radii of the atoms 21,53. For the 11 Pro variants with increased stability, including the two double mutants, we plotted ΔΔG (measured) as a function of ΔΔG (predicted) and found: ΔΔG (measured) = (0.38 ± 0.07)*ΔΔG (predicted) + (0.29 ± 0.12) with a correlation coefficient of 0.87. (One clear outlier (G133P RNase HI) was omitted.) The good correlation suggests that changes in the conformational entropy of the denatured state probably make the major contribution to the stability changes. The fact that the measured values are significantly smaller than the predicted values suggests that less conformational space is available in unfolded proteins than suggested by this approach. This is consistent with the many experimental results that show that the denatured state ensemble is much more compact than this model of the denatured state. This model is similar to the upper boundary model for a denatured state ensemble suggested by Creamer et al.54. These results are also in accord with the results of Street et al.55 who showed convincingly that ΔS for unfolding decreases as the number of prolines in a protein increases. The differences between the ΔΔH and ΔΔS values in Table II show that factors other than the entropy of the denatured state surely contribute to the observed ΔΔG values.

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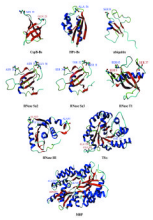

Figure 1.

Ribbon diagram showing the candidates for mutation in β-turn sequences in the 9 proteins studied. Residues in blue were mutated to Pro and those in red were mutated to Gly. The following crystal structures were used: 1CSP (CspB-Bs) 56, 2HPR (HPr-Bs) 57, 1ZW7 (ubiquitin)58, 1PYL (RNase Sa2) 59, 1MGR (RNase Sa3) 60, 9RNT (RNase T1) 61, 2RN2 (RNase HI) 62, 1WQ5 (Tryptophan Synthase α subunit) 63, and 3MBP (Maltose Binding Protein) 64. The program YASARA was used to generate the figures 65.

Table I

Positional preferences of wild-type residues and favored Pro or Gly residues

Protein (# res)	Residue	Turn-Type	Turn-Position	Wild-Type Preference	Favored Preference [a]	Increase in Preference
CspB-Bs	Asn 55	I	i+1	Asn (0.71)	Pro (4.29)	3.58
(67)	Gln 23	IV	i+3	Gln (0.81)	Gly (1.96)	1.15
Ubiquitin (76)	Ser 19	I	i+1	Ser (1.61)	Pro (4.29)	2.68
HPr-Bs (88)	Ala 56	II	i	Ala (1.07)	Pro (1.91)	0.84
RNase Sa2	Asn 33	I	i+1	Asn (0.71)	Pro (4.29)	3.58
(97)	Gly 50	II	i	Gly (0.97)	Pro (1.91)	0.94
	Asn 51	II	i+1	Asn (0.47)	Pro (4.92)	4.45
RNase Sa3	Ser 34	I	i+1	Ser (1.61)	Pro (4.29)	2.68
(99)	Ser 51	II	i	Ser (0.65)	Pro (1.91)	1.26
	Thr 52	II	i+1	Thr (0.71)	Pro (4.92)	4.21
RNase T1	Ser 8	IV	i+3	Ser (1.03)	Gly (1.96)	0.93
(104)	Ser 37	I	i+3	Ser (0.94)	Gly (2.57)	1.63
	Ser 63	I	i+1	Ser (1.61)	Pro (4.29)	2.68
RNase HI	Ala 93	I	i+1	Ala (1.07)	Pro (4.29)	3.22
(155)	Gly 123	I	i+1	Gly (0.42)	Pro (4.29)	3.87
	Ala 125	I	i+3	Ala (0.90)	Gly (2.57)	1.67
TSα	Thr 24	II	i	Thr (0.94)	Pro (1.91)	0.97
(268)	Ala 158	I	i+3	Ala (0.59)	Gly (2.57)	1.98
	Ala 189	IV	i+3	Ala (0.83)	Gly (1.96)	1.13
MBP	Gly 13	I	i+1	Gly (0.42)	Pro (4.29)	3.87
(370)	Asn 205	IV	i+3	Asn (1.24)	Gly (1.96)	0.72
	Ala 206	I	i+1	Ala (1.07)	Pro (4.29)	3.22

Positional preference as determined by Guruprasad and Rajkumar 17.

[a]Favored refers to glycine or proline preferences that are statistically significant as determined by Guruprasad and Rajkumar 17.

Table II

Parameters characterizing the thermal denaturation of β-turn protein variants

Protein	Variant	Turn-type	Position	ΔH_m [a]	$\Delta\Delta H_m$ [b]	ΔS_m [c]	$\Delta\Delta S_m$ [d]	T_m [e]	ΔT_m [f]	$\Delta\Delta G$ [g]
CspB-Bs	WT			44.9±1.9		137		55.0±0.1		
	N55P	I	i+1	48.3±0.1	3.4	144	7	62.4±0.1	7.4	1.0
	Q23G	IV	i+3	40.0±0.5	−4.9	122	−15	54.7±0.1	−0.3	0.0
HPr-Bs	WT			76.7±3.0		223		73.0±0.6		
	A56P	II	i	87.7±4.0	11.0	250	27	78.2±1.6	5.2	0.6
RNase Sa2	WT			69.1±0.5		220		41.4±0.1		
	N33P	I	i+1	72.9±1.5	3.8	230	10	43.8±0.2	2.4	0.5
	G50P	II	i	73.2±0.3	4.2	229	9	46.7±0.1	5.3	1.2
	N51P	II	i+1	71.9±1.0	2.8	226	7	44.4±0.1	3.0	0.7
	G50P/N51P	II	i/i+1	72.6±1.4	3.5	226	6	48.4±0.2	7.0	1.5
RNase Sa3	WT			122±3.0		366		59.2±0.1		
	S34P	I	i+1	120±2.0	−1	359	−7	61.7±0.1	2.5	0.9
	S51P	II	i	121±2.0	−1	362	−3	61.7±0.1	2.5	0.9
	T52P	II	i+1	118±1.0	−4	353	−12	60.6±0.1	1.4	0.5
	S51P/T52P	II	i/i+1	119±4.0	−3	365	−11	62.2±0.1	3.0	1.1
RNase T1	WT			99.7±1.4		306		52.3±0.1		
	S8G	IV	i+3	93.5±1.8	−6.2	289	−18	50.8±0.1	−1.5	−0.5
	S37G	I	i+3	93.2±0.3	−6.5	285	−21	53.5±0.2	1.2	0.4
	S63P	I	i+1	92.7±1.0	−7.0	282	−22	55.0±0.1	2.7	0.8
RNase HI	WT			69.9±1.7		216		50.3±0.3		
	A93P	I	i+1	68.8±5.1	−1.1	213	−3	49.8±0.2	−0.4	−0.1
	G123P	I	i+1	73.4±1.0	3.5	226	10	51.7±0.3	1.4	0.3
	A125G	I	i+3	70.0±2.5	0.1	217	1	49.9±0.2	−0.4	−0.1

Protein	Variant	Turn-type	Position	ΔH_m [a]	$\Delta\Delta H_m$ [b]	ΔS_m [c]	$\Delta\Delta S_m$ [d]	T_m [e]	ΔT_m [f]	$\Delta\Delta G$ [g]
TSα	WT			88.3±2.5		266		59.4±0.1		
	T24P	II	i	73.3±3.0	−15.1	222	−44	56.9±0.2	−2.5	−0.7
	A158G	I	i+3	81.3±2.1	−7.0	247	−19	56.0±0.1	−3.4	−0.9
	A189G	IV	i+3	81.1±1.6	−7.2	244	−22	59.5±0.2	0.1	0.0
MBP	WT			199±3.0		593		62.5±0.1		
	G13P	I	i+1	198±5.0	−1	589	−4	62.4±0.1	0.0	0.0
	N205G	IV	i+3	186±9.0	−14	555	−38	61.3±0.2	−1.2	−0.7
	A206P	I	i+1	198±11.0	−1	589	−4	62.4±0.2	−0.1	−0.1

[a] ΔH_m, enthalpy of unfolding at T_m (kcal/mol). The thermal denaturation curves were analyzed as previously described to determine ΔH_m, ΔS_m, and T_m 47. The errors given are based on at least three measurements of ΔH_m, and are larger than the errors obtained from a fit of the data from a single experiment.

[b] $\Delta\Delta H_m = \Delta H_m$(variant)-$\Delta H_m$(wild-type) (kcal/mol).

[c] $\Delta S_m = \Delta H_m / T_m$ (cal/mol/K).

[d] $\Delta\Delta S_m = \Delta S_m$(variant)-$\Delta S_m$(wild-type) (cal/mol/K).

[e] T_m=T (in °C), where $\Delta G°=0$.

[f] $\Delta T_m = T_m$(variant)-T_m(wild-type) (°C).

[g] $\Delta\Delta G = \Delta S_m$(wild-type)*$\Delta T_m$ (kcal/mol). A positive value indicates an increase in stability.

Table III

Parameters characterizing the urea denaturation of wild-type and S19P yeast ubiquitin

Variant	Turn – type	Position	$[Urea]_{1/2}$[a]	$\Delta G°(H_2O)$[b]	$\Delta\Delta G°$[c]
wild-type	-	-	5.44±0.05	5.4±0.1	-
S19P	I	i+1	6.33±0.10	6.3±0.1	0.9

Experiments were done at 25°C, 30 mM NaOAc (pH 5.0), and the results analyzed as described previously 48.

[a] $[Urea]_{1/2}$ (in M) is the urea concentration at the midpoint of the unfolding curve.

[b] $\Delta G° (H_2O) = m*[Urea]_{1/2}$ (kcal/mol), the wild-type *m* value of 1.00 (kcal/mol/M) was used.

[c] $\Delta\Delta G° = \Delta G°$ (variant) - $\Delta G°$ (wild-type), in kcal/mol. A positive value indicates an increase in stability.

Table IV

Structural parameters and expected entropic changes for turn variants

Protein (# res)	Mutation	B-factor[a]	Side Chain Burial%[b]	$-T\Delta\Delta S_{BB}$[c]	$\Delta\Delta G°_m$[d]
Pro Substitutions					
CspB-Bs (67)	N55P	34	6.5	1.3	1.0
Ubiquitin (76)	S19P	7	5.7	1.2	0.9
HPr-Bs (88)	A56P	16	18.3	1.4	0.6
RNase Sa2 (97)	N33P	34	−7.2	1.3	0.5
	G50P	27	(24.6)	2.0	1.2
	N51P	31	6.3	1.3	0.7
RNase Sa3 (99)	S34P	15	16	1.3	0.9
	S51P	30	6.6	1.3	0.9
	T52P	30	22.5	0.7	0.5
RNase T1 (104)	S63P	10	38.6	1.3	0.8
RNase HI (155)	A93P	20	18.8	1.3	−0.1
	G123P	29	(20.6)	2.1	0.3
TSα (268)	T24P	25	61.8	0.7	−0.7
MBP (370)	G13P	19	(71.9)	2.1	0.0
	A206P	24	68.6	1.3	−0.1
Gly Substitutions					

Protein (# res)	Mutation	B-factor[a]	Side Chain Burial%[b]	$-T\Delta\Delta S_{BB}$[c]	$\Delta\Delta G°_m$[d]
CspB-Bs (67)	Q23G	56	55.4	−0.9	0.0
RNase T1 (104)	S8G	16	−3.1	−0.8	−0.5
	S37G	12	24.1	−0.8	0.4
RNase HI (155)	A125G	30	45.7	−0.8	−0.1
TSα (268)	A158G	36	100	−0.8	−0.9
	A189G	36	17.2	−0.8	0.0
MBP (370)	N205G	27	31	−0.8	−0.7

[a] Average B-factors for backbone atoms (Å^2).

[b] Accessibility values for the wild-type residue. For Gly residues, the average for the two neighboring residues is given in parentheses. The program pfis was used to calculate the % burial of the side chains 36.

[c] ΔΔG (kcal/mol) contribution due to the difference in backbone conformational entropy calculated according to Nemethy et al. 50, as explained in Matthews et al.20. Temperatures used are T_m values for the variants and 25 °C for S19P ubiquitin.

[d] Measured stability change from Table II (kcal/mol).

Table V

Changes in stability for the Pro variants of RNases Sa, Sa2, and Sa3[a]

RNase	Mutation	ΔΔG[b]	Mutation	ΔΔG[b]	Mutation	ΔΔG[b]
Sa[c]	S31P	0.7	S48P	1.3	Y49P	−0.2
Sa2[d]	N33P	0.5	G50P	1.2	N51P	0.7
Sa3[d]	S34P	0.9	S51P	0.9	T52P	0.5

[a] These mutations are in the equivalent β-turns in the three proteins.

[b] Units are kcal/mol.

[c] From Trevino et al.13

[d] From Table II.