Article No. mb981944 *J. Mol. Biol.* (1998) **281**, 227–234

JMB

COMMUNICATION

P22 Tailspike Folding Mutants Revisited: Effects on the Thermodynamic Stability of the Isolated β-Helix Domain

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The folding of the trimeric phage P22 tailspike protein is influenced by amino acid substitutions of two types, virtually all of which affect residues in the central domain, a large parallel β-helix. Temperature sensitive folding (*tsf*) mutations lead to drastically decreased folding yields at elevated temperature. Their phenotype can be alleviated by global suppressor (*su*) mutations. Both types of mutations appeared to have no influence on the stability of the native protein at the time of their first isolation and were thus suggested to carry information needed for the folding pathway exclusively. The monomeric β-helix of tailspike, expressed as an isolated domain, exhibits freely reversible unfolding and refolding transitions, allowing us to analyse the effects of two well-characterised *tsf* and all four known *su* mutations on its thermodynamic stability. We find a marked decrease in stability for the *tsf* mutants and a striking increase in stability for all *su* mutants. This leads to the conception that the isolated β-helix domain, although active in receptor-binding and native-like in its spectroscopic properties, is close in conformation to a crucial monomeric folding intermediate whose thermolability is responsible for the kinetic partitioning between productive folding and irreversible aggregation during the maturation process of P22 tailspike protein.

Keywords: protein folding; beta-helix; folding transitions; protein stability; folding intermediate

The folding of P22 tailspike protein

The question of how proteins reach their native and functional conformation, and which factors contribute to the stability of this conformation, has been a long standing puzzle in protein chemistry. Among the attempts to elucidate the influence of single amino acid residues on the folding of proteins was the genetic approach by Jonathan King and co-workers using the tailspike protein of *Salmonella* phage P22. Three to six of these homotrimeric proteins made of 72 kDa subunits have to be attached to each phage capsid to form infectious virus particles. P22 tailspikes bind to and hydrolyse lipopolysaccharide, the phage receptor at the host cell surface (Israel *et al.*, 1967; Baxa *et al.*, 1996). The atomic structure of the tailspike protein has been determined and the receptor binding site has been identified by X-ray crystallography (Steinbacher *et al.*, 1994, 1996, 1997). The remarkable thermostability and resistance of tailspike trimers against SDS made it possible to analyse their maturation pathway, misfolding, and misassembly *in vivo* (Goldenberg *et al.*, 1982; Goldenberg & King, 1982) and *in vitro* (Fuchs *et al.*, 1991; Seckler, 1997). Tailspike maturation consists of subunit folding, subunit association, and a rate-limiting folding reaction at the trimer level. Accordingly, two intermediates accumulate in the pathway: a monomeric precursor detectable by native gel electrophoresis or size-exclusion HPLC, and an assembly intermediate termed the protrimer, distinguishable from the native trimer by its sensi-

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Abbreviations used: wt, wild-type; *tsf*, temperature sensitive for folding; *su*, global suppressor; CD, circular dichroism; TSPΔN, tailspike protein lacking the amino-terminal domain.

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0022–2836/98/320227–08 $30.00/0

tivity to proteolysis and to dissociation by SDS (Goldenberg & King, 1982; Fuchs *et al*., 1991; Danner *et al*., 1993).

Tailspike folding yields decrease dramatically with increasing temperature, with very similar dependencies *in vivo* and *in vitro* (Haase-Pettingell & King, 1988; Danner *et al*., 1993; Seckler, 1997). The temperature dependence is even more drastic for a type of mutants designated temperature sensitive for folding or *tsf* (Smith *et al*., 1980; Betts *et al*., 1997). These *tsf* mutations prevent the formation of the native trimer at high growth temperature, but once assembled at a lower permissive temperature, the mutant tailspike trimers have full biological activity at the restrictive temperature (Goldenberg & King, 1981; Haase-Pettingell & King, 1997). *tsf*-mutations have been identified at more than 40 independent sites almost exclusively in the central β-helix domain (Yu & King, 1984, 1988; Villafane & King, 1988). A second class of mutations, isolated as global suppressors (*su*) of the *tsf* phenotype, have the opposite effect: they increase the yield of correctly assembled tailspikes at higher temperature, thus compensating for the effect of *tsf* mutations (Fane *et al*., 1991; Mitraki *et al*., 1991). Only two such mutations were repeatedly isolated after random mutagenesis in the *tsf* background: V331A and A334V (Fane & King, 1991; Fane *et al*., 1991), but by site-directed mutagenesis at these two sites, V331G and A334I were also found to suppress the *tsf* phenotype (Lee *et al*., 1991).

Biophysical studies of tailspike folding *in vitro* confirmed the *tsf* mutations to affect tailspike folding by destabilising thermolabile intermediates in the folding and assembly pathway and suggested the *su* mutations to stabilise such intermediates (Danner & Seckler, 1993). Whether the interactions affected by the mutations are unique to folding intermediates (''foldons'') or native-like had remained an open question (King *et al*., 1987). Early denaturation experiments had indicated that the thermal stability of the native trimer was largely unaffected by *tsf* or *su* mutations (Goldenberg & King, 1981; Sturtevant *et al*., 1989; Mitraki *et al*., 1991). More detailed experiments, however, revealed marked effects of both types of mutations on thermal unfolding kinetics (Chen & King, 1991; Danner & Seckler, 1993; Beißinger *et al*., 1995). The quantitative analysis of thermal unfolding was facilitated by the preparation of a truncated tailspike (TSPΔN), in which the amino-terminal, head-binding domain had been removed (Danner *et al*., 1993; Miller *et al*., 1998a). For all *tsf* mutations analysed, an increase in the rate of unfolding at high temperature was found, indicating decreased stability. In the case of the *su* mutants, only V331G and V331A were found to stabilise the completely folded protein, whereas A334V and A334I accelerated unfolding at high temperature compared to wt. Supported by the then available crystal structure of TSPΔN (Steinbacher *et al*., 1994, 1997), this led to the hypothesis that although the large sidechains V and I at position 334 lead to steric conflict in the native structure, improved hydrophobic stacking might stabilise the less rigid conformation of early folding intermediates (Beißinger *et al*., 1995).

A reversibly folding β-helix

All these conclusions had to be drawn exclusively from kinetic and therefore indirect experiments, as unfolding of both tailspike and TSPΔN is irreversible at high temperature or elevated concentrations of denaturant, probably due to the pronounced subunit interdigitation at the C terminus. In order to obtain a protein that unfolds reversibly and thus permits a thermodynamic analysis of the effects of *tsf* and *su* mutations by means of denaturation transitions, the central part of tailspike, consisting of residues 109 to 544, was expressed as an isolated domain (Miller *et al*., 1998b). In the crystal structure of TSPΔN, this central domain, which contains virtually all of the known folding mutation sites, is a large parallel right-handed β-helix of 13 coils. Figure 1 shows a graphic representation of the tailspike β-helix as derived from the crystal structure of TSPΔN. The isolated β-helix domain in solution is a monomer at submicromolar concentrations. It is structurally very similar to the conformation of the β-helix domain in the complete, native tailspike as indicated by

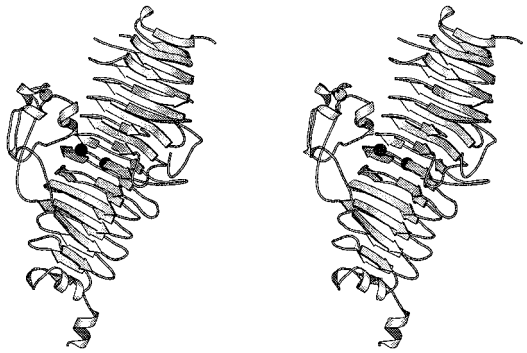

Figure 1. Stereo ribbon drawing of the β-helix domain of tailspike as derived from the crystal structure of TSPΔN (Steinbacher *et al*., 1997) using Molscript (Kraulis, 1991). The suppressor sites 334 and 331 (dark spheres) are located in the substrate binding groove, the *tsf* sites 238 and 244 investigated here (light spheres) are located in the dorsal fin subdomain. The mutations were introduced into pBHX, the plasmid bearing the gene for the β-helix domain, by restriction fragment exchange from the genes of the corresponding mutants in TSPΔN (Miller *et al*., 1998a). Expression and purification of the isolated β-helix and its mutants *via* an N-terminally attached hexahistidine tag was performed as described (Miller *et al*., 1998b). All protein solutions were stored at −70°C in 50 mM sodium phosphate buffer (pH 7) containing 0.5 M sodium chloride and dialysed against the same buffer additionally containing 50 mM EDTA before use. Protein concentrations were determined using an extinction coefficient at 280 nm of $\varepsilon = 57.6 \times 10^3\ M^{-1}\ cm^{-1}$ and the calculated molecular mass of 48,032 Da (Miller *et al*., 1998b).

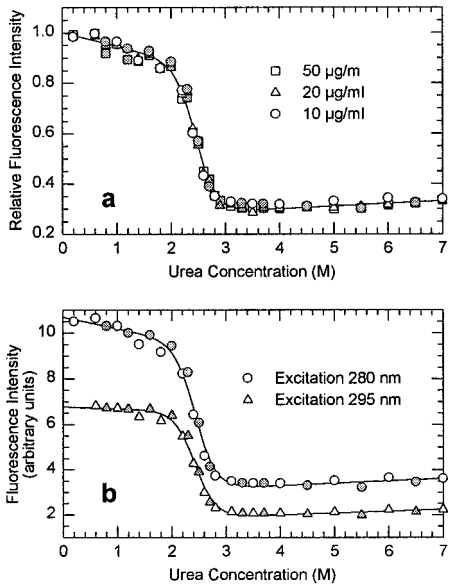

Figure 2. Urea-induced unfolding and refolding curves of the isolated β-helix domain at different protein concentrations (a, the extrapolated fluorescence of the native protein was set to 1) and using different fluorescence excitation wavelengths (b, raw data shown). All urea transitions reported here were performed at a final protein concentration of 10 µg/ml in 50 mM sodium phosphate buffer (pH 7.0) at 10°C (if not indicated otherwise). Fluorescence was measured in a Spex FluoroMax or a Perkin-Elmer MPF-3L fluorescence spectrophotometer with thermostatted cell holder. Excitation wavelength was 280 nm, emission wavelength 338 nm (if not indicated otherwise). To assure reversibility, both unfolding (white symbols) and refolding (grey symbols) transitions were measured and then analysed together by non-linear regression as described by Santoro & Bolen (1988). Assuming that the small structural changes brought about by the point mutations investigated here do not influence the cooperativity of the denaturation curves significantly, we used the same m value of -12.6 kJ mol^{-1} M^{-1} for the analysis of all transitions and obtained the estimated conformational stability of the respective protein at zero denaturant concentration by multiplication with the urea concentration at the denaturation midpoint (Table 1; Pace 1986). The m value used was a weighted average of the mean cooperativities of all mutants studied, employing the variance of the means as a weight factor as usual.

spectroscopic data and receptor oligosaccharide binding. Most important of all though, its denaturant-induced unfolding is completely reversible (Miller *et al.*, 1998b), which allows us to analyse the influence of *tsf* and *su* mutations on equilibrium folding transitions and thus on the thermodynamic stability of this domain.

We exploited the decrease in tryptophan fluorescence upon denaturation of the tailspike β-helix domain to monitor unfolding. Extrapolation of pre- and post-transition baselines to zero denaturant concentration gives a ratio of the specific fluorescence intensities of native to denatured protein of about 3 (Figure 2) at an excitation wavelength of 280 nm and an emission wavelength of 338 nm. As the transition in guanidinium hydrochloride takes place at concentrations far below 1 M denaturant, and as the protein aggregates upon thermal unfolding, we employed urea at pH 7.0 to induce denaturation. At low protein concentrations and low temperatures, aggregation could be avoided, such that stable folding/unfolding equilibria were reached. Denaturation and renaturation transitions were coincident and the transition curves of all protein variants had similar slopes of pre- and post-transition baselines, similar ratios of specific fluorescence of native to denatured protein, and similar cooperativities (Figures 2 to 4). The six tryptophan residues of the tailspike β-helix domain are located in its central part; three of them are part of the caudal fin insertion. The cooperative fluorescence change and the emission spectra observed for the denatured protein ($\lambda_{max} = 353$ nm) thus suggest that the tertiary structure of the β-helix is lost in the transition. In addition, CD data indicate little secondary structure remaining at urea concentrations beyond the folding transition observed by fluorescence (Miller *et al.*, 1998b), suggesting that this transition leads to the urea denatured state and not to an equilibrium folding intermediate.

To test the applicability of a two-state analysis of the folding transitions, experiments with varying protein concentrations and using different excitation wavelengths of fluorescence were performed (Figure 2). The concentration-dependent transitions are congruent within experimental error, and the data obtained at different fluorescence excitation wavelengths fit a two-state folding model with the same cooperativity m and denaturation midpoint $c_{1/2}$. In far-UV CD, the amplitude of the negative band around 217 nm exhibited a cooperative decrease between 2 and 3 M urea. However, for two reasons we refrained from a quantitative analysis of the CD data: first, the β-helix domain begins to oligomerise at protein concentrations necessary for reliable CD measurements of β-structures; second, we observed significant aggregation during refolding at low urea concentrations and protein concentrations >1 µM, not allowing us to determine reliable pre-transition baselines. Thus, although the concentration- and wavelength-dependent data from the fluorescence transitions support a two-state analysis, the existence of equilibrium intermediate states not obvious from fluorescence measurements cannot be ruled out completely. Partially folded states not observed here may be stabilised under different conditions or could be of sufficient influence at high protein concentration to play a part in the folding and aggregation properties of the protein. Consequently, we confine ourselves to a discussion of the very reliable changes in $c_{1/2}$ brought about by the mutations, and use the two-state model only to determine the transition midpoints and to obtain an estimate of the order of magnitude of the corre-

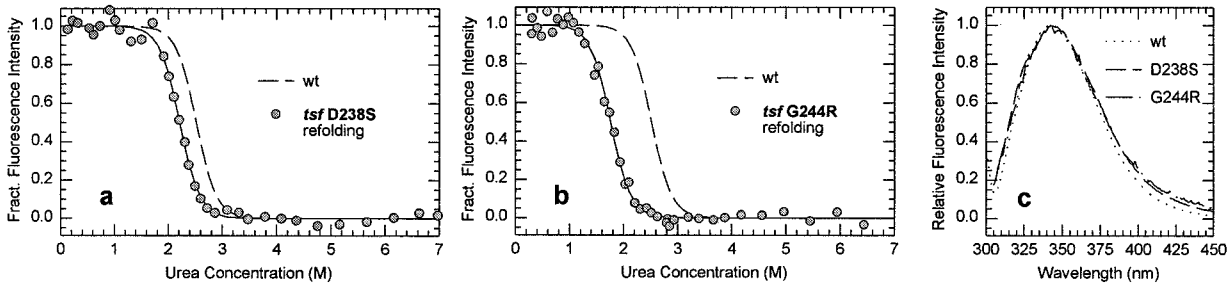

Figure 3. Baseline-corrected urea refolding transitions of the two *tsf* mutants investigated (a, b). The two-state fit from Figure 2a is included as a broken line to indicate the location of the wt transition for comparison. Additionally, the fluorescence spectra of native wt and refolded *tsf* mutants at an excitation wavelength of 280 nm are shown to confirm the native structure of the refolded proteins (c). Although the baseline slopes were similar for all mutants, the folding transitions in Figure 3 and 4 are baseline-corrected for clarity of presentation. Experimental conditions as described in Figure 2.

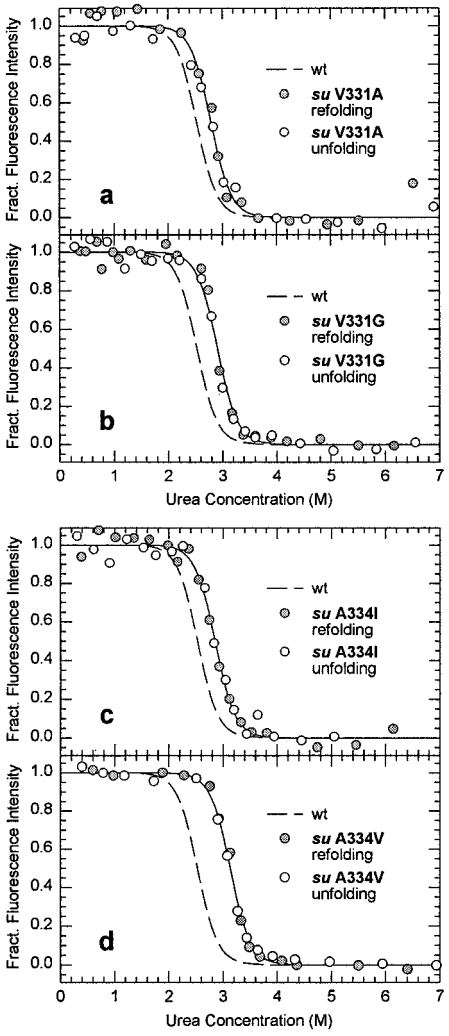

Figure 4. Baseline-corrected urea unfolding (white symbols) and refolding (grey symbols) transitions of the suppressor mutants with amino acid substitutions at position 331 (a, b) and 334 (c, d). The wt two-state fit from Figure 2a is included as a broken line to indicate the location of the wt transition for comparison. Experimental conditions as described in Figure 2.

sponding changes in the free energy of folding ΔG_0.

Effects of *tsf* mutations

For the *tsf* mutants D238S and G244R, significant shifts of the transition midpoints to lower urea concentrations by about 0.3 M and 0.8 M, respectively, compared to wild-type (wt) protein were observed. This clearly indicates a destabilisation of the β-helix domain by both amino acid substitutions (Table 1). Accordingly, a drastically increased tendency to aggregate even in the absence of denaturants was observed during protein purification. It was thus not possible to obtain a solution of native protein from our preparations devoid of small aggregates or misfolded protein. This coincided with a significant decrease of the fluorescence of these protein solutions compared to solutions of protein refolded from 6 M urea at low protein concentration, indicating the presence of non-native protein. These complications made it impossible to determine adequate unfolding transitions, which is why our data for D238S and G244R are obtained from refolding transitions only. For all other mutants and the wild-type, sufficiently aggregate-free protein solutions could be obtained under optimised purification and storage conditions, although aggregation was observed upon prolonged incubation at room temperature, high protein concentrations, intense stirring or introduction of air bubbles. To verify the native structure of refolded *tsf* mutant proteins, we recorded fluorescence spectra (Figure 3) and measured binding to an octasaccharide fragment of the tailspike receptor structure by fluorescence titration as described (Baxa *et al.*, 1996). The values of the dissociation constants K_D and the fractional decreases in fluorescence $\Delta F/F_0$ upon saturation with saccharide are identical to wt within experimental error (Table 1) and the fluorescence spectra are well superimposable. This proves that the

Table 1. Effects of *tsf* and *su* mutations on the stability of the tailspike β-helix domain

Mutant	wt	D238S	G244R	V331A	V331G	A334I	A334V
Original designation		*ts* U2	*ts* H304				
Type of mutation[a]		*tsf*	*tsf*	*su*	*su*	*su*	*su*
$c_{1/2}$ (M)[b]	2.52 ± 0.06	2.20 ± 0.02	1.76 ± 0.06	2.8 ± 0.1	3.0 ± 0.1	2.91 ± 0.07	3.09 ± 0.04
$\Delta c_{1/2}$ (M)[c]	–	−0.32	−0.76	+0.3	+0.5	+0.39	+0.57
ΔG_0 (kJ/mol)[d]	−32	−28	−22	−35	−38	−37	−39
$\Delta\Delta G_0$ (kJ/mol)[e]	–	+4	+10	−3	−6	−5	−7
Global suppressor effect							
in vitro[f]	–	–	–	++	+++	+	++
in vivo[g]	–	–	–	+++	+++	++	+++
k_{Denat} at 71°C (min^{-1})[h]	0.06	0.2	0.2	0.01	0.02	0.2	0.08
K_D (μM) in 40 mM urea[i]	12	14	18	–	–	–	–
$\Delta F/F_0$[k]	0.17	0.15	0.16	–	–	–	–

[a] Temperature-sensitive for folding (*tsf*) or global suppressor (*su*).
[b] The data represent averages of two to nine determinations ± one standard deviation. $c_{1/2}$ was mainly used in the discussion of the results due to the small error in determination of this parameter compared to ΔG_0. Non-linear regression of the transitions was performed as described by Santoro & Bolen (1988).
[c] Difference between $c_{1/2}$ of the respective mutant and the wt value.
[d] The estimated free energy of folding of the respective protein at zero denaturant concentration was calculated by multiplication of the common slope *m* of all transitions with the urea concentration at the denaturation midpoint, as described in Figure 1.
[e] Difference between ΔG_0 of the respective mutant and the wt value.
[f] Taken from Beißinger *et al*. (1995).
[g] Taken from Lee *et al*. (1991).
[h] Rate constant of the thermal denaturation reaction measured in TSPΔN at 71°C taken from Miller *et al*. (1998a).
[i] Dissociation constants of oligosaccharide binding were determined by fluorescence titration as described (Baxa *et al*., 1996) after refolding to a final protein concentration of 10 μg/ml and a urea concentration of 40 mM in 50 mM sodium phosphate buffer (pH 7.0). For wt, native protein was used under identical buffer conditions.
[k] The fractional decrease in fluorescence $\Delta F/F_0$ upon saturation of the protein with oligosaccharide was obtained from fluorescence titration data as described (Baxa *et al*., 1996), and is used here as a measure of quantitative refolding of denatured protein in the case of the *tsf* mutants investigated, as only refolding transitions are available for these mutants.

refolded β-helices have native structure, and that no significant amount of protein lacking octasaccharide binding activity is formed during refolding of the mutant proteins. Therefore, and in the light of the comparable shape of all measured unfolding and refolding transitions of wild-type and mutant proteins, the refolding curves of the *tsf* mutant proteins can be taken to represent equilibrium transitions, and a comparison of transition midpoints is justified. The observed destabilisation brought about by both *tsf* mutants is in agreement with the folding phenotypes of the mutants *in vivo* and *in vitro*, causing drastically decreased folding or reconstitution yields of tailspike and TSPΔN at temperatures above 25°C, and parallels the accelerated denaturation kinetics of TSPΔN at 71°C found by Miller *et al*. (1998a, see Table 1). The destabilising effects of the *tsf* substitutions can be rationalised on the basis of the X-ray structure (Figure 1). Both substitution sites are located in the so-called dorsal fin of tailspike, a subdomain formed from a long loop inserted between the third and fourth coil of the β-helix. The dorsal fin is packed against one face of the β-helix burying Asp238 that forms a hydrogen-bond to Tyr232, which is lost upon mutation to serine. Exchange of glycine at position 244 by arginine leads to increased steric hindrance due to very unfavourable dihedral angles (ϕ: +96°, ψ: −7°) for residues containing long side-chains. The unified picture emerging from the available data is that *tsf* mutations lead to a global destabilisation of both the single domain and the complete tailspike protein, which suggests that a similarly destabilised intermediate of native-like conformation accounts for the increased aggregation tendency of *tsf* mutants during folding (King *et al*., 1987; Danner & Seckler 1993).

Effects of global suppressor mutations

Similarly obvious conclusions as for the *tsf* mutants can be drawn from the effects of the *suppressor* mutations at position 331. For both V331A and V331G, the shifts of the midpoints of their denaturation curves to higher urea concentrations by 0.3 M and 0.5 M, respectively, indicate an increased thermodynamic stability of the β-helix domain. Val331 is located in the substrate binding groove in a turn region between two of the β-sheets (Figure 1), with the side-chain being partly solvent exposed. The stabilising effect in V331A and particularly V331G is presumably due to the removal of steric strain by introduction of an amino acid compatible with the unfavourable backbone dihedral angles at this site (Beißinger *et al*., 1995). Again, the increased thermodynamic stability measured here, the retarded denaturation kinetics measured at 71°C in TSPΔN, and the folding phenotypes show an obvious correlation (Table 1). The stabilising effects brought about by these amino acid substitutions apparently are similar for the native trimer, the monomer, and the folding intermediate crucial for kinetic partitioning during folding.

A more surprising result arises from the data for the *suppressor* mutants A334V and A334I. Although these mutants suppress misfolding caused by *tsf* mutations *in vivo* and *in vitro*, they accelerate the unfolding kinetics of TSPΔN at high temperature (Table 1), and thus apparently destabilise the native, trimeric structure. This seeming discrepancy was hypothesised to arise from an effect on the native structure and a folding intermediate into opposite directions. A334 is located in the β-sheet in the binding groove (Figure 1) with its side-chain pointing into the interior of the β-helix. The introduction of a bulky side-chain of Val or Ile has been proposed to improve hydrophobic stacking in the core of the β-helix of an early but largely structured folding intermediate, but to lead to steric interference in the rigid trimeric structure of tailspike (Beißinger *et al*., 1995). In the isolated β-helix domain investigated here, both mutations lead to a large shift of the transition midpoint to higher urea concentrations, with a $\Delta c_{1/2}$ for A334V of about +0.4 M and about +0.6 M for A334I. This increase in thermodynamic stability is just the effect expected for the postulated folding intermediate with native-like structure but less rigid compaction compared to the completely folded tailspike trimer. This confirms the suggested differences in the mode of action of the *su* mutations at position 331 and 334, respectively (Beißinger *et al*., 1995). A frequent isolation of other mutants with a mechanism analogous to A334V and A334I would still not be expected as most residues of tailspike appear to be highly optimised for stability and folding. Correspondingly, the known suppressor mutation sites are located in the active site region, where function is known to often compromise stability or folding, allowing for the improvement of these properties at the cost of protein function (Meiering *et al*., 1992; Shoichet *et al*., 1995; Baxa, U. & R.S., unpublished observations).

A model for a crucial folding intermediate

It would appear, then, that in structure space the β-helix as an isolated domain is located close to the folding intermediates that play the crucial role at the switch of kinetic partitioning into folding or misfolding on the assembly pathway of tailspike. Not only do the *tsf* mutations investigated destabilise, and all known *su* mutations stabilise the isolated β-helix, but the mutations at position 334 also exert the differential effect expected specifically for an early, but native-like monomeric intermediate. Moreover, several other lines of evidence indicate that the β-helix is formed early during tailspike refolding, before subunit association takes place, and that the folding mutations affect the global stability of a highly structured intermediate. Much of the change in far-UV CD occurring during tailspike folding happens in the dead time of manual mixing, which indicates rapid secondary structure formation (Fuchs *et al*., 1991). The fluorescence changes during subunit folding of the complete tailspike and during the folding of the isolated β-helix are very similar in both their amplitude and time course (Miller *et al*., 1998b). The sites of known *tsf* mutations are distributed throughout the β-helix domain, and all *tsf* mutations investigated have been found to accelerate thermal unfolding of the native structure in tailspike lacking a folded head-binding domain (Chen & King, 1991; Danner & Seckler, 1993; Miller *et al*., 1998a). The temperature-dependence of the partitioning between productive folding and off-pathway aggregation resembles a cooperative melting transition and is thus difficult to explain on the basis of differences in activation energies of unfolding (Haase-Pettingell & King, 1988). Similarly, the effects of *su* mutations, which accelerate subunit refolding kinetics only at high temperature and in the background of *tsf* mutations, are most readily explained by their stabilisation of a cooperatively melting productive folding intermediate (Jaenicke & Seckler, 1997). Lastly, the effects of both types of mutations can be rationalised on the basis of the structure of the β-helix domain in the crystal structure of the native protein. The isolated β-helix domain thus meets the criteria for a native and functional structure (Miller *et al*., 1998b), but still retains conformational properties that allow the effects of folding mutations to be similar to those on the labile intermediate leading to aggregation at restrictive temperature. Using a two-state analysis to estimate the changes in conformational stability from the urea-induced folding transitions, we obtain values for the differences in the free energy of stabilisation $\Delta\Delta G_0$ between mutants and wt in the range of 6 kJ/mol at 10°C (Table 1). According to a model involving an early thermolabile intermediate in rapid equilibrium with an aggregation-prone denatured state (Danner & Seckler, 1993), changing the free energy of stabilisation of the intermediate by a few kJ/mol, as observed for the folding mutants in the isolated β-helix domain, would be sufficient to account for the observed acceleration of the off-pathway reaction of this denatured intermediate towards irreversible aggregation at restrictive temperature (Danner & Seckler, 1993).

Our findings with P22 tailspike contrast with observations on bacterial luciferase and interleukin-1 (Sugihara & Baldwin, 1988; Chrunyk & Wetzel, 1993). In the case of the heterodimeric luciferase, several mutants that exhibit reduced refolding yields at high temperature were found to have unaltered thermodynamic stabilities when compared to the wild-type enzyme in urea-induced equilibrium transitions at 18°C. The luciferase mutations appear to affect subunit association kinetics, thus favouring competing off-pathway reactions (T. O. Baldwin, personal communication). For a *ts* mutant of interleukin-1, Wetzel and co-workers actually observed an increase in the free energy of unfolding relative to the wild-type in urea transitions at 25°C. The same mutant, however, appeared destabilised in thermal melts.

To sum up, our results confirm the notion that P22 tailspike folding mutants act by globally stabilising or destabilising thermolabile monomeric folding intermediates in which the large central β-helix is structured as in the native, trimeric protein. Nevertheless, this intermediate appears to be less rigidly packed than the native structure, which accounts for the different effects of some of these mutants on the conformational stability of the protein at different stages during folding and assembly. The β-helix of tailspike, expressed as an isolated domain, exhibits many of the properties expected for this thermolabile intermediate and might thus prove a valuable model for further investigation into the early stages of tailspike and β-helix folding.

Acknowledgements

We thank Stefan Miller for discussion and for providing plasmid material. The current work has been supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie.

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Edited by A. R. Fersht

(Received 28 January 1998; received in revised form 11 May 1998; accepted 20 May 1998)