Synergism in Folding of a Double Mutant of the α Subunit of Tryptophan Synthase†

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Received August 1, 1986; Revised Manuscript Received August 27, 1986

ABSTRACT: The urea-induced unfolding of the inactive single mutants Tyr-175 → Cys and Gly-211 → Glu and the active double mutant Cys-175/Glu-211 of the α subunit of tryptophan synthase from *Escherichia coli* was examined by using ultraviolet difference spectroscopy. Equilibrium techniques were used to determine the equilibrium free energies of unfolding for the mutant proteins to permit comparison with the wild-type protein. The sum of the changes in stability for the single mutants is not equal to the change seen in the double mutant. This inequality is evidence for a structural interaction between these two residues. Kinetic studies show that this synergism, which destabilizes the native form by 1.5–2.0 kcal/mol at pH 7.8, 25 °C, occurs only after the final rate-limiting step of domain association.

The roles that particular amino acids play in the function of proteins can now be elucidated by using single amino acid replacements (Craik et al., 1985). A logical extension of the mutagenic approach is the determination of functional and structural interactions between two amino acids in a protein. Examples of such interactions can be found in classical genetic studies of second-site revertants, e.g., the α subunit of tryptophan synthase (Yanofsky, 1967) and, more recently, the λ repressor/operator system (Hecht et al., 1985), where loss of binding due to substitution of one amino acid can be recovered by substitution at a second site.

Ackers and Smith (1985) have suggested that the "functional interaction" between two residues can be measured by quantitatively comparing the properties of the two singly substituted proteins with the protein containing both mutations. This approach has been used to determine the interaction energy between two amino acids in the transition state of the rate-limiting step in catalysis by tyrosyl-tRNA synthetase (Carter et al., 1984). The existence and magnitude of a synergistic effect on synthetase function were found to depend on the particular pair of residues studied.

The functional interaction or synergism approach can also be used to monitor interactions that stabilize the native conformation. If the sum of the effects of two single substitutions on the stability of the wild-type protein equals the change seen in the double mutant, then the residues at these two positions do not interact. If the effects are not additive, the two amino acids must interact either directly or indirectly. Stabilities of single and double mutants of T4 lysozyme have been determined in order to detect interactions in the native state (Becktel et al., 1986a,b; Baase et al., 1986); however, the effects of the mutations were additive, and thus, no synergistic effects on the stabilities were found. Studies on the guanidine hydrochloride denaturation of single and double mutants of staphylococcal nuclease demonstrate nonadditivity, but the origin of these effects is unknown (Shortle, 1986).

The synergism approach can be extended to both folding and stability by studying double mutants in a well-defined system. The α subunit of tryptophan synthase from *Escherichia coli* (*E. coli*)[1] is an excellent candidate:

(1) Over 30 single and 15 double mutants have been isolated (Yanofsky, 1967; Murgola & Yanofsky, 1974; Murgola & Hijazi, 1983).

(2) Equilibrium and kinetic urea-induced unfolding experiments have been performed (Crisanti & Matthews, 1981; Matthews & Crisanti, 1981), and a folding model has been proposed (Matthews et al., 1983).

(3) A methodology for the analysis of folding data from the mutant strains has been developed (Beasty et al., 1986).

(4) The $\alpha_2\beta_2$ complex from *Salmonella tymphimurium* has been crystallized, and the structure is being determined (Ahmed et al., 1985). The high homology with the α (85%) and the β (96%) subunits from *E. coli* suggests that the conformations of these two complexes will be quite similar (Nichols & Yanofsky, 1979; Crawford et al., 1980).

The particular system discussed in this paper involves the amino acid substitutions Tyr-175 → Cys and Gly-211 → Glu. The α subunit converts indole-3-glycerol phosphate to indole and glyceraldehyde 3-phosphate; while the single mutants are inactive in this reaction, the double mutant Cys-175/Glu-211 is active (Helinski & Yanofsky, 1963). Since the two positions show functional interdependence, structural interactions in the native state may occur as well.

Materials and Methods

α Subunit of Tryptophan Synthase. The wild-type α subunit of tryptophan synthase from *E. coli* (EC 4.2.1.20) and the Tyr-175 → Cys, Gly-211 → Glu, and Cys-175/Glu-211 mutant α subunits were isolated from strains BN55, PR8, A46, and A46PR8, respectively, using previously described methods with appropriate modifications (Matthews et al., 1983). The abilities of the wild-type α subunit and the mutant proteins to stimulate the activity of the wild-type β_2 subunit, the condensation of indole and serine to tryptophan, were determined as previously described (Beasty et al., 1986). The wild-type α subunit and the mutant proteins Tyr-175 → Cys, Gly-211 → Glu, and Cys-175/Glu-211 had β_2 activities of 4500, 3300, 4000, and 1100 units/mg, respectively, with errors of ±10%. The maximum specific activity of the wild-type protein is 5500 units/mg in this assay (Kirschner et al., 1975).

Protein concentrations for the wild-type and Gly-211 → Glu mutant were determined by using the specific absorption

†This work was supported by U.S. Public Health Service Grant GM23303. C.R.M. is the recipient of Research Career Development Award 1 K04 AG00153.

[1] Abbreviations: *E. coli*, *Escherichia coli*; EDTA, ethylenediaminetetraacetic acid.

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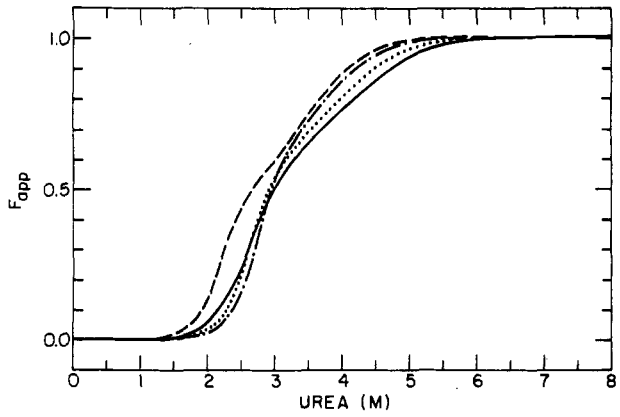

FIGURE 1: Dependence of the fractional change, F_{app}, in $\Delta\epsilon_{286}$ on urea concentration for the Tyr-175 → Cys (···) and Cys-175/Glu-211) (---) α subunits. Lines represent fits to data sets of >20 points as described in the paper. Fits previously presented (Beasty et al., 1986) for the wild-type (—) and Gly-211 → Glu (-·-) α subunits are also shown. The buffer used in these experiments is 10 mM potassium phosphate, 0.2 mM EDTA, and 1 mM β-mercaptoethanol, pH 7.8, 25 °C.

$E^{1\%}_{278nm}$ = 4.4 (Adachi et al., 1974). Due to the loss of a chromophore in the Tyr-175 → Cys and Cys-175/Glu-211 mutants, the specific absorptions for these mutants were both determined to be 3.3 using the Bio-Rad protein assay (Bradford, 1976) with wild-type controls (data not shown).

Chemicals. Ultrapure urea was purchased from Schwarz/Mann and used without further purification. All other chemicals were reagent grade. The buffer used in these studies was 10 mM potassium phosphate, pH 7.8, 0.2 mM EDTA, and 1 mM β-mercaptoethanol.

Spectroscopy. Ultraviolet difference spectroscopy measurements at 286 nm were made by using the tandem cell technique (Herskovits, 1967) on a Cary 118CX spectrophotometer. Equilibrium and kinetic experiments were performed and analyzed as previously described (Beasty et al., 1986).

Results

Equilibrium Experiments. The dependence of the apparent fraction of unfolded protein, $F_{app} = (\epsilon_{obsd} - \epsilon_n)/(\epsilon_u - \epsilon_n)$, for the α subunit on the urea concentration for the wild-type, Tyr-175 → Cys, Gly-211 → Glu, and Cys-175/Glu-211 proteins is shown in Figure 1. The inflection at $F_{app} \sim 0.6$ reflects the highly populated intermediate forms for the multistate denaturation process (Matthews & Crisanti, 1981; Yutani et al., 1979, 1984). The conversion of the native to the intermediate forms occurs at urea concentrations between 1.5 and 3 M urea, while the conversion of intermediate to unfolded forms occurs between 3 and 6 M urea. These intermediate forms contain a folded amino domain, residues 1–188, and an unfolded carboxyl domain, residues 189–268 (Miles et al., 1982).

Figure 1 shows that for the N ↔ I transition, the denaturation curves for the two single mutants are nearly identical with the wild-type protein; however, the curve for the double mutant is shifted to lower urea concentration. The midpoints (see Analysis) of the wild-type, Tyr-175 → Cys, Gly-211 → Glu, and Cys-175/Glu-211 proteins are 2.62, 2.58, 2.73, and 2.20 M urea, respectively. Thus, the double mutant is significantly less stable.

For the I ↔ U transition, the midpoints of the wild-type, Tyr-175 → Cys, Gly-211 → Glu, and Cys-175/Glu-211 proteins are 4.18, 3.71, 3.72, and 3.49 M urea, respectively. The Tyr-175 → Cys replacement destabilizes the intermediates of both the wild-type (Gly-211) and Glu-211 proteins with respect to their unfolded forms. The decrease in stability for the Gly-211 → Glu replacement is thought to be due to electrostatic interactions between the unfolded carboxyl domain, containing residue 211, and the folded amino domain (Beasty et al., 1986).

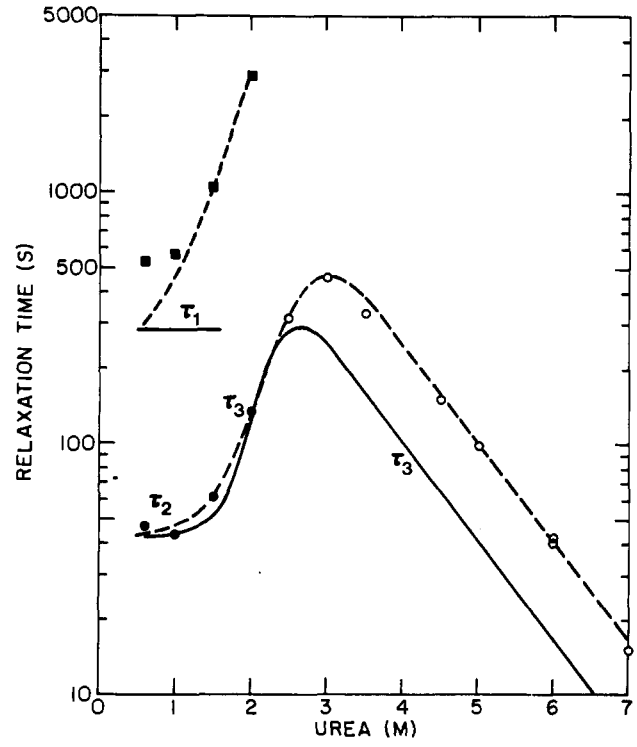

FIGURE 2: Relaxation times at the indicated final urea concentration for the single phase of unfolding (○) and the two slow phases of refolding (●, ■) for the Tyr-175 → Cys α subunit. Fits to this and previously published (Beasty et al., 1986) data, as described in the paper, are also shown for the Tyr-175 → Cys (---) and wild-type (—) α subunits. Unfolding jumps were initiated from 0 M urea and refolding jumps from 6 M urea. Assignments for relaxation times of the folding model (Figure 4) are also shown. Buffer is same as that described in Figure 1.

Kinetic Experiments. The urea-induced unfolding of the wild-type α subunit follows a single kinetic phase, while refolding is more complex (Crisanti & Matthews, 1981). Two urea-independent relaxation times, τ_1 and τ_2, of 282 and 44 s are seen in refolding to low urea concentrations. As the final urea concentration increases, the faster τ_2 phase becomes rate limited by a urea-dependent phase, τ_3. The relaxation time for this phase increases with urea concentration, reaching a maximum of approximately 280 s at ~2.5 M urea. The relaxation time of the single phase of unfolding connects smoothly with this τ_3 phase and decreases with increasing urea concentration. These results are shown graphically in Figure 2. The inverted V shape formed by the urea-dependent refolding and unfolding reactions is characteristic of reversible two-state folding reactions (Tanford, 1970). In the case of the α subunit, this phase corresponds to a rate-limiting, domain association step (Beasty et al., 1986).

The dependences of the observed relaxation times on the final urea concentration for the Tyr-175 → Cys and Cys-175/Glu-211 proteins are shown in Figures 2 and 3, respectively. Figure 2 demonstrates the effect of the mutation Tyr-175 → Cys on the wild-type protein (Crisanti & Matthews, 1981), which has Gly at position 211. Figure 3 illustrates the effect of the same mutation on the protein containing Glu at position 211 (Matthews et al., 1983).

The Tyr-175 → Cys replacement in the wild-type protein (Figure 2) does not affect the τ_2 phase of refolding, but the τ_1 phase becomes urea dependent. The τ_3 refolding phase is

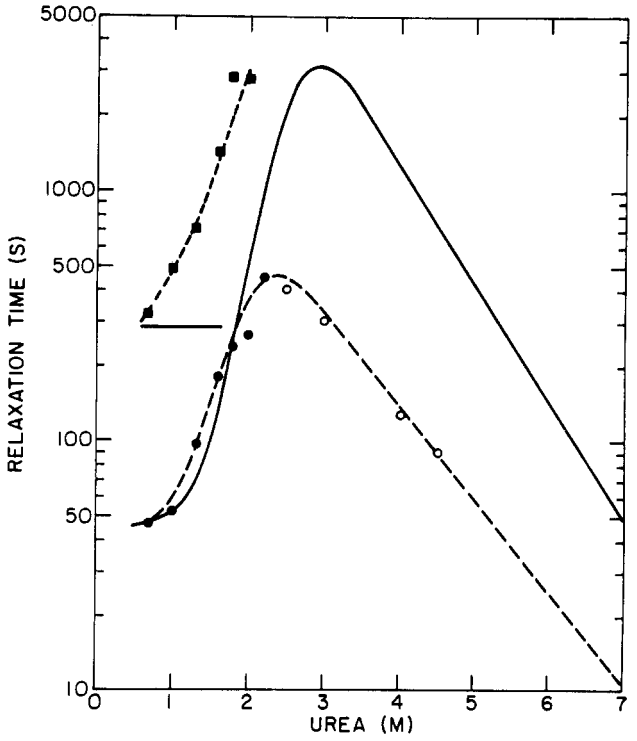

FIGURE 3: Relaxation times at the indicated final urea concentration for the single phase of unfolding (○) and the two slow phases of refolding (●, ■) for the Cys-175/Glu-211 α subunit. Fits to this and previously published (Beasty et al., 1986) data, as described in the paper, are also shown for the Cys-175/Glu-211 (---) and Gly-211 → Glu (—) α subunits. Unfolding jumps were initiated from 0 M urea and refolding jumps from 6 M urea. Buffer used is same as that described in Figure 1.

also unaffected, but the unfolding relaxation time is *increased*. The same substitution in the Gly-211 → Glu protein (Figure 3) causes the same effects on refolding as in the wild-type protein. However, in contrast to the effect on the wild-type protein, the unfolding relaxation time of the Gly-211 → Glu protein is *decreased* by the Cys-175 substitition.

The effects of the mutation at position 175 on all observable phases of refolding are independent of the amino acid at position 211. In contrast, the effects on unfolding depend upon the amino acid at position 211. The relation between these results and the equilibrium results will be examined further under Discussion.

ANALYSIS

Equilibrium Model. The equilibrium data for the urea-induced unfolding transition of the α subunit can be fit to a three-state model, N ↔ I ↔ U. The appropriate equation (Beasty et al., 1986) is

$$F_{app} = \frac{K_{NI}(Z + K_{IU})}{1 + K_{NI} + K_{IU}}$$

where K_{NI} and K_{IU} are the equilibrium constants for the N ↔ I and I ↔ U reactions, respectively. Also, $Z = (\epsilon_I - \epsilon_N)/(\epsilon_U - \epsilon_N)$, where ϵ_N, ϵ_I, and ϵ_U are the extinction coefficients of the native, intermediate, and unfolded forms, respectively.

The dependence of the equilibrium constants obtained from this fit, K_{NI} and K_{IU}, on the urea concentration can be described by a linear dependence of the associated free energy change on the urea concentration (Beasty et al., 1986; Schellman, 1978):

$$\Delta G_{xy} = \Delta G^\circ_{xy} + A_{xy}[\text{urea}]$$

where ΔG_{xy} is the free energy difference between species x and y at a given urea concentration, ΔG°_{xy} is the free energy difference in the absence of urea, and A_{xy} is a parameter that reflects the cooperativity of the transition. The parameters ΔG°_{xy} and A_{xy} can then be determined by using the nonlinear least-squares fitting program NLIN (SAS Institute, 1985), with $Z = 0.59$ for most α-subunit proteins (Beasty et al., 1986). For the Tyr-175 → Cys and Cys-175/Glu-211 mutants, the effect of removing the Tyr-175 chromophore on the value of Z was determined by fitting Z as a variable. The resulting value of 0.48 was then included as a constant, and both data sets were refit. This decrease in the value of Z implies that the Tyr-175 chromophore becomes exposed to solvent in the native to intermediate transition. Since Tyr-175 is located in the amino domain, which remains folded in the intermediate, this residue is most likely located at the domain interface in the native protein.

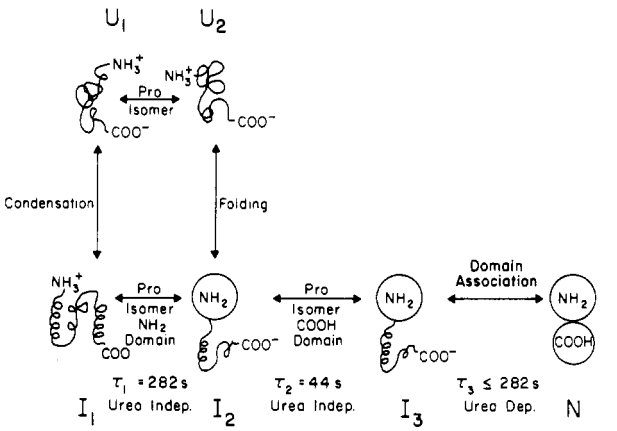

FIGURE 4: Proposed folding model for the α subunit.

The free energy changes can be compared in the absence of denaturant by evaluating differences in ΔG°_{xy}. However, the linear extrapolation assumed to obtain ΔG°_{xy} may not be correct at low denaturant concentrations (Pace & Vanderburg, 1979). Furthermore, the magnitude of the extrapolation decreases the accuracy of the value. An alternative approach is to compare free energy changes at the concentration of denaturant that corresponds to the midpoint of the unfolding transition in the wild-type protein (Beasty et al., 1986; Cupo & Pace, 1983). The midpoint of the transition, $C_{m_{xy}}$, is the concentration of urea at which $\Delta G_{xy} = 0$:

$$C_{m_{xy}} = -\Delta G^\circ_{xy}/A_{xy}$$

These reference states are 2.62 and 4.18 M urea for the N ↔ I and I ↔ U transitions in the wild-type protein at 25 °C and pH 7.8. The effect of a given mutation, $\Delta\Delta G_{xy}$, is then computed by using the fitted values of ΔG°_{xy} and A_{xy} for that mutant and the urea concentration for the appropriate reference state. $C_{m_{xy}}$ and $\Delta\Delta G_{xy}$ are relatively insensitive to variations in the value of Z. Values for the wild-type protein and the three mutants are shown in Table I.

Kinetic Model. The structural folding model for the α subunit is shown in Figure 4 (Beasty et al., 1986). U_1 and U_2 are two unfolded forms that probably differ by the state of isomerization of an "essential" proline residue (Crisanti & Matthews, 1981; Jullien & Baldwin, 1981). Proline isomerization is also likely to be responsible for the three intermediate species, I_1, I_2, and I_3. The urea-independent relaxation time, τ_1, reflects the isomerization of an amino-domain proline. This process controls folding of the collapsed intermediate I_1 to the intermediate I_2, which contains a folded amino domain. The nonnative carboxyl-domain proline in I_2 isomerizes with relaxation time τ_2 to the native isomeric form in I_3. This reaction

Table I: Midpoints and Changes in Free Energy of Unfolding at Standard States Defined by the Wild-Type Protein[a]

species	$C_{m_{NI}}$	A_{NI}	$C_{m_{IU}}$	A_{IU}	$C^{K}_{m_{12}}$	$A^{\ddagger}_{u}$	$A^{\ddagger}_{r}$	$\Delta\Delta G_{NI}{}^{b}$	$\Delta\Delta G_{IU}{}^{c}$	$\Delta\Delta G^{\ddagger}_{u}{}^{d}$	$\Delta\Delta G^{\ddagger}_{r}{}^{d}$
wild type	2.62 ± 0.02	−2.2 ± 0.2	4.18 ± 0.06	−1.2 ± 0.2	2.48 ± 0.13	−0.54 ± 0.03	2.4 ± 0.5				
Tyr-175 → Cys	2.58 ± 0.04	−3.0 ± 0.5	3.71 ± 0.06	−1.1 ± 0.1	2.89 ± 0.14	−0.54 ± 0.03	1.6 ± 0.3	−0.1 ± 0.1	−0.5 ± 0.1	0.5 ± 0.1	−0.4 ± 0.2
Gly-211 → Glu	2.73 ± 0.02	−3.0 ± 0.2	3.72 ± 0.04	−1.5 ± 0.1	2.81 ± 0.17	−0.65 ± 0.11	2.2 ± 0.3	0.3 ± 0.1	−0.7 ± 0.1	1.7 ± 0.3	0.7 ± 0.2
Cys-175/Glu-211	2.20 ± 0.03	−3.0 ± 0.4	3.49 ± 0.04	−1.4 ± 0.1	2.21 ± 0.33	−0.51 ± 0.18	1.9 ± 0.7	−1.3 ± 0.2	−1.0 ± 0.1	0.2 ± 0.3	0.8 ± 0.7

[a] Values defined under Analysis. Units are as follows: C_m, [urea]; A, kcal mol^{-1} [urea]$^{-1}$; $\Delta\Delta G$, kcal mol^{-1}. [b] Standard state: 2.62 M urea. [c] Standard state: 4.18 M urea. [d] Standard state: 2.48 M urea.

precedes folding of the carboxyl domain and the subsequent rate-limiting association to the folded amino domain, with relaxation time τ_3.

The folding model provides an explanation for the urea dependence of the τ_1 phase in the Tyr-175 → Cys and Cys-175/Glu-211 mutants. Because the $U_1 \leftrightarrow I_1$ equilibration is fast compared to the $I_1 \rightarrow I_2$ relaxation (Crisanti & Matthews, 1981), the apparent rate constant for this phase, $k_{app} = \tau_1^{-1}$, is given by $k_{app} = kK/(1 + K)$. In this equation, k is the urea-independent rate constant for the $I_1 \rightarrow I_2$ process and $K = [I_1]/[U_1]$. When $K \gg 1$, the collapse of the U_1 form to the I_1 form is highly favored, and thus, $k_{app} \approx k$ and τ_1 is urea independent. However, if K approaches unity, the urea dependence of this equilibrium constant results in urea dependence of τ_1. As the I_1 form becomes less stable, the relaxation time of the τ_1 phase increases. Thus, the Tyr-175 → Cys mutation appears to destabilize the collapsed I_1 form with respect to the unfolded form U_1.

As has been discussed (Beasty et al., 1986), τ_3 is a function of the urea-dependent unfolding (k_u) and refolding (k_r) rate constants: $\tau_3^{-1} \approx k_u + k_r$. The free energies of activation of unfolding and refolding derived from the Eyring formulation have been observed to be linearly dependent on the urea concentration (Beasty et al., 1986) and can be described by

$$\Delta G^{\ddagger}_{x} = \Delta G^{\circ\,\ddagger}_{x} + A^{\ddagger}_{x}[\text{urea}]$$

where $\Delta G^{\ddagger}_{x}$ is the free energy of activation, $\Delta G^{\circ\,\ddagger}_{x}$ is the free energy of activation in the absence of urea, and $A^{\ddagger}_{x}$ is the dependence of this activation free energy on urea concentration. For unfolding, $A^{\ddagger}_{u} < 0$, and for refolding, $A^{\ddagger}_{r} > 0$. Again, comparison of the effects of the mutations on these kinetic parameters can be made either in the absence of denaturant or at the midpoint of the wild-type transition. The equilibrium parameters, ΔG^{K}_{12} and A^{K}_{12}, for the $N \leftrightarrow I_3$ reaction can be determined since $\Delta G^{K}_{12} = \Delta G^{\ddagger}_{u} - \Delta G^{\ddagger}_{r}$ and $A^{K}_{12} = A^{\ddagger}_{u} - A^{\ddagger}_{r}$. The transition midpoint, $C^{K}_{m_{12}}$, is determined from these parameters as in the equilibrium case. Unfolding and refolding activation free energies for the $N \leftrightarrow I_3$ step are then compared at the wild-type midpoint, 2.48 M urea at 25 °C, and are expressed as $\Delta\Delta G^{\ddagger}_{u}$ and $\Delta\Delta G^{\ddagger}_{r}$, respectively. These parameters can be evaluated for the wild-type and three mutant proteins and are also shown in Table I.

The near-equivalence of the equilibrium parameters obtained from kinetic studies of the $N \leftrightarrow I_3$ reaction and those from the equilibrium study of the $N \leftrightarrow I$ reaction for nearly all mutant α subunits studied thus far (Beasty et al., 1986) has led to the conclusion that the major part of the apparent $N \leftrightarrow I$ equilibrium is due to the $N \leftrightarrow I_3$ reaction. Since the accuracy of equilibrium data is much greater when measured directly rather than from kinetic experiments (Beasty et al., 1986), the equilibrium values from the $N \leftrightarrow I$ transition, $\Delta\Delta G_{NI}$, will be used in discussing the effects of mutations on the $N \leftrightarrow I_3$ equilibrium.

Discussion

Synergistic Effects. For the $N \leftrightarrow I$ equilibrium transition, the large destabilization, −1.3 kcal/mol ($\Delta\Delta G_{NI}$ in Table I), seen *only* in the double mutant clearly demonstrates that the residues at these two positions interact. This structural interaction parallels the effects of these mutations on function; both single mutants are inactive while the double mutant is active (Helinski & Yanofsky, 1963).

From the kinetic experiments in Figures 2 and 3, it can be seen that the effects of mutations at positions 175 and 211 are independent in refolding. However, the effect of each mutation

on the unfolding kinetics clearly depends on the specific amino acid at the other position. Therefore, these two residues interact. The kinetic experiments thus provide a more refined view of how the mutations alter the folding.

These effects can be put on a quantitative basis by using the following approach (Carter et al., 1984; Ackers & Smith, 1985). The free energy change due to the double mutation, $\Delta\Delta G_{AB}$, can be written as the sum of the changes due to the two single mutations, $\Delta\Delta G_A$ and $\Delta\Delta G_B$, plus the interaction energy between the two mutations, ΔG_I:

$$\Delta\Delta G_{AB} = \Delta\Delta G_A + \Delta\Delta G_B + \Delta G_I$$

From the data in Table I, the interaction energies for the equilibrium and kinetic experiments can be calculated. The interaction energy for the N $\leftrightarrow$ I equilibrium is

$$\Delta G_I = -1.3 - (-0.1) - 0.3 = -1.5 \pm 0.2 \text{ kcal/mol}$$

Similarly, interaction energies calculated from the activation free energies for the N $\rightarrow$ I_3 and I_3 $\rightarrow$ N kinetic transitions are -2.0 ± 0.4 and 0.5 ± 0.8 kcal/mol, respectively. The observation that only the unfolding step, N $\rightarrow$ I_3, has a significant interaction energy demonstrates that interaction between residues 175 and 211 occurs *after* the final rate-limiting step of domain association. The absence of additional interactions in the rest of the folding pathway is confirmed by the additivity of the effects of mutations on the I $\leftrightarrow$ U transition ($\Delta G_I = 0.2 \pm 0.1$ kcal/mol). This same conclusion is reached whether free energy changes at the wild-type midpoint (Table I) or at zero molar denaturant concentrations (data not shown) are used as a basis for comparison.

In addition to the 175/211 pair, second-site revertant studies showed that loss of activity due to a double mutation in the carboxyl domain at positions 211 and 213 is restored by a third mutation at position 177, which is located in the amino domain (Yanofsky, 1967). This pattern of reversion suggests that regions 175–177 and 211–213 are in close proximity at the domain interface and that the reason for the 175/211 interaction may be direct physical contact between two strongly interacting domains. Further experiments with other sets of 175/211 double mutants may help to answer this question.

Replacements at residue 211 also affect the energy of the transition state of the domain association step (Matthews et al., 1983); however, the present study has shown that interaction with residue 175 occurs after this step. Therefore, the structural change that occurs in the transition state of the domain association step is not an interface-wide phenomenon.

Conclusion. Analysis of the folding behavior of a double mutant has been used to quantitate the existence of an interaction between residues 175 and 211 in the native state of the α subunit. No such interaction exists in the intermediate state, in the transition state between the native and intermediate states, or in the unfolded state. This experimental approach provides a method for determining the existence of such interactions, which even with the benefit of a crystal structure could not be identified with *certainty* using our current knowledge of the forces stabilizing protein structure. Further experiments with other double mutants will determine the extent of other residue–residue interactions in the α subunit and refine our understanding of the structural aspects of its folding pathway.

ACKNOWLEDGMENTS

We are grateful to Dr. Charles Yanofsky, Stanford University, for samples of the Tyr-175 $\rightarrow$ Cys, Gly-211 $\rightarrow$ Glu, and Cys-175/Glu-211 mutant α-subunit strains and to Dr. Brian Nichols, University of Illinois, Chicago, for samples of the plasmid pBN55, which produces the wild-type α subunit.

REFERENCES

Ackers, G. K., & Smith, F. R. (1985) *Annu. Rev. Biochem. 54*, 597.

Adachi, O., Kohn, L. D., & Miles, E. W. (1974) *J. Biol. Chem. 249*, 7756.

Ahmed, S. A., Miles, E. W., & Davies, D. R. (1985) *J. Biol. Chem. 260*, 3716.

Baase, W. A., Muchmore, D. C., & Becktel, W. J. (1986) *Biophys. J. 49*, 109a.

Beasty, A. M., Hurle, M. R., Manz, J. T., Stakhouse, T., Onuffer, J. J., & Mathews, C. R. (1986) *Biochemistry 25*, 2965.

Becktel, W. J., Baase, W. A., Chen, B. L., Muchmore, D. C., Schellman, C. G., & Schellman, J. A. (1986a) *Biophys. J. 49*, 572a.

Becktel, W. J., Baase, W. A., Wetzel, R., & Perry, L. J. (1986b) *Biophys. J. 49*, 109a.

Bradford, M. M. (1976) *Anal. Biochem. 72*, 248.

Carter, P. J., Winter, G., Wilkinson, A. J., & Fersht, A. R. (1984) *Cell (Cambridge, Mass.) 38*, 835.

Craik, C. S., Langman, C., Fletcher, T., Roczniak, S., Barr, P. J., Fletterick, R., & Rutter, W. J. (1985) *Science (Washington, D.C.) 228*, 291.

Crawford, I. P., Nichols, B. P., & Yanofsky, C. (1980) *J. Mol. Biol. 142*, 489.

Crisanti, M. M., & Matthews, C. R. (1981) *Biochemistry 20*, 2700.

Cupo, J. F., & Pace, C. N. (1983) *Biochemistry 22*, 2654.

Hecht, M. H., Hehir, K. M., Nelson, H. C. M., Sturtevant, J. M., & Sauer, R. T. (1985) *J. Cell. Biochem. 29*, 217.

Helinski, D. R., & Yanofsky, C. (1963) *J. Biol. Chem. 238*, 1043.

Herskovits, T. T. (1967) *Methods Enzymol. 11*, 748.

Jullien, M., & Baldwin, R. L. (1981) *J. Mol. Biol. 145*, 265.

Kirschner, K., Wiskocil, R. L., Loehn, M., & Rezeau, L. (1975) *Eur. J. Biochem. 60*, 513.

Matthews, C. R., & Crisanti, M. M. (1981) *Biochemistry 20*, 784.

Matthews, C. R., Crisanti, M. M., Manz, J. T., & Gepner, G. L. (1983) *Biochemistry 22*, 1445.

Miles, E. W., Yutani, K., & Ogasahara, K. (1982) *Biochemistry 21*, 2586.

Murgola, E. J., & Yanofsky, C. (1974) *J. Mol. Biol. 86*, 775.

Murgola, E. J., & Hijazi, K. A. (1983) *Mol. Gen. Genet. 191*, 132.

Nichols, B. P., & Yanofsky, C. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 5244.

Pace, C. N., & Vanderburg, K. E. (1979) *Biochemistry 18*, 288.

SAS Institute Inc. (1985) *SAS User's Guide: Statistics, Version 5 Edition*, p 575, SAS Institute Inc., Cary, NC.

Schellman, J. A. (1978) *Biopolymers 17*, 1305.

Shortle, D. (1986) *J. Cell. Biochem. 30*, 281.

Tanford, C. (1970) *Adv. Protein Chem. 24*, 1.

Yanofsky, C. (1967) *Harvey Lect. 61*, 145.

Yutani, K., Ogasahara, K., Suzuki, M., & Sugino, Y. (1979) *J. Biochem. (Tokyo) 85*, 915.

Yutani, K., Ogasahara, K., Aoki, K., Kakuno, T., & Sugino, Y. (1984) *J. Biol. Chem. 259*, 14076.