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Fluorescence and folding properties of Tyr mutant tryptophan synthase α-subunits from *Escherichia coli*☆

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Abstract

The fluorescence of tyrosine has been used to monitor a folding process of tryptophan synthase α-subunit from *Escherichia coli*, because this protein has 7 tyrosines, but not tryptophan. Here to assess the contribution of each Tyr to fluorescence properties of this protein during folding, mutant proteins in which Tyr was replaced with Phe were analyzed. The result shows that a change of Tyr fluorescence occurring during folding of this protein is contributed to ~40% each by Tyr^4 and Tyr^{115}, and to the remaining ~20% by Tyr^{173} and Tyr^{175}. Y173F and Y175F mutant proteins showed an increase in their fluorescence intensity by ~40% and ~10%, respectively. These increases appear to be due to multiple effects of increased hydrophobicity, quenching effect of nearby residue Glu^{49}, and/or energy transfer between Tyrs. Two data for Y173F α-subunit of urea-induced unfolding equilibrium monitored by UV and fluorescence were different. This result, together with ANS binding and far UV CD, shows that folding intermediate(s) of Y173F α-subunit, contrary to that of wild-type, may contain self-inconsistent properties such as more buried hydrophobicity, highly quenched fluorescence, and different dependencies on urea of UV absorbance, suggesting an ensemble of heterogeneous structures.

Keywords: Folding intermediate; Protein folding; Tryptophan synthase α-subunit; Tyrosine fluorescence; Tyrosine mutant

Tryptophan synthase α-subunit (αTS) from *Escherichia coli* has been used to study protein folding and stability [1–7]. It consists of 268 residues and has no disulfide bond or prosthetic group. The X-ray structure of highly homologous αTS from *Salmonella typhimurium* showed $(\beta\alpha)_8$ barrel motif, which is most frequently found in protein folds [8].

The folding process of αTS has been studied by various structure-monitoring techniques including fluorescence (FI), UV absorbance, CD, and NMR [2–7]. Aromatic residues of αTSs from both *E. coli* and *S. typhimurium* consist of 7 Tyrs and 11 Phes without any single Trp. Because the FI intensity of Tyr is much more prominent than that of Phe [9], this property of Tyrs in αTS has been utilized to monitor its folding properties. The three-dimensional structure shows that three Tyrs at 102, 169, and 203 are highly exposed to solvent, while the other 4 Tyrs are buried in protein (Fig. 1). Considering the fact that only buried Tyrs may possibly experience changes in spectroscopic properties in the folding/unfolding, only part of 7 Tyrs may be implicated in folding process of this protein.

In the present study, wild-type (WT) and mutant αTS proteins in which Tyr was substituted with Phe were examined for their FI property in order to evaluate the contribution of each chromophore to the FI properties. As a result, Tyrs at 4, 115, 173, and 175 were shown to contribute to different extent to FI during the folding process of the protein. In addition, the characterization of Y173F mutant protein revealed

☆ *Abbreviations:* ANS, 1-anilino-8-naphthalene sulfonate; αTS, tryptophan synthase α-subunit; N, I, and U, native, intermediate, and unfolded forms, respectively; FI, fluorescence; WT, wild-type.

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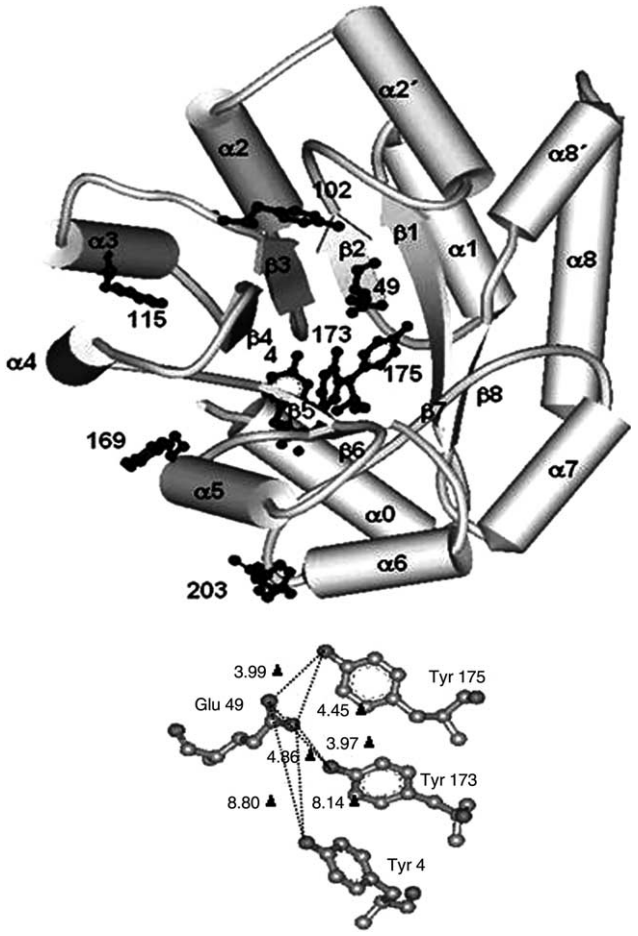

Fig. 1. Schematic structure of tryptophan synthase α-subunit from *S. typhimurium*. It has $(\beta\alpha)_8$ barrel motif with additional three helices (α0, α2′, and α8′). The diagram is generated with WebLab ViewerLite 3.7 from a refined version of PDB file, 1wsy [8]. Seven Tyrs and Glu^{49} residues are also depicted. At the bottom, expanded view of side chains of Tyr^4, Tyr^{173}, Tyr^{175}, and Glu^{49} are shown.

various intriguing features of native and folding intermediate forms.

Materials and methods

Enzymes, chemicals, and buffer. All chemicals were of reagent grade or ultrapure quality and purchased from Sigma (St. Louis, MO). Enzymes for mutagenesis and sequencing were purchased from Amersham Pharmacia Biotech (Uppsala, Sweden) or New England BioLabs (Bervery, MA). Oligonucleotides were obtained from Research Genetics (Huntsville, AL). Buffer F contains 10 mM potassium phosphate buffer (pH 7.8), 0.2 mM EDTA, and 1 mM β-mercaptoethanol.

Mutagenesis and cloning. Oligonucleotide-directed mutagenesis of the *trpA* gene was carried out according to the method of Kunkel et al. [10]. To substitute the Tyr residues at 4, 102, 115, 169, 173, 175, and 203 positions with Phe, the oligonucleotides 5′-ATG GAA CGC TTC GAA TCT CTG-3′, 5′-CTG TTG ATG TTT GCC AAT CTG-3′, 5′-GAT GAG TTT TTT GCC CAG TGC-3′, 5′-ATA GCC TCT TTC GGT CGT GGT-3′, 5′-GGT CGT GGT TTC ACC TAT TTG-3′, 5′-GGT TAC ACC TTT TTG CTG TC-3′, and 5′-CGT AAA GAG TTC AAC GCT GCA-3′ were used, respectively. The sequence alterations from the WT sequence are underlined. The altered sequences were confirmed by dideoxy chain termination method using T7 Sequencing Kit (Amersham Pharmacia Biotech). Preparation of E49G mutant protein was described before [3].

Purification and activities of αTSs. The WT and mutant proteins were purified to high homogeneity as described previously [11,12]. Purified WT αTS was measured by extinction value $E^{1\%}_{278\,nm} = 4.4$ [13] and mutant αTSs were estimated by microbiuret assay using WT αTS as a standard [14]. SDS–PAGE was performed according to the method of Laemmli [15]. Enzymatic activity of WT and mutant αTS was measured in the β reaction; synthesis of L-Trp from indole plus L-Ser [16].

Thermal denaturation. This was performed by using UV difference spectroscopy (Varian, Cary 4E) equipped with a thermoelectric cell holder. Proteins (1 mg/ml) in butter F were degassed just before use. Absorbance at 287 nm was monitored with temperature increasing at a rate of 1 °C/min, after the solution temperature was equilibrated to the initial temperature. Temperature was monitored by temperature probe inside cuvette, while the solution was being stirred by a magnetic bar. When the proteins were cooled down rapidly on ice for 1 h after thermal denaturation and were rescanned under the same condition, about 80% intensity was recovered.

FI, UV absorbance, and far UV CD spectrophotometry. All measurements were carried out in buffer F. Urea-induced equilibrium unfolding measurements were performed 12 h after incubating proteins with the various concentrations of urea. FI intensities of proteins (0.15–0.2 mg/ml) were scanned in the range or measured at 303 nm with an excitation at 275 nm at ambient temperature by a Hitachi FI spectrophotometer F-4500. UV difference absorbance of proteins (1–1.2 mg/ml) was measured at 287 nm at 25 °C by Cary 4E spectrophotometer (Varian). The CD spectra of proteins (0.2 mg/ml) were monitored over the wavelength from 270 to 180 nm using a 0.05 cm pathlength cell at ambient temperature by a JASCO J-715 spectropolarimeter. The CD signal was converted to MRE using the following equation: $\mathrm{MRE} = \theta/(10 \cdot l \cdot C \cdot N_A)$, where θ is the ellipticity in mdeg, l is the pathlength in centimeters, C is the molar concentration, and N_A is the number of residue.

Curve fit. The equilibrium unfolding data were fit to three-state model [17]. The observed changes of FI and UV spectroscopic measurement were normalized to the apparent fraction of U form, F_{app}, by $F_{app} = (Y_{obs} - Y_N)/(Y_U - Y_N)$, where Y_{obs} is the observed value at a given urea concentration, and Y_N and Y_U are the values of N and U, respectively. Data were fit using following equations for a three-state model with a non-linear least-squares program, Sigma plot 5.0 (SPSS). $F_{app} = K_{NI}(Z + K_{IU})/[1 + K_{NI}(1 + K_{IU})]$, where $Z = (Y_I - Y_N)/(Y_U - Y_N)$ and K_{NI} and K_{IU} are the equilibrium constants for the $N \leftrightarrow I$ and $I \leftrightarrow U$ transition, respectively. $\Delta G_{NI} = \Delta G_{NI(H_2O)} - m_{NI}[\mathrm{urea}]$ and $\Delta G_{IU} = \Delta G_{IU(H_2O)} - m_{IU}[\mathrm{urea}]$, where $\Delta G_{NI(IU)}$ and $\Delta G_{NI(IU)(H_2O)}$ are the free energy differences between N (I) and I (U) in the presence and absence of the given urea concentration, respectively, and $m_{NI(IU)}$ is the dependence of $\Delta G_{NI(IU)}$ on urea concentration.

The fractions of the native (f_N), intermediate (f_I), and unfolded (f_U) forms at various urea concentrations were calculated by the following equations:

$$\exp[-\Delta G_{NI(H_2O)}/RT] = K_{NI} = f_I/f_N,\ \exp[-\Delta G_{IU(H_2O)}/RT] = K_{IU} = f_U/f_I$$

$$\text{and } f_N + f_I + f_U = 1.$$

ANS binding. The concentration of 1-anilino-8-naphthalene sulfonate (ANS) was determined by the absorbance at 370 nm using a molar extinction coefficient of 6800 $M^{-1}\,cm^{-1}$. Proteins were pre-equilibrated for 12 h in the presence of urea and then incubated for additional 2 h with 50-fold molar excess of ANS. ANS binding FI was monitored at 490 nm with an excitation at 370 nm by a Hitachi FI spectrophotometer model F-4500.

Trypsin treatment and size-exclusion chromatography. The proteins (1 mg/ml) were incubated with 10 μg trypsin for 15 min at room

temperature. Trypsin-treated proteins were eluted with buffer F at 0.5 ml/min through a size-exclusion (Shodex KW-804 column) HPLC (Waters). The elution was monitored by absorbance at 280 nm.

Results

Global structure of mutant αTSs

To check a possibility that the replacement of Tyr by Phe in αTS could disturb its global structure, examined were enzymatic activities and far UV CD, which monitor three-dimensional and secondary structures, respectively. Seven Tyr mutant αTSs showed 80–108% of WT activity and far UV CD spectra of all these mutant proteins remained nearly unchanged as well (data not shown). These indicate that none of the mutant αTSs show appreciable deviation from WT structure.

Fluorescence (FI) properties of Tyr mutant αTSs

FI emission spectra of WT and 7 Tyr mutant αTSs were measured with an excitation at 275 nm (Fig. 2).

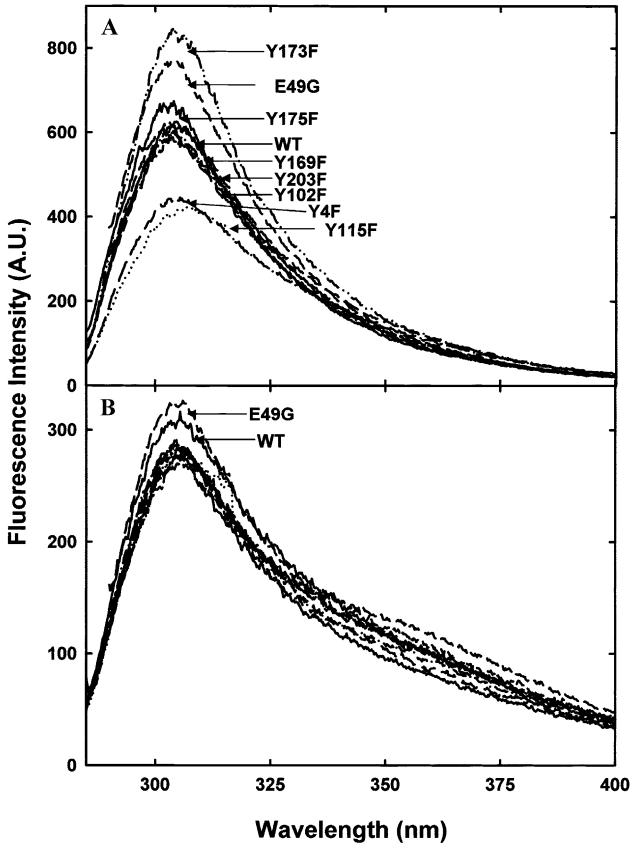

Fig. 2. Fluorescence emission spectra of wild-type and mutant α-subunits. Proteins were pre-equilibrated in buffer F in the absence (A) or presence of 6 M urea (B). Fluorescence intensity was scanned from 285 to 400 nm with an excitation at 275 nm. Unmarked spectra in B are for 7 Tyr mutant proteins. A.U., arbitrary unit.

With an excitation at 275 nm, FI is virtually emitted from only remaining Tyrs, but not from Phes of each mutant protein, because Phe is insignificantly excited at 275 nm [18].

Maximum FI emissions in the native forms of all Tyr αTSs are at ~303 nm with varied FI intensities (Fig. 2A). Y4F or Y115F mutant αTS showed ~30% decrease in FI intensity compared to WT, but FI intensity of Y102F, Y169F or Y203F virtually remains unchanged. Unexpectedly, Y173F and Y175F mutant proteins showed an increase in FI intensity by ~40% and ~10%, respectively.

The unfolded states of all mutant proteins with the addition of 6 M urea showed similar FI intensities, but all of which were less than that of WT protein in unfolded state (Fig. 2B). The FI intensity at 303 nm of each Tyr could be calculated. Using this value and linear dependence of Tyr FI intensity on urea concentration [19], contributions of three exposed Tyr^{102}, Tyr^{169}, and Tyr^{203} on Fl intensities at 303 nm of native forms of WT and all mutant proteins were estimated. Thus, the contribution of Tyrs to total FI of native form of αTS could be deduced: Tyr115, ~30%; 3 Tyrs at 4, 173, and 175 together, ~50%; and 3 Tyrs at 102, 169, and 203, ~20%. The FI difference occurring during unfolding/refolding could be ascribed to Tyr 115 for 40% and to Tyrs at 4, 173, and 175 for 60%, because FI intensities of the exposed Tyrs were not changed.

Thermal denaturation and urea-induced unfolding equilibrium

Tyr FI intensity may increase, as its environment is more rigid and hydrophobic. In the three-dimensional structure of αTS [8], three of Tyr^{4}, Tyr^{173}, and Tyr^{175} are clustered inside the protein (Fig. 1). Therefore, Y173F or Y175F substitution may improve hydrophobicity so as to increase FI intensity of other nearby Tyr(s). To test this, the stability of mutant αTSs was measured by thermal denaturation (Fig. 3A). The thermal unfolding curves of the proteins show that melting temperatures (T_m) of Y173F and Y175F were 56.3 and 60.3 °C, respectively, while that of WT was 54.3 °C. Given the almost constant dependency of transition on heat, the stabilities of Y175F and Y173F mutant αTSs were increased. Therefore, it is concluded that FI increase in Y173F and Y175F mutant αTSs can be ascribed to the hydrophobicity improvement of the residues.

The stabilities of proteins were also examined by urea-induced unfolding equilibrium (Fig. 3B). Two data by FI and UV of WT αTS fit very well to a three-state model of native (N), intermediate (I), and unfolded form (U). The thermodynamic parameters from the curve fits for WT UV and FI data are also in good agreement within experimental error (Table 1) and are similar to those reported by others [2,4,20].

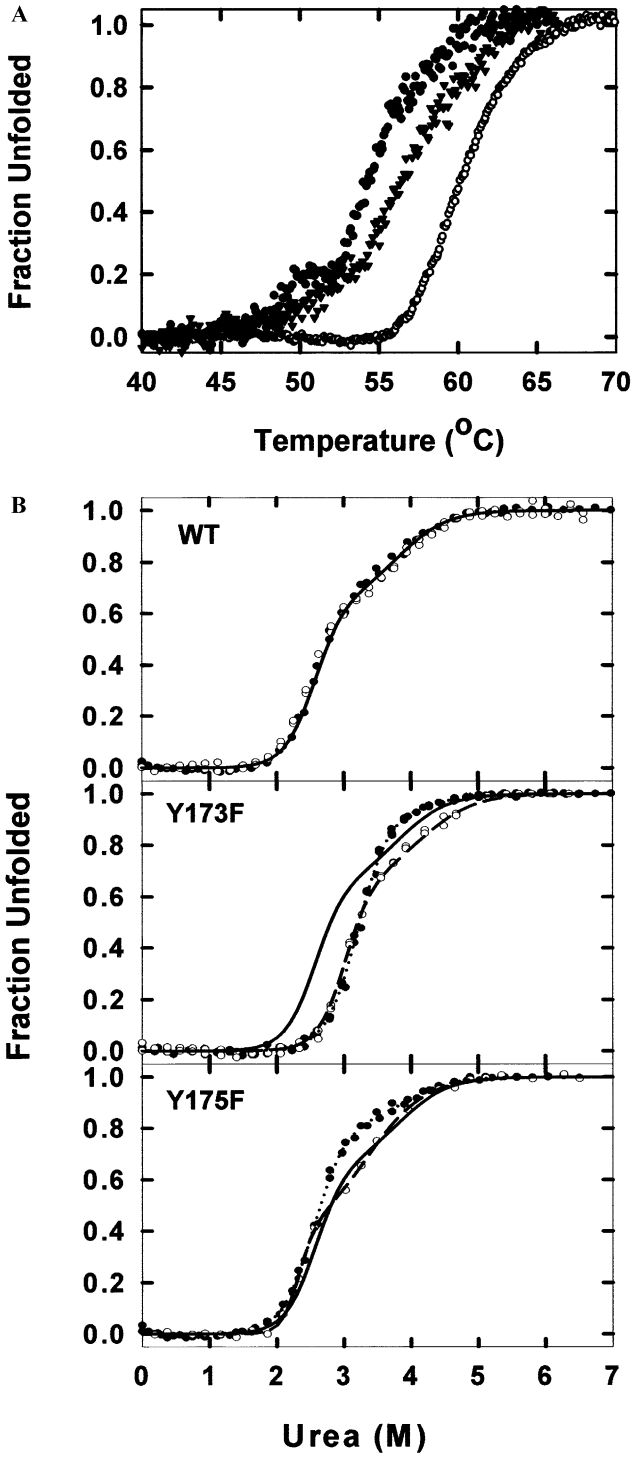

Fig. 3. Thermal denaturation (A) and urea-induced equilibrium unfolding (B) of proteins. (A) Thermal denaturation was obtained by monitoring the change of absorbance at 287 nm of wild-type (closed circle), Y173F (triangle), and Y175F (open circle) proteins with temperature increasing at a rate of 1 °C. (B) For urea equilibrium, proteins were equilibrated for 12 h in a buffer (pH 7.8) with various concentrations of urea. Fluorescence intensity (closed circle) of proteins was measured at 303 nm with an excitation at 275 nm at ambient temperature. UV absorbance (open circle) of proteins was measured at 287 nm at 25 °C. Curve fit was obtained using three-state model: solid line, wild-type; dot, fluorescence; and dashed line, UV.

To the contrary, two data monitored by UV and FI for Y173F or Y175F mutant αTS showed manifest disagreements (Fig. 3B). For these data, a three-state model also fit better than a two-state model (data not shown). But, Z value was varied for the best fit in case of FI data for Y173F. The thermodynamic parameters are shown in Table 1. While both N ↔ I and I ↔ U transitions of Y173F were stabilized by ~2 kcal/mol in FI data, UV data showed that only N ↔ I transition was stabilized by ~2.9 kcal/mol, with I ↔ U transition remaining unchanged. The stabilities for N ↔ I and I ↔ U steps of Y175F were changed by ~2.8 and ~0.5 kcal/mol relative to WT protein from UV data, whereas only I ↔ U transition was altered by ~1 kcal/mol from FI data. The overall stabilities of Y173F and Y175F αTSs appear to increase by about 3–4 and 1–3 kcal/mol, respectively. This is consistent with the results of thermal denaturation.

Structure of Y173F mutant αTS

Urea-induced unfolding equilibrium data for Y173F and Y175F mutant αTSs were varied according to UV or FI, as mentioned above. The fractional distributions of N, I, and U forms for WT and Y173F proteins depending on urea concentration are given in Fig. 4A. The concentration of I form for WT αTS reaches a maximum at 3.2 M urea, regardless of monitoring methods. In contrast, the I form of Y173F αTS is most populated at 3.8 M urea with FI, but at 3.5 M urea with UV. Both FI and UV data for Tyr mutant proteins cannot be directly compared with WT data, because Tyr, which is a source for FI and UV absorbance monitored here, was in effect removed in mutant proteins. Therefore, secondary structure contents of αTSs with the addition of various urea concentrations were examined by far UV CD in order to resolve the discrepancy for the I form of Y173F (Fig. 4B). The Y173F mutant αTS showed nearly identical secondary structure contents in N (at 0 M urea) and U states (at 6 M urea) to those of corresponding WT state. The secondary structure content of WT at 3.25 M urea was comparable to that of Y173F protein at 3.9 M urea, but much different from that of Y173F protein at 3.48 M urea (Fig. 4B). This suggests that the I structure of Y173F monitored by FI rather than by UV may be similar to that of WT.

The I forms of proteins tend to enable their hydrophobic core to be more accessible to solvent. ANS binding to Y173F and WT proteins was examined (Fig. 5). The extent of ANS binding to the N state of Y173F αTS was much lower than that of WT protein. Moreover, maximum binding of ANS to WT protein was observed at ~2.5 M urea, while it was at ~3.2 M urea for Y173F protein. The FI extent for Y173 protein at this urea concentration was lower than that for WT. These indicate that hydrophobic portion is less exposed in

Table 1
Thermodynamic parameters for urea-induced unfolding transition at 0 M Urea[a]

αTS	$\Delta G_{NI(H_2O)}$	m_{NI}	$\Delta G_{IU(H_2O)}$	m_{IU}	Z	$\Delta\Delta G_{NI(H_2O)}$	$\Delta\Delta G_{IU(H_2O)}$	$\Delta\Delta G_{NU(H_2O)}$
Fluorescence								
Wild-type	6.35 ± 0.21	−2.46 ± 0.06	6.15 ± 0.28	−1.57 ± 0.07	0.64 ± 0.03			
Y173F	8.38 ± 0.21	−2.61 ± 0.07	8.27 ± 0.84	−1.87 ± 0.12	0.91	2.03	2.12	4.15
Y175F	6.30 ± 0.18	−2.47 ± 0.08	7.11 ± 1.29	−1.79 ± 0.29	0.83 ± 0.02	−0.05	0.96	0.91
Absorbance								
Wild-type	5.56 ± 0.15	−2.19 ± 0.07	6.22 ± 0.50	−1.54 ± 0.11	0.67 ± 0.02			
Y173F	8.43 ± 0.62	−2.79 ± 0.21	6.33 ± 0.25	−1.52 ± 0.06	0.66 ± 0.02	2.87	0.11	2.98
Y175F	8.34 ± 0.80	−3.46 ± 0.36	6.72 ± 1.18	−1.84 ± 0.28	0.56 ± 0.05	2.78	0.50	3.28

[a] Units are as follows: $\Delta G_{xy(H_2O)}$, free energy at 0 M urea, kcal per mole; m, kcal per mole per mole (urea); $\Delta\Delta G_{NI}$ and $\Delta\Delta G_{IU}$, kcal per mole; and $\Delta\Delta G_{xy(H_2O)} = \Delta G_{xy(H_2O)}$ (Mutant) − $\Delta G_{xy(H_2O)}$ (WT).

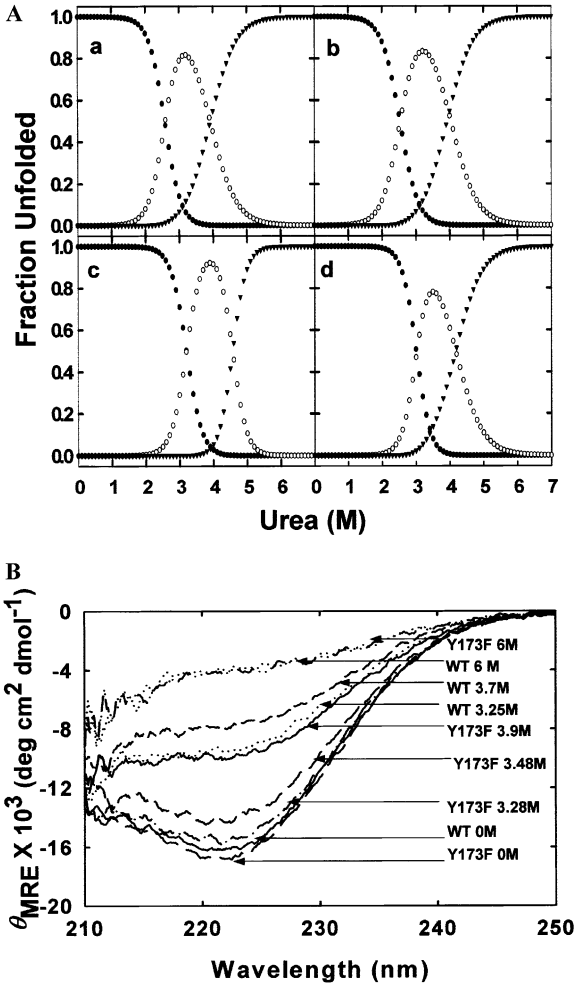

Fig. 4. Dependence of N, I, and U forms on urea (A) and far UV CD spectra of α-subunits (B). (A) N (closed circle), I (open circle), and U (triangle) forms were calculated as in Materials and methods for FI data (a, c) and UV (b, d) of WT (a, b) and Y173F proteins (c, d). (B) Proteins were equilibrated for 12 h in buffer F with various concentrations of urea. The CD spectra were measured from 270 to 180 nm using a 0.05 cm pathlength cell. The CD signal was converted to MRE.

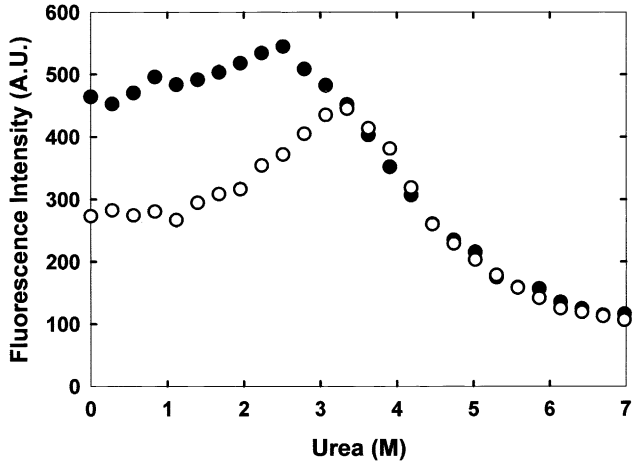

Fig. 5. ANS binding fluorescence of wild-type and Y173F α-subunits.The wild-type (●) and Y173F (○) proteins were pre-equilibrated for 12 h in the presence of urea and then incubated for 2 h with the addition of 50-fold molar excess of ANS. ANS binding FI was monitored at 490 nm with an excitation at 370 nm.

both N and I states of Y173F αTS. For WT αTS, the maximum FI intensity for ANS binding appeared at ~0.7 M urea lower than the urea concentration for maximum amount of I state. Similarly, ~0.6 M urea difference was shown for Y173F αTS. This is consistent with a possibility that I form of Y173F monitored by FI rather than by UV may be similar to that of WT.

Sensitivity to trypsin was measured to check the compactness of protein (Fig. 6). Major cleavage site to trypsin is at the C-terminal peptide bond of Arg^{188} [21,22]. The region 178–191 between β6 and α6 encompassing Arg^{188} is too flexible to be resolved in X-ray crystal structure [8]. The N state of Y173F mutant protein showed very high resistance to trypsin digestion, indicating that the region including Arg^{188} might be compact even in the N form. This result is in good agreement with ANS binding result.

Discussion

In the present paper, it is shown that a change of Tyr FI intensity during a folding/unfolding process of WT

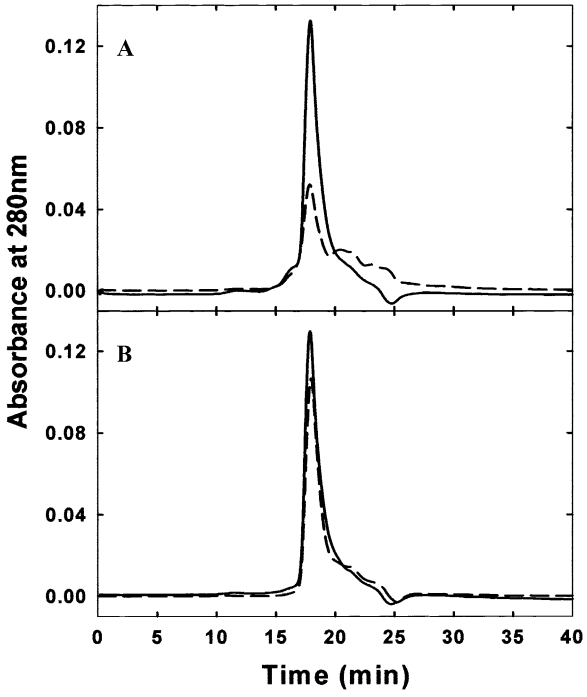

Fig. 6. Size-exclusion chromatography of trypsin-treated wild-type and Y173F α-subunits. The wild-type (A) and Y173F α-subunits (B) at 1 mg/ml were treated with 10 μg trypsin for 15 min at room temperature. α-Subunits without (solid lines) and with the treatment (dashed lines) were eluted at 0.5 ml/min through a size-exclusion HPLC, with monitoring absorbance at 280 nm.

αTS is contributed dominantly by Tyr^{4} and Tyr^{115}, and to a minor extent by Tyr^{173} and Tyr^{175}. Therefore, the folding process of WT TS monitored by Tyr FI may be regarded grossly as environmental changes around Tyr^{4} and Tyr^{115}. This is surprising because WT αTS folding process monitored by far UV CD also showed the identical transition pattern. This indicates that environments of mainly two internal chromophores, Tyr^{4} and Tyr^{115}, in the intermediate form of equilibrium folding, are exposed somehow to the same extent as the secondary structure content changes.

Replacement of either Tyr^{173} or Tyr^{175} with Phe resulted in a substantial increase of FI intensity (Fig. 2). A quenching effect may be involved beside the hydrophobic effect, because Glu^{49} is located close to Tyrs in the αTS structure (Fig. 1). The carboxylate groups of weak acids that serve as acceptors for the phenolic proton in the excited state are known as Tyr FI quencher [18]. To examine a possible quenching effect of Glu^{49}, FI intensity of E49G mutant αTS was measured; its intensity in native structure increased by ~20% compared to that of WT (Fig. 2). Considering the fact that the stability of E49G mutant protein is almost similar to that of WT [3], it is suggested that Glu^{49} may have quenching effect on neighboring Tyrs. Moreover, it has been shown that energy transfers can occur between aromatic residues in protein [18]. It is, therefore, highly possible that energy may be transferring between spatially stacked Tyr^{173} and Tyr^{175} (and possibly Tyr^{4}) residues of αTS. Taken together, a combined effect of multiple factors seems to be responsible: a hydrophobicity increase, quenching of neighboring Glu^{49}, and energy transfer between Tyr^{173} and Tyr^{175} (and Tyr^{4}). A similar case can be found in human carbonic anhydrase II, where W5F substitution increased Trp FI intensity [23]. It was suggested that energy from Trp^{16} is transferred to nearby Trp^{5} and that neighboring His^{64} quenches FI.

In the extracellular ribonuclease barnase, quenching effect of His^{18} on Trp^{94} in native form successfully served as a probe of the folding pathway [24,25]. In the case of αTS, a native structure formation of 173/175(/4) residues would yield dramatic increase of total FI intensity contrary to decrease in barnase. Dramatic FI increase of Tyr(s) in Y173F mutant αTS can be utilized to monitor folding process in a similar way.

Y173F and Y175F mutant αTSs showed a marked disagreement between two data measured by FI and UV. The Z value (the fractional change in UV or FI intensity of intermediate form) of FI data was much larger than that of UV data for these mutants (0.91 vs. 0.66 for Y173F, 0.83 vs. 0.56 for Y175F) (Table 1). Removal of an internal chromophore makes it difficult to compare the denaturant-induced unfolding of Tyr mutants with WT data. For the comparison, far UV CD was measured. The result suggests that the intermediate of FI data may similarly represent WT intermediate concerning secondary structure content and exposed hydrophobic region. However, the compactness and quenching extent in FI intensity of Y173F intermediate are not in agreement.

This disagreement might suggest the mixture of various intermediate structures. The equilibrium intermediate (I1) was known from the early studies [26,27]. Various models for this intermediate have been suggested; folded α-2 domain with unfolded α-1 [28], a molten globule [29], and a heterogeneous mixture of Pro *cis*/*trans* isomers [2,30] or partially unfolded form of α-1 attached to either a completely folded or completely unfolded form of α-2 [20]. The result reported here is compatible with the latter two models. The intermediate dealt with in this paper seems to be clearly different from another intermediate (I2) recently observed at 5 M urea, as this intermediate cannot be detected by FI and UV, but can be detected by FI anisotropy and NMR [6].

It is noteworthy that the N state of Y173F protein seems to have different structure from that of WT. The presence of various conformers for αTS such as active and inactive conformers has been known [31]. Especially flexible region including Arg^{188} is known to play a role in a series of catalysis with fine allosteric communication of $\alpha_2\beta_2$ complex [31]. Therefore, it is also suggested here that N form of Y173F αTS might be one of such conformers playing in allostery.

Replacement of Tyr with Phe at 173 as in Y173F proteins resulted in very dramatic effects on folding and stability properties of αTS. An overall increase in stability of Y173F αTS indicates that 173 residue may be important to stability of native form of this protein as well. The further detailed characterization of Tyr mutant proteins could be useful for the elucidation of folding mechanism.

Acknowledgments

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