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SCANNING CALORIMETRIC STUDY OF TRYPTOPHAN SYNTHASE α-SUBUNITS FROM *ESCHERICHIA COLI*, *SALMONELLA TYPHIMURIUM*, AND AN INTERSPECIES HYBRID

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Summary

Denaturation temperatures (Td) and denaturation enthalpies (ΔH) for α-subunits from *Escherichia coli*, *Salmonella typhimurium*, and an interspecies hybrid in which the C-terminal domain comes from *E. coli* and N-terminal domain comes from *S. typhimurium* were determined by scanning microcalorimetry (DASM4) at various pH values. The Td of the *E. coli* protein was the highest at each pH and the Td of the hybrid protein was close to that of *S. typhimurium*. ΔH values of the three proteins were similar to each other at the same temperature. The results suggest that the difference in the stabilities between *E. coli* and *S. typhimurium* proteins is caused by the difference of hydrophobicity with replacements of Val to Ile at positions 52 and 166, which are buried in the interior of the molecule.

Introduction

Many mutants of the tryptophan synthase α-subunit from *Escherichia coli* have been used for studies of the effects of amino acid substitutions at a single site on folding and stability (1-4). Although the x-ray crystallographic analysis of the α-subunit from *E. coli* has not yet been determined, the structure of the α-subunit in the $\alpha_2\beta_2$ complex from *Salmonella typhimurium* has been elucidated(5). We tried to determine which amino acids affect the stability of the α-subunit from *E. coli* by comparing the stabilities of the proteins from *E. coli* and *S. typhimurium* and correlating differences with amino acids which can be located in the structure of the α-subunit from *S. typhimurium*.

The α-subunit from *E. coli* consists of two folding domains; a N-terminal domain (188 residues) and a C-terminal domain (80 residues) (6). The sequences of both proteins from *E. coli* and *S. typhimurium* show homologies of 92.0 % in the N-terminal domain and 68.8 % in the C-terminal domain (7, 8). In order to determine the stability of each of these domains, we have previously used spectroscopic techniques to compare the guanidine hydrochloride (GuHCl) denaturation and thermal denaturation of α-subunits from *E. coli*, *S. typhimurium*, and from an interspecies hybrid in which the C-terminal domain comes from *E. coli* and the N-terminal domain comes from *S. typhimurium* (9). We now use scanning calorimetry to get additional information on the thermodynamic properties.

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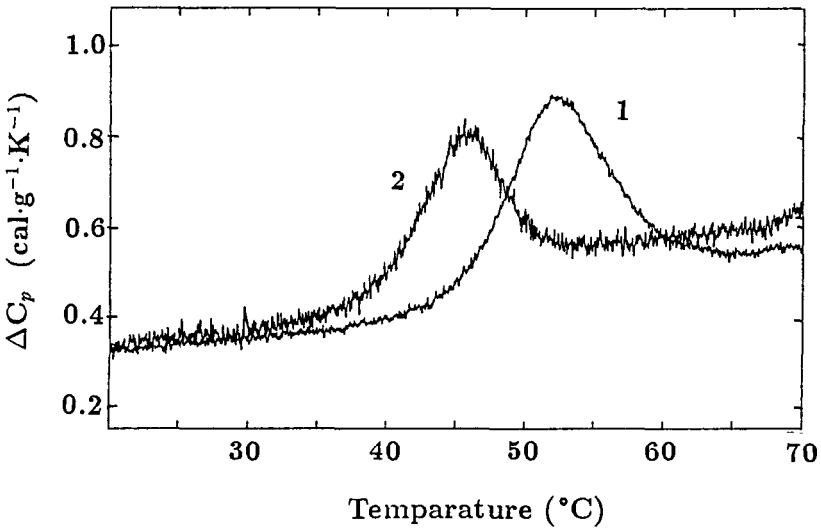

Figure 1: Instrument traces of excess heat capacity curves for the α-subunits from *E. coli* and *S. typhimurium*. (1);the protein from *E. coli*, concentration of 2.47 mg/ml, scan rate of 0.5 degree/minute, at pH 9.21. (2);the protein from *S. typhimurium*, concentration of 1.04 mg/ml, scan rate of 0.5 degree/ml, at pH 9.08.

Material and Methods

The α-subunits of tryptophan synthase from *E. coli*, *S. typhimurium*, and the interspecies hybrid were obtained as described (9). Protein concentrations were estimated from the absorbance at 278.5 nm, assuming $E^{1\%}_{1cm} = 4.4$ (10).

Calorimetric measurements were carried out with a scanning microcalorimeter, DASM4, at the scan rate of 0.25, 0.5 or 1.0 degree/minute, equipped with NEC personal computer. Each memory unit in the computer stored one datum obtained over 5 seconds. The protein concentrations used were 0.8-3.3 mg/ml. Just before measurements, protein solutions were dialyzed with an Amicon YM10 membrane against 1 mM sodium tetraborate containing 1mM EDTA. The pH of the solvent was adjusted with 1 N HCl or 1 N KOH. The pH values reported in this paper were determined after calorimetric measurements. Calorimetric and Van't Hoff enthalpies were calculated by a computer program developed by Kidokoro and Wada (11). The reversibilities of the thermal denaturation were examined by reheating the protein solution in the calorimeter cell immediately after cooling from the first run.

Results

Typical excess heat capacity curves for the α-subunits from *E. coli* and *S. typhimurium* are shown in Fig. 1, suggesting that α-subunit from *E. coli* is more heat stable than that from *S. typhimurium*. Figure 2 shows the pH dependence of the denaturation temperatures of the three α-subunits from *E. coli*, *S. typhimurium*, and the interspecies hybrid. These denaturation temperatures (Td) correspond to peak temperatures in calorimetric measurements. The figure shows that the α-subunit from *E. coli* is the most stable among three proteins at each pH. These results are similar to those obtained from GuHCl denaturation (9).

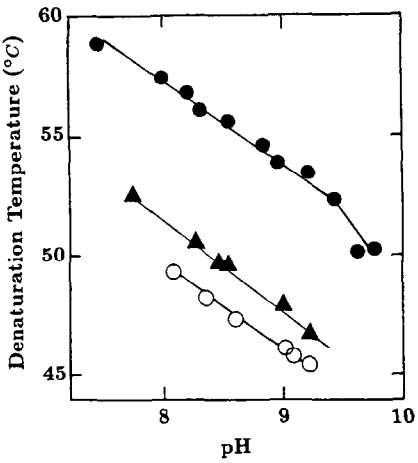

Figure 2: pH Dependence of denaturation temperatures for α-subunits from *E. coli* (●), *S. typhimurium* (○), and hybrid (▲) at various pHs.

Protein	pH	T_d (°C)	$\Delta_d h^{cal}$ (cal/g)	$\Delta_d h^{vH}$ (cal/g)	Ratio $\Delta_d h^{cal}/\Delta_d h^{vH}$	Average
EC	8.98	54.2	4.09	3.15	1.28	1.25±0.09
ST	9.01	46.2	3.03	3.68	0.78	0.80±0.10
HY	9.00	47.6	3.11	3.18	0.98	1.00±0.11

Table 1: Thermodynamic parameters of three α-subunits from *E. coli* (EC), *S. typhimurium* (ST), and the hybrid (HY) near pH 9.0. Average represents average values measured in various pHs.

Figure 3 shows the denaturation calorimetric enthalpies for the three proteins as a function of Td. The measurements of the α-subunits from *S. typhimurium* and the hybrid were done at a scan rate of 0.25 or 0.5 degree/minute. The denaturation enthalpy values obtained at a scan rate of 1.0 degree/minute were lower than those obtained at lower scan rates. These results indicate that the thermal denaturations did not reach equilibrium at each temperature when they were done at a scan rate of 1.0 degree/minute. The denaturation enthalpies for the three proteins seem to be a linear function of Td. The slope of the straight line corresponding to the heat capacity change of denaturation was 0.15 $cal \cdot g^{-1} \cdot K^{-1}$. This was similar to values obtained from the individual calorimetric recordings. The linear relationship suggests that the denaturation calorimetric enthalpies of the three proteins coincide with each other at the same temperature.

The van't Hoff enthalpies of the three proteins were also obtained from the excess heat capacity curves. The van't Hoff enthalpies for *E. coli* α-subunit were different from values for *S. typhimurium* protein at the same denaturation temperature. Thermodynamic parameters of three α-subunits from *E. coli*, *S. typhimurium*, and the hybrid near pH 9.0 are listed in Table I. The average ratios of calorimetric and van't Hoff enthalpies ($\Delta H^{cal}/\Delta H^{vH}$) were 1.25±0.09, 0.80±0.10, and 1.00±0.10 for each protein from *E. coli*, *S. typhimurium*, and hybrid. The dif-

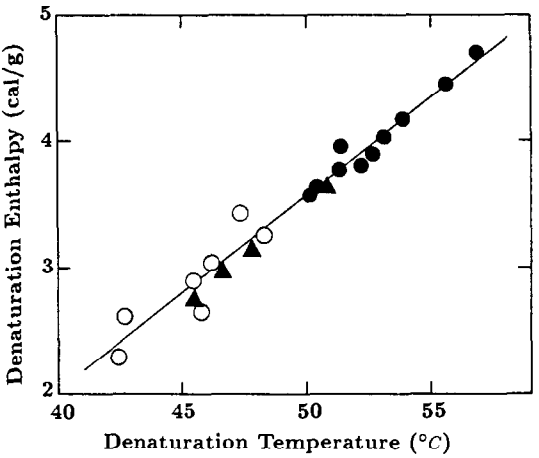

Figure 3: Denaturation specific enthalpy for α-subunits from *E. coli* (●), *S. typhimurium* (○), and hybrid (▲) as a function of denaturation temperatures. A line was given by a least-square fit to the experimental points for the three proteins.

ferent ratios among three proteins mean that the thermal denaturation mechanism of *E. coli* α-subunit is different from that of *S. typhimurium* protein.

Discussion

If the ratio of $\Delta H^{cal}/\Delta H^{vH}$ departs from unity, we can not employ the two-state model to describe the denaturation process. When the ratio is greater than unity there may be intermediates between the native and denatured states: a ratio of 0.5 indicates that the cooperative unit of a protein is a dimer (12). The ratio of $\Delta H^{cal}/\Delta H^{vH}$ for the α-subunit from *E. coli* was 1.25±0.09, suggesting that there are some intermediates in the denaturation process. Our previous report that the ratio for the α-subunit from *E. coli* was close to unity (13, 14) may have been caused by measurement of the calorimetric enthalpies at a scan rate of 2 degree/minute which was too fast to allow unfolding to reach equilibrium. The present suggestion that there are some intermediates in the denaturation process agrees with results obtained by GuHCl denaturation (6, 7). In the first step of the denaturation process by GuHCl, only the C domain (C-terminal 80 residues) unfolds, but the N domain (N-terminal 188 residues) remains native, resulting in a stable intermediate form, and in the second step, the N domain also unfolds. Each domain of *E. coli* α-subunit may also unfold separately in the thermal denaturation.

The ratio of $\Delta H^{cal}/\Delta H^{vH}$ for *S. typhimurium* α-subunit was significantly less than unity, suggesting the presence of some interaction between the protein molecules. However, the measurements of the molecular weight of the *S. typhimurium* α-subunit at various concentrations of the protein by a low-angle laser light-scattering photometry coupled with high-performance gel chromatography (15) did not show any association of the protein at room temperature (unpublished data). Although the protein does not form a dimer at room temperature, it may associate at higher temperatures near denaturation temperature. The ratio of $\Delta H^{cal}/\Delta H^{vH}$ for the hybrid protein was close to unity (1.00±0.10).

Denaturation enthalpies (ΔH^{cal}) of three proteins were similar to each other at the same temperatures (Fig. 3), although denaturation temperatures (Td) of *E. coli* protein were considerably higher than those of the *S. typhimurium* protein at the same pH (Fig. 2). These results mean that *E. coli* protein is more stable than the other one and it is caused mainly by entropic factor. The stability of the hybrid protein was close to that of the *S. typhimurium* protein, suggesting that the destabilization of the *S. typhimurium* is mainly due to amino acid replacements in the N domain. Among the 15 residue replacements between the N domains of the two proteins, 11 residues are completely exposed on the surface of the molecule, and 4 residues are buried in the interior of the molecule, judging from the accessible surface area of their residues (5). The four buried residues are Val52, Asn68, Ile148, and Val166 in the protein from *S. typhimurium* and Ile52, Thr68, Val148, and Ile166 in the other protein. The replacements on the surface might not affect the stability. It has been reported that the stability of the *E. coli* α-subunit increases with increasing hydrophobicity of residues at position 49 which is buried in the interior of the molecule (4) and the increase of the stability is caused by entropic factor (13). The hydrophobicities of Ile, Val, Thr, and Asn are estimated to be 2.97, 1.69, 0.44, and -0.01, respectively (16). The difference in hydrophobicities of the four buried residues (positions: 52, 68, 148, and 166) between the two proteins is 1.73 kcal/mol. The difference in hydrophobicity at position 68 is a few (0.45 kcal/mol) and Asn68 is not completely buried since the accessibility is 11.2 %. Then, we can conclude that the *E. coli* α-subunit is stabilized mainly by the increase of hydrophobicity in the interior with replacements at position 52 and 166, although one of them is compensated by the replacement at 148.

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