A Hyperthermophilic Protein Acquires Function at the Cost of Stability†

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Received May 8, 2006; Revised Manuscript Received August 8, 2006

ABSTRACT: Active-site residues are not often optimized for conformational stability (activity−stability trade-offs) in proteins from organisms that grow at moderate temperature. It is unknown if the activity−stability trade-offs can be applied to proteins from hyperthermophiles. Because enzymatic activity usually increases at higher temperature and hyperthermophilic proteins need high conformational stability, they might not sacrifice the stability for their activity. This study attempts to clarify the contribution of active-site residues to the conformational stability of a hyperthermophilic protein. We therefore examined the thermodynamic stability and enzymatic activity of wild-type and active-site mutant proteins (D7N, E8A, E8Q, D105A, and D135A) of ribonuclease HII from *Thermococcus kodakaraensis* (*Tk*-RNase HII). Guanidine hydrochloride (GdnHCl)-induced denaturation was measured with circular dichroism at 220 nm, and heat-induced denaturation was studied with differential scanning calorimetry. Both GdnHCl- and heat-induced denaturation were highly reversible in these proteins. All the mutations of these active-site residues, except that of Glu8 to Gln, reduced the enzymatic activity dramatically but increased the protein stability by 7.0 to 11.1 kJ mol^{-1} at 50 °C. The mutation of Glu8 to Gln did not seriously affect the enzymatic activity and increased the stability only by 2.5 kJ mol^{-1} at 50 °C. These results indicate that hyperthermophilic proteins also exhibit the activity−stability trade-offs. Therefore, the architectural mechanism for hyperthermophilic proteins is equivalent to that for proteins at normal temperature.

Proteins fold into unique three-dimensional structures to acquire function. The structures reflect two opposite tendencies: (i) the overall structures are organized to be stable; and (ii) their active sites often have flexibility, leading to the local instability of the active sites. Some experimental studies indicate that active-site residues are not optimized for the conformational stability. Yutani and co-workers (*1*, *2*) constructed a complete set of 19 mutant proteins at Glu49 of the α subunit of tryptophan synthase (TSase α) from *Escherichia coli* and demonstrated that every mutation reduced activity significantly but stabilized the protein. In functional residues of T4 lysozyme, the mutations increased the conformational stability up to 8.4 kJ mol^{-1} (*3*). Similar results were found in barnase, citrate synthase, and ribonuclease HI (RNase[1] HI) (*4*−*6*). Beadle and Shoichet (*7*) reported that substitutions of catalytic residues of AmpC β-lactamase from *E. coli* decreased the enzyme activity by 10^3 to 10^5 compared to those of the wild-type, and concomitantly increased the stability of the enzymes significantly by up to 19.7 kJ mol^{-1}. However, a mutation far from the active site resulted in activity gain and stability loss. They termed this relationship "activity−stability trade-offs." It is unclear, however, whether the activity−stability trade-offs are applicable in highly stable proteins from hyperthermophiles.

Generally, microorganisms can be divided into psychrophiles, mesophiles, thermophiles, or hyperthermophiles based on the differences in their optimal growth temperatures. The stability of proteins from these microorganisms exhibits different properties. Proteins from hyperthermophiles have greater stability than those from mesophiles (*8*−*10*). However, enzymatic activities usually increase with temperature. Thus, hyperthermophilic proteins, which require high stability, may have to optimize the stability but not their function. The activity−stability trade-offs observed in mesophilic proteins may not apply to hyperthermophilic proteins. To address this question, we examined the thermodynamic stability and enzymatic activity of the active-site mutant proteins of ribonuclease HII from a hyperthermophilic archaeon, *Thermococcus kodakaraensis* (*Tk*-RNase HII).

RNase H endonucleolytically cleaves RNA of RNA/DNA hybrids at the PO-3′ bond (*11*). The enzyme is ubiquitously

† This work was supported in part by a Grant-in-Aid for National Project on Protein Structural and Functional Analyses and by a Grant-in-Aid for Scientific Research (No. 16041229) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, and by an Industrial Technology Research Grant Program from the New Energy and Industrial Technology Development Organization (NEDO) of Japan.

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[1] Abbreviations: CD, circular dichroism; DSC, differential-scanning calorimetry; GdnHCl, guanidine hydrochloride; RNase, ribonuclease; SPMP, stability profile of mutant protein; *Tk*, *Thermococcus kodakaraensis*; TSase α, α subunit of tryptophan synthase.

10.1021/bi060907v CCC: $33.50
Published on Web 10/03/2006

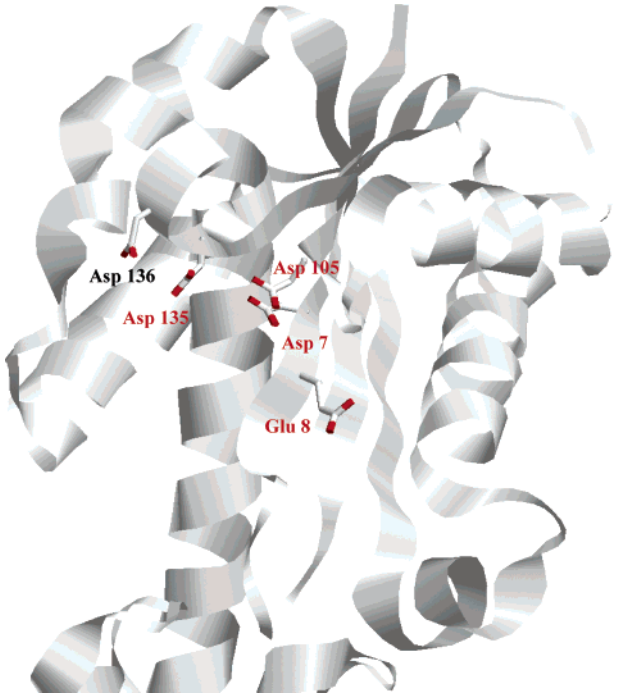

FIGURE 1: Crystal structure of *Tk*-RNase HII. Side chains around the active site are shown. The figure was created by Ras Top.

present in various organisms and is involved in DNA replication and repair. *Tk*-RNase HII is a monomer and consists of 228 amino acid residues (*12*). The crystal structure has already been determined (*13*), and *Tk*-RNase HII has high conformational stability (*14*). The active sites of all RNase H consist of acidic residues (*15*). In *Tk*-RNase HII, Asp7, Glu8, Asp105, and Asp135 are the active-site residues (Figure 1). Percent buried for Asp7, Glu8, Asp105, and Asp135 is 92.2%, 84.5%, 89.0%, and 72.5%, respectively, indicating that these residues are considerably buried. In *E. coli* RNase HI, most of the active-site mutant proteins were stabilized (*16*).

This study analyzed the conformational stability and enzymatic activity of the active-site mutant proteins (D7N, E8A, E8Q, D105A, and D135A) and compared them to those of the wild-type. Guanidine hydrochloride (GdnHCl)-induced unfolding was measured with circular dichroism (CD) at 220 nm, and heat-induced denaturation was done by differential scanning calorimetry (DSC). Most of the mutations in these active-site residues reduced the enzymatic activity dramatically but increased the conformational stability. These results indicate that the stabilization mechanism of hyperthermophilic proteins is similar to that of proteins from organisms that grow at moderate temperature. This implies that the basic principle of protein folding is not dependent on protein stability and evolution of life.

MATERIALS AND METHODS

Preparation of the Protein. The active-site mutants of *Tk*-RNase HII, E8A, D105A, and D135A were previously constructed (*13*). The active-site mutants D7N and E8Q were constructed as described for E8A, D105A, and D135A using Quick Change Site-Directed Mutagenesis kit (Stratagene). Overproduction and purification of *Tk*-RNase HII and its active-site mutant proteins were performed as described by Haruki et al. (*12*). The purity of the proteins was analyzed by sodium dodecyl sulfate (SDS)−polyacrylamide gel electrophoresis (PAGE) on a 12% polyacrylamide gel. The protein concentration was estimated by assuming A_{280nm} of 0.63 for 1 mg mL^{-1} protein (*12*).

Enzymatic Activity. The RNase H assay was performed at 50 °C in 20 mM Tris-HCl containing 10 mM $MgCl_2$ at pH 9.0 using M13 DNA/RNA hybrid as a substrate, as described by Kanaya et al. (*16*). Tris-HCl buffer, which is pH 9 at 50 °C, was adjusted at 25 °C (*17*). The experimental temperature, 50 °C, at which GdnHCl-induced unfolding experiments were performed, was chosen because M13 DNA/RNA hybrid is unstable at higher temperatures.

Equilibrium Experiments on GdnHCl Induced Unfolding and Refolding. CD measurements were carried out on a J-725 automatic spectropolarimeter (Japan Spectroscopic Co., Ltd.) as described by Mukaiyama et al. (*14*). The GdnHCl-induced unfolding curves were determined, and a nonlinear least-squares analysis (*18*) was used to fit the data to

$$y = \{(b_n^0 + a_n[\mathrm{D}]) + (b_u^0 + a_u[\mathrm{D}])\exp[\Delta G(\mathrm{H_2O}) - m[\mathrm{D}]]\}/\{1 + \exp[\Delta G(\mathrm{H_2O}) - m[\mathrm{D}]]\} \quad (1)$$

$$C_m = \Delta G(\mathrm{H_2O})/m \quad (2)$$

where y is the observed CD signal at a given concentration of GdnHCl, and [D] is the concentration of GdnHCl. b_n^0 is the CD signal for the native state, and b_u^0 is the CD signal for the unfolded states. a_n is the slope of the pretransition of the baseline, and a_u is the slope of the posttransition of the baseline. $\Delta G(\mathrm{H_2O})$ is the Gibbs energy change (ΔG) of the unfolding in the absence of GdnHCl, m is the slope of the linear correlation between ΔG and the GdnHCl concentration [D], and C_m is the GdnHCl concentration at the midpoint of the curve. The experimental raw data was directly fit with eq 1 using SigmaPlot (Jandel Scientific). All equilibrium experiments were performed in 20 mM Tris-HCl, at pH 9.0. Each Tris-HCl buffer, which is pH 9 at 50 °C, was adjusted at 25 °C (*17*). The protein concentration when CD measurements were taken was 0.16 mg mL^{-1}. The difference in stability between the wild-type and mutant proteins, $\Delta\Delta G(\mathrm{H_2O}) = \Delta G(\mathrm{H_2O})(\text{mutant}) - \Delta G(\mathrm{H_2O})(\text{wild-type})$, was calculated according to the equation

$$\Delta\Delta G(\mathrm{H_2O}) = \Delta G(\mathrm{H_2O})(\text{mutant}) - \Delta G(\mathrm{H_2O})(\text{wild-type}) = m_{av}(C_m(\text{mutant}) - C_m(\text{wild-type})) \quad (3)$$

where m_{av} is the average value of m for the wild-type and all mutant proteins.

Heat-Induced Unfolding Experiments. DSC measurements were carried out as described by Mukaiyama et al. (*14*). The buffer used was 20 mM Gly−NaOH at pH 9.0. The protein concentrations used for the measurements were 0.27 to 0.73 mg mL^{-1}. In the previous work, the peak temperatures on the DSC curves of *Tk*-RNase HII depended on the heating rate (*14*), so experiments were carried out at the different scan rates (30, 60, 90 °C h^{-1}). The excess heat capacity curves for the protein were corrected by subtracting the corresponding buffer baseline and then normalized by the protein concentration using the Origin software package

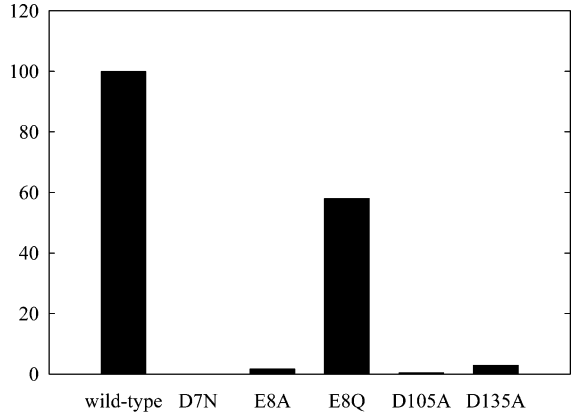

FIGURE 2: Relative enzymatic activity of the active-site mutant proteins at 50 °C. The activity of the wild-type is set to 100 (%). The errors, which represent 67% confidence limits, are all at or below ±20% of the values reported.

(Microcal Inc.). The reversibility of thermal denaturation was verified by reheating the samples.

RESULTS

Enzymatic Activity. The enzymatic activity of the wild-type and active-site mutant proteins (D7N, E8A, E8Q, D105A, and D135A) of *Tk*-RNase HII was determined at 50 °C, pH 9.0, using M13 DNA/RNA hybrid as a substrate. Relative activity of D7N, E8A, E8Q, D105A, and D135A was 56%, 0.5%, 12%, 1.7%, and 14.5%, respectively, as summarized in Figure 2. The mutations of Asp7 to Asn, Glu8 to Ala, Asp105 to Ala, and Asp135 to Ala dramatically reduced the enzymatic activity. These results are consistent with those previously reported by Muroya et al. (*13*), although the enzymatic activity of D135A was slightly higher than that previously reported. This decrease in activity was not caused by protein denaturation because they were not denatured at 50 °C (see below). In contrast, the mutation of Glu8 to Gln did not seriously affect the enzymatic activity. The mutations of the corresponding residues of *Archaeoglobus fulgidus* RNase HII (*19*) and *E. coli* RNase HI (*20*) to Gln, however, almost fully inactivate the enzyme. The reason why the effect of this mutation varies for different RNase H enzymes remains to be determined.

Conformational Stability. To determine the contribution of the active-site residues to the conformational stability of *Tk*-RNase HII, we examined the equilibrium GdnHCl-induced unfolding and refolding of the active-site mutant proteins at 50 °C, pH 9.0. GdnHCl-induced denaturation was monitored using a change of the far-UV CD signal at 220 nm. The denaturation by GdnHCl was completely reversible at 50 °C in all proteins, and the unfolding and refolding reactions attained a two-state equilibrium within three weeks for all mutant proteins. Previously, we reported that the unfolding and refolding reactions of *Tk*-RNase HII attained equilibrium in two weeks (*14*). The reactions of the mutant proteins were slightly slower than those of the wild-type protein, because of their higher stability (see below). Figure 3 exhibits the GdnHCl-induced unfolding and refolding curves of the wild-type and active-site mutant proteins at 50 °C. The thermodynamic parameters for GdnHCl-induced equilibrium unfolding (as listed in Table 1) were calculated using eqs 1 and 2. The difference in stability between the

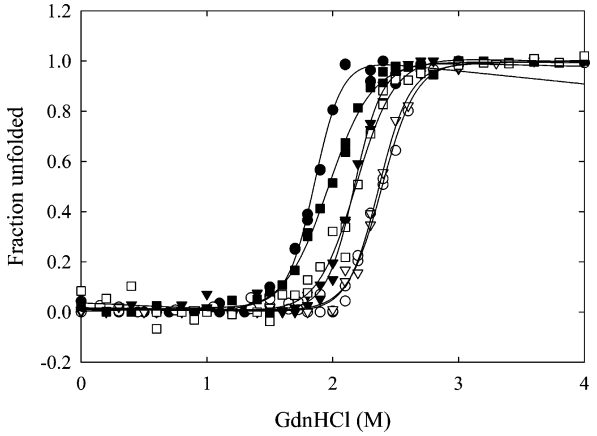

FIGURE 3: GdnHCl-induced unfolding and refolding curves of the wild-type (closed circle), D7N (open square), E8A (open circle), E8Q (closed square), D105A (closed triangle), and D135A (open triangle) at 50 °C. The lines are best fits to a two-state equation.

Table 1: Thermodynamic Parameters for GdnHCl-Induced Unfolding of the Wild-Type and Active-Site Mutant Proteins of *Tk*-RNase HII at 50 °C

	C_m[a] (M)	m[b] (kJ mol^{-1} M^{-1})	$\Delta\Delta G(H_2O)$[c] (kJ mol^{-1})
wild-type	1.85	23.6	
D7N	2.18	18.6	7.0
E8A	2.39	19.8	11.1
E8Q	1.97	15.6	2.5
D105A	2.17	23.7	6.6
D135A	2.37	20.3	10.7

[a] The error is ±0.1 M. [b] The error is ±2.5 kJ mol^{-1} M^{-1}. [c] Equation 3. $\Delta\Delta G(H_2O) = m_{av}(C_m(\text{mutant}) - C_m(\text{wild-type}))$. $m_{av} = 20.2$ kJ mol^{-1} M^{-1}.

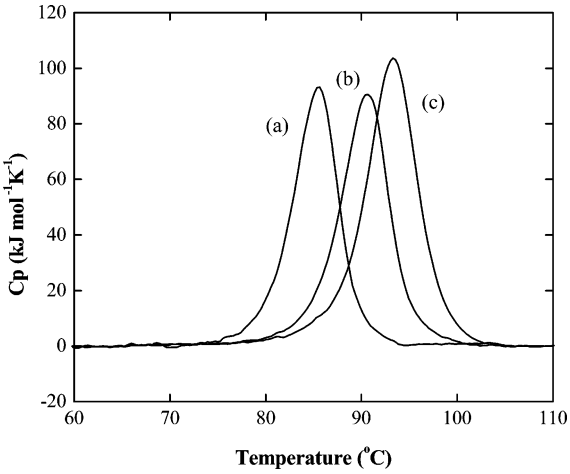

FIGURE 4: Representative excess heat capacity curves of (a) the wild-type, (b) D135A, and (c) E8A at a scan rate of 1 °C min^{-1} at pH 9.0.

wild-type and mutant proteins, $\Delta\Delta G(H_2O)$, was calculated by using eq 3. All active-site mutant proteins, except for the E8Q mutant protein, were stabilized by 7.0 to 11.1 kJ mol^{-1}. The mutation of Glu8 to Gln increased the stability by only 2.5 kJ mol^{-1} at 50 °C.

DSC measurements were also taken to examine the heat-induced denaturation of the active-site mutant proteins. The reversibility was verified by reheating experiments. Figure 4 illustrates typical heat-capacity curves of the wild-type protein and active-site mutant proteins at a scan rate of 1 °C

Table 2: Thermodynamic Parameters for Heat-Induced Denaturation of the Wild-Type and Active-Site Mutant Proteins of *Tk*-RNase HII at the Scan Rate of 1 °C min^{-1}

	T_m[a] (°C)	ΔT_m[b] (°C)	ΔH_{cal}[c] (kJ mol^{-1})	ΔH_{vH}[c] (kJ mol^{-1})
wild-type	85.2		573	682
D7N	88.3	3.1	573	682
E8A	93.2	8.0	715	628
E8Q	85.9	0.6	661	682
D105A	89.4	4.2	711	678
D135A	90.4	5.2	615	640

[a] The error is ±0.3 °C. [b] $\Delta T_m = T_m$(mutant) − T_m(wild-type). [c] The error is ±70 kJ mol^{-1}.

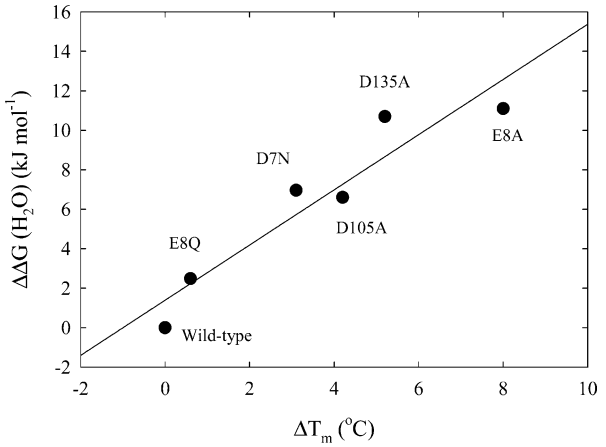

FIGURE 5: Correlation between the $\Delta\Delta G(H_2O)$ values from GdnHCl-induced unfolding and the ΔT_m values from heat-induced denaturation. The solid line represents a linear fit.

min^{-1} at pH 9.0 using Gly−NaOH buffer. The transition curves appear to have a single peak and approximate a two-state unfolding. Table 2 summarizes the thermodynamic parameters for heat-induced denaturation for the wild-type protein and active-site mutant proteins. Thus, the increase in conformational stability of the active-site mutant proteins relative to the wild-type was also confirmed by DSC. A previous report indicates that the denaturation temperature of the wild-type protein in DSC measurements shifts as a function of the scan rate because of remarkable slow unfolding (*14*). When the scan rate dependence of the T_m value of the wild-type protein and the most stabilized mutant protein, E8A, was examined, the difference in T_m value between the wild-type and mutant proteins at the scan rate 30 °C h^{-1} and 90 °C h^{-1} (ΔT_m: 7.9 and 7.5 °C, respectively) was similar to that at the scan rate 60 °C h^{-1} (8.0 °C). This result indicated small kinetic effect on the changes in stability upon the mutations. Strong correlation was observed between the $\Delta\Delta G(H_2O)$ values at 50 °C from GdnHCl-induced unfolding and the ΔT_m values from heat-induced denaturation (Figure 5).

DISCUSSION

Contribution of the Active-Site Residues to the Conformational Stability and Enzymatic Activity. A favorable charge−charge interaction stabilizes proteins (*21*−*24*), indicating the unfavorable contribution of charge repulsion to protein stability (*25*). However, it is not yet examined in detail at the active-site in highly stable proteins like hyperthermophilic ones. Local instability in active sites due to

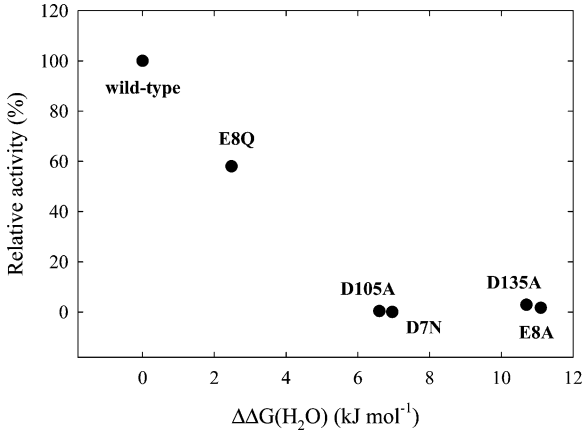

FIGURE 6: Correlation between the $\Delta\Delta G(H_2O)$ values from GdnHCl-induced unfolding and the relative activity.

charge repulsion is believed necessary for the activity of RNase H family (*6*). The active site of *Tk*-RNase HII consists of four acidic residues (Asp7, Glu8, Asp105, and Asp135) (*13*). We examined the contribution of the active-site residues to the conformational stability and enzymatic activity by constructing some active-site mutant proteins. Figure 6 presents the correlation between the $\Delta\Delta G(H_2O)$ values from GdnHCl-induced unfolding and the relative activity. The active-site mutant proteins with less activity were stabilized compared with the wild-type protein, indicating that *Tk*-RNase HII exhibits the activity−stability trade-offs. Mutations at Asp7, Asp105, and Asp135 diminished the enzymatic activity, but greatly increased the stability (7.0 kJ mol^{-1} for D7N, 6.6 kJ mol^{-1} for D105A, and 10.7 kJ mol^{-1} for D135A). Such a decrease in activity and increase in stability of D7N is accomplished by the elimination of the charge repulsion in the active site. D7A of *Tk*-RNase HII also exhibited little detectable activity at 30 °C (*13*). In D105A and D135A, the negative charge and/or packing of the side chains of Asp105 and Asp135 contribute favorably to the activity, but unfavorably to the stability. In *E. coli* RNase HI, two Asp residues corresponding to Asp7 and Asp105 of *Tk*-RNase HII are important for binding to Mg^{2+} ion (*26*), and the charge-neutralization mutation at this site stabilizes the protein (*6*). In addition, the stability of D135A of *Tk*-RNase HII was higher than those of D7N by 3.7 kJ mol^{-1} and of D105A by 4.1 kJ mol^{-1}. This may be due to the unfavorable electrostatic interaction between Asp135 and the adjacent Asp136 (Figure 1). On the other hand, charged residues which are buried in the interior of a protein sometimes contribute to the conformational stability unfavorably (*27*, *28*). For example, Asp79 in RNase Sa, which is 85% buried, makes an unfavorable contribution to the conformational stability, and the mutation of Asp79 to Ala increased the stability by 13.8 kJ mol^{-1} at pH 8.5 (*27*). The V66E and V66K mutations in staphylococcal nuclease decreased the stability dramatically (*28*). This site is in the hydrophobic core of the protein. These buried charged residues exhibit higher pK_a values, resulting in the destabilization of proteins (*27*−*30*). In *Tk*-RNase HII, all active-site residues are considerably buried, so just the removal of charge may increase the stability. The mutation of Glu8 to Ala also decreased the enzymatic activity and increased stability, but the mutation of Glu8 to Gln did not seriously affect the activity and stability. This suggests that the side

Table 3: Relationship between Activity and Stability

protein		species	residues	T_m (°C)	ΔT_m (°C)	$\Delta(\Delta G(H_2O))$ (kJ mol^{-1})	rel act. (%)	ref
RNase HII	wild-type	*T. kodakaraensis*	228	85.2			100	
	D7N			88.3	3.1	7.0	0.5	
	E8A			93.2	8.0	11.1	12	
	D105A			89.4	4.2	6.6	1.7	
	D135A			90.4	5.2	10.7	14.5	
RNase HI	wild-type	*E. coli*	155	47.0			100	6
	D10N			53.8	6.8	8.2	<0.1	
	D10A			60.7	13.7	16.9	<0.1	
TSase α	wild-type	*E. coli*	268				100	1, 2
	E49L					30.5	<0.1	
	E49Q					15.1	<0.1	
lysozyme	wild-type	bacteriophage T4	164	66.5			100	3
	E11F				4.3	1.7	<0.01	
	D20N				3.1	1.3	<0.01	
	S117I				4.2	1.7	5	
barnase	wild-type	*Bacillus amyloliquefaciens*	110	54.1			100	4
	R59A					2.7	15	
	E73A					9.6	2	
AmpC lactamase	β-wild-type	*E. coli*	358 (homodimer)	54.6			100	7
	S64G				6.5	15.1	0.001	
	K67Q				2.0	4.6	0.01	
RNase Sa	wild-type	*Streptomyces aureofaciens*	96	49.0			100	35
	Q38A			52.5	3.5	4.2	7.8	
citrate synthase	wild-type	pig	464	47.8			100	5
	D375G			53.8	6.0		0.06	
	D375Q			57.6	9.8		0.016	
SNase	wild-type	*Staphylococcal aureus*	149	53.6			100	43
	E43Q			56.3	2.7		0.01	
	E43S			58.6	5.0		0.02	

chain of Glu8 has an unfavorable interaction, such as steric strain of the side chain, for stability, but plays an important role for activity, such as substrate binding. The crystal structure of *Tk*-RNase HII indicates that the side chain of Glu8 does not face the other acidic side chains (Figure 1). Therefore, the acidic nature of Glu8 is not as essential for enzymatic activity.

The stability profile of mutant protein (SPMP) proposed by Ota et al. (*31*) estimates the change in conformational stability due to single amino acid substitutions and reflects mainly short-range interactions (*32*). This method was applied to the mutant proteins of several proteins, *E. coli* RNase HI and human lysozyme (*31*, *33*, *34*). Correlations between the experiment and calculations of *E. coli* and human lysozyme are expected to 0.5 and 0.6, respectively. When SPMP was applied to *Tk*-RNase HII, it was estimated that all active-site mutant proteins examined in this study were stabilized compared with the wild-type (2.5 kJ mol^{-1} for D7N, 2.6 kJ mol^{-1} for E8Q, 4.6 kJ mol^{-1} for E8A, 3.6 kJ mol^{-1} for D105A, and 0.9 kJ mol^{-1} for D135A). At the position 8, Ala was predicted to be better for stability than Gln, which agrees with the experimental results. These results suggest that the local conformation around the active site is unfavorable for stability in terms of stereochemistry.

Activity−Stability Trade-Off. It has been reported that active-site residues are not optimized for conformational stability in some proteins. Table 3 summaries the data concerning the relationship between activity and stability reported in various proteins with activity−stability trade-offs. Here, the wild-type of *Tk*-RNase HII exhibits remarkably high stability by 20 to 30 °C in T_m value relative to other proteins in which activity−stability trade-off is applicable. *Tk*-RNase HII is a hyperthermophilic protein, while others are from organisms that grow at moderate temperatures. We believe this is the first example that reveals the activity−stability trade-off in hyperthermophilic proteins. These results indicate that hyperthermophilic proteins also acquire function at the cost of stability.

In contrast, a mutation in active-site residues sometimes decreases both activity and stability. Miering et al. (*4*) reported that, in barnase, some mutations in the active site increased stability and decreased enzymatic activity, but other mutations decreased both stability and activity. Yakovlev et al. (*35*) demonstrated that RNase Sa has six residues that are important for function, with two of the residues actively involved in catalysis and one involved in substrate specificity, making important favorable contributions to the stability. They conclude that because RNase Sa is one of the smallest enzymes (96 residues), it cannot tolerate an unfavorable contribution to the stability as well as a larger enzyme might. Barnase is also a small protein (110 residues). This suggests that activity−stability trade-offs are dependent on the size of proteins rather than the robustness.

Structural Principle of (Hyperthermophilc) Proteins. Enzymatic activities usually increase with temperature. Psychrophilic proteins need to acquire high activity at lower temperature (*36*−*38*). To do so, the global structure of these proteins is thought to be highly flexible at low temperature, resulting in low stability (*39*, *40*). In contrast, hyperthermophilic proteins exhibit high conformational stability, although the stabilization mechanism is not fully understood. Considering that enzymatic activities are generally higher at higher temperature, the activity−stability trade-offs might not be applicable for hyperthermophilic proteins. We demonstrated that they also have the same activity−stability trade-offs as mesophilic proteins. This indicates that all proteins have an unfavorable contribution of the active site to the stability, or the strategy of folding and activity gain is

the same in all proteins, irrespective of the conformational stability and species of organisms in which they are present, except for small proteins as described above.

Activity−Stability Trade-Offs from the Viewpoint of Enzyme Evolution. Pace (*41*) proposed a (hyper)thermophilic common ancestor of the extant life on earth. Wang et al. (*42*) demonstrated that enzyme evolution has a stability cost. If the thermophilic common ancestor hypothesis is correct, methophilic and psychrophilic proteins with higher activity at normal and lower temperatures were evolved from (hyper)-thermophilic proteins requiring stability loss. Because active-site residues are usually conserved within homologous proteins and the active site in hyperthermophilic proteins has an unfavorable contribution to the stability, as indicated here, methophilic and psychrophilic proteins maintain an unfavorable contribution of the active site to the stability. Consequently, proteins in various organisms exhibit the activity−stability trade-offs.

CONCLUDING REMARKS

In this study we demonstrated the activity−stability trade-offs of a hyperthermophilic protein. This provides important knowledge for understanding the stabilization mechanism of hyperthermophilic proteins and folding and evolution problems of proteins in various organisms. These results imply that the principle of protein architecture is fundamentally the same in every protein.

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BI060907V