Thermostable valyl-tRNA, isoleucyl-tRNA and methionyl-tRNA synthetases from an extreme thermophile *Thermus thermophilus* HB8: protein structure and Zn^{2+} binding

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Thermostable valyl-tRNA, isoleucyl-tRNA and methionyl-tRNA synthetases have been purified from an extreme thermophile, *Thermus thermophilus* HB8. Valyl-tRNA and isoleucyl-tRNA synthetases are found to be monomer proteins (M_r 108000 and 129000, respectively), while methionyl-tRNA synthetase is a dimer protein (M_r 150000). These enzymes are very similar with respect to amino acid compositions and α-helix contents as estimated by circular dichroism analyses. Furthermore, two Zn^{2+} are tightly bound to each of these synthetases. These data suggest that valyl-tRNA and isoleucyl-tRNA synthetases consist of two domains, each corresponding to the subunit of methionyl-tRNA synthetase.

Valyl-tRNA synthetase *Isoleucyl-tRNA synthetase* *Methionyl-tRNA synthetase*
Thermus thermophilus *Amino acid composition* *Zinc content*

1. INTRODUCTION

Proteins from *Thermus thermophilus* HB8, an extreme thermophile, are known to be thermostable. In fact, we have found that the polypeptide chain elongation factor Tu (EF-Tu) from this extreme thermophile is remarkably more stable than EF-Tu from *Escherichia coli* and have successfully made proton NMR analyses in combination with photo-oxidation experiments [1,2]. Thus, we found that a histidine residue of EF-Tu·GTP is involved in the binding site for aminoacyl-tRNA.

Here, in a series of physicochemical studies on the molecular mechanism of protein biosynthesis, we have purified valyl-tRNA, isoleucyl-tRNA and methionyl-tRNA synthetases from *T. thermophilus* HB8. We found that these 3 synthetases are very similar, with respect to the amino acid compositions, secondary structures and the number of tightly bound Zn^{2+}, although the subunit structure of methionyl-tRNA synthetase (MetRS) is different from those of valyl-tRNA synthetase (ValRS) and isoleucyl-tRNA synthetase (IleRS). This set of 3 thermostable synthetases from the same source will be useful for detailed physicochemical analyses (including NMR analyses) of the strict recognitions of cognate amino acid and tRNAs in the aminoacylation reactions. reactions.

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2. MATERIALS AND METHODS

T. thermophilus HB8 was cultured at 75°C. ValRS, IleRS and MetRS were purified by the combination of chromatographic procedures with DEAE–cellulose, DEAE–Sephacel, phosphocellulose, Mātrex Red A (Amicon) and hydroxyapatite columns (to be reported separately in detail). The aminoacylation activity of enzyme preparations was assayed at 65°C using unfractionated tRNA from *T. thermophilus* HB8.

M_r values of purified synthetases were obtained

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by high-pressure liquid chromatography (HPLC) gel filtration with a TSK-Gel G3000SW column (Toyo Soda) equilibrated with 0.2 M potassium phosphate buffer at pH 6.8 (nondissociating condition). Subunit M_r values were obtained by electrophoresis on 5% polyacrylamide gels containing 0.1% SDS (dissociating condition).

Amino acid analyses of synthetases were performed with a Hitachi 835-10 amino acid analyzer. The enzyme was hydrolyzed in 6 M HCl at 110°C for 24 h. However, for the analysis of cysteine, the enzyme was oxidized with performic acid and then hydrolyzed in 6 M HCl at 110°C for 18 h. Finally, for the analysis of tryptophan, the enzyme was hydrolyzed with 3 M mercaptoethanesulfonic acid at 110°C for 22 h. Corrections for the yield were made for serine (90%), threonine (95%), cysteinic acid (90%) and tryptophan (95%).

From the amino acid compositions obtained, the extinction coefficients of synthetases at 280 nm were estimated as 2.1, 2.3, and 2.0 $ml \cdot mg^{-1} \cdot cm^{-1}$ for ValRS, IleRS and MetRS, respectively, and were used for the determination of enzyme concentrations. The number of Zn^{2+} tightly bound to the enzyme was determined by the measurement of atomic absorption at 213.9 nm, with a Seiko SAS atomic absorption spectrophotometer. The enzyme (1 mg) was dialyzed at 4°C for 24 h, 3 times against 100 ml of 10 mM Tris–HCl buffer (pH 7.9) containing 1 mM 2-mercaptoethanol and 10 μM EDTA. The circular dichroism (CD) of synthetases was measured at 60°C with a JASCO J-40S dichrograph. The CD spectra in the region 195–240 nm were analyzed by the FORTRAN program of [3].

3. RESULTS AND DISCUSSION

Previously, IleRS was partially purified from *T. thermophilus* HB8 and found to be stable at temperatures as high as 77°C [4]. Here, we have succeeded in the purification to homogeneity of IleRS as well as ValRS and MetRS from the same extreme thermophile.

Using SDS–PAGE, M_r values of ValRS, IleRS and MetRS in the dissociating condition have been determined as 108000, 129000 and 75000, respectively (table 1). In comparison, M_r values of enzymes in the nondissociating condition have been approximately obtained as 100000 (ValRS), 115000 (IleRS) and 175000 (MetRS) by HPLC gel filtration. Therefore, ValRS and IleRS are found to be monomer proteins (α) while MetRS is a dimer protein (α_2). M_r values and subunit structures of these 3 synthetases (table 1) are similar to those of corresponding synthetases from *E. coli* [5].

Table 1

Structural properties of aminoacyl-tRNA synthetases from *T. thermophilus* HB8

	M_r	Zinc content	α-helix content (%)
ValRS	108000 ± 3000 (α)	2.1 ± 0.1	60
IleRS	129000 ± 3000 (α)	2.1 ± 0.1	54
MetRS	150000 ± 4000 (α_2)	2.0 ± 0.1	50

The amino acid compositions (mole fractions) of ValRS, IleRS and MetRS from *T. thermophilus* HB8 are shown in table 2. For each of the 3 synthetases, the differences in amino acid compositions between the *T. thermophilus* and *E. coli* enzymes are also shown in table 2. As a common tendency for the 3 synthetases, the mole fractions of Leu and Arg residues are higher and that of Asx lower in *T. thermophilus* as compared with *E. coli* enzymes. Such tendencies of amino acid compositions of synthetases have also been found for most of the other proteins from thermophiles [9] and are possibly related to the thermostability of thermophilic proteins.

The 3 synthetases, ValRS, IleRS and MetRS from *T. thermophilus*, are remarkably similar, with respect to the mole fractions (%) of amino acid residues (table 2). For any pair of 3 synthetases, the root-mean-squared difference in mole fractions of amino acid residues is less than 0.8% (slightly more than 0.3%, the error of amino acid analysis [10]). The α-helix contents of the 3 synthetases estimated by analyses of CD spectra are often as high as 50–60% (table 1).

The numbers of Zn^{2+} tightly bound to ValRS, IleRS and MetRS from *T. thermophilus* HB8 are shown in table 1. These 3 synthetases are found here to bear two Zn^{2+}. The binding of two Zn^{2+} has been reported for MetRS from *E. coli* [11]. However, this is the first report on the exact number of Zn^{2+} tightly bound to ValRS and IleRS,

Table 2

Amino acid compositions and mol fractions (%) of ValRS, IleRS and MetRS from *T. thermophilus* HB8

Amino acid	ValRS			IleRS			MetRS		
Asx	63[a]	6.6%	↓↓	77[a]	6.7%	↓↓	86[a]	6.5%	↓↓
Thr	41	4.3	↓	50	4.4		50	3.8	
Ser	37	3.9		40	3.5	↓	30	2.2	↓↓
Glx	121	12.7		141	12.4	↑↑	162	12.3	
Pro	57	6.0	↑	75	6.6	↑	84	6.3	
Gly	66	6.9		81	7.1		102	7.7	
Ala	103	10.8		115	10.1		134	10.2	↑
Cys	11	1.2		11	1.0		8	0.5	↓
Val	59	6.2	↓	76	6.7		98	7.3	↑
Met	21	2.2	↓	14	1.2	↓	24	1.8	↓
Ile	28	2.9	↓↓	34	3.0	↓↓	50	3.8	
Leu	112	11.7	↑↑	136	11.9	↑↑	152	11.5	↑↑
Tyr	28	2.9	↑	53	4.6	↑	66	5.0	↑
Phe	34	3.6		47	4.1		46	3.5	↓
Lys	52	5.4		66	5.8		78	6.0	
His	19	2.0		23	2.0		28	2.1	
Trp	24	2.5		19	1.7		22	1.7	
Arg	79	8.3	↑↑	83	7.3	↑↑	102	7.8	↑↑

[a] mol amino acid residues/mol enzyme

The differences in mole fractions between the *T. thermophilus* and *E. coli* enzymes [6–8] are shown as ↑↑ (>2%), ↑ (1–2%), ↓ (−(1–2)%), ↓↓ (< −2%)

which is due to the extraordinary stability of the synthetases from *T. thermophilus* HB8.

The inhibition of aminoacylation activity by the binding of 1,10-phenanthroline has been reported for MetRS from *E. coli* [11]. Here, such an inhibition experiment has been made of *T. thermophilus* ValRS, and one molecule of this inhibitor has been found to bind to the active center with the binding constant of $K_i = 0.5\ mM^{-1}$. We also found that ValRS, IleRS and MetRS from *T. thermophilus* are very similar with respect to amino acid composition and α-helix content. Therefore, the tight binding of two Zn^{2+} to ValRS and IleRS suggests that these synthetases are composed of two structural domains, each bearing one active center for the aminoacylation reaction. This model is consistent with the results of enzyme kinetics and substrate binding studies on ValRS [6,12] and IleRS [13–15]. The two structural domains of ValRS and IleRS probably correspond to the subunits of MetRS.

It has been proposed, from several criteria, that ValRS, LeuRS, IleRS and MetRS form a family among the 20 synthetases [16]. Nevertheless, each of these synthetases strictly recognizes the cognate amino acid and tRNAs. In order to gain insight into the molecular mechanism of such strict recognition in the aminoacylation reactions, we are now isolating domains of *T. thermophilus* synthetases by limited proteolysis. Thermostable fragments of synthetases from an extreme thermophile will be useful for detailed NMR analyses in combination with enzymological studies.

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