Journal of the Korean Magnetic Resonance Society **2005**, *9*, 110 - 121

Structural Studies on the E. *coli* Methionyl-tRNA Synthetase and Their Interaction with E. *coli* $tRNA^{fMet}$

Ji-Hun Kim, Hee-Chul Ahn, Sung-Jin Park, Sung-Hoon Kim and Bong-Jin Lee*

Research Institute of Pharmaceutical sciences, College of Pharmacy, Seoul National University, 151-742, Korea

Received September 1, 2005

Abstract : E.coli methionyl tRNA synthetase consist of 676 amino acids and plays a key role in initiation of protein synthesis. The native form of this enzyme is a homodimer, but the monomeric enzyme truncated approximately C-terminal 120 amino acids retains the full enzymatic activities. X-ray crystal structure of the active monomeric enzyme shows that it has two domains. The N-terminal domain is thought to be a binding site for acceptor stem of tRNA, ATP, and methionine. The C-terminal domain is mainly α-helical and makes an interaction with the anticodon of $tRNA^{Met}$. Especially it is suggested that the region of helix-loop-helix including the tryptophan residue at the position 461 may be the essential for the interaction with anticodon of $tRNA^{Met}$. In this work the structure and function of E. *coli* methionyl-tRNA synthetase was studied by spectroscopic method (NMR, CD, Fluorescence). The importance of tryptophan residue at the position 461 was investigated by fluorescence spectroscopy. Tryptophan 461 is expected to be an essential site for the interaction between E. coli methionyl-tRNA synthetase and E. coli $tRNA^{Met}$. Proton and heteonuclear 2-dimensional NMR spectroscopy were also used to elucidate the protein-tRNA interaction.

Keyword: methionyl-tRNA synthetase, $tRNA^{Met}$, anticodon, NMR, circular dichroism, fluorescence, protein-tRNA interaction.

INTRODUCTION

The aminoacyl-tRNA synthetases catalyze the aminoacylation of transfer RNAs with their cognate amino acid. It activates the amino acid ATP-dependently and transferes the activated amino acid to the 3' terminus of appropriate cognate tRNAs.

* To whom correspondence should be addressed. E-mail : lbj@nmr.snu.ac.kr

$$AA + ATP \rightarrow AA\text{-}AMP + PP_i$$
$$AA\text{-}AMP + tRNA^{AA} \rightarrow AA\text{-}tRNA^{AA} + AMP$$

The aminoacyl-tRNA synthetases are classified into two groups. Each group consisting of ten enzymes has the conserved sequence motif that corresponds to specific three-dimensional structural elements.[1-7] The class I synthetases contain signature sequences such as HIGH tetrapeptide[1, 3] and KMSKS pentapeptide.[2] These sequence elements are found in the N-terminal catalytic domain. On the other hand the class II synthetases share degenerate sequence motifs at three regions along the protein sequence.

All tRNAs adopting a similar L-shaped tertiary structure have two domains. One domain consists of the acceptor stem, the TΨC stem and loop. The other domain consists of the D-stem loop and the anticodon stem and loop.

Aminoacyl-tRNA synthetases in both classes contain nonconserved domains in addition to the conserved active site domains. It has been thought that the conserved catalytic domain interacts with the tRNA acceptor-TΨC stem and the second nonconserved domain interacts with the region of the tRNA distal to the acceptor stem. This second domain provides for interactions with the tRNA anticodon in many instances.[8-11]

Native *E. coli* MetRS is a dimeric enzyme containing indentical subunits of molecular weight 76,000.[12] Controlled proteolysis of the native enzyme releases amino acids from the carboxyl terminus of each protomer and yields a monomeric fragment of molecular weight 64,000 which retains full biological activity.[13] The crystal structure of this form of the enzyme complexed to ATP has been refined to 2.5 Å resolution (Fig. 1).[14] The class-defining catalytic domain is within the N-terminal half of the structure. The N-terminal domain contains a nucleotide-binding fold and is involved in methionine activation and transferring the activated methionine to the acceptor end of the bound $tRNA^{Met}$.[15] The C-terminal domain is mainly α-helical and makes a major interaction with the anticodon of $tRNA^{Met}$.[8, 16-17]

Fig. 1. Crystal structure of E.coli methionyl-tRNA synthetase

Tryptophan residue is located at the junction between the surface α-helix and loop in the C-terminal domain. Although it is suggested that W461 may be essential for the interaction with the anticodon of $tRNA^{Met}$ [16] it is not clearly understood how this residue achieves its specific interaction with the anticodon of $tRNA^{Met}$.

In this work the structure-activity relationship of *E. coli* MetRS was investigated by spectroscopic methods. Fluorescence, circular dichroism and NMR spectroscopy could give the structural information of E. coli MetRS. Fluorescence and circular dichroism spectra showed the importance of tryptophan residue in the interactions with $tRNA^{fMet}$.

In NMR spectroscopy, *E. coli* MetRS and mutant *E. coli* methionyl-tRNA synthetase W461Y (MetRS W461Y) which was replaced with tyrosine at the position of W461 in native enzyme was used in order to assign the peak of W461 and to elucidate the importance of W461. *E. coli* MetRS W461Y is a active mutant with less affinity for *E. coli* $tRNA^{fMet}$ than native MetRS. To investigate the interaction between the synthetase and $tRNA^{fMet}$, each enzyme were labeled with $_{L}$-[indole-2-^{13}C] Trytophan and accomplished ^{1}H-^{13}C heteronuclear single quantum correlation spectroscopy.

MATERIALS AND METHODS

Materials

L-[indole-2-^{13}C]Trp was purchased from ICON Service Inc. (Summit, NJ, USA). The isotope enrichment is 95% for L-[indole-2-^{13}C]Trp. D_2O (99% pure) and *E. coli* $tRNA^{fMet}$ was purchased from Sigma (St. Louis, MO, USA). Ampicillin was purchased from Chongkundang (Seoul, Korea). Other chemicals were of biological grade.

Overexpression and Purification of methionyl-tRNA synthetase

E. coli strain MV1184 harvoring pAG112 which contains a gene encoding the monomeric *E. coli* methionyl-tRNA synthetase of 547 amino acids was innoculated to LB medium (ampicillin 50μg/mℓ) and incubated overnight at 37℃. When the culture reached 0.5 OD_{550nm} the cell was induced with 1mM IPTG and further grown 4 hours.

The cell culture was harvested by centrifugation. The crude extract was lysed and disrupted by sonication in 200mℓ of 50mM potassium phosphate buffer, pH 7.0, 1mM EDTA, 0.1mM PMSF, 5% glycerol, and 14mM β-mercaptoethanol and then centrifuged (8,000g, 30min, 4℃). The supernatant was diluted and loaded onto a column of DEAE-sepharose (Pharmacia) equilibrated with buffer A (50mM potassium phosphate buffer, pH 7.0). The crude protein was eluted by the linear salt gradient with the same buffer containing 0 to 600mM KCl. The eluted proteins were detected by SDS-PAGE and pooled. The protein eluted at 300mM KCl was further purified on a Blue-sepharose CL-6B (Pharmacia) column using a gradient of 0-600mM KCl buffer A. The protein was eluted at 200mM KCl and concentrated by centrifugation using Centriprep and Centricon (Amicon). The buffer containing the enzyme was exchanged during concentration with 100mM KCl buffer A.

The concentration of *E. coli* MetRS was estimated by the methods of Bradford et al.. *E. coli* MetRS 1mg/mℓ yielded 1.72 Abs_{280nm}. *E. coli* MetRS W461Y was expressed in *E. coli* strain TG1/pJB104. The mutant MetRS was purified by same procedure as that of the native protein. For the preparation of NMR sample, *E. coli* strain ME5395 was used for Trp-auxotroph. E. coli were grown at 37℃ in M9 minimal medium containing glucose as a carbon source, NH_4Cl as a nitrogen source and amino acids except tryptophan. When the cell culture was induced with 1mM IPTG, 35μg/mℓ L-[indole-2-^{13}C]Trp was added.

Other conditions of expression and purification were identical to those of *E. coli* MetRS grown in LB medium.

Circular dichroism measurement

Circular dichroism spectra were recorded on a JASCO J-715 spectropolarimeter. Circular dichroism spectra of *E. coli* MetRS and MetRS W461Y were recorded in 200 ~ 250㎚ region and band width was 1.0㎚, resolution 0.5㎚, sensitivity 50mdeg and scanning speed was 100㎚/min. All experiments were repeated 3 times and the average value was acquired. The concentration of the 3 kinds of proteins was identically 0.2nM. The effects on substitution of tryptophan at the position of 461 residue by tyrosine were investigated by comparing the spectra of the mutant enzyme with that of the native *E. coli* enzyme. Then various amounts of *E. coli* $tRNA^{fMet}$ were added to all proteins and the structural changes by MetRS-tRNA interaction were examined.

Fluorescence measurement

Fluorescence emission spectra were recorded on a JASCO FP-777 spectrofluorometer. Tryptophan excitation was conducted at 280㎚ with emission spectra monitored from 300 to 500㎚. The concentrations and the other conditions of 3 kinds of enzymes and *E. coli* $tRNA^{fMet}$ were equal to those of CD measurement. The scanning speed was 200㎚/min and the data interval is 0.5㎚. *E. coli* $tRNA^{fMet}$ was titrated to all enzymes respectively to investigate the changes of intensity of tryptophan residues.

NMR measurement

^{1}H-NMR spectra and ^{1}H-^{13}C heteronuclear single quantum correlation (HSQC) spectra were recorded on a 500㎒ Bruker AMX-500 FT NMR spectrometer.

^{1}H-NMR measurement were carried out in a neutral solution (pH 7.0, 100mM KCl, 50mM potassium phosphate, 1mM NaN_3) at 27℃ in H_2O (10% D_2O). Protein samples for ^{1}H-NMR spectra in H_2O were concentrated to 0.3 ~ 0.4mM. 1-1 echo water suppression pulse was used to remove the H_2O peaks. ^{1}H-NMR spectra was recorded with a spectral width of 8064.52 Hz and 16384 real data points. Prior to Fourier transformation, the data obtained were multiplied by exponential function and zero-filled to 32768 real data points.

In order to acquire HSQC spectra, the solution containing 0.5mM ~ 1mM enzyme in the same buffer in D_2O was used. The spectral width was 8064.52Hz for ^1H and 3144.25 Hz for ^{13}C. The data points were 1024 and 128 respectively. The free induction decay (FID) were multiplied by squared-signbell function in t2 and t1 and zero-filled to yield a matrix of 2048 (F2) × 256 (F1) real data points. Chemical shifts were given in parts per million relative to calibrated resonance of an internal dioxane in buffer solution. The probe temperature was controlled at 27℃ throughout the experiments.

E. coli $tRNA^{fMet}$ corresponding to 1/3 amount of the enzymes was titraed and the complex spectra of enzyme and tRNA were recorded.

RESULTS

CD study on methionyl-tRNA synthetase

In crystal structure, MetRS has 7 β-sheets and several α-helices in their N-terminal domain. C-terminal domain predominantly consists of α-helices. As shown in Fig. 2, CD spectra of *E. coli* MetRS showed that the values of around 208 and 222㎚ was strongly negative. It represents a typical α-helix pattern of protein. In the structure of *E. coli* methionyl-tRNA synthetase, β-sheets are buried in the hydrophobic core of the enzyme and α-helices are exposed to the hydrophilic environments. CD spectra of *E. coli* MetRS W461Y was identical to that of *E. coli* MetRS. It implies that the mutation does not affect the structure of enzyme (Fig. 3).

In the experiments of *E. coli* $tRNA^{fMet}$ titration, the CD intensities of two enzymes were reduced. It seems that the structure of the enzyme was slightly changed or the α-helicity of the enzyme was reduced by the protein-tRNA interaction. The degree of reduced intensity of CD was greater in native MetRS, than in MetRS W461Y.

Fluorescence study on methionyl-tRNA synthetase

In order to investigate the importance of tryptophan 461 in the interaction between *E. coli* MetRS and anticodon of $tRNA^{fMet}$, we used fluorescence spectroscopy. The same concentration of native MetRS and mutant MetRS W461Y was prepared. The intensity of

mutant *E. coli* MetRS W461Y at 340㎚ was reduced 3-folds to that of the native protein. It implies the most intensity of fluorescence in *E. coli* MetRS is from tryptophan 461. When *E. coli* $tRNA^{fMet}$ was titrated to the native enzyme, the relative intensity was decreased by the degree of 20%, while that of mutant enzyme was not significantly changed. Therefore, tryptophan 461 seems to be an important residue for the interaction with $tRNA^{fMet}$ (Fig. 4).

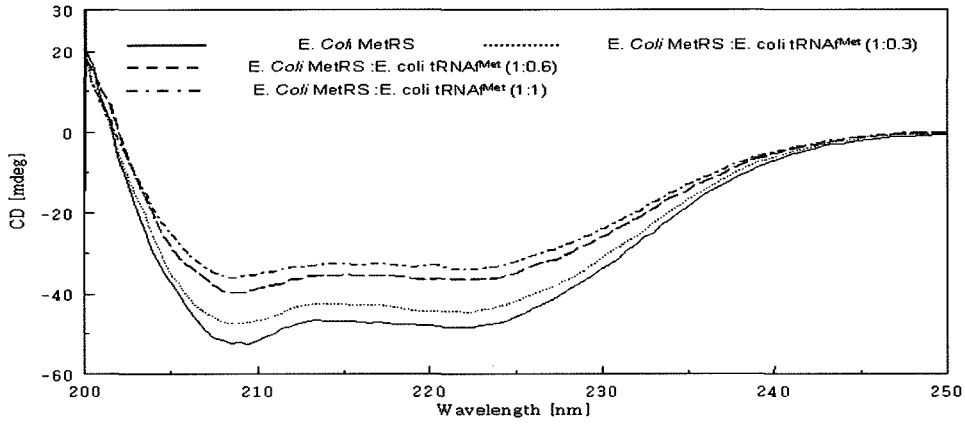

Fig. 2. CD spectra of E.*coli* MetRS and its complex with E.*coli* $tRNA^{fMet}$

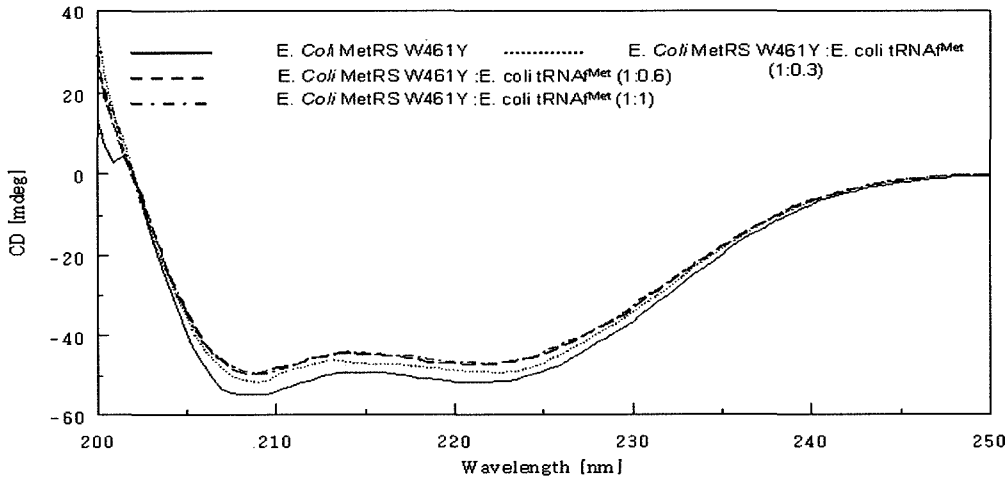

Fig. 3. CD spectra of E.*coli* MetRS W461Y and its complex with E.*coli* RNA^{fMet}

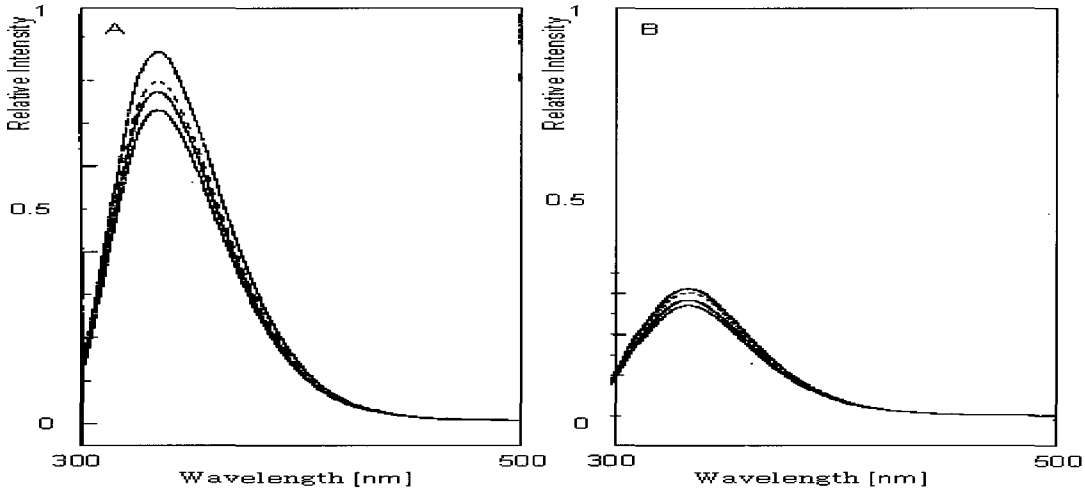

Fig. 4. Fluorescence spectra of E.coli MetRS, E.coli MetRS W461Y (A)Fluorescence spectra of E. coli. MetRS and its complexes with E. coli tRNAfMet (B) Fluorescence spectra of E. coli MetRS W461Y and its complexes with E.coli $tRNA^{fMet}$

Peak assignment of indole-imino proton of tryptophan 461

The mutant enzyme was replaced with tyrosine at the position of tryptophan 461. So the comparison of ^{1}H-NMR spectra of the native and mutant enzyme of was useful to assign the imino-proton originated from indole-ring of tryptophan 461. In the spectra of the mutant enzyme, a peak around 12ppm was disappeared. Not only CD data but also the high field region and low field region of NMR spectra shows that the substitution of tryptophan with tyrosine at the residue of 461 does not affect the structure of *E. coli* MetRS. The peak around 12ppm could be assigned as tryptophan461(Fig. 5).

HSQC spectra analysis

We used tryptophan residue as probes for the structural analysis of MetRS. The native and mutant enzyme were enriched with $_{L}$-[indole-2^{13}C] tryptophan. *E. coli* MetRS has 11 tryptophan residues, the mutant enzyme has 10 tryptophan residues. HSQC spectra of MetRS showed only 6 cross-peaks. Because of the overlapping of peaks and the assignment of tryptophan 461 peak could not be accomplished (Fig. 6).

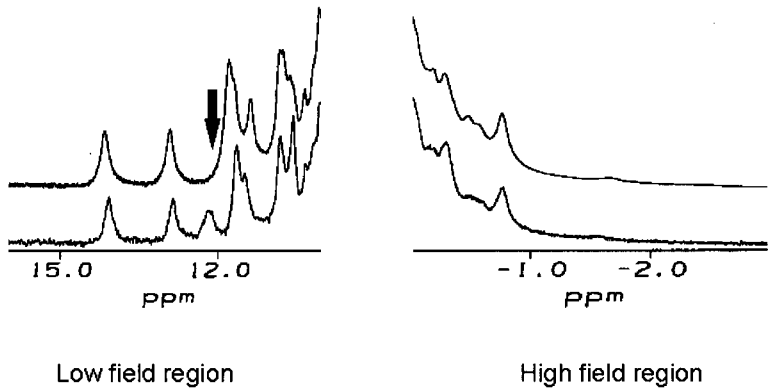

Fig. 5 proton NMR spectra of E.coli MetRS and E.coli MetRS W461Y (A) High field region of NMR spectra, (B) Low field region of NMR spectra. The arrow indicates the peak of imino-proton of tryptophan 461

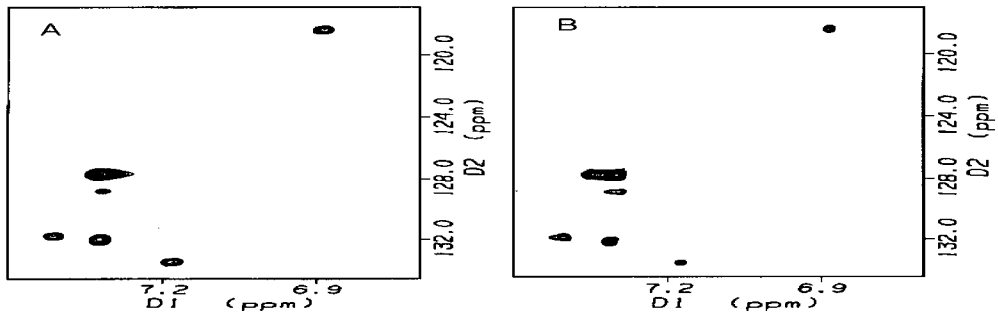

Fig. 6. HSQC spectrum of *E. coli* MetRS labeled with [indole-2^{13}C]-Trp (A) and mutant *E. coli* MetRS W461Y [indole-2^{13}C]-Trp (B)

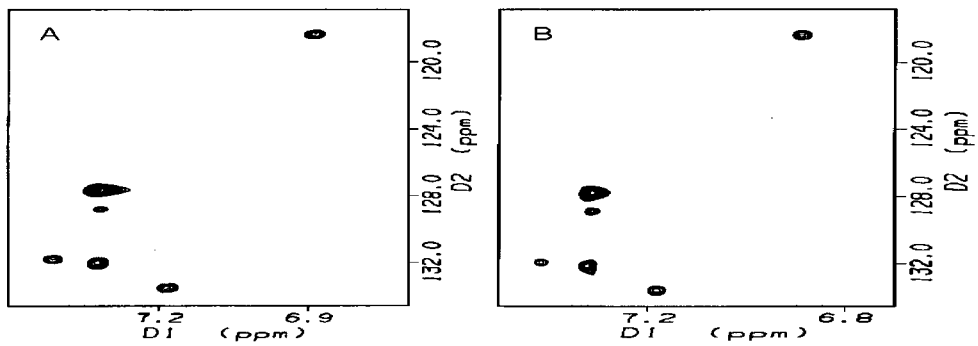

Fig.7. HSQC spectrum of *E. coli* MetRS labeled with [indole-2^{13}C]- Trp (A) and its complex with *E. coli* tRNA^{fMet} (B)

The changes of the peaks were not significant by titration of *E. coli* $tRNA^{fMet}$(Fig 7) And NMR peaks of *E. coli* MetRS W461Y was not changed either by the titration of E. *coli* $tRNA^{fMet}$(data not shown). It seems that 461 tryptophan peak was included in the overlapped peaks and the peak could not be assigned and could not be monitored by titration experiment (Fig. 6 B, 7 B).

DISCUSSION

In order to elucidate the structure-activity relationship of MetRS, the spectroscopic methods of circular dichroism, fluorescence and NMR were used. CD spectra of the enzymes revealed that they predominantly consist of α-helices. In HSQC measurement it was impossible to assign the peak of tryptophan 461 of *E. coli* MetRS and to elucidate the detail of interaction of tryptophan with the anticodon of *E. coli* $tRNA^{fMet}$.

By using *E. coli* MetRS W461Y we tried to investigate the similarity of the interaction of two enzymes with *E. coli* $tRNA^{fMet}$. Structural change was not occurred by substitution. Fluorescence measurement revealed that the interaction site with *E. coli* $tRNA^{fMet}$ was a tryptophan residue at the position of 461 in *E. coli* MetRS and suggested tryptophan residue should play a key role in the interaction of MetRS with $tRNA^{fMet}$.

The binding affinities of the enzymes with $tRNA^{fMet}$ were investigated by CD and fluorescence measurement. Mutant *E. coli* MetRS W461Y has a little binding affinity to *E. coli* $tRNA^{fMet}$ compared to native enzyme. This result means with the fact that the Van der Waals volume of the amino acid which interacts with the anticodon of $tRNA^{fMet}$ would be a significant factor in aminoacylation.[18] We knew the fluorescence intensity of the enzyme was from 461 tryptophan of *E. coli* MetRS by using the mutant enzyme which was replaced with tyrosine in the position of 461. Although it is not clearly understood how the enzyme interacts with $tRNA^{fMet}$, it seems that tryptophan 461 is a binding site with the anticodon of $tRNA^{fMet}$. To resolve the problem of overlapping of peaks and to elucidate the protein-tRNA interaction of aminoacylation in NMR measurement, the protein labeled with 15N enriched amino acid would give more informations in structural analysis. Side chain nitrogen and nitrogen-bound proton is a direct candidate for the interaction with the anticodon of $tRNA^{fMet}$.

Acknowledgement

This study was supported by the National Research Laboratory Program (M1-0412-00-0075) and 2005 BK21 project for Medicine, Density, and Pharmacy.

REFERENCES

1. Webster, T. A., Tsai, H., Kula, M., Mackie, G. A. and Schimel, P. *Science* 226, 1315-1317 (1984).
2. Hountondji , C., Dessen, P. and Blanquet, S. *Biochimie* 68, 1071-1078 (1986).
3. Ludmerer, S. W. and Schimmel, P. *J. Biol. Chem. 262*, 10801-10806 (1987).
4. Cusack, S., Berthet-Colominas, C., Härtlein, M., Nassar, N. and Leberman, . *Nature* 347, 249-255 (1990).
5. Eriami, G., Delarue, M., Poch, O., Gangloff, J. and Moras, D. *Nature 347*, 203-206 (1990).
6. Nagel, G. M. and Doolittle, R. F. *Proc. Natl. Acad. Sci. USA* 88, 8121-8125 (1991).
7. Burbaum, J.J. and Schimmel, P. *Proteins: Struc. Funct., Genet.* 7, 99-111 (1991).
8. Perona, J.J., Rould, M. A., Steitz, T. A., Risler, J. -L. Zelwer, C. and Brunie, S. *Proc. Natl. Acad. Sci. USA* 88, 2903-2907 (1991).
9. Rould, M. A., Perona, J.J., Söll, D. and Steitz, T. A. *Science* 352, 213-218 (1991).
10. Cavarelli, J., Rees, B., Ruff, M., Thierry, J. -C. and Moras, D. *Nature* 362, 181-184 (1993).
11. Kim, S., Landro, J. A., Gale, A. J. and Schimmel, P. *Biochemistry* 32, 13026-12031 (1993).
12. Kawaminami, S., Ozaki, K., Sumitomo, N., Hayashi, Y., Ito, S., Shimada, I. and Arata, Y. *J. Biol. Chem.* 269, 28752-28756, (1994)
13. Cassio, D. and Waller, J. -P. *Eur. J. Biochem.* 20, 283-300 (1971).
14. Brunie, S., Zelwer, C. and Risler, J.-L. *J. Mol. Biol.* 216, 411-424 (1990).
15. Hountondji , C., Schmitter, J. M., Beavallet, C. and Blanquet, S. *Biochemistry* 29, 8190-8198 (1990).
16. Ghosh, G., Pelka, H. and Schulman, L. H. *Biochemistry* 29, 2220-2225 (1990).

17. Valenzuela, D. and Schulman, L. H. *Biochemistry* 25, 4555-4561 (1986).
18. Kim, S. *J. Biochem. Mol. Biol.* 28, 363-367 (1995).