

# A three-dimensional structure model of the complex of glutamyl-tRNA synthetase and its cognate tRNA

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Received 18 October 1995

**Abstract** A docking model of glutamyl-tRNA synthetase (GluRS) and tRNA<sup>Glu</sup> was constructed, on the basis of the distinguished similarity between the X-ray crystallographic three-dimensional structures of the N-terminal halves of the *Thermus thermophilus* GluRS in the free state and the *Escherichia coli* glutamyl-tRNA synthetase in a complex with tRNA<sup>Gln</sup>. The modeled structure is energetically favorable and is also well consistent with the results of site-directed mutagenesis studies. The model indicates that the GluRS-specific insertions 2 and 3 fit and bind to the acceptor stem and the D arm, respectively, of the cognate tRNA without affecting other contacts. In particular, insertion 3 strongly interacts with the two D-stem base pairs that are essential for the tRNA·GluRS recognition.

**Key words:** Aminoacyl-tRNA synthetase; Computer modeling; Molecular mechanics; Molecular superposition

## 1. Introduction

Aminoacyl-tRNA synthetases (aaRS's) strictly recognize and ligate the specific tRNA(s) and amino acids. The specificity of a tRNA toward an amino acid (the tRNA identity) depends on the recognition of characteristic nucleotides (identity determinants) in the tRNA molecule by aaRS's [1–6]. Based on the sequences and the three-dimensional (3D) structures of the catalytic domains, the aaRS's are divided into two classes, each with ten synthetases [7]. In general, every class I synthetase has catalytic domains containing the classical nucleotide-binding (Rossmann) fold [8].

As for class I, *Escherichia coli* glutamyl-tRNA synthetase (GlnRS) is the only one for which a crystal structure has been determined for the complex with the cognate tRNA [9,10]. Recently, we have solved the 3D structure of another class I synthetase, glutamyl-tRNA synthetase (GluRS) from *Thermus thermophilus* [11]. While it had been suggested that GluRS is closely related to GlnRS, at the level of their overall primary structures, we found a striking similarity in the 3D structures of the N-terminal halves (catalytic domains) of GluRS and GlnRS [11]. Simply by substituting GluRS for GlnRS in the complex with tRNA<sup>Gln</sup> (the coordinates of the phosphates only were available in this analysis), we suggested that the tRNA<sup>Gln</sup> structure fits quite well in the GluRS structure, and furthermore, we found that the specifically inserted secondary struc-

tural elements of GluRS are likely to have access to the bound tRNA [11]. Actually, our site-directed mutagenesis analyses demonstrated that some amino acid residues in the insertions are important for the aminoacylation of tRNA<sup>Glu</sup> by GluRS [11].

However, in the preliminary docking model of GluRS and tRNA<sup>Gln</sup> (including the coordinates of all the atoms), we detected a steric clash between the two molecules when the energy of the complex was calculated. Therefore, it was still necessary to clarify whether the insertions of GluRS could actually contact tRNA<sup>Glu</sup> without any energetic discrepancy. In this study, we constructed a much more sophisticated model of the GluRS·tRNA<sup>Glu</sup> complex, by automatic molecular superposition and computer modeling coupled with molecular mechanics calculation. This in fact allowed us to examine how well the GluRS-specific insertions contact the identity-determining base pairs of tRNA<sup>Glu</sup>.

## 2. Materials and methods

### 2.1. Molecular modeling

Molecular mechanics calculations were performed using the united atom force field of AMBER 4.0 [12]. A distance-dependent dielectric function ( $\epsilon = 4r$ ) was used to approximate the solvent screening effect in the energy calculations. A 10-Å cutoff distance for non-bonded interactions was used. Energy minimizations were carried out by a conjugate gradient method until the gradient of the RMS force reached 0.1 kcal·mol<sup>-1</sup>·Å<sup>-2</sup>. These calculations were performed on a DEC3000/700 computer. The molecular display and the molecular modeling for the structural modifications were carried out on a Silicon Graphics Indigo<sup>2</sup> workstation using the Insight II molecular graphics program [13].

### 2.2. Construction of the initial structure of the *E. coli* tRNA<sup>Glu</sup> moiety

For the template of the acceptor arm of *Escherichia coli* tRNA<sup>Glu</sup>, the atomic coordinates of *Escherichia coli* GlnRS-bound tRNA<sup>Gln</sup> [10], a gift from Dr. Steitz to M.G., were used. The coordinates of the anticodon arm of yeast tRNA<sup>Phe</sup> [14], and those of the D- and T-arms, and the variable loop of yeast tRNA<sup>Asp</sup> [15] were obtained from the Brookhaven Protein Data Bank [16].

The nucleotide sequence of each part was changed to that of *E. coli* tRNA<sup>Glu</sup> as follows. First, the coordinates of the phosphate moieties, the ribose moieties, the C4, C8, and N9 atoms of the purine residues, and the N1, C2, and C6 atoms of the pyrimidine residues were taken from the templates. The coordinates of the other atoms were generated using the internal coordinates of AMBER 4.0 by the EDIT module of the program package. Next, for each part, energy minimization was performed with the MINMD module in four steps, first for the base moieties, secondly for the ribose moieties, thirdly for the backbone moieties (the phosphate moieties and the ribose C5' and O3' atoms) by the IBELLY option of the MINMD module, and finally for the entire structure.

The D- and T-arms and the variable loop were joined to the acceptor stem, and then to the anticodon arm. G1 was added, since the coordinates of tRNA<sup>Gln</sup> lack those of residue 1. Next, energy minimization

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**Abbreviations:** GluRS, glutamyl-tRNA synthetase; aaRS, aminoacyl-tRNA synthetase; GlnRS, glutamyl-tRNA synthetase.

was carried out around the phosphate groups at the junctions. Finally, energy minimization was performed by the four-step procedure used for the energy minimization of each part.

### 2.3. Superposition of the N-terminal halves of GluRS and GlnRS

The 2.5 Å refined crystal structures of GluRS [11] and GlnRS were used for the superposition (the coordinates of GlnRS were a gift from Dr. Steitz to M.G. [10]).

C and FORTRAN programs implementing algorithms by the Rossmann and Argos method [17] for the identification of corresponding atoms were developed (Kaneda et al., in preparation) and combined with the least-squares fitting routine by McLachlan [18]. Using these programs, the coordinates of GluRS were translated and rotated so as to superimpose the C $\alpha$  atoms of the GluRS N-terminal half (1–322; trial structure) with those of the GlnRS N-terminal half (8–342; reference structure). In this way, GluRS was naturally docked with the modeled tRNA<sup>Glu</sup>, since the acceptor stem of the tRNA was originally derived from that of tRNA<sup>Gln</sup> bound to GlnRS.

### 2.4. Molecular mechanics refinement of the docking model of GluRS and tRNA<sup>Glu</sup>

In order to find the overlap of atoms between GluRS and tRNA<sup>Glu</sup> in the initial docking model, for each nucleotide residue, the surrounding amino acid residues were elucidated and the atomic distances between the nucleotide and the amino acid residues were calculated. When the distances were smaller than the sum of the van der Waals radii of the two atoms, energy minimization of the amino acid residues was carried out locally, in order to reduce the high-energy contact. Next, we performed energy minimization for all of the amino acid residues with at least one atom within 4.0 Å from the tRNA<sup>Glu</sup> molecule. This energy minimization was carried out further by using a distance 10.0 Å instead of 4.0 Å. In these four processes, the atoms of tRNA<sup>Glu</sup> were fixed. Then, the energy of the whole tRNA<sup>Glu</sup> molecule was minimized by the four-step procedure described above (see section 2.2). Finally, the energy of the entire GluRS·tRNA<sup>Glu</sup> complex was minimized.

## 3. Results and discussion

The 3D structures of the N-terminal halves of GluRS and GlnRS are so similar to each other that we could well superimpose them manually [11]. In this study, we have carried out a more precise and quantitative superposition in an automatic manner with the use of a computer program (Table 1). Thereby, GluRS was translated and rotated so as to best-fit the corresponding C $\alpha$  atoms. Through this analysis, it was found that more than 80% of the C $\alpha$  atoms in the GluRS N-terminal half could be superimposed with those of the GlnRS N-terminal half. Furthermore, three insertions specific to GluRS were automatically identified; the positions of these insertions in the GluRS sequence correspond well with those of the insertions found by the previous manual superposition, except for a few amino acid residues around the boundaries of insertion 2. Thus, we could definitely determine the inserted sequences specific to GluRS. Similarly, for GlnRS, the specific insertions were unambiguously identified.

Every class I synthetase has the conserved motifs 'KMSK' and 'HIGH'. These motifs were found to be superimposed between the GluRS and GlnRS molecules. In addition, about fifty amino acid residues of the GlnRS N-terminal half contact tRNA<sup>Gln</sup>, and among these amino acid residues, more than forty were found to correspond with those of the GluRS N-terminal half by the superposition. Thus, it was shown that these functional amino acid residues are conserved between the two synthetases. This preservation of the function–structure relationship between the two synthetases naturally led us to consider that the interactions between the acceptor stem of tRNA<sup>Glu</sup> and the GluRS N-terminal half are also similar to

those of the Gln system. The acceptor stem of the tRNA<sup>Gln</sup> molecule bound to GlnRS is changed to the active form, i.e. the base pair of G1·U72 is disrupted and the CCA-extremity forms a hair-pin turn toward the catalytic site of GlnRS [9]. Our assumption is that these interactions are essentially conserved in the Glu system. Accordingly, we used the acceptor stem of the tRNA<sup>Gln</sup> molecule as the template for that of tRNA<sup>Glu</sup>. The sequence of the template was exchanged according to that of tRNA<sup>Glu</sup>, and then the structure was carefully optimized by means of several molecular mechanics steps.

However, for the other parts of tRNA<sup>Glu</sup>, we did not use the coordinates of the GlnRS-bound tRNA<sup>Gln</sup> as the template in our study, for the following reasons. First, a characteristic feature is found in the variable loop of tRNA<sup>Glu</sup>, i.e. it has four nucleotide residues, in contrast to the five found in most tRNAs, such as *E. coli* tRNA<sup>Gln</sup> and yeast tRNA<sup>Phe</sup>. The number of nucleotides in the variable loop is expected to influence the 3D structure of the 'augmented D helix', which is composed of the D stem, the variable loop, and their neighboring nucleotide residues [15,19]. In addition, the conformation of the augmented D helix of tRNA<sup>Glu</sup> could, essentially, be conserved through interactions with GluRS. Consequently, we used the coordinates of yeast tRNA<sup>Asp</sup> (the free form) as the template for the D- and T-arms and the variable loop of tRNA<sup>Glu</sup>, since tRNA<sup>Asp</sup> has four nucleotide residues in the variable loop [15]. Second, conformational changes of the anticodon loop of tRNA<sup>Gln</sup> are induced through the interaction with GlnRS [10]. Accordingly, we used the anticodon arm of yeast tRNA<sup>Phe</sup>, which is considered to be the standard form of the various tRNAs, as the template for that of tRNA<sup>Glu</sup>. We exchanged the sequences of the template according to that of tRNA<sup>Glu</sup>, and then optimized their structures by performing several molecular mechanics steps. The D- and T-arms and the variable loop of tRNA<sup>Glu</sup> were joined to the acceptor stem, and then the anticodon arm was attached to the D arm and the variable loop. The structures around the junctions were relaxed by molecular mechanics calculation.

In this way, we constructed a starting structure of tRNA<sup>Glu</sup> to investigate its interactions with GluRS. Thus, we obtained the starting structure of the docking model of GluRS and tRNA<sup>Glu</sup> (note that the modeled acceptor stem is derived from that of the tRNA<sup>Gln</sup> bound to GlnRS). In order to optimize the interactions between the two molecules in the model, we examined whether there were high-energy contacts between atoms of the GluRS and tRNA<sup>Glu</sup> molecules. Although there was no serious steric clash, slight overlaps of atoms between the two molecules were found by the distance calculations. We eliminated the high-energy contacts by performing the molecular mechanics locally around those residues including the overlapped atoms. Finally, we carefully carried out several steps of energy minimization for relaxing the structures of the two molecules, and thus obtained the model of the GluRS·tRNA<sup>Glu</sup> complex (Fig. 1).

The total energy of the complex was significantly decreased (~150,000 kcal/mol) through the relaxation of the modeled structure. In particular, the van der Waals energy of the complex was decreased and became remarkably low (~–5,000 kcal/mol), indicating that the refinement reduced the overlaps and the high-energy contacts of atoms between the two molecules in the starting structure of the docking model. In order to determine which residues were affected and how largely the structures

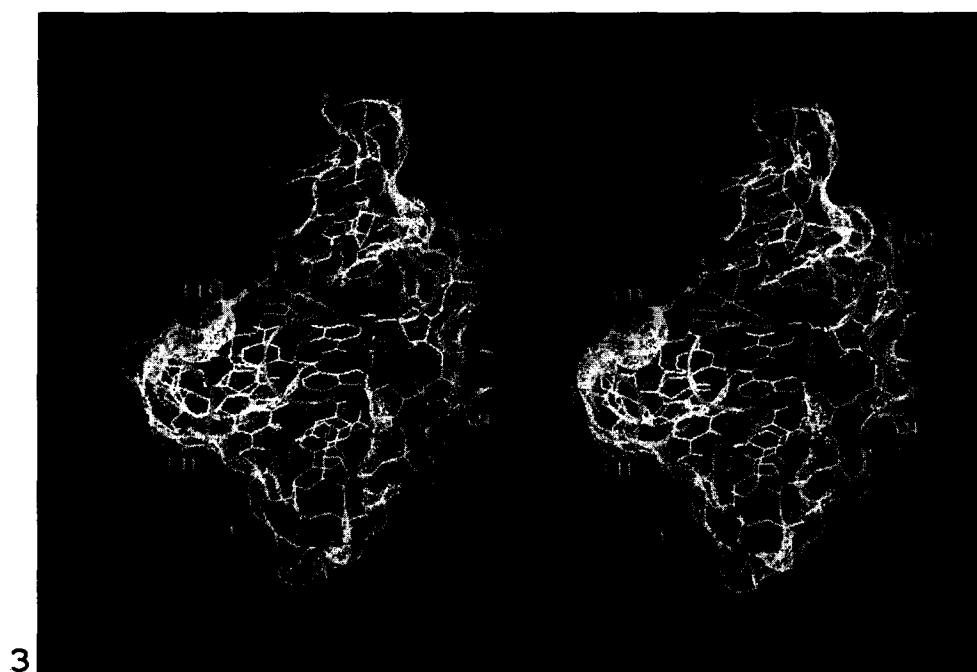


Fig. 1. (stereo view) The model of the GluRS-tRNA<sup>Glu</sup> complex. GluRS and tRNA<sup>Glu</sup> are represented by the trace of the C $\alpha$  atoms (light green) and the CPK model (purple), respectively. Characteristic secondary structural elements specific to GluRS are shown by the CPK model in orange (insertion 2) and red (insertion 3). The molecular graphics picture was produced utilizing the Insight II program [13].

Fig. 3. (stereo view) Interactions between GluRS (represented by the C $\alpha$  trace) and the determinants (colored in yellow) in the D stem of tRNA<sup>Glu</sup> (represented by the wire model on which the solvent-accessible surface is superimposed). Amino acid residues that contact the determinants are represented by ball-and-stick models in red. The molecular graphics picture was produced utilizing the Insight II program [13].

were refined, we calculated the r.m.s.d. before and after the refinement for each residue of GluRS and tRNA<sup>Glu</sup> (Fig. 2). It was found that the deviations are all within 0.25 Å, and their average values are as small as 0.06 Å and 0.07 Å for GluRS and tRNA<sup>Glu</sup>, respectively. In addition, the residues with r.m.s.d

values larger than the average are not localized, but are scattered throughout the two molecules. These results indicate that only slight structural deviations from the starting conformations for the scattered residues were sufficient to avoid all the atomic overlaps, and the consequence was a remarkable de-



pothesis that some conformational change of the interdomain region between domains III and IV allows both of these domains to interact properly with the anticodon arm of tRNA<sup>Glu</sup> [11]. On the basis of the model constructed in this study, we are now investigating, by molecular dynamics simulation, the large conformational changes of tRNA<sup>Glu</sup> and GluRS that are necessary for specific recognition of the anticodon and other determinants.

**Acknowledgements:** We are grateful to Dr. T.A. Steitz for providing M.G. with the coordinates of the GluRS·tRNA<sup>Glu</sup> complex. This work was supported by Grants-in-Aid for Science Research on Priority Areas from the Ministry of Education, Science and Culture of Japan (S.Y.).

## References

- [1] Normanly, J., Ogden, R.C., Horvath, S.J. and Abelson, J. (1986) *Nature* 321, 213–219.
- [2] Yarus, M. (1988) *Cell* 55, 739–741.
- [3] Normanly, J. and Abelson, J. (1989) *Annu. Rev. Biochem.* 58, 1029–1049.
- [4] Schimmel, P. (1989) *Biochemistry* 28, 2747–2759.
- [5] Schulman, L.H. (1991) *Progr. Nucleic Acids Res. Mol. Biol.* 41, 23–87.
- [6] Giegé, R., Puglisi, J.D. and Florentz, C. (1993) *Progr. Nucleic Acids Res. Mol. Biol.* 45, 129–206.
- [7] Eriani, G., Delarue, M., Poch, O., Gangloff, J. and Moras, D. (1990) *Nature* 347, 203–206.
- [8] Rossmann, M.G., Moras, D. and Olsen, K.W. (1974) *Nature* 250, 194–199.
- [9] Rould, M.A., Perona, J.J., Söll, D. and Steitz, T.A. (1989) *Science* 246, 1135–1142.
- [10] Rould, M.A., Perona, J.J. and Steitz, T.A. (1991) *Nature* 352, 213–218.
- [11] Nureki, O., Vassylyev, D.G., Katayanagi, K., Shimizu, T., Sekine, S., Kigawa, T., Miyazawa, T., Yokoyama, S. and Morikawa, K. (1995) *Science* 267, 1958–1965.
- [12] Pealman, D.A., Case, D.A., Caldwell, J.C., Seibel, G.L., Singh, U.C., Weiner, P. and Kollman, P.A. (1991) *AMBER 4.0*, University of California, San Francisco.
- [13] Biosym Technologies (1993) *Insight II User Guide*, version 2.3.0. San Diego: Biosym Technologies.
- [14] Sussman, J.L., Holbrook, S.R., Warrant, R.W., Church, G.M. and Kim, S.H. (1978) *J. Mol. Biol.* 123, 607–630.
- [15] Westhof, E., Dumas, P. and Moras, D. (1985) *J. Mol. Biol.* 184, 119–145.
- [16] Bernstein, F.C., Koetzle T.F., Williams, G.J.B., Meyer, E.F., Brice, M.D., Rodgers, J.R., Kennard, O., Shimanouchi, T. and Tasumi, M. (1977) *J. Mol. Biol.* 112, 535–542.
- [17] Rossmann, M.G. and Argos, P. (1975) *J. Mol. Biol.* 250, 7525–7532.
- [18] McLachlan, A.D. (1979) *J. Mol. Biol.* 128, 49–79.
- [19] Ladner, J.E., Jack, A., Robertus, J.D., Brown, R.S., Rhodes, D., Clark, B.F.C. and Klug, A. (1975) *Proc. Natl. Acad. Sci. USA* 91, 8670–8674.
- [20] Sekine, S., Nureki, O., Sakamoto, K., Niimi, T., Tateno, M., Go, M., Kohno, T., Brisson, A., Lapointe J. and Yokoyama, S., submitted to *J. Mol. Biol.*
- [21] Kern, D. and Lapointe, J. (1979) *Biochemistry* 18, 5819–5826.
- [22] Sylvers, L.A., Rogers, K.C., Shimizu, M., Ohtsuka, E. and Söll, D. (1993) *Biochemistry* 32, 3836–3841.
- [23] Rogers, K.C. and Söll, D. (1993) *Biochemistry* 32, 14210–14219.