산업기술연구(강원대학교 산업기술연구소 논문집), 제24권 B호, 2004.
Journal of Industrial Technology, Kangwon Natl. Univ., Korea, No. 24 B, 2004.

Three Common Subunits in the Editing Domains of Class Ia tRNA Synthetases.

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Abstract

To identify conserved structural or functional subunit(s) in the CP1 (editing) domains of class Ia tRNA synthetases, five available structures were compared and analyzed. Through sequence alignments of the CP1 domains, three conserved regions were found near the amino acid binding site in the editing domain. Structural overlapping of the three subunits clearly showed that there exist three common structural subunits in all of the five editing RS structures. The new alignment suggests a translocation movement of the CP1 domain caused by the binding with tRNA. Based on the experimental and modeling results, it is proposed that subunits 1 and 3 accommodate the incoming amino acid binding, while subunit 2 contributes to the interactions with the adenosine ring of the A76 to stabilize the overall tRNA binding.. Since these subunits are critical for the editing reaction, we expect that these key structures should be conserved through all class Ia editing RSs.

Keywords : *aminoacyl-tRNA synthetase (aaRS), editing domain, modeling*

1. Introduction

Correct aminoacylation of an amino acid to its cognate tRNA is critical for accurate protein synthesis. This very important reaction is controlled by a family of enzymes called aminoacyl-tRNA synthetases (aaRSs)[1]. Among them, some class Ia RSs including leucyl-, valyl-, and isoleucyl-RSs (LeuRS, ValRS, and IleRS) have developed highly accurate molecular machinery to discriminate their cognate amino acids against structurally similar amino acids[1]. The real error rate with which IleRS distinguishes its cognate isoleucine from valine, differing by only one methyl group, was demonstrated to be fewer than 1/3000[2], although previously Pauling predicted it to be 1/5 based on thermodynamic calculations[3]. This high accuracy is achieved by employing two separate active sites, namely, the activation (aminoacylation) site and the editing (proofreading) site[4]. The specificity of the amino acid activation and the editing activity of the editing RSs has been described as a double sieve[5]. The activation site (sieve 1) is in the ATP-binding Rossman fold that is common to all class I aaRSs while the editing active site (sieve 2) is located in a large inserted domain called connective polypeptide 1 (CP1)[6,7,8,9].

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2. Modeling

Structural and functional insights into the editing domain of the RSs were provided by four X-ray structures[6,7,8,9] and one homology modeled structure[10]. In 1998, the first X-ray structure of the class Ia editing RS was reported by Nureki *et al.*[6] for the *T. thermophilus* IleRS, and then the tRNA complexed *S. aureus* IleRS structure followed from Silvian *et al.*[7]. Later, the *T. thermophilus* IleRS structure was revealed by Fukai *et al.*[8] and finally Cusack *et al.*[9] solved *T. thermophilus* LeuRS structures with and without an activation substrate. In parallel with the achievements in structural biology, a number of mutagenesis experiments were independently performed on the editing domains of *E. coli* LeuRS and IleRS[11, 12]. Considering those experimental results performed in *E. coli*, Lee and Briggs recently built and refined an *E. coli* LeuRS structure via a homology modeling method[10] using the *T. thermophilus* LeuRS X-ray structure as a template[9].

The five structures (Figure 1) show that they have a common, large inserted domain (CP1, colored in blue) with distances between the two active sites, one in the middle of main body and the other in the center of the CP1 domain, of about 30Å[.6,7,8,9,10].

Our goal in this study was to elucidate common structural or functional elements in the editing site of the editing RSs. These aaRSs should have similar structural subunits in their editing sites since they share the same or very similar substrates. Considering *T. thermophilus* and *S. aureus* IleRS, even though they exist in different organisms, they share the same substrate, isoleucine, and the editing domain has the same function, i.e. distinguishing Ile from Leu and Val.

3. Results

To identify common structural subunits in the CP1 domain, we focused on the residues located near the editing active site. Sequence alignment of the five CP1 domains showed that there exist three conserved sequences near the amino acid binding sites (Table 1), which includes the highly conserved threonine rich region (subunit1) and another established region where the universally conserved aspartic acid exists (subunit3).

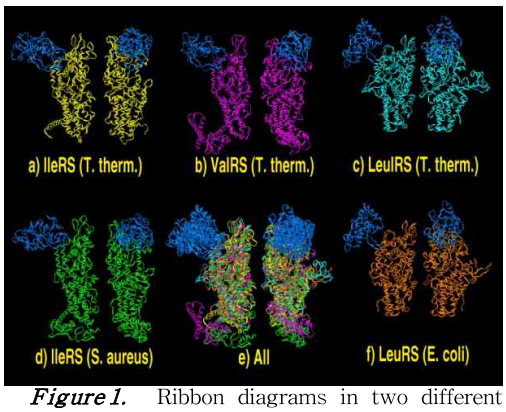

Figure 1. Ribbon diagrams in two different views are shown for the currently available five class Ia tRNA synthetase (RS) structures: four X-ray structures (a-d) and one homology modeled structure (f). All editing domains (CP1) are highlighted in blue for clarity. All five structures were also superimposed (e) by overlapping the homologous residues identified by a multiple sequence alignment scheme embedded in the HOMOLOGY module of the INSIGHTII program[13].

Table 1. Sequence alignment for the CP1 domains of the five class Ia aaRSs showing the three common conserved sequences near the amino acid binding site. The eighteen residues in three units were used for structural alignment and the five key residues are labeled. Sa, Tt, and Ec in the parentheses represent for *S. aureus*, *T. thermophilus*, and *E. coli*, respectively.

	Subunit1	Subunit2	Subunit3
IleRS(Sa)	**T**TTPW**T**	AG**T**GC	H**G**ED**D**YI
	231 236	321	331 334
IleRS(Tt)	**T**TTPW**T**	DG**T**GI	F**G**AE**D**LE
	228 233	315	325 328
ValRS(Tt)	**T**VRPE**T**	FG**T**GA	H**D**PLD**E**
	214 219	266	276 279
LeuRS(Tt)	**T**TRPD**T**	YG**T**GA	H**D**QR**D**YE
	247 252	334	344 347
LeuRS(Ec)	**T**TRPD**T**	YG**T**GA	H**D**QR**D**YE
	247 252	332	342 345

The eighteen residues listed in Table 1 were used for the structural alignments of the three subunits. The root mean square deviations (RMSD) of the alpha carbons were measured for the available combinations and the average RMSD value for the ten measurements was 1.01 Å. A slightly better result was obtained when only the five labeled residues were used for the alignment, resulting in an average RMSD of 0.79 Å. Figure 2 clearly shows that the shapes of the three subunits are very similar and that the structures are well aligned (Figure 2e). In particular, the two established regions, subunits 1 and 3, are almost perfectly aligned through all five structures. The existence of common structural subunits implies that the three subunits are functionally important for the editing reaction and should be conserved for the rest of the editing RS structures.

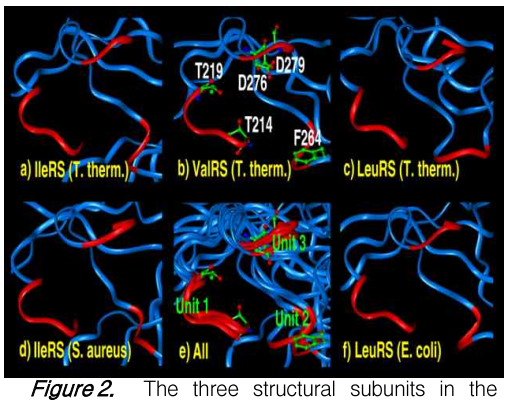

Figure 2. The three structural subunits in the editing domains of the five RSs. Ribbon diagrams of the CP1 domains were built for the four currently available X-ray structures (a-d) and a homology modeled structure (f) for comparison. The three common structural subunits are in red for clarity all three units were superimposed together (e) using the five residues labeled in Table 1.

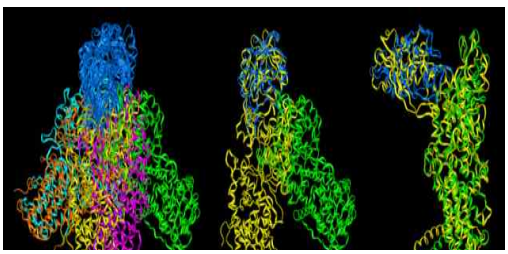

Figure 3. Overlapping of the editing RSs. The five editing RSs are superimposed (a) for the aligning using the three structural subunits in the CP1 domain (blue color). The coloring and structure IDs in panel a are the same in Figure 1. The *T. thermophilus* IleRS (yellow) and *S. aureus* IleRS's (green) are aligned by the three structural subunits (b) and main body (c). In b and c, the CP1 domain of the *T. thermophilus* IleRS is in yellow rather than in blue (a) for clarity.

The overall RS structures showed huge distortions in their main bodies after alignment of the three structural subunits (Figure 3a). Translocation of the CP1 domain, resulting from the binding with the tRNA, has been an intriguing question in the reaction mechanism of these proteins. This new alignment approach can provide some clues about this interesting conformational transition. For direct comparison, a pair of RS structures with and without tRNA is required. From the five available RS structures, two X-ray structures were solved with their cognate tRNAs, *T. thermophilus* ValRS (Figure 1b) and *S. aureus* IleRS (Figure 1d), and the rest were without their tRNA partners. Unfortunately, therefore, the precise pair is not yet available. However, the next best pair is that of *T. thermophilus* IleRS (Figure 1a) and *S. aureus* IleRS (Figure 1d) because they share high levels of structural homology. For the two structures, the sequence similarities of the CP1 domains and the main bodies are ca. 68% and 62%, respectively. Although structural alignments achieved by making use of the entire structures (Figures 1e and 3c) did not show any significant translocation movement of the CP1 domain, the newly aligned structures (i.e. via the three conserved functional subunits) clearly show the rotation of the CP1 domain with respect to the main bodies of the structures (Figure 3b). The results demonstrate that the CP1 domain may rotate clockwise by ca. 40-50 degrees after binding with tRNA.

4. Conclusions

In summary, to identify common structural or functional unit(s) in the editing domain of class Ia RSs, the editing active sites of the five available RS structures were compared and analyzed. Through sequence alignments of the

CP1 domains, three conserved regions were found near the active sites in the editing domains. The structural overlapping of those three subunits clearly showed that there exist three common structural subunits in the editing active sites in the five different CP1 structures. Our alignment also resulted in some clues about the translocation movement of the CP1 domain caused by the binding with tRNA. Finally, it is proposed that the three structural subunits are essential for the editing reaction (the role of each subunit is discussed in the supporting information) and we, therefore, expect that these key structures should be conserved through all class Ia editing RSs.

5. Acknowledgment

This research was supported by Texas Advanced Research Program (003652-0016-1999) and the National Institutes of Health (GM63789). Gratitude is expressed to Accelrys. Inc. for providing software to the Institute for Molecular Design at the University of Houston.. YJ.Kwon is also grateful to Kangwon National University for its partial support.

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