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The Seryl-tRNA synthetase/tRNA^{Ser} acceptor stem interface is mediated *via* a specific network of water molecules

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ABSTRACT

tRNAs are aminoacylated by the aminoacyl-tRNA synthetases. There are at least 20 natural amino acids, but due to the redundancy of the genetic code, 64 codons on the mRNA. Therefore, there exist tRNA isoacceptors that are aminoacylated with the same amino acid, but differ in their sequence and in the anticodon. tRNA identity elements, which are sequence or structure motifs, assure the amino acid specificity. The Seryl-tRNA synthetase is an enzyme that depends on rather few and simple identity elements in tRNA^{Ser}. The Seryl-tRNA-synthetase interacts with the tRNA^{Ser} acceptor stem, which makes this part of the tRNA a valuable structural element for investigating motifs of the protein–RNA complex. We solved the high resolution crystal structures of two tRNA^{Ser} acceptor stem microhelices and investigated their interaction with the Seryl-tRNA-synthetase by superposition experiments. The results presented here show that the amino acid side chains Ser151 and Ser156 of the synthetase are interacting in a very similar way with the RNA backbone of the microhelix and that the involved water molecules have almost identical positions within the tRNA/synthetase interface.

1. Introduction

The precise interaction between tRNA and the correct aminoacyl-tRNA synthetase is one of the crucial steps for assuring the correct transfer of information from genes to proteins. As there are 20 natural amino acids, but 64 codons on the messenger RNA, the genetic code is redundant. Therefore, there exist isoacceptor tRNAs for most amino acids [1], that differ in sequence but code for the same amino acid. The tRNA isoacceptors are aminoacylated by specific aminoacyl-tRNA synthetases. The unique synthetase recognises the specific isoacceptor-tRNAs and has to assure the correct aminoacylation on one hand, but has to avoid misaminoacylation of non-cognate tRNAs on the other hand. This requires precise tRNA-synthetase interactions. The tRNA/aminoacyl-tRNA systems have been divided into two classes, class I and class II [2]. In class I, the tRNA identity elements that assure the specificity are complex and consist of sequence/structure motifs, which are located in different regions of the tRNAs, mostly including the anticodon. In contrast, within class II, the determinants are more simple and most of them are located in the region of the aminoacyl stem, often including the discriminator base at position 73 [2]. The tRNA identity elements in the class II system can be so simple as to consist of only one base pair [3]. Within the here investigated serine system, tRNA isoacceptors have to translate six specific serine mRNA codons, whereas the aminoacylation is governed by only one Seryl-tRNA synthetase (SerRS). The SerRS depends on rather simple tRNA structure motifs and has been assigned to the class II system [2].

The tRNA^{Ser}–SerRS complex has been investigated in detail by biochemical methods and by structural analysis. The main part of the tRNA, which is in contact with the synthetase, is the aminoacyl stem. Nucleotides that determine the specificity for serine consist of the A3-U70 and G2-C71 base pairs, the nucleotide C72, the discriminator base G73, and the C11-G24 base pair in the D-stem [4–7]. Nucleotides at the position 67, 68, 69 and 70 in the acceptor stem and additionally a part of the T-stem were proven to be in contact to the SerRS, which could be shown by footprinting analysis [8]. tRNA^{Ser} possesses an extra element, the long extra arm, which also contributes to SerRS binding, but rather by structure and length than by sequence [6]. This interaction has also been described for the archaebacterial Seryl-tRNA synthetase from *Methanosarcina barkeri* [9,10]. As the tRNA^{Ser}/SerRS belongs to the 'class II' system, tRNA^{Ser} acceptor stem microhelices can be recognised and aminoacylated by Seryl-tRNA synthetase [11].

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The crystal structure of the $tRNA^{Ser}$/Seryl-tRNA synthetase, which has been solved in 1994 provided detailed information concerning the RNA–protein contacts on the structural level [12]. The SerRS interacts with the inside of the L-shaped three-dimensional structure of $tRNA^{Ser}$. In coherence with the results from biochemical studies, it could be shown that most of the interactions are built up by the acceptor stem and the long extra arm of $tRNA^{Ser}$. The resolution of the aminoacyl region has been improved within a second crystal structure of the complex. By this, it could be shown that several amino acids from the so-called motif 2 loop, which is a conserved region of class II synthetases, are in direct interaction with the aminoacyl stem [13]. A conformational switch of the loop 2 has been described upon $tRNA^{Ser}$ binding. First, the 'A-conformation', which represents the ATP-bound structure and second, the 'T-conformation' which shows the tRNA-bound state of the SerRS.

Due to a lack in the electron density map at the very terminal part of the $tRNA^{Ser}$ acceptor stem, this region could not be examined in the crystal structure of the complex. Recently, we performed superposition experiments of our 1.8 Å resolution crystal structure of a $tRNA^{Ser}$ aminoacyl stem [14] into the complex [12] and visualised the interface between $tRNA^{Ser}$ and SerRS [15]. The nucleotides U66, C67, U68, C69 and U70 from the 3′-strand and the G1 from the 5′-strand of $tRNA^{Ser}$ acceptor stem helix contact the 'loop 2' region of the synthetase, which is in agreement with an earlier report [13]. Ser 261, Phe 262, Lys 264 and Arg 267 could be identified to directly contact the tRNA acceptor stem.

Nevertheless, all these investigations have been undertaken without regarding the hydration of the tRNA and the synthetase. The extensive solvation of RNA is additionally governed by the specific hydration of the ribose 2′-OH group. It is well accepted, that the hydration of RNA plays an important role in RNA–protein interactions and that the extensive solvent content of the minor groove has a special function in RNA [16,17]. We solved the high resolution crystal structure of a further $tRNA^{Ser}$ microhelix at 1.2 Å recently [18], in which we could describe a detailed hydration pattern. Both $tRNA^{Ser}$ microhelices have been compared in detail regarding not only the RNA structure, but also the defined hydration patterns [19]. In the manuscript presented here, we show superposition experiments of both microhelices [14,18] with the SerRS complex, focussing on the role of water molecules and on the hydration of the RNA/protein interface.

2. Materials and methods

2.1. The Escherichia coli $tRNA^{Ser}$ acceptor stem microhelices

Both *E. coli* $tRNA^{Ser}$ microhelix sequences were derived from the tRNA compilation data base [1]. The $tRNA^{Ser}$ acceptor stem isoacceptor with the data base ID: RS 1660, which structure has been solved to 1.8 Å resolution [14], possesses the sequence 5′-$G_1G_2A_3$-$G_4A_5G_6A_7$-3′ and 5′-$U_{66}C_{67}U_{68}C_{69}U_{70}C_{71}C_{72}$-3′. The second $tRNA^{Ser}$ microhelix structure, isoacceptor RS 1661, has been solved to 1.2 Å resolution [18] and was adapted from the sequence 5′-$G_1G_2U_3G_4A_5G_6G_7$-3′ and 5′-$C_{66}C_{67}U_{68}C_{69}A_{70}C_{71}C_{72}$-3′. The two 7mer duplexes are tRNA isoacceptor helices and possess a naturally occurring change of two base pairs by simultaneously maintaining the specificity for the amino acid serine.

2.2. Structure calculation and superposition of the $tRNA^{Ser}$ acceptor stem microhelices with the Seryl-tRNA synthetase

The RNA strands were purchased from IBA (Göttingen, Germany) with HPLC-purification grade. The complementary strands of the $tRNA^{Ser}$ microhelices were hybridized in water at a

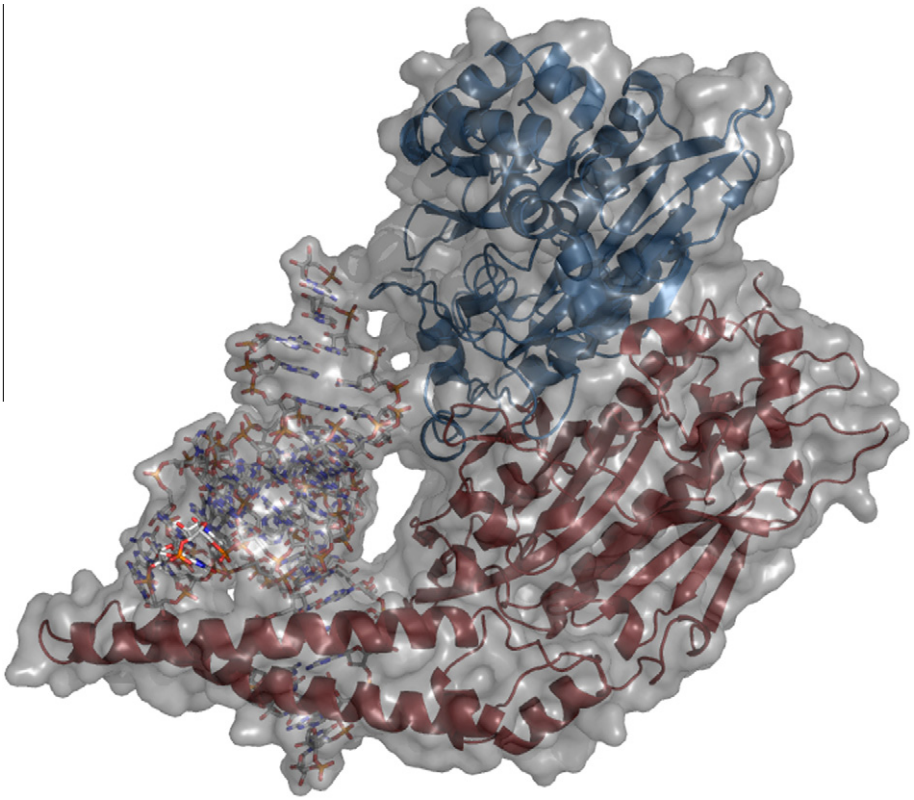

Fig. 1. General overview of the $tRNA^{Ser}$/Seryl-tRNA synthetase complex adapted from [12] (PDB-ID: 1SER). The synthetase subunits are presented in blue and red, the $tRNA^{Ser}$ is coloured by atoms. The $tRNA^{Ser}$ aminoacyl stem is in contact to the 'loop 2' region (part of the blue coloured subunit) of the synthetase. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)