CHEMISTRY LETTERS, pp. 1561-1564, 1982.

RECOGNITION OF THE AMINOACYL-tRNA-SYNTHETASE BY THE COGNATE U SHAPED tRNA AND ITS RELATION TO THE GENETIC CODE

Mikio SHIMIZU

The Institute of Space and Astronautical Science
Komaba 4-6-1, Meguro-ku, Tokyo 153

The U shaped tRNA can fit to the cognate aminoacyl-tRNA-synthetase, by making a covalent adduct of its uracil at position 8 to the C-terminal half of the synthetase. Then the complex of three anitcodon bases with a discriminator base (C4N, complex of four nucleotides) comes over the Rossmann fold of the synthetase and many features concerning with the genetic code can be explained, including the membrane transport of the amino acids by the help of the uncharged cognate tRNAs.

The three dimensional structure of the tRNA in the crystalline state is well established to be of the L letter shape through the X ray analysis.[1] However, whether the tRNA has the L shaped structure in the solution or during interaction with proteins and ribosomes is a matter of debate. Many investigators pointed out that the aminoacylated tRNA had a more extended structure (possibly exposing the TψC bases) than the uncharged tRNA, by measuring sedimentation rate[2,3], circular dichroism of 4-thiouridine[4], oligo-C binding[5], electron spin resonance spectra[6], and laser scattering[7]. Olson et al.[8] found by a laser scattering experiment that the diffusion coefficient of the tRNA was large in a dense solution of Mg^{++}, suggesting a compact U conformation of the tRNA as proposed by Doctor et al.[9]. Reid also suggested from a nuclear magnetic resonance experiment[10] that the U structure was possible and conjectured that the discriminator base would combine with the second anticodon base by the help of A at position 76 and U at position 33, which might be important for the recognition of amino acid by the tRNA. Ehrenberg et al.[11] found by using the ethidium-labeled fluorescence method that the tRNA in the solution could have at least two conformations, possibly L and U, the transition time between them being comparable with the time of amino acid transfer from the aminoacyl adenylate to the tRNA. The same investigators also suggested that the breaking of several hydrogen bonds was involved in this transition by the study of Mg^{++} binding effect. The presence of aminoacyl-tRNA-synthetase increased the transition rate[12].

In reality, the X ray crystallographic studies give only the average position of composit atoms over thermal fluctuation. The L shaped structure could be the major form of the tRNA in the solution but the presence of the minor U shaped form in the solution and during the aminoacylation process is quite possible. One of the evidence for this is the finding of a mitochondrial $tRNA^{Ser}$ which lacks [13] the D loop,because this tRNA may not take the L structure.

I have already pointed out[14] that the discriminator base at the 4th position from the 3'end of a tRNA can combine with the stacked anticodon bases simultaneously to form a complex of four nucleotides (C4N) and that the pocket on the C4N could accept the cognate L-type amino acid by a lock and key relation. The general features of the "universal" and mitochondrial genetic codes can be explained in terms of this model, as well as the chilarity of amino acids and the reason why twenty kinds of protein amino acids were selected from more than two hundred kinds of amino acids. There could be two ways for the discriminator base to approach the anticodon bases: (1) A set of two L shaped tRNAs in a head and tail position and (2) the U shaped tRNA. Starzyk et al.[15] found that the uracil in the D stem at position 8 can make a covalent adduct to the C-terminal half of the alanyl-tRNA-synthetase. In the 2L case, the D stem should attach to the central part of the synthetase, thus to the N-terminal half. Consequently the 2L structures may be less likely. (Similarly the L shaped tRNA cannot be used for the recognition[1] of the synthetase.) Other difficulties for the 2L structure are that the probability to find another cognate tRNA on the synthetase is low and that the number of the tRNA on the synthetase is frequently measured to be one. I have constructed the U shaped $tRNA^{Phe}$ in the HGS molecular model, by cutting several hydrogen bonds between the D loop and the TψC loop,and by keeping the two axis of the L straight. The tRNA was separated into two parts: (1) the anticodon loop + the D loop and (2) the acceptor stem + the TψC loop. The two parts are connected by a bridge composed of the D stem and the variable loop. The uracil at position 8 is just in the center of this bridge. The C4N of yeast $tRNA^{Phe}$ is easily formed[14] at the open head of U. Fig. 1 shows the relation of the U shaped tRNA with a tyrosyl-tRNA-synthetase. The grooves of the tRNA just fit to the alpha helixes of the synthetase. The fitting is much better than in the 2L case , since the number of alpha helixes in the C-terminal half of the synthetase is more than those in the N-terminal. The C4N comes just on the Rossmann fold on the synthetase where the aminoacyl adenylate is waiting to be charged with the CCA chain. Although the used $tRNA^{Phe}$ and tyrosyl and alanyl tRNA-synthetases are not cognate with each other, the similarity of the structure[1,15,16] of these apparatus through all the species of twenty protein amino acids, respectively, may support the generality of this good fitting. The good fitting of other parts than the anticodon loop of the tRNA to the synthetase solves the dissected molecule problem[16].

It is recently found that the amino acids are transported through the sphero plasts of E.coli by the help of uncharged cognate tRNAs (isoacceptor species)[18,19,20]. Aminoacylation, hypermodification of the base next to the third anticodon base, and the presence of the synthetase all inhibit the transport. The simplest interpretation is that the compact U shaped tRNA with the amino acid can be transported through the membrane. The extended aminoacylated tRNA, which is made by the help of the synthetase or hypermodification[14], cannot do this. So far there is no experiment to show the direct interaction between the amino acid and the cognate tRNA. Consequently this is a good evidence for the C4N hypothesis.[14] This experiment also support the dynamical behavior of the tRNA in the aminoacylation process[11],possibly the transition among three conformation, L→U→Ψ(extended)→L.[14]

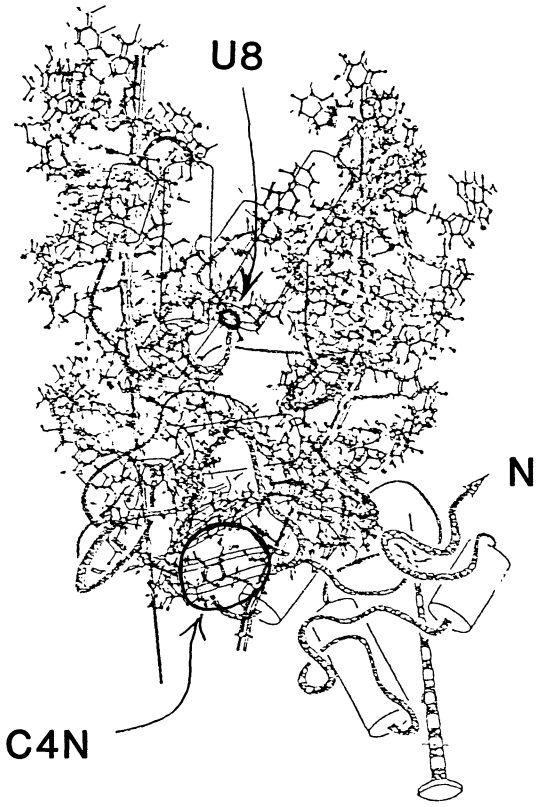

Fig.1. The U shaped tRNA on the aminoacyl-tRNA-synthetase.[23] The uracil at position 8 is in the center of the bridge or almost at the center of U. By this reason, this conformation should be called the H shaped structure. However, the primitive tRNA which might not have the D loop and the TψC loop would have an U shaped conformation[14]. Consequently I hope to leave the name of the U structure. The left axis of U is composed of the anticodon loop + the D loop and the right axis is from the acceptor stem + the TψC loop. The C4N (a complex of four nucleotides, namely that of three anticodon bases and a discriminator base at 4th position from the 3' end) is formed in the lower part of the figure, namely over the Rossmann α/β structure.

The main form of the charged tRNA in the solution could be of the L type, since the shielding of the TψC nucleotides prevents the uncharged tRNA from the misattachment to the ribosome.

In conclusion, I emphasize that the primitive tRNA could have the U shaped structure[14] and so the C4N could have been used throughout the history of the biosystem. Various phenomena concerning with the nucleic acids can be elucidated in terms of this model[14]: the necessity of ribose (namely its 2' OH), hypermodification and other modification of the tRNA bases, inhibitory effect of the synthetase by polynucleotides[21], the necessity of the C4N position over the Rossmann fold on the synthetase, and the amino acid charging mechanism. Other evidence to support the C4N theory will further be presented[22]: chemical editing or two shieves mechanism to discriminate the amino acid, the effect of amino acid analogues on protein synthesis in microorganisms, suppressor problems, aminoacylation of plant virus tRNA analogues etc..

The author thanks to the fruitful discussions with Drs. K. Miura of National Institute of Genetics, K.Watanabe of Univeristy of Tokyo, and M. Kawakami of Nagoya University.

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(Received July 27, 1982)