A DYNAMIC MOLECULAR MODEL FOR TRANSFER RNA

A. DANCHIN
Institut de Biologie Physico-chimique, 13 rue Pierre Curie, Paris Ve, France

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1. Introduction

Many chemical, physical, and biological studies of the tertiary structure of transfer ribonucleic acid have been reported, several of which were based on the highly probable clover leaf secondary structure [1–8]. However, these models were more or less conceived as rigid lattices and no unequivocal picture of the microscopic shape and geometrical variation of tRNA molecules was evolved, and little was described about the likely movement of the nucleic acid during aminoacylation or protein synthesis. Furthermore, except for one case [4] no conformational differences were proposed for the initiator tRNA and for the aminoacylated tRNA, and the observation that some tRNAs have several conformations dependent on cation concentration was not taken into account. The model of tRNA which we describe here exhibits at least two stable structures compatible with experimental evidence showing that aminoacyl tRNA differs from uncharged tRNA. We describe these two forms as the P form, corresponding to the most stable structure of FMet-tRNA^{FMet} and peptidyl-tRNA and the A form corresponding to the structure of aminoacylated tRNA during protein synthesis.

In the absence of definite crystallographic data, assumptions have been made on the local structure of the highly ordered regions and of the loops. This is why, although built within 0.1Å, the model presented does not claim to describe the tRNA structure at the 1 Å level, but only suggests overall features explaining some aspects of tRNA behavior.

2. The model

E. coli tRNA$_1^{Val}$ (fig. 1) was built by using Corey–Pauling–Koltun components [9]; the ribose ring of the nucleotides are in the C2′ endoconformation and in the *anti* conformation with respect to the bases. Nineteen different tRNAs were checked for correlated pairs of nucleotides common to all the sequences and different from those already appearing in the clover-leaf structure [review 10, 10–14]. We examined the correlation which enables the tRNA to assume two different structures locked by stable intramolecular associations.

The criteria used for building the model were as follows:

The largest possible continuous double helical structure was built to account for the cooperative melting of the secondary structure in the presence of divalent ions: the aminoacyl arm is stacked on the TΨC arm as in several other models (main helix).

The maximum stacking of bases is assumed: this favors the building of another continuous double helix: the anticodon stem is a continuation of the hU stem; the connecting purine (at about position 26), between the hU and the anticodon stem is stacked in the helix and not complemented so that a translation of the anticodon stem axis occurs easily with respect to the hU stem axis. The double helices were built assuming an RNA 10 structure [15].

The maximum number of phosphate groups are turned towards the outside of the molecule. Thus, the double helices are accessible to the solvent and diva-

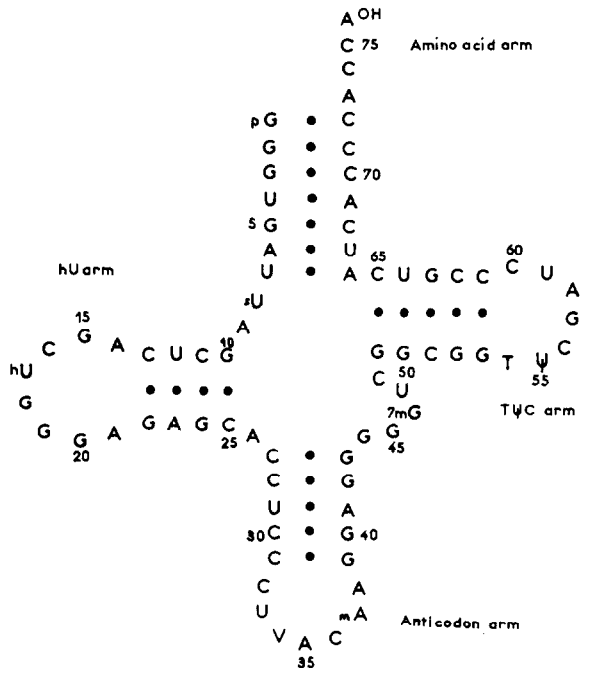

Fig. 1. *E. coli* $tRNA_1^{Val}$ according to Yaniv, Favre and Barrel [16]. The V in the anticodon arm is uridine-5-oxyacetic acid.

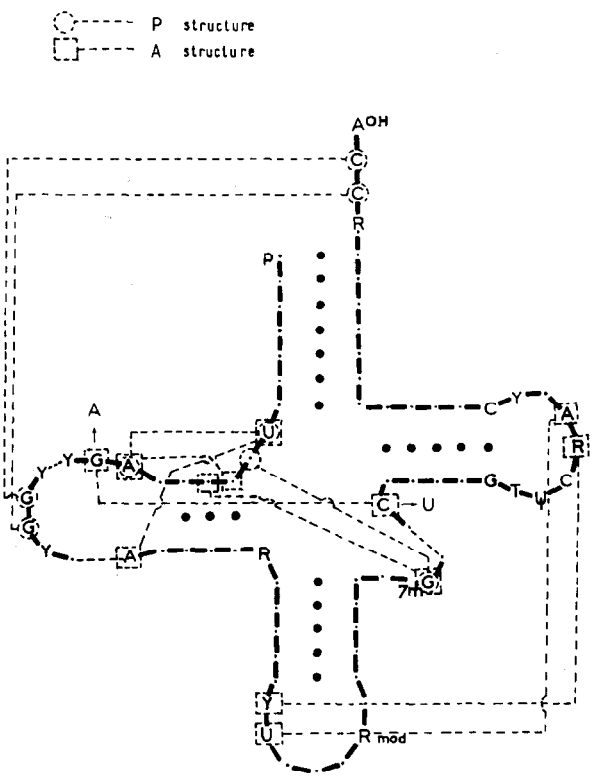

Fig. 2. Constant nucleotides in tRNA primary structures. Y is pyrimidine and R is purine. The circles show the interaction found in the P structure; the squares the interaction found in the A structure.

lent ions can bind two phosphate groups in complementary strands without changing the position of the bases; this increases the accessibility of the large groove to reagents, exposing the possible recognition sites localized in the double helices.

Yaniv et al. have shown that, in several *E. coli* tRNAs [16] a photoreaction occurs between $4sU_8$ and C_{13}; on the contrary, Pochon et al. [17] have shown that no such reaction occurs in the copolymer (4sU, C), even when complexed with poly G. This suggests both that $4sU_8$ and C_{13} are neighbors, and that they are forced into a specific geometrical relationship by the tertiary structure: they are located in the large groove of the main helix.

The displacement of a region containing two consecutive G differentiates acylated neutral tRNA from uncharged tRNA [18]. The motion of the hU loop affects the possibility of aminoacylation: it has been shown that a small change in the stereochemistry of the hU base results in a long lag phase before acylation of tRNA [19].

It has been found that some structure of the ribose backbone of tRNA remains after all the base pairs have been melted [20]; it has also been shown that tRNA is partially resistant to degradation by polynucleotide phosphorylase [21]; this specific stability of tRNA requires a particular topological feature.

These criteria demand that the two double helical regions be maintained in very close contact by a unique topology. These features have all been taken into account in the model presented where the hU loop is bent back and wrapped around the aminoacyl arm, as in a slip knot: the two double helices are parallel, and the hU-anticodon helix can slide in the large groove of the main helix. This movement is favored both by the size of the hU loop and by the flexibility of the nucleotides forming the 'extra' arm [22]. The hU loop can only adhere to the main double helix if the repulsion between phosphate groups is lessened by the presence of cations: there are at least four sites with three or four PO_4^- which must be

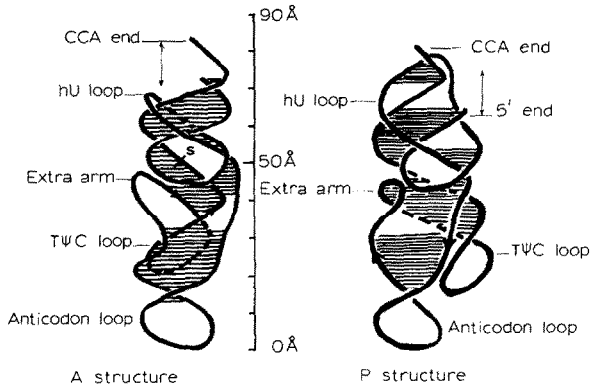

Fig. 3. The ribose phosphate backbone of the Corey–Pauling–Koltun models as drawn from photographs. *Left*: the A structure shows that the 4sU is imbedded in the large groove of the main helix, making an angle of about 45° with its axis. *Right*: the P structure shows the interaction between the G–G of the hU loop and the C–C of the 3'OH terminus.

saturated by divalent ions before allowing a compact structure resulting in a binding pattern of the 'all or none' type, as is experimentally observed [23].

The slip knot allows the movement of the molecule between two extreme structures (P and A) (figs. 2, 3).

2.1. *P structure*

The hU loop is bound to the aminoacyl arm by two normal antiparallel G–C pairs (G_{18}–C_{75}; G_{19}–C_{74}) and the two helical axes are only separated by 3Å; the 'extra' arm adheres to the main structure because $7mG_{46}$ binds to phosphate 10. In the absence of $4sU_8$–C_{13} interaction, A_{14} might be paired to $4sU_8$.

2.2. *A structure*

The hU loop slips down the main helix and the anticodon axis is slightly translated (2 to 3Å), A_{14} and G_{15} flip to the *syn* conformation so that A_{14} can now bind to phosphate 11 and G_{15} pair with C_{48}; $7mG_{46}$ binds to phosphate 12. In addition, in the absence of a $4sU_8$–C_{13} interaction, A_{21} may be paired with $4sU_8$. An interaction (not necessary for the model) between the anticodon and the TΨC loop may also take place: G_{57}–C_{32} (normal base pair) and A_{58}–U_{33} (Hoogsten pair), forcing the anticodon loop pyrimidine to unstack, thus allowing a Fuller–Hodgson type anticodon structure [25].

The structure of uncharged tRNA which might also be that of aminoacyl tRNA kept of acidic pH would usually be an intermediate one where the hU loop is free to move.

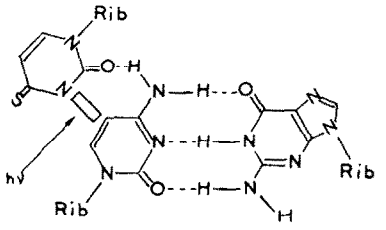

Fig. 4. The 4sU and C_{13}–G_{22} as found in the model. An interaction between nitrogen-1 of 4sU and carbon-5 of cytosine is likely upon the action of a photon.

3. Correlation of experimental results with the theoretical model

4sU is imbedded in the large groove of the main helix and makes a 45–50° angle with its axis. In the native conformation 4sU can be hydrogen bonded with C_{13} (fig. 4) so that a photoreaction between nitrogen-1 of the thiouridine and carbon-5 of the cytosine is possible, which would only slightly affect the stability of the A and P structures; such a reaction would be highly improbable in any other structure: for instance 4sU is not fluorescent in tRNA devoid of Mg^{2+}, and this shows that there is no interaction between 4sU and C_{13} (A. Favre, private communication).

The eighteen other primary structures examined show that both A and P conformations are possible (tRNAs with a long extra arm might have additional intermediate conformations), though in *E. coli* $tRNA^{Met}$ and rat liver $tRNA^{Ser}$ interactions between TΨC and the anticodon loop in the A form are not possible. The stability of the A form would therefore be decreased for the initiator tRNA; moreover, this tRNA is *the only one* which has three pyrimidines between A_{14}–G_{15} and G–G in the hU loop, and this greatly enhances its ability to assume the P structure.

A_{73} (or the corresponding purine in certain tRNA sequences) may be sterically protected by the hU loop against chemical modifications [5]; likewise the ability of the 5' end to be enzymatically phosphorylated will depend on the position of the hU loop [26].

The N^4-acetylcytidine present in several tRNAs and imbedded in the large groove of the main helix, should also be protected against chemical reagents in the native conformations, and indeed reduction does not readily occur [27]. The 7mG present in several tRNAs should likewise be protected by zwitterionic interaction with a phosphate. Finally, the guanosines in the hU loop should not all exhibit the same reactivity with chemicals.

Yeast $tRNA^{Ala}$ is the only tRNA where the bases homologous to A_{14} and G_{15} are displaced to an A_{16} and G_{17} positions; this should destabilize the hU arm in the absence of magnesium, possibly break the base pairs in this arm, and thus result in the 20% hyperchromicity observed in the absence of magnesium at room temperature [28, 29].

The function of the hU loop should be highly affected by mutations, especially on the guanosines since they lock the stable extreme structures: this has been experimentally observed [30].

A slip knot might appear as an odd feature in a biological molecule; however, this model is the only one so far which assigns a function to the tRNA nucleotidyl transferase which positions the –CCA end in all tRNA molecules: –CCA-less tRNA could be visualized, folded in such a way that the pG terminal and its complementary strand would be located just under the center of the hU loop, the transferase would then position the –CCA from above, thus completing the knot. Moreover, the aminoacyl synthetases might act in two directions instead of one: attaching the proper amino acid on tRNA, and pulling down the hU loop. The tRNA would then act like a compressed spring when the hU loop changes position from the A structure to the P structure.

The slip knot model might not possess the fine features we have assumed, but it does explain several properties of tRNA such as importance of divalent cations, and suggest a behavior for tRNA on ribosomes. It also suggests that several kinds of denaturation are possible due to interaction with salts or enzymes, according to the method used [31].

Acknowledgements

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