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tRNA tertiary structure in solution as probed by the photochemically induced 8-13 cross-link

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A B S T R A C T

The conformation of ten purified tRNAs from Escherichia Coli has been investigated by means of the photo-induced cross-linking of ^{4}Srd8 and Cyd13, which is sensitive to the juxtaposition of the two bases. Three tRNAs photoreact abnormally slowly, $tRNA^{Phe}$, $tRNA^{Met}_{m}$ and $tRNA^{Val}_{2}$; a comparison with normally reacting species suggests that base 47 (Urd or modified Urd) is involved in a tertiary interaction in Class I tRNAs with the triplet 8, 14, 28.

The UGA suppressor $tRNA^{Trp}$ photoreacts significantly slower than the wild type. Thus the single base change Gua 24 to Ade induces a conformational change that alters the rate constant for the cross-linking reaction.

I N T R O D U C T I O N

A detailed knowledge of tRNA structure is needed for an understanding of its functioning. The models of tRNA which have emerged from X-ray crystallographic studies [1,2] are invaluable in this respect ; it is clear nevertheless that in solution, and also presumably during its functioning in the cell, tRNA displays a dynamic structure which is only partly represented by a rigid model. Furthermore, crystallographic data have been obtained so far for a single species and we know little about the structural repercussions of small changes in base sequence, which may play a determining role in the recognition process between tRNA and other macromolecules. An approach to this problem is to measure a parameter related to the relative position of two residues adjacent in the native tRNA structure. This is possible using the method of covalent cross-linking which we have described previously [3]. Irradiation at 335 nm induces the formation of a covalent link between ^{4}Srd(8) and Cyd(13) in a number of E. coli tRNAs and this finding provided the first feature of tRNA tertiary structure to be definitively established[4,5]. More recently the kinetics of the photochemically induced cross-linking have been used as a probe of tRNA conformation : the photoreaction rate differs significantly in different tRNA species ($tRNA^{Val}_{1}$, $tRNA^{Met}_{f}$, $tRNA^{Trp}$)

and more strikingly in response to the single base substitution m^7Gua → Ade in $tRNA_f^{Met}$ [6,7]. The rate of cross-link formation should be influenced by the relative distance and orientation of the reacting residues in the tRNA strucure. For example, if a thietane intermediate is involved, the C-S bond of ^{4}Srd(8) should be approximately parallel to the C_5-C_6 bond of Cyd(13) for optimal reactivity [8]. Recent studies of ^{4}Srd luminescence in tRNAs suggest that the alternative possible influence, the presence of groups able to quench ^{4}Srd excited state, plays at most a minor role (A. Favre and G. Thomas, unpublished work).

We have undertaken a systematic investigation of the rate and plateau of cross-linking in various E. coli tRNA species. Here we present our data concerning the wild-type and suppressor $tRNA^{Trp}$ which are shown to differ in conformation. We have also identified a class of tRNAs which cross-links at an abnormally slow rate. These results are discussed with respect to our knowledge of tRNA sequences and tertiary structure, and could be explained by an involvement of base 47 in a tertiary structural interaction.

MATERIAL AND METHODS

Irradiation procedure

Typically three purified samples and one control (mixed E. coli tRNA) in 5.10^{-2} NaCl, 5.10^{-3} $MgCl_2$ and 5.10^{-1} sodium cacodylate buffer pH 7 (A260 between 0.45 and 2.0), were simultaneously irradiated with the Lantern Cunow System using a MTO J 324a filter [4]. The cuvettes were held in a thermostated holder during irradiation and the temperature was maintained between 18 and 20°C.

Kinetic determination

The amount of cross-link was assessed after reduction with $NaBH_4$ as described previously [9]. 50µl aliquots of the tRNA solution were taken after various times of irradiation and diluted with 500 µl of 0.1 N NaCl, 0.1 N sodium cacodylate buffer pH 7. Then, 50µl of 1 M NH_4OH and 50µl of 1 M $NaBH_4$ were added successively. The solution was mixed and left 12 h in the dark at 4°C. The fluorescence intensity (λ exc 390 nm - λ em 450 nm) of the irradiated reduced samples was read at 20°C, pH 9.7 with a Jobin-Yvon spectrofluorimeter [9].

tRNA samples

$tRNA^{Trp}(su^-)$ was prepared as previously described [10] from E. coli B tRNA (General Biochemicals). tRNA from the UGA suppressor strain CAJ64 was prepared as before [11] and $tRNA^{Trp}$ (su^+) was purified by the same method as for the wild-type species, except for a preliminary chromatography on Sephadex A50 according to Nishimura et al [12].

The purified samples of $tRNA^{His}$, $tRNA^{Asn}$ and $tRNA^{Pro}$ were kindly given to us by Dr S. Nishimura. The samples of $tRNA^{Met}_m$ were gifts of Drs Petrissant and J.P. Waller. $tRNA^{Val}_2$ was obtained from Dr M. Yaniv. $tRNA^{Met}_f$ (lot 10-85), $tRNA^{Phe}$ (lot 2), $tRNA^{Val}_1$ (lot 15-179) were obtained from Oak Ridge Laboratories by courtesy of Dr Kelmers. Another set of these purified tRNA species were products from Boehringer.

RESULTS

All purified E. coli tRNAs so far investigated which have a ^{4}Srd(8) and a Cyd(13) residue in their sequence undergo the cross-linking reaction under conditions where they assume their functional structure but not their denatured form [3,4,6,7]. All such tRNAs of known sequence fall into class 1A (4 base pairs in the D stem and 5 bases in the extra-loop). Recently all E. coli tRNAs susceptible to being cross-linked have been indirectly identified[5] including the tRNAs of unknown sequence respectively acylated by Thr, Asn and Pro. Here we confirm directly that purified $tRNA^{Asn}$ and $tRNA^{Pro}$ preparations are cross-linkable.

The ^{4}Srd photochemistry of ten purified species has been examined. Amongst these, one can make a distinction between certain species which cross-link significantly faster than the average ($tRNA^{Met}_f$, $tRNA^{Pro}$, $tRNA^{Asn}$ and $tRNA^{Trp}$), a group with a "normal" photoreaction rate ($tRNA^{His}$ and $tRNA^{Val}_1$) and several tRNAs which photoreact slowly ($tRNA^{Phe}$, $tRNA^{Met}_m$ and $tRNA^{Val}_2$). The relative cross-linking rates for some of these tRNAs are presented in Table 1. The last five species listed above, the normal and slowly reacting tRNAs, afford an interesting comparison because they have identical dihydrouridine stems.

As juged by the ratio A_{330}/A_{260} the ^{4}Srd content of these molecules is higher than 0.9. The kinetics of cross-linking are first order, except possibly in the case of $tRNA^{Phe}$, and the yield of conversion of ^{4}Srd into the 8-13 link, estimated by the changes in the absorption spectra (300-400nm), is in each case higher than 85 %, as reported for $tRNA^{Val}_1$ (refs 3 and 4).

TABLE 1

Relative t 1/2 values for some purified E. coli tRNA species.

MET f_1 MET f_3	0.78	0.72 * 1.40 *
MIXED	1.00	1.00 *
HIS VAL_1 PHE MET_m VAL_2	1.05 1.10 1.40 1.45 1.70	1.06 *

Time $t_{1/2}$ for 50 % cross-linking (relative to that observed in mixed tRNAs) of various purified E. coli tRNA species. Conditions were those of Methods. Also included (*) are the data of Delaney et al (7).

This is important because it might otherwise be possible that the apparent differences in rate of cross-linking arise from a competing side reaction :

$$8 - 13 \text{ cross-link} \xleftarrow{k_1} {}^4\text{Srd} \xrightarrow{k_2} \text{other photoproducts}$$

In such a case the amount of cross-link after time t of irradiation is

$$\frac{k_1}{k_1 + k_2}\left[1 - e^{-(k_1 + k_2)t}\right] \text{ with a final yield } \frac{k_1}{k_1 + k_2}, \text{ and}$$

a time period for 50 % of the final yield, $t_{1/2} = \frac{\ln 2}{k_1 + k_2}$, which is what we have measured. From the point of view of the juxtaposition of bases 8 and 13 the parameter of interest is k_1, though k_2 might reflect other factors in the environment of ^{4}Srd, such as accessibility to solvent. The absence of important differences in the final yield of conversion eliminates this complication in comparing values of $t_{1/2}$.

An unambiguous interpretation of the behaviour of the tRNAs investigated above is necessarily complicated by the fact that their sequences, though related, differ in many parts of the structure. Much more straightforward are cases in which two tRNAs differ by a single base. One such example has been described by Delaney et al [7]. We have investigated a second case in which two tRNAs differ by a single base, the wild-type $tRNA^{Trp}$ from E. coli and the UGA suppressor $tRNA^{Trp}$, in which the Gua 24 in the dihydrouridine arm is replaced by an Ade (ref. 13) thus changing a G.U base pair to A.U (Fig. 1). Both tRNAs possess a single ^{4}Srd in position 8 and a Cyd in position 13 in their sequence [13], and it has already been shown that the wild-type molecule can be cross-linked in the native, but not in the stable denatured form, even in the presence of 10^{-2}M $MgCl_2$ [14].

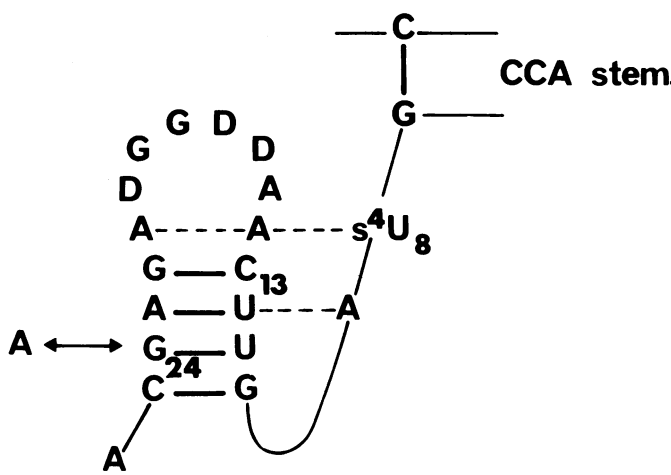

Fig. 1 Dihydrouridine arm of the wild-type $tRNA^{Trp}$. The arrow indicates the substitution of G by A that occurs in the su^+ mutant. Also indicated by dotted lines are the tertiary interactions involving several hydrogen bonded bases (refs. 1,2).

Our $tRNA^{Trp}$ preparations were first examined for their ^{4}Srd content : the absorbance ratio at 260 and 330 nm was determined in our samples as described under Methods and compared to that measured in a number of other E. coli tRNAs. We found that the mutant molecule contains 0.95 ± 0.05 ^{4}Srd residue per molecule although the wild-type content was only 0.65 ± 0.05. We do not know whether the latter value is due to a lower efficiency of in vivo thiolation or to oxidation of ^{4}Srd during the tRNA purification.

The kinetics of cross-linking formation were determined using the fluorescence of the photoproduct reduced with $NaHB_4$ [9]. Tne results of two experiments conducted under slightly different conditions of Mg^{2+} and ionic strength, are presented in Table II. In all cases the photoreaction was

TABLE 2

Relative $t_{1/2}$ values for wild-type and Su^+ $tRNA^{Trp}$

	$tRNA^{Trp}$		unfractionated tRNA	
	wild-type	su^+	wild-type	su^+
exp 1	0.65 ± 0.05	0.85 ± 0.05	1.00 ± 0.05	
exp 2	0.47 ± 0.05	0.67 ± 0.05	1.00 ± 0.05	1.00 ± 0.05

The rates are defined by the time $t_{1/2}$ necessary to obtain 50 % of the final cross-linking level and are normalized with respect to unfractionated tRNA.

In experiment 1 the conditions described in Material and Methods were used and the concentrations of the differents samples were respectively (in A_{260} units) 1.710 for total 0.630 for the su^+ and 0.460 for the wild-type tRNA.

In experiment 2 we used a 0.01 M $MgCl_2$ 0.025 M NaCl, 0.025 M sodium cacodylate pH 7 buffer. The 260 nm absorbancies were 1.9 for the total tRNAs and respectively 1.790 and 1.840 for the wild-type and su^+ $tRNA^{Trp}$.

found to be first order with both wild-type and su^+ $tRNA^{Trp}$, and in both experiments the mutant tRNA was cross-linked significantly slower than the wild-type species (Fig. 2). Unfractionated tRNA from E. coli B or E. coli CAJ64, used as controls, photoreacted more slowly than either $tRNA^{Trp}$. Some effect on the relative rate of photoreaction is apparent on changing the solution conditions but the difference in rate between wild-type and suppressor species remains. The value for the wild-type tRNA is in agreement with the results of Delaney et al [7].

As discussed above, the possibility must be considered that the apparent difference in rate constant k_1 arises through a competing side

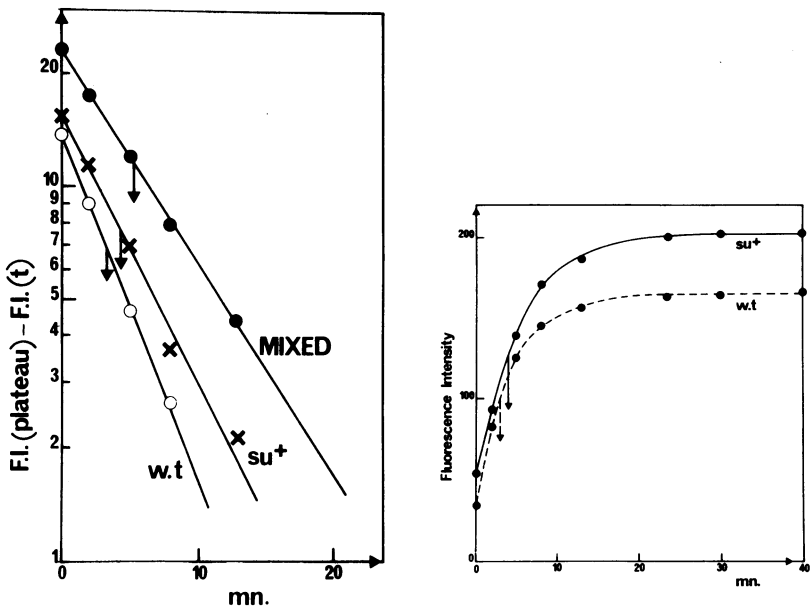

Fig. 2 Kinetics of 8-13 link formation in wild-type and su^+ $tRNA^{Trp}$ in conditions of exp 1 (Table 1). The irradiation procedure and the fluorimetric assay of photoproduct concentration are described under Methods.
a) Linear representation
b) Logarithmic representation.

reaction. We have therefore measured the efficiency of conversion of ^4Srd to the 8-13 cross-link. As judged by the 300-400 nm absorption spectra of the $tRNA^{Trp}$ species before and after irradiation the yield of conversion is close to one (a difference greater than 10 % between the two species is excluded). Thus this single base substitution induces a conformational change that significantly alters the rate constant of the cross-linking reaction.

D I S C U S S I O N

Our results on the kinetics of cross-linking of a variety of tRNA species show that several photoreact significantly slower than the average. We will consider this in terms of base differences between the tRNAs.

Several regions of the tRNA molecules appear to have little influence, if any, on the rate of cross-linking. Removal of the CCA end in total *E. coli* $tRNA$[5], a nick in position 71 in $tRNA_f^{Met}$ [15] or in the anticodon loop of

$tRNA_1^{Val\ 16}$ show no detectable effect. Similarly no deviation from first-order kinetics can be detected in $tRNA_2^{Val}$, a mixture of 2 subspecies A (60 %) and B (40 %) that differ in several base pairs : one in the TΨC arm, two, including the 7.66 pair, in the CCA arm[17]. On the other hand single-base substitutions can affect the rate of cross-linking. Thus the base change $m^7 Gua_{46} \rightarrow Ade_{46}$ in $tRNA_f^{Met}$ results in a lower photoreactivity and a decreased stability of the 8-13 region [7,18]. These results, together with the finding [5] that in E. coli tRNAs the presence of m^7 Gua is always accompanied by Cyd 13 demonstrate that the triple interaction between residues in position 13, 22 and 46 found in $tRNA^{Phe}$ crystal [1,2] indeed exist in solution for class 1 tRNAs. As shown here, the wild type and Su^+ $tRNA^{Trp}$, in which the G.U. and A.U. pairs in position 11.24 are stereochemically non-equivalent, [19] provide a second case where a single base change modifies photoreactivity.

Although they differ in other regions of the molecule the average and slowly photoreacting species have the same dihydrouridine stem (Fig. 3 and Table 3). It is striking that all slowly cross-linked tRNAs but none of the average and rapidly cross-linked species possess in position 47 a uridine residue substituted in the N_3 position. The presence or absence of side chain may influence the rate through an interaction with the triplet 8, 14, 28. Such interaction has not yet been observed in crystallographic studies [1,2].

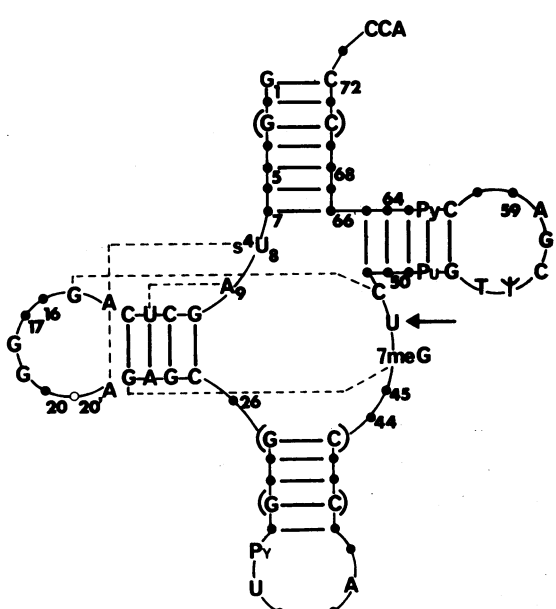

Fig. 3

A composite structure showing the similarities between the tRNAs which cross-link with an average and slow rate. ($tRNA^{His}$ has an additional nucleotide at the 5' end paired with the base adjacent to the CCA.)

TABLE 3

Differences in the sequence of the average and slowly cross-linking tRNAs

Positions	7	16	17	20	20'	26	44	45	47	59	60
HIS	A	D	D	D		C	U	U	U	A	U
VAL_1	U	C	D	G		A	G	G	U	U	C
MET_m	G	U	D	D	D	A	G	G	U^*	A	U
VAL_2	G	D	D	D	D	A	G	G	U^*	G	U
PHE	A	D	C	D		A	G	U	U^*	U	U

Only positions close to the 8-13 region in the tRNA tertiary structure have been tabulated. The position numbering is indicated on Fig. 3. D and U^* represent respectively dihydrouridine and N_3 substituted uridine residues. The data are taken from ref. 22.

It is noteworthy that the residues adjacent to Urd_{47}, i.e. $m^7 Gua_{46}$ and Cyd_{48} are involved in tertiary interactions with bases adjacent to the 8, 14, 28 triplet. It is unlikely that the side chain is directly involved because of its hydrophylic character[20] and its reactivity towards chemical reagents [21]. The other possibility, namely the formation of an "unusual" hydrogen bond between residue 47 and the 8, 14, 28 triplet in all class I tRNAs, is strengthened by the fact that a Urd (or modified Urd) is always found in that position[22]. The accessibility of Urd_{47} to carbodiimide in $tRNA^{Phe}$ from yeast [1,2] does not totally eliminate this possibility : the hydrogen bond may form transiently in the dynamic state of tRNA in solution and/or Urd_{47} may not be well protected by stacking interactions.

The su^+ $tRNA^{Trp}$ translates UGA nonsense codons *in vivo* and *in vitro* much more efficiently than the wild-type tRNA despite the fact that both tRNAs possess the same anticodon sequence [13,23]. Until now no physical measurement has been able to discriminate between the two tRNAs in their active forms. For example the anticodons of the two molecules cannot be distinguished by their binding either to the UGA triplet[24] or to the complementary $tRNA^{Pro}$

anticodon (R.H.B. unpublished results). They are, however, very different in their behaviour under denaturing conditions : the wild-type tRNA has a stable denatured form which is not found in the mutant [11]. It has been suggested that the conformational stability of the tRNA may be altered by the mutation in the dihydrouridine stem, so as to facilitate a change in conformation required on the ribosome to unmask a common binding site, such as the TΨC loop [25]. Correct binding might thus become more probable to the nonsense codon UGA[26]. Structural differences are shown by the quite dissimilar behaviour of the two tRNAs on degradation by polynucleotide phosphorylase (R.H. Buckingham, L. Dondon and M.N. Thang unpublished results). The experiments described here on the rate of photochemical cross-linking provide, however, the most direct evidence of a structural difference between the species at a certain distance from the mutation site.

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