MUTUAL ADAPTATION OF YEAST $tRNA^{Phe}$ AND PHENYLALANYL-tRNA SYNTHETASE

Possible role of tryptophan residues and long range interactions

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1. Introduction

In [1] we showed that upon interaction between $tRNA^{Phe}$ and phenylalanyl-tRNA synthetase from yeast, conformational changes occur in both macromolecules.

We characterized a conformational change in the anticodon loop of the tRNA, leading to the expulsion of the wybutine from a stacked region. This phenomenon only takes place in the complex with the cognate synthetase and is triggered by a critical $[Mg^{2+}]$ around 1 mM. In [2] a similar effect of Mg^{2+} has been described in the phenylalanine system of *Escherichia coli*.

In the enzyme moiety, the conformational changes, upon the complex formation, are revealed by an important quenching of the tryptophan fluorescence and a loss of the ellipticity at 222 nm.

The specific conformational changes induced by complexation might be related to the adaptation step postulated in the tRNA–enzyme recognition mechanism [3,4].

Here we have studied the mechanism leading to the reciprocal adaptation of tRNA and synthetase:

(i) The effect of some model peptides (Lys–Trp–Lys and Lys–Ala–Lys–*O*-methyl) on the conformation of the anticodon loop in native $tRNA^{Phe}$ and $tRNA^{Phe}$ fragments;

(ii) The interaction between the synthetase and different $tRNA^{Phe}$ fragments or recombined molecules;

(iii) The effects of modifications of the CCA end, either by aminoacylation or by chemical or enzymatic procedures, on the conformation of the anticodon loop, in the isolated or complexed tRNA.

2. Materials and methods

Yeast phenylalanyl tRNA synthetase was prepared and its activity controlled as in [5]. Yeast $tRNA^{Phe}$ was prepared according to [6] and accepted $\geqslant$1600 pmol amino acid/A_{260} unit. Different fragments or modified molecules of $tRNA^{Phe}$ were obtained by methods in [6]. These molecules were: $tRNA^{Phe}$ox, $tRNA^{Phe}$ lacking m^7Gua 46, $tRNA^{Phe}$ (18–76) and $tRNA^{Phe}$ (18–76) lacking m^7 Gua 46. The preparation of other $tRNA^{Phe}$, modified at the 3′-terminus has been described: tRNA pCpCp [7] and tRNA pCpC [8] – $[^{14}C]tRNA^{Phe}$ was prepared by exchange of the terminal adenosine with $[^{14}C]$ATP in the presence of trinucleotidyl transferase as in [9].

All tRNAs tested were treated to remove Mg^{2+} and

Abbreviations: $tRNA^{Phe}$, transfer ribonucleic acid specific for phenylalanine; $tRNA^{Phe}_{ox}$, periodate oxidized $tRNA^{Phe}$; $tRNA^{Phe}$ (1–17), fragment of $tRNA^{Phe}$ extending from 5′-end to nucleotide in position 17; $tRNA^{Phe}$ (18–76), fragment of $tRNA^{Phe}$ extending from nucleotide in position 18 to the 3′-end; $tRNA^{Phe}$ pCpCp, $tRNA^{Phe}$ deprived of 3′-terminal adenosine; $tRNA^{Phe}$ pCpC, preceding tRNA deprived of 3′-phosphate; YWye, modified base at position 37 of yeast $tRNA^{Phe}$ (formerly called Y base) and called wybutine as proposed by W. E. Cohn; m^7Gua, 7-methylguanine found at position 46 of $tRNA^{Phe}$

Enzymes: Yeast phenylalanyl-tRNA synthetase, EC 6.1.1.20; micrococcus luteus polynucleotide phosphorylase, EC 2.7.7.8

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renatured as in [1]. The recombined molecules $tRNA^{Phe}$ (18–76) + $tRNA^{Phe}$ (1–17) and $tRNA^{Phe}$ (18–76) lacking m^7 Gua 46 + $tRNA^{Phe}$ (1–17) were prepared by renaturing both fragments in the presence of each other. Amino acylation experiments were according to [10] and Phe $tRNA^{Phe}$ preparation according to [11].

Polynucleotidyl phosphorylase from *Micrococcus luteus* was purchased from P. L. Biochemical and used under conditions of limited hydrolysis as in [12]. The tripeptides Lys–Trp–Lys and Lys–Ala–Lys–*O*-methyl were from Dr C. Helene. The concentrations of the tripeptides were determined by absorption measurements for Lys–Trp–Lys and amino acid analysis for Lys–Ala–Lys, respectively.

Fluorescence intensity measurements were performed with an absolute spectrofluorimeter FICA 55. Polarization degree was measured with a highly sensitive apparatus as in [1].

Complex formation between $tRNA^{Phe}$, modified $tRNA^{Phe}$ or $tRNA^{Phe}$ fragments and phenylalanyl-tRNA synthetase, were done considering the different K_d values. Enzyme concentration was then fixed in order to obtain ⩾80% of complexed tRNA molecules. All solutions contained 20 mM potassium phosphate buffer (pH 7.2).

3. Results and discussion

3.1. *Interaction between $tRNA^{Phe}$ and the model peptides Lys–Trp–Lys and Lys–Ala–Lys–O-methyl*

The quenching of tryptophan fluorescence in the tRNA–enzyme complex [1] suggests the possible participation of these residues in the recognition process. Different mechanisms could lead to a tryptophan fluorescence quenching in a nucleic acid–protein complex, one of them being the stacking of aromatic amino acids with the nucleic acid bases [13–16]. Thus we studied the conformational changes in the anticodon loop of $tRNA^{Phe}$ which may occur upon the interaction with model peptides containing a tryptophanyl residue.

The binding of Lys–Trp–Lys to $tRNA^{Phe}$ is accompanied by a quenching of tryptophanyl fluorescence (data not shown) and the study of the wybutine mobility by fluorescence depolarization showed (fig.1) that the interaction with the tripeptide leads to an increase of the minor base mobility. Indeed, the observed decrease of the polarization degree can only be interpreted as an increase of rotational freedom, since the life time of the excited state of wybutine slightly decreases (data not shown). Furthermore, no energy transfer from tryptophan to wybutine can take place, since the excitation is performed at 313 nm, outside the tryptophan absorption band. A control experiment was carried out with a similar tripeptide lacking tryptophan residue (Lys–Ala–Lys–*O*-methyl) to mimic the electrostatic effect due to the binding of the tripeptide to the tRNA. As shown in fig.1, an increase of the polarization of wybutine emission was observed, reflecting a decrease in the fluorophore mobility. This effect is most likely similar to that observed upon addition of Mg^{2+} to tRNA solution [1] and results from an increased stacking of wybutine. We are therefore led to the conclusion that the expulsion of wybutine observed above could be due to tryptophan stacking.

3.2. *Interaction between $tRNA^{Phe}$ (18–76) lacking m^7 Gua_{46} and the model peptides*

Whereas the interaction of Lys–Trp–Lys with the

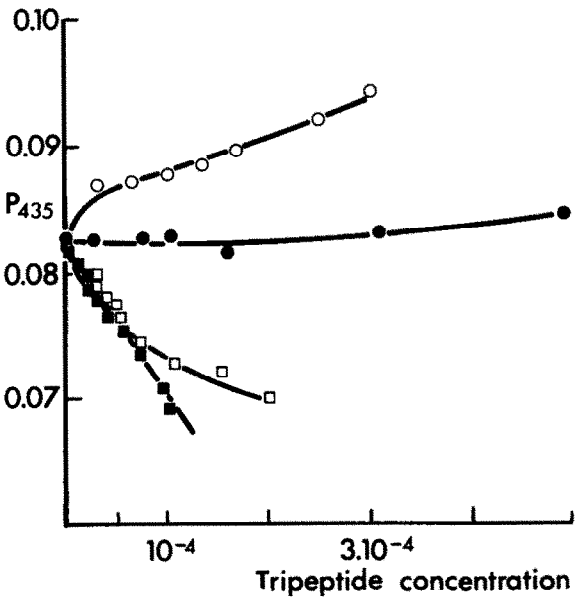

Fig.1. Variation of the polarization degree of YWye luminescence in intact $tRNA^{Phe}$ (open symbols) and in $tRNA^{Phe}$ (18–76)–m^7Gua_{46} (closed symbols) against tripeptide concentration: Lys–Trp–Lys (□,■); Lys–Ala–Lys–*O*-methyl (○,●). Excitation and emission at 313 nm and 435 nm, respectively. tRNA 6.6 . 10^{-6} M. Phosphate buffer, 20 mM (pH 7.2).

modified fragment always results in an expulsion of wybutine (fig.1) the effect of Lys–Ala–Lys–*O*-methyl appears different from that observed with native tRNA. The increase of wybutine stacking in the presence of the control peptide is no longer observed in the modified fragment or at least appears to be shifted to higher concentrations of Lys–Ala–Lys–*O*-methyl. As shown in section 3.3, a similar effect is observed for Mg^{2+}.

Given the loss of cooperativity between the different residual structural domains of the modified tRNA fragment observed [6], the difference between the behaviour of Lys–Trp–Lys and Lys–Ala–Lys–*O*-methyl, can be understood in the following way. Electrostatic effects (like those of Mg^{2+} and Lys–Ala–Lys–*O*-methyl) are perceived at the level of the anticodon loop as a consequence of modifications of the overall tRNA conformation. They will thus be affected by any important alteration of the tRNA structure. On the contrary, the specific effect of tryptophan stacking could be a local effect at the level of the anticodon loop and therefore might subsist even in a deeply altered fragment. Tryptophan stacking preferentially occurs in double helical regions [14].

3.3. *Interaction between phenylalanyl-tRNA synthetase and modified $tRNA^{Phe}$, $tRNA^{Phe}$ fragments and recombined molecules*

The biological activities of $tRNA^{Phe}$ lacking m^7Gua_{46}, of the fragments $tRNA^{Phe}$ (18–76), $tRNA^{Phe}$ (18–76) lacking m^7Gua_{46} as well as of the recombined molecules [$tRNA^{Phe}$ (18–76) + $tRNA^{Phe}$ (1–17) and $tRNA^{Phe}$ (18–76) lacking m^7Gua_{46} + $tRNA^{Phe}$ (1–17)] were described in [6].

The study of the effect of Mg^{2+} on the conformation of the anticodon loop in these molecules, showed (fig.2) that the main phenomenon is similar to that observed in the intact $tRNA^{Phe}$: Mg^{2+} appear to favour a conformer where the wybutine residue is stacked between adjacent bases (see [1]). However, the apparent affinity constants of the different modified tRNAs for Mg^{2+} were found somewhat different, in the order:

$$K_a\ [tRNA^{Phe}] \simeq K_a\ [tRNA^{Phe} - m^7Gua] >$$

$$K_a\ [tRNA^{Phe}\ (18–76) + tRNA^{Phe}\ (1–17)] \simeq$$

$$K_a\ [tRNA^{Phe}\ (18–76) - m^7Gua + tRNA^{Phe}\ (1–17)] > K_a\ [tRNA^{Phe}\ (18–76)] \simeq$$

$$K_a\ [tRNA^{Phe}\ (18–76) - m^7Gua]$$

The apparent affinity constants range from 10^4–$3 \cdot 10^2\ M^{-1}$.

The shift of the apparent affinity constant for Mg^{2+} can be accounted for by the following hypotheses:

(i) Modification or loss of possible strong binding sites for Mg^{2+} in the altered molecules;

(ii) A simple electrostatic treatment [16] could explain the decrease of the apparent affinity constant by an overall decrease of the charge density of the modified tRNAs;

(iii) A decrease or a loss of long-range interactions in the fragments and recombined molecules.

The modification of the conformation of the anticodon loop in the modified tRNAs upon complex formation with the cognate synthetase was also investigated. Table 1 summarizes the results obtained, together with the biological properties of the modified

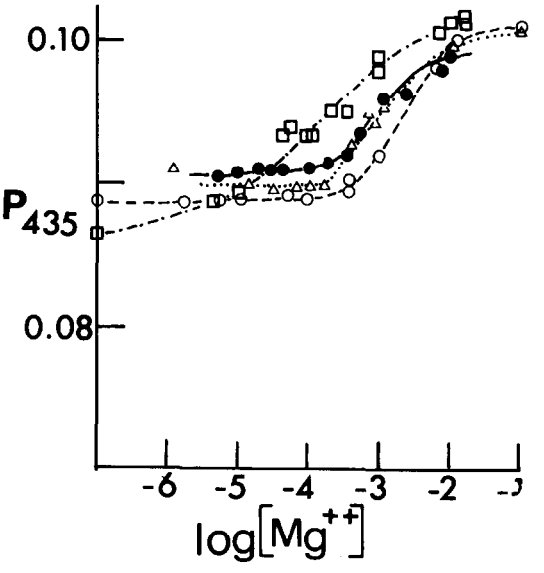

Fig.2. Variation of the polarization degree of YWye luminescence against [Mg^{2+}] in: (□–·–□) native $tRNA^{Phe}$; (△–·–△) $tRNA^{Phe}$ (18–76)–m^7Gua_{46} + $tRNA^{Phe}$ (1–17); (●——●) $tRNA^{Phe}$ (18–76) + $tRNA^{Phe}$ (1–17); (○– – –○) $tRNA^{Phe}$ (18–76)–m^7Gua_{46}. Excitation and emission at 313 nm and 435 nm, respectively. Phosphate buffer, 20 mM (pH 7.2).

Table 1

Conformational change of the anticodon loop upon complexation and biological properties of intact and modified $tRNA^{Phe}$, $tRNA^{Phe}$ fragments and recombined molecules

tRNA molecule	Conformation change in anticodon loop[a]	Affinities of tRNAs for phenylalanyl-tRNA synthetase[b] k_d modified tRNA / k_d intact tRNA	Aminoacylation parameters[b]	
			Maximal extent of aminoacylation	V modified tRNA / V intact tRNA
Intact $tRNA^{Phe}$	+	1[c]	100	1
$tRNA^{Phe}$–m^7Gua_{46}	+	1	80	1.2
$tRNA^{Phe}$ (18–76) + $tRNA^{Phe}$ (1–17)	+	n.d.	80	n.d.
$tRNA^{Phe}$ (18–76)–m^7Gua_{46} + $tRNA^{Phe}$ (1–17)	–	n.d.	60	n.d.
$tRNA^{Phe}$ (18–76)	–	7	–	n.d.
$tRNA^{Phe}$ (18–76)–m^7Gua_{46}	–	70	30	0.1

[a] Expulsion of wybutine upon complexation with synthetase at $>10^{-3}$ M Mg^{2+}

[b] Affinities and aminoacylation parameters are taken from [6]

[c] k_d of intact $tRNA^{Phe}$ for phenylalanyl-tRNA synthetase at $2 \cdot 10^{-7}$ M

n.d., not determined

molecules in [6]. As can be seen, the presence of the 5′-quarter of the molecule is required for the conformational change in the anticodon loop to occur. Furthermore, the different behaviours observed for the recombined molecules, suggest that the propagation of constraints, between the different structural domains of the recombined tRNA molecules, requires the integrity of some ternary hydrogen bonds. Indeed, the removal of m^7Gua residue eliminates the hydrogen bond with the C_{13}–G_{22} basepair which, in the tertiary structure, interlocks two domains which are distant in the cloverleaf arrangement and results in the disappearance of the conformational transition of the anticodon loop in recombined molecules.

3.4. *Study of the conformational changes in the anticodon loop of tRNA^{Phe} modified at the 3′-terminus*

In all cases studied [$tRNA^{Phe}_{ox}$, $tRNA^{Phe}_{pCpCp}$, $tRNA^{Phe}_{pCpC}$, either isolated or complexed by the cognate synthetase] no differences could be found with respect to the behaviour of the native tRNA molecule under the same conditions, as judged by the wybutine emission properties. Similarly, we observed that the limited hydrolysis of the 3′-end of tRNA^{Phe} by polynucleotide phosphorylase does not affect the conformation of the anticodon in the isolated tRNA, since the polarization of wybutine emission remained constant during hydrolysis (fig.3a).

Furthermore, we observed that the emission properties of wybutine do not change during the aminoacylation reaction, in the presence of catalytic amounts of the synthetase. Conversely, during the enzymatic deacylation of phenylalanyl-tRNA^{Phe}, in the presence of stoichiometric amount of phenylalanyl-tRNA synthetase, no changes of the polarization of wybutine luminescence could be detected (fig.3b,c).

We can therefore conclude, that whereas the anticodon loop of tRNA^{Phe} undergoes a conformational modification upon interaction with the cognate synthetase, it recovers its earlier conformation upon release from the enzyme whether it is aminoacylated or not.

4. Conclusion

These results show that the stacking of aromatic residue and particularly tryptophan groups of the

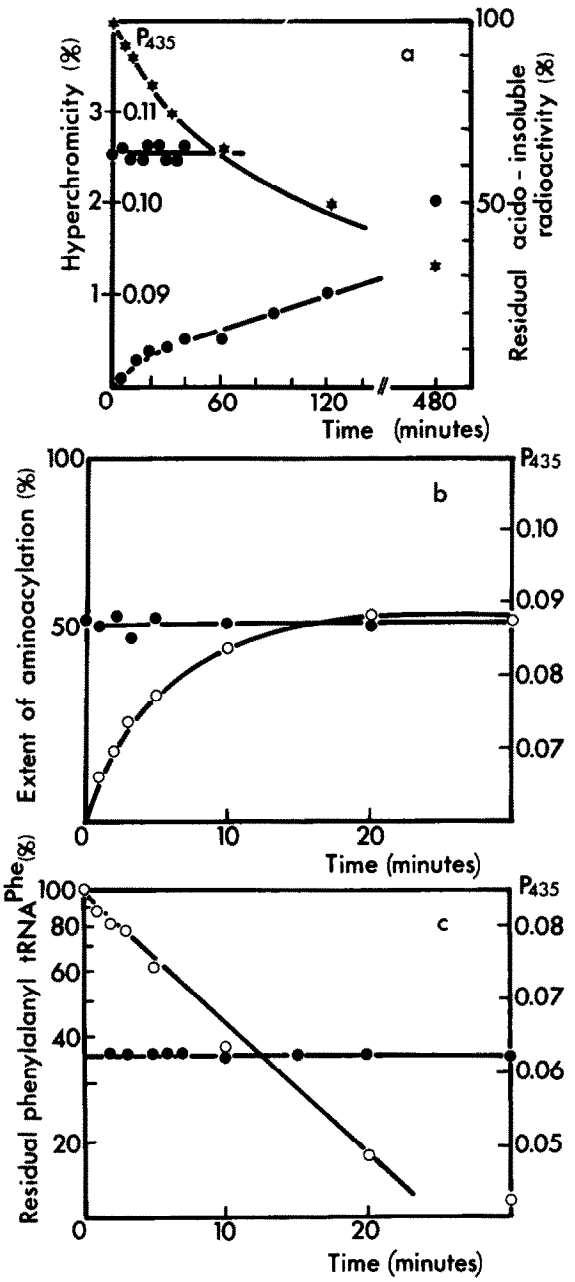

Fig.3. Study of the polarization degree (•——•) of YWye luminescence upon: (a) hydrolysis of tRNA^{Phe} by polynucleotide phosphorylase – (□——□) hyperchromicity at 260 nm, (*——*) loss of radioactive 3′-terminal adenosine; (b) aminoacylation in the presence of catalytic amount of phenylalanyl-tRNA synthetase – (○——○) incorporation of [^{14}C]phenylalanine in tRNA^{Phe}; (c) deacylation of [^{14}C]-Phe-tRNA^{Phe} in the presence of stoichiometric amount of phenylalanyl-tRNA synthetase – (○——○) residual phenylalanyl-tRNA^{Phe}. In all experiments, the excitation was performed at 313 nm.

enzyme between adjacent bases of the tRNA might be responsible for the conformational change of the anticodon loop of $tRNA^{Phe}$ upon complexation by yeast phenylalanyl-tRNA synthetase. The study of wybutine expulsion, in altered $tRNA^{Phe}$ molecules, suggests that the stacking of tryptophanyl residues, leading to the transconformation of the anticodon loop, most likely occurs in this region. On the contrary, electrostatic effects, like those observed in the presence of Mg^{2+} or lysyl groups of the tripeptides studied, arise from an overall modification of the tRNA tertiary structure and require a relative integrity of the tRNA molecule.

The stacking of tryptophanyl residues of the enzyme might result from the mutual adaptation of tRNA and protein which could be the origin of aminoacylation kinetic specificity [17,18], since we could show that wybutine expulsion did not occur upon complex formation with non-cognate synthetases like aspartyl- and valyl-tRNA synthetases [1]. Furthermore, the studies carried out with modified $tRNA^{Phe}$ showed that the anticodon loop transconformation depended upon the integrity of acceptor and D stems suggesting that the adaptation of tRNA to enzyme requires the whole set of interaction sites with the synthetase. One may question whether the interdependence observed between different regions of the tRNA during the adaptation process is a property of tRNA molecule per se or is the consequence of a concerted modification of the enzyme structure. Finally, the link between structural adaptation and biological activity remains to be clearly established, since Mg^{2+} were found necessary for structural modifications as well as for the transfer of the amino acid from the adenylate to the tRNA [20].

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