THE TERTIARY STRUCTURE OF YEAST tRNA^{Phe} IN SOLUTION STUDIED BY PHOSPHODIESTER BOND MODIFICATION WITH ETHYLNITROSOUREA

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1. Introduction

Chemical modification of tRNAs with base-specific reagents was successfully used for investigation of their structure in solution [1]. Using base-specific reagents allows the identification of bases involved in hydrogen bond formation in tRNA structure. The development of a chemical modification method that would provide more information could be the use of reagents able to modify the riboses or the phosphates common to all nucleotide residues in tRNA. Studying the reactivity of these residues scattered throughout the entire tRNA molecule would then provide information about the backbone interactions in the tRNA and about the folding of the tRNA polynucleotide chain.

We present here results of chemical modification of yeast tRNA^{Phe} with ethylnitrosourea, a reagent alkylating phosphodiester bonds in nucleic acids [2,3]. In the unfolded tRNA^{Phe} all phosphates were reactive toward this reagent. In contrast in the folded tRNA^{Phe} phosphates in different sites of the molecule were remarkably different in their reactivities toward the reagent. Phosphates in the exposed helical regions of tRNA^{Phe} (for instance in the anticodon arm) readily react with ethylnitrosourea, whilst the reactivity of some phosphates in the regions known to be involved in the tertiary structure formation is strongly reduced. The most pronouced effects were observed for the phosphates in positions 9,10,11,19,49,58,59 and 60.

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2. Materials and methods

Yeast tRNA^{Phe} was isolated from total brewer's yeast tRNA (Boehringer, Mannheim) as described in [4]. Ethylnitrosourea was synthesized in a similar manner as in [5]. 5′-Labelling and 3′-labelling of tRNA^{Phe} was performed according to [6].

Alkylation of tRNA^{Phe}, under conditions stabilising the tRNA macrostructure, was performed at 20°C for 3 h in 0.3 M sodium cacodylate (pH 8.0), 0.02 M $MgCl_2$, 0.1 m NaCl, 2×10^{-3} M EDTA. Alkylation of tRNA^{Phe}, under conditions providing the unfolded tRNA structure, was performed at 80°C for 2 min in 0.3 M sodium cacodylate (pH 8.0), 2×10^{-3} M EDTA. In both cases ethylnitrosourea was added as a saturated ethanol solution (5 μl) to 20 μl aqueous buffer containing 2 μg tRNA^{Phe}. The final concentration of reagent was 150 mM. In control experiments, ethanol was substituted for the ethylnitrosourea–ethanol solution. After alkylation, the reaction mixture was supplemented with 2 μl of a carrier ribosomal RNA solution (5 mg/ml) and 3 μl 3 M sodium acetate (pH 6.0). The tRNA was then precipitated by addition of 100 μl ethanol. After pelletting, the modified tRNA was redissolved in 10 μl 0.3 M sodium acetate (pH 6.0) containing 1×10^{-2} M EDTA and precipitated with ethanol. The precipitated tRNA^{Phe} was further dissolved in 10 μl 0.1 M Tris–HCl (pH 9.0) and incubated for 5 min at 50°C to split the phosphotriesters. After the incubation, 7 μl 3 M sodium acetate (pH 6.0) were added to the solution and tRNA was precipitated by addition of 60 μl ethanol, rinsed with ethanol, and vacuum dried.

The samples of 3′- and 5′-labelled tRNA^{Phe} after modification were analysed by electrophoresis on 15% and 20% acrylamide/8 M urea gels essentially as

in [7] (fig.1–3). The assignment of the bands was done by comparison with partial T_1 RNase digests of labelled $tRNA^{Phe}$ electrophoresed in parallel. The X-ray films were scanned with a Transidyne 2955 scanning densitometer (Transidyne General Corporation, Ann Arbor, MI using the linear scale of the instrument.

Under the conditions used, the total extent of alkylation of tRNA was low; only ~0.2 mol ethyl groups were incorporated/mol tRNA. Therefore the extent of modification of a given phosphate and the intensity of the corresponding band on the electrophoregram should be directly related to the phosphate reactivity in the tRNA structure, unchanged by the modification. The treatment of alkylated tRNA includes ethanol precipitation which results in a partial loss of short oligonucleotides. Therefore the reactivity of the terminal phosphates, 7–10 residues apart from each end of the molecule, could not be determined quantitatively. Reactivity of phosphates 33 and 35 could not be determined due to the natural methylation of the 2′-hydroxyl groups in riboses 32 and 34 of $tRNA^{Phe}$ which prevent splitting at these phosphates.

Due to its limited solubility in water, ethylnitrosourea was added to the reaction mixtures as saturated ethanol solution so that the final reaction mixtures contained 20% ethanol. In order to find out whether this concentration of ethanol influences the tRNA structure and the pattern of alkylation, we investigated the alkylation of $tRNA^{Phe}$ in 10% and in 5% ethanol solution. The decrease in the reagent concentration resulted in a lower extent of tRNA alkylation; however the patterns of phosphate alkylation were identical in all cases.

3. Results and discussion

Ethylnitrosourea was found to be an efficient reagent for alkylation of phosphotriester bonds in nucleic acids [2,3]. In the case of RNA phosphodiesters formed are unstable and splitting of the alkylated polymer could be achieved under mild alkaline conditions [3]. Therefore alkylation of phosphates in the case of end-labelled tRNA can easily be followed by gel electrophoresis after splitting of the partially modified polymer.

We investigated the alkylation of phosphodiester bonds in yeast $tRNA^{Phe}$ with ethylnitrosourea under

Fig.1. Autoradiogram of a 20% acrylamide gel of phosphate alkylation experiments with 3′-labelled $tRNA^{Phe}$: (1) $tRNA^{Phe}$ incubated in the absence of the reagent under conditions stabilising tRNA structure; (2) $tRNA^{Phe}$ alkylated with ethylnitrosourea in the folded state; (3) $tRNA^{Phe}$ alkylated with ethylnitrosourea in the unfolded state; (4) $tRNA^{Phe}$ incubated in the absence of the reagent under conditions providing the unfolded tRNA.

conditions stabilising the tRNA structure, at 20°C in the presence of 0.02 m Mg^{2+} and 0.1 M Na^{+}. We also studied the alkylation of the tRNA in an unfolded state, at 80°C in the absence of Mg^{2+}, to reveal the effect of the tertiary structure on the reactivity of the various phosphates. After alkylation the modified $tRNA^{Phe}$ was split by incubation at pH 9.0 and the obtained fragments were analysed by gel electrophoresis. Fig.1–3 display typical cleavage patterns of alkylated $tRNA^{Phe}$ revealed by gel electrophoresis. The densitometer tracings of the electrophoretic patterns demonstrate the relative intensities of the electrophoretic bands (fig.4). It can be seen that the degradation of $tRNA^{Phe}$, alkylated in the unfolded state, occurs nearly uniformly at all phosphates. The

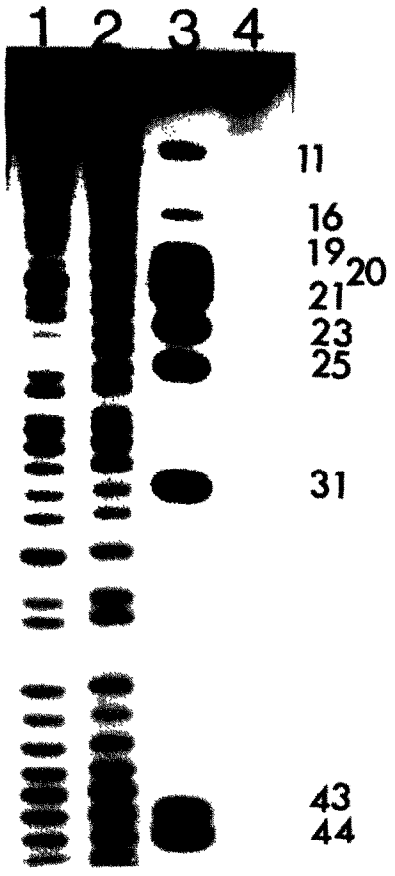

Fig.2. Autoradiogram of a 15% acrylamide gel of phosphate alkylation experiments with 3'-labelled $tRNA^{Phe}$: (1) $tRNA^{Phe}$ alkylated with ethylnitrosourea in the folded state; (2) $tRNA^{Phe}$ alkylated with ethylnitrosourea in the unfolded state; (3) Partial T_1 ribonuclease digest of $tRNA^{Phe}$; (4) $tRNA^{Phe}$ incubated in the absence of the reagent.

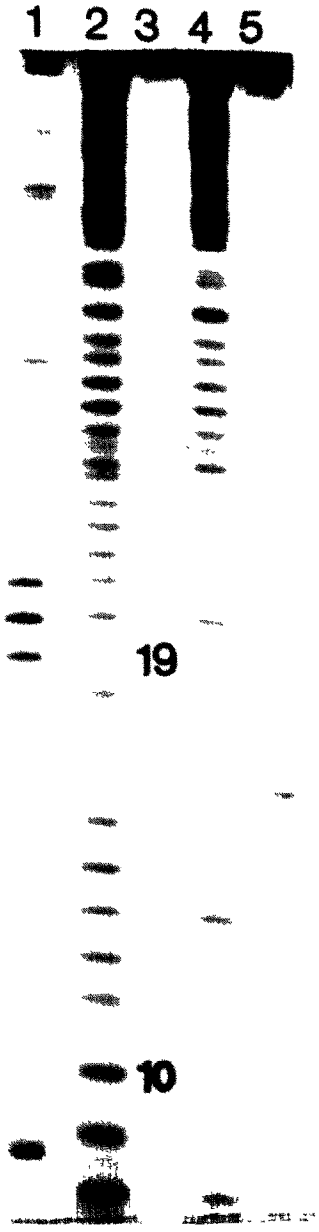

Fig.3. Autoradiogram of a 20% acrylamide gel of phosphate alkylation experiment with 5'-labelled $tRNA^{Phe}$: (1) Partial T_1 ribonuclease digest of $tRNA^{Phe}$; (2) $tRNA^{Phe}$ alkylated with ethylnitrosourea in the unfolded state; (3) $tRNA^{Phe}$ incubated in the absence of the reagent under conditions providing the unfolded tRNA; (4) $tRNA^{Phe}$ alkylated with ethylnitrosourea in the folded state; (5) $tRNA^{Phe}$ incubated in the absence of the reagent under conditions stabilising tRNA structure.

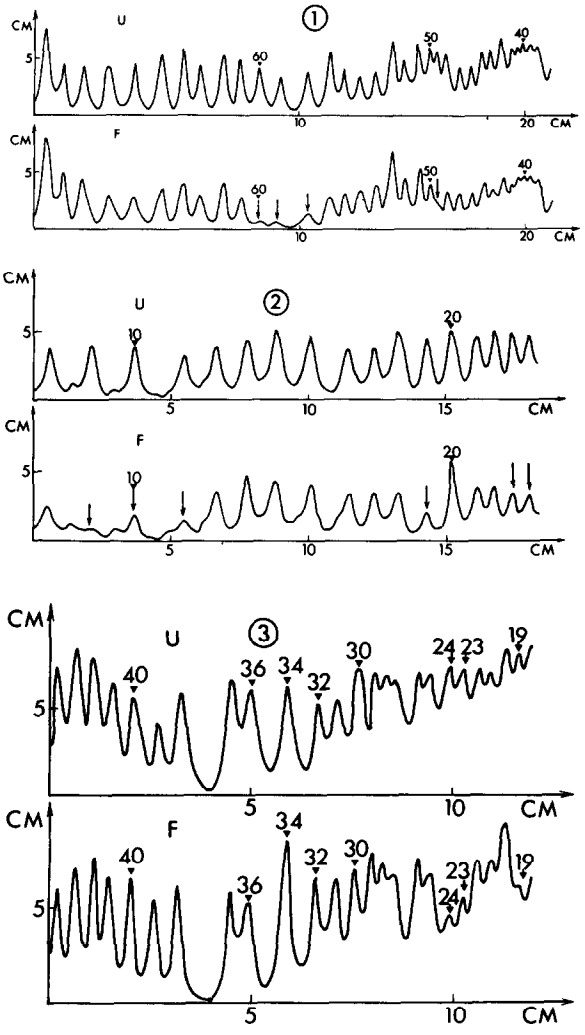

Fig.4. Densitometric tracing of the patterns of phosphate alkylation in unfolded (U) and in folded (F) $tRNA^{Phe}$. Panels 1,2 and 3 represent tracings of the patterns in the experiments shown in fig.1,2 and 3, respectively. Numbers refer to phosphate positions in $tRNA^{Phe}$. Arrows show the positions of low reactive phosphates.

observed slight differences in phosphate reactivities in unfolded $tRNA^{Phe}$ could be attributed to the influence of neighbouring bases. In contrast, when the modification was performed under conditions stabilising the tRNA structure, the intensities of some electrophoretic bands was strongly decreased. This means that in the native $tRNA^{Phe}$ some phosphates were alkylated much less than the others. The less reactive phosphates are those in positions 9,10,11, 19,58,59 and 60. They are essentially located in the

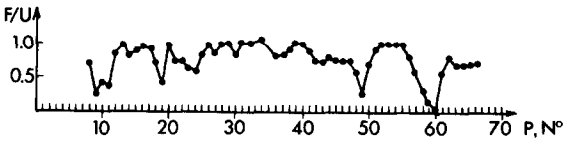

Fig.5. Reactivities of the phosphates in yeast $tRNA^{Phe}$ towards ethylnitrosourea. The F/U values are the ratios between the intensities of the corresponding electrophoretic bands of alkylated folded (F) and unfolded (U) $tRNA^{Phe}$. The intensities were measured as the peak hights of the densitometric tracings in fig.4. An F/U ratio of <1 means that alkylation of the given phosphate is inhibited in the folded $tRNA^{Phe}$. The values are reproducible within 20%.

D- and T-loop regions of the tRNA. Some decrease in reactivity was also observed for phosphates 23 and 24. The phosphates in the helical regions and in the anticodon loop show similar reactivities and in the native $tRNA^{Phe}$ they are practically as reactive as in the unfolded molecule. All these results are summarized in fig.5.

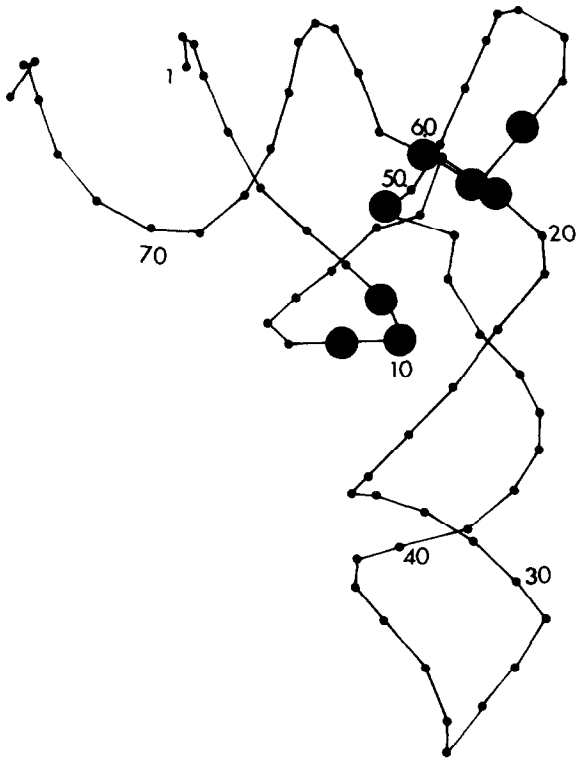

Fig.6. ORTEP drawing of the three-dimensional structure of yeast $tRNA^{Phe}$ (calculated according to coordinates given in [13]) showing positions of low reactive phosphates (enlarged points).

Most of the phosphates exhibiting low reactivity towards ethylnitrosourea in folded $tRNA^{Phe}$ are those known to interact with other functional groups in the crystal structure of $tRNA^{Phe}$. Thus for instance phosphates 9,23,49,58 and 60 participate in hydrogen bond formation with other nucleotides and phosphates 9,10,11 and 19 are known to interact with Mg^{2+} [8–11]. Hence these interactions result in a shielding of these phosphates from solvent which explains their decreased reactivity. The calculation of phosphate availabilities for water in the crystal structure of $tRNA^{Phe}$ [12] showed a general picture similar to that observed in our experiments.

It appears that the protection pattern we observe is the reflection of the inaccessibility of certain phosphates in the crystal structure of $tRNA^{Phe}$. Therefore our results demonstrate the similarity of the solution structure of the $tRNA^{Phe}$ with its crystal structure.

Alkylation of phosphates with ethylnitrosourea provides a useful tool for investigating the structure of tRNA in solution. The reaction is unspecific with regard to the nature of the nucleotides but sensitive to their positions in the tRNA structure. Therefore information about the entire tRNA molecule can be obtained. As the unfolding of the molecule results in an increase of reactivities of certain phosphates, the reaction could be used to follow conformational changes of tRNA. Finally, the use of this reaction could provide an informative method to define the precise localization of contact areas in complexes of tRNA with other macromolecules.

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