CONFORMATIONAL DIFFERENCES BETWEEN THE NATIVE AND DENATURED FORMS OF $tRNA^{Ser}$ AND $tRNA^{Phe}$ FROM YEAST

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1. Introduction

A number of tRNAs can be reversibly denatured, as was first found by Lindhal, Adams, and Fresco for $tRNA^{Leu}_{III}$ from yeast [1] and by Gartland and Sueoka for $tRNA^{Try}$ from *E. coli* [2]. Heating native tRNAs in the presence of EDTA renders them inactive with respect to amino acid acceptance and other activities. The denatured tRNAs can be completely renatured by heating in the presence of Mg^{2+}. A number of physical and biological properties of the native and denatured forms of tRNA have been investigated [3, 4]. The denaturation must be caused by a change in the tRNA conformation, but it was not known which parts of the tRNA structure are involved.

In this communication, we report the results of partial T1 RNase digestion experiments with the native and denatured forms of two tRNAs. Several differences were found, e.g. the stems of the miniloop of $tRNA^{Ser}$ (fig. 1a) and of the dihydrouridine loop of $tRNA^{Phe}$ (fig. 1b) are exposed in the denatured forms while they are protected against the nuclease

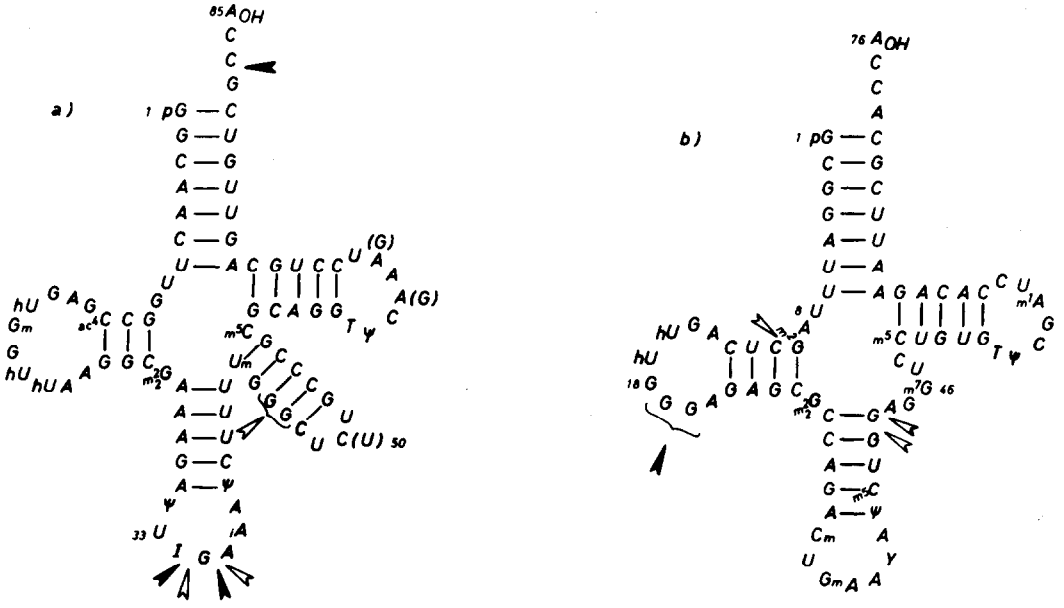

Fig. 1. Cloverleaf models of $tRNA^{Ser}_{I(II)}$ (a) and $tRNA^{Phe}$ (b) from yeast. Full arrows indicate positions of preferential T1 RNase splitting of native tRNAs, open arrows splits in the denatured tRNAs.

attack in the native forms. These parts of the molecules, therefore, must have different conformations in the biologically inactive and active forms of the two tRNAs.

2. Materials and methods

$tRNA^{Ser}_{I+II}$ [5] and $tRNA^{Phe}$ [6] were prepared from brewer's yeast tRNA as in the references. pCpA depleted [7] $tRNA^{Ser}$ and $tRNA^{Phe}$ were assayed as in [8]. 200-Fold purified seryl and phenylalanyl-tRNA synthetases from yeast were kindly provided by R.Hirsch of this laboratory.

Denaturation: the tRNAs were dialysed for 20 hr against 1 M NaCl, 5 mM EDTA, pH 7.0, 10 hr against 5 mM EDTA, pH 7.0, and 20 hr against quartz distilled water. The tRNAs were lyophilized and stored at −20°. Denaturation was effected by heating 10–100 A_{260} units $tRNA^{Ser}$ and $tRNA^{Phe}$ per ml of 1 mM EDTA, 10 mM potassium cacodylate, pH 6.8, for 5 min to 60° and 90°, respectively, cooling to 0° and addition of $MgCl_2$ to 10 mM. All samples of native tRNA were prepared by heating the $MgCl_2$ containing solutions of the denatured tRNAs for 2 min at 60°. The degree of denaturation was determined by performing the standard amino acid acceptance assay [9] at 0°. $tRNA^{Ser}$ was denatured to an extent of 90%, while in these experiments only 50% of $tRNA^{Phe}$ were stabilized in the denatured form (a higher degree of denaturation can be reached under certain conditions). Both tRNAs regained full activity after renaturation.

Partial nuclease digestion: the solutions of native and denatured tRNAs were incubated at 0° with potassium acetate, pH 6.0 (final concn. 0.1 M), and 2–40 units T1 RNase per ml for various lengths of time. The rates of renaturation of the denatured tRNAs are such that only negligible renaturation should have occurred during the nuclease digestions. For analytical purposes the digestions were stopped [10] and submitted to disc electrophoresis [11]. In preparative experiments the digests were extracted with phenol; the tRNA fragments were then separated by electrophoresis in 11–14% acrylamide gels.

3. Results

The fragmentation patterns of the native and denatured forms of $tRNA^{Ser}$ were clearly different (fig. 2). In the native form the first and predominant split was in the anticodon yielding Ser 1–34 and Ser 36–85 (fig. 2a; [12] and F.Fittler, unpublished). At later times the 3′-terminal CpCpA was removed from part of the tRNA and Ser 36–85 molecules (fig. 2b). In the denatured form the additional fragment Ser 1–45/47 was found (fig. 2c,d). It appeared in amounts about equal to those of Ser 36–85 throughout the kinetics. There are good indications that the supplementary fragment Ser 46/48–82/85 migrated together with Ser 1–34 (fig. 2c,d), but this has not yet been investigated in detail.

In the oligonucleotide analysis of fragment Ser 1–45/47 (fig. 3a), all splitting products from the pGp end to position 45 were found in about equimolar amounts. This was confirmed by a second analysis (not shown here) in which, however, the amount of Gp rather corresponded to the chain length 1–47. No trace of the following pentanucleotide 48–52 was detected in either analysis.

The primary T1 RNase split in native $tRNA^{Phe}$ occurred in the dihydrouridine loop leading to fragments Phe 1–18/20 and Phe 19/21–76 (fig. 4a; R.Thiebe, unpublished). These two fragments were also found in the digests of denatured $tRNA^{Phe}$ (fig. 4b), but their formation can largely be ascribed to the about 50% native $tRNA^{Phe}$ in the denatured $tRNA^{Phe}$ samples. The other peaks of the densitogram (fig. 4b) are derived from the denatured fraction of the sample. The following fragments have been fully analysed: Phe 11–76 (fig. 3b), the supplementary fragment Phe 1–10, and the mixture Phe 11–42/43 with Phe 43/44–76 (analyses not shown). The last fragment has nucleotide 44 to about 70% and nucleotide 43 to about 30% as 5′-terminus according to phosphorylation with ^{32}P-phosphate by polynucleotide kinase [13]. The peak following Phe 19/21–76 and the one preceding Phe 1–10 corresponded to fragments which, according to oligonucleotide analyses, contained the complete dihydrouridine loop.

In kinetic experiments, the fragments Phe 11–42/43 and Phe 43/44–76 seemed to appear somewhat later than fragment Phe 11–76. It could

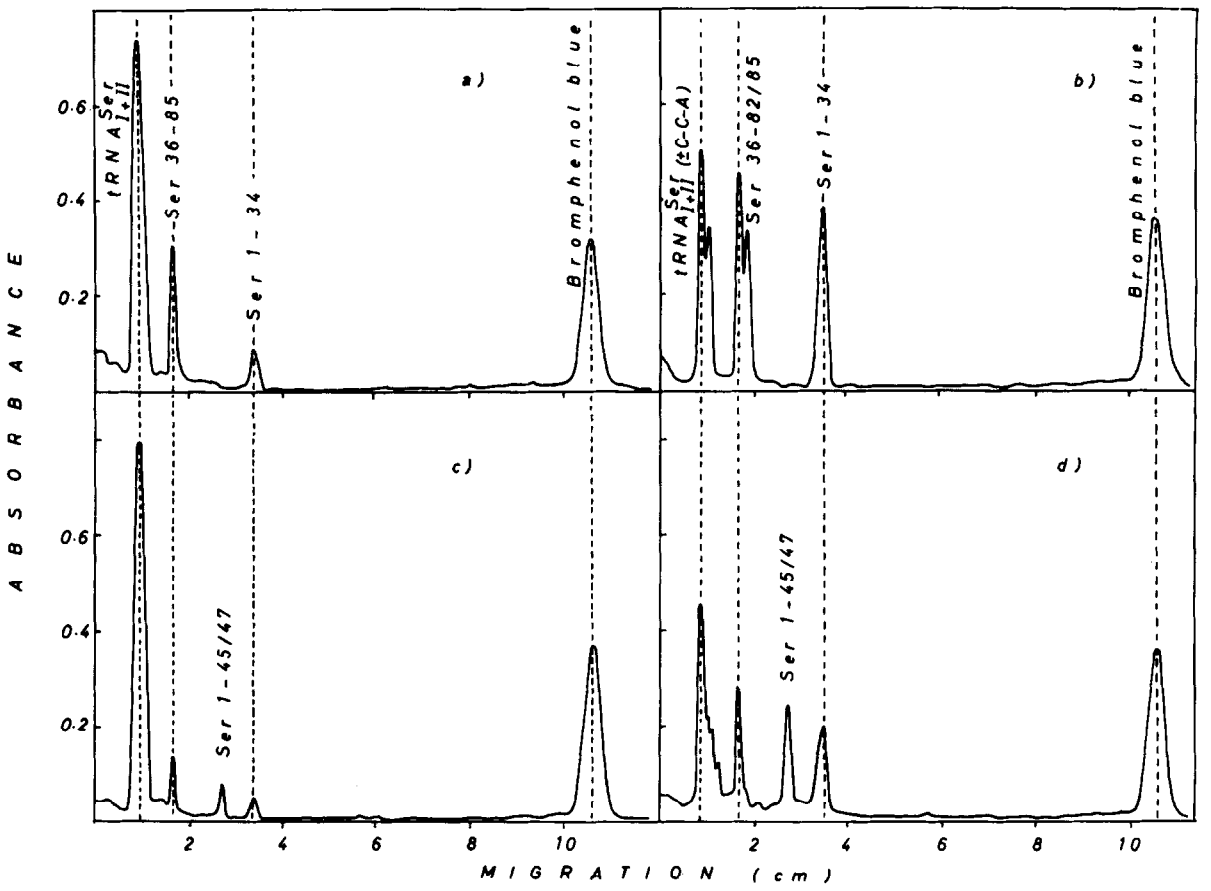

Fig. 2. Densitograms of parallel disc electrophoretic separations of partial T1 RNase digests of native (a, b) and denatured (c, d) $tRNA^{Ser}$. 0.5 A_{260} units $tRNA^{Ser}$ and 0.25 units T1 RNase in 50 µl, 5 min (a, c) 30 min (b, d); 10 µl aliquots were submitted to electrophoresis in 18% acrylamide gels [11].

not be shown, however, that the two fragments were formed only by a secondary split in Phe 11–76. It is therefore possible that the chain scission at position 42/43 is as much a primary split in denatured $tRNA^{Phe}$ as the one at position 10/11.

The small differences in the tRNA regions of the T1 RNase fragmentation patterns (fig. 2b,d) may indicate conformational differences around the 3′-terminal sequence between the native and at least some of the denatured tRNA molecules. Results from partial T2 RNase and snake venom phosphodiesterase digestions of $tRNA^{Ser}$ and $tRNA^{Phe}$ point in the same direction. The 3′-terminal pCpA is not essential, however, for the stabilization of the denatured conformation. pCpA depleted $tRNA^{Ser}$ and $tRNA^{Phe}$ can be denatured as well as the pCpA containing tRNAs. This was shown by ^{14}C-CTP incorporation with CpCpA pyrophosphorylase (as in [8] but at 0°). In addition the characteristic T1 RNase fragmentation patterns of the native and denatured tRNAs were obtained also after removal of pCpA.

4. Discussion

The pronounced differences between the fragmentation patterns of native and denatured tRNAs demonstrate that partial T1 RNase digestion is a valuable probe of the three-dimensional structure of

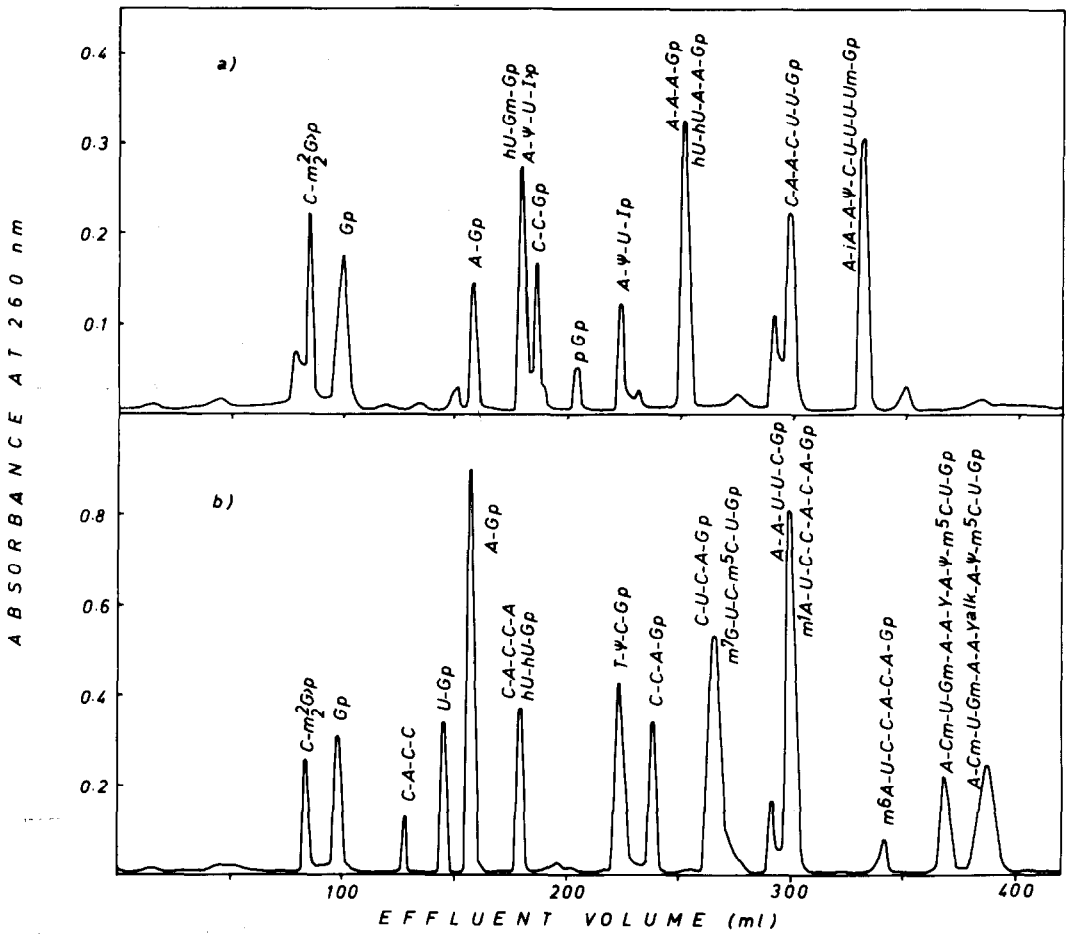

Fig. 3. Elution diagrams of T1 RNase digests of the fragments Ser 1–45/47 (a) and Phe 11–76 (b). Digestion (11 and 20 A_{260} units of the fragments with 200 and 250 units T1 RNase) and DEAE cellulose/urea chromatography were as in fig. 6 of [13]. All oligonucleotides were characterized by UV spectra and paper electrophoresis. $Y^{alk.}$ in (b) refers to an alkali conversion product of Y (R.Thiebe, unpublished).

tRNA. T1 RNase may recognize, through certain subsites, elements of the primary structure in the neighborhood of the guanylic acids [14], but the availability of these elements will depend again on the three-dimensional structure.

Is it obvious from the fragmentation patterns of the denatured tRNAs that they also possess defined three-dimensional structures. It may not be a single structure for one denatured tRNA but a group of structures, in agreement with an earlier observation [15].

It is noteworthy that in a denatured form of $tRNA^{Ser}$ the most stable part of the molecule changes its conformation, i.e. the stem of the miniloop which, at least in the two-dimensional cloverleaf model, is stabilized by 4 G:C pairs. In looking for alternative possibilities to stabilize the GC containing sequence of the miniloop region, the C's and G's of the hU stem may be considered as partners (fig. 1a) and to a lesser degree the 3′-terminal CpCpA. The fact that some denatured $tRNA^{Ser}$ molecules were split in the anticodon and others in the stem of the miniloop

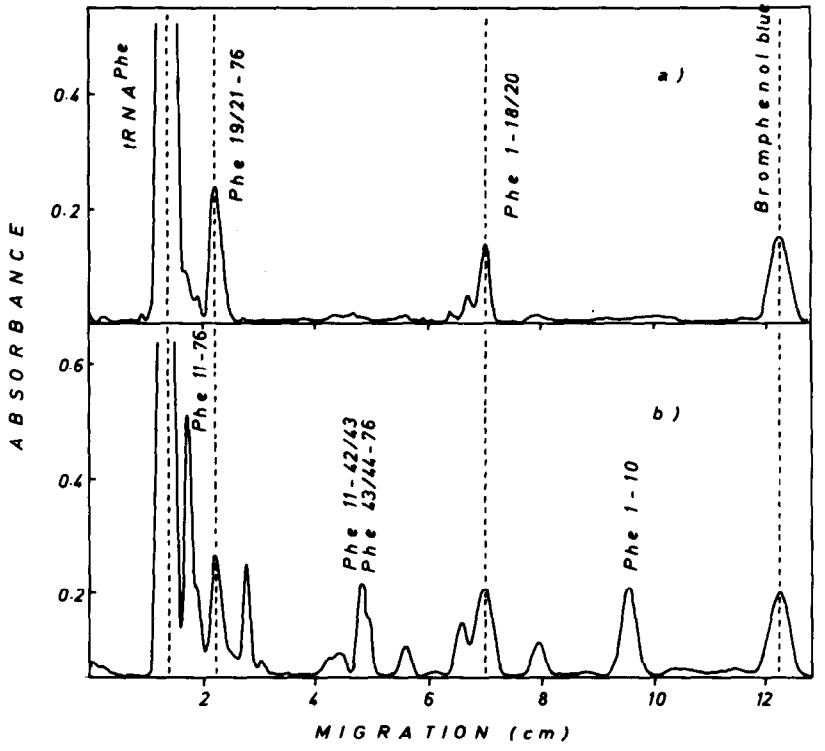

Fig. 4. Densitograms of parallel disc electrophoretic separations of partial T1 RNase digests of native (a) and denatured (b) $tRNA^{Phe}$. 5.4 A_{260} units $tRNA^{Phe}$, 2 units T1 RNase in 65 µl, 5 min; electrophoresis as in fig. 2 with 2.5 µl aliquots.

could be due either to the occurrence of two denatured forms or to the presence of two about equally exposed regions in one denatured form. In $tRNA^{Phe}$ the m^2G (no. 10) and possibly also the G's in positions 42 and 43 become exposed and, hence, also the complementary C rich sequence 25–28 (fig. 1b). The latter may be paired to the G's in position 18–20, which become protected in the denatured form. Alternative pairing schemes also are conceivable.

The experiments indicate that the stem of the mini-loop in $tRNA^{Ser}$ and the hU stem in $tRNA^{Phe}$ have to be in a specific conformation to ensure the correct interaction of the tRNAs with the cognate aminoacyl-tRNA synthetases. Attempts to localize the recognition site with the help of denaturation experiments encounter, however, the same difficulties as many experiments on chemical or enzymatic modification: definite conclusions can only be drawn when the complete three-dimensional structures of the tRNA and its denaturation (or modification) products are known.

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