ASSOCIATION AND DISSOCIATION OF HALF MOLECULES OF PHENYLALANINE SPECIFIC tRNAs FROM YEAST AND WHEAT GERM

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1. Introduction

From a number of tRNAs, half molecules have been prepared which by themselves are inactive but after recombination can be charged with amino acids in the in vitro acceptance assay [1–4]. Recently, active combinations were also obtained when half molecules from tRNAs of two different species, tRNA\textit{Phe}^\textit{yeast} and tRNA\textit{Phe}^\textit{wheat}, were combined [5]. In the preceding paper the mechanism of the helix-coil transitions in half molecules of tRNA\textit{Phe}^\textit{yeast} is described [6]. In the present paper, the results of thermodynamic and kinetic measurements on the half molecules of tRNA\textit{Phe}^\textit{yeast} and tRNA\textit{Phe}^\textit{wheat} and their homologous and heterologous combinations are reported. Data on the stability of double helical structures at various temperatures in the presence and absence of magnesium are given. In contrast to the tRNA\textit{Phe}^\textit{yeast} halves [6] the half molecules from tRNA\textit{Phe}^\textit{wheat} possess base pairs additional to those normally written in cloverleaf models. This complicates the interpretation of the thermodynamic data on the recombination of the half molecules. The kinetic analysis, however, shows that in a heterologous combination the number of base pairs is lower than in the homologous combinations. The observations of the preceding [6] and the present paper are discussed with regard to the acceptor activity for phenylalanine.

2. Materials and methods

tRNA\textit{Phe}^\textit{yeast}, tRNA\textit{Phe}^\textit{wheat} and the acid conversion products tRNA\textit{Phe}^\textit{yeast} (HCl) and tRNA\textit{Phe}^\textit{wheat} (HCl) were the same preparations as previously described [5]. The splitting of tRNA\textit{Phe} (HCl) with aniline into half molecules was carried out under standard conditions [2]. 450 A\textsubscript{260} units of a mixture of half molecules of tRNA\textit{Phe}^\textit{yeast} were separated at room temperature on a DEAE-Sephadex A-25 column (1.0 X 200 cm) with a linear gradient of 500 ml each of 0.2 and 0.5 M sodium chloride in 7 M urea, pH 3. The tRNA\textit{Phe}^\textit{wheat} halves were separated at pH 3.3 under otherwise identical conditions. The CCA-half was almost quantitatively recovered, while a considerable loss was observed with the pG-half. The loss may be related to the very low solubility of this fragment [5]. The thermodynamic and kinetic measurements were carried out as described previously [6,7].

3. Results

The half molecules were isolated from aniline treated tRNA\textit{Phe}^\textit{yeast} (HCl) by column chromatography (fig. 1). Peaks 1 and 2, according to previously described experiments [5], contain pure CCA- and pG-halves, respectively. Peak 3, which is separated
Fig. 1. Chromatographic separation of half molecules of tRNA\textsubscript{\text{Phe}}\text{\textsubscript{yeast}}, see Methods.

Fig. 2. Differential melting curves at 260 nm in 0.01 M sodium cacodylate, 0.1 M NaCl, 0.005 M MgCl\textsubscript{2}, pH 6.8; (a) tRNA\textsubscript{\text{Phe}}\text{\textsubscript{yeast}} intact and half molecules, (b) tRNA\textsubscript{\text{Phe}}\text{\textsubscript{wheat}} intact and half molecules, (c) CCA-half from tRNA\textsubscript{\text{Phe}}\text{\textsubscript{wheat}} and pG-half from tRNA\textsubscript{\text{Phe}}\text{\textsubscript{yeast}} separate and recombinant, (d) CCA-half from tRNA\textsubscript{\text{Phe}}\text{\textsubscript{yeast}} and pG-half from tRNA\textsubscript{\text{Phe}}\text{\textsubscript{wheat}} separate and recombinant, \(\Delta\triangle\triangle\) CCA-half; \(\bigcirc\bigcirc\) pG-half; \(\bullet\bullet\) 1:1 mixture (by \(A_{260}\) units) of CCA- and pG-half; \(\bigcirc\bigcirc\) intact tRNA\textsubscript{\text{Phe}}.
Fig. 3. Differential melting curves at 260 μm in 0.01 M sodium cacodylate, 0.5 M NaCl, pH 6.8; —— CCA-half of tRNA\textsuperscript{Phe\textsubscript{wheat}}, —— pG-half of tRNA\textsuperscript{Phe\textsubscript{yeast}}, ⋅⋅⋅ 1:1 mixture (by A\textsubscript{260} units) of these half molecules.

Fig. 4. Simplified scheme for association and dissociation of the CCA-half of tRNA\textsuperscript{Phe\textsubscript{wheat}} and the pG-half of tRNA\textsuperscript{Phe\textsubscript{yeast}}. See text.
itself. Three additional base pairs have to be present in the CCA-half to account for the difference between the activation enthalpies of the homologous and heterologous recombinations.

An estimate of the number of base pairs between the CCA-half of tRNA\textsubscript{Phe} \texttext{Wheat} and the pG-half of tRNA\textsubscript{Phe} \texttext{Yeast} can be obtained from the activation enthalpy of dissociation $\Delta E_D$, which is not affected by the pre-equilibrium, and therefore represents a measure for comparing homologous and heterologous combinations. This $E_D$ is about 15 kcal/mole lower than the corresponding value for the tRNA\textsubscript{Phe} \texttext{Yeast} halves (82 kcal/mole; table 1 in [6]). Assuming the same nucleation length [11] for the bimolecular step in the homologous and heterologous recombinations one has to conclude that in heterologous recombination about two base pairs less are formed than in the homologous case. A difference of 4 base pairs between homologous and heterologous combinations [5] is not excluded.

The melting curve of the other heterologous combination, pG-half of tRNA\textsubscript{Phe} \texttext{Wheat} versus CCA-half of tRNA\textsubscript{Phe} \texttext{Yeast}, shows a low and broad peak between 15° and 40° (fig. 2d). This is definitely a recombination peak since also a relaxation time in the range of seconds is found. The fact that the peak is small may be explained either by extensive structure of the pG-half itself in addition to the base pairs written in the cloverleaf model or to the possibility that the number of base pairs formed on recombination with the CCA-half is smaller than expected.

In the presence of Mg\textsuperscript{2+} the complexes of homologous halves dissociate only about 10° below the transition midpoints of the corresponding intact tRNAs (79° and 69°, fig. 2a,b). The heterologous combinations, on the other hand, dissociate at about 30° and 40°, respectively. In the absence of Mg\textsuperscript{2+} the pG-half of tRNA\textsubscript{Phe} \texttext{Yeast} and the CCA-half of tRNA\textsubscript{Phe} \texttext{Wheat} do not recombine even at 10° in 0.1 M NaCl but do so in 0.5 M NaCl (fig. 3). This demonstrates again the equivalence of low concentrations of Mg\textsuperscript{2+} with higher NaCl concentrations. Mg\textsuperscript{2+} is not absolutely required for the combination of tRNA\textsubscript{Phe} halves ([6] and fig. 3) as stated for the halves of tRNA\textsubscript{Ala} \texttext{Yeast} II [4].

Under the conditions of the standard amino acid acceptance assay the various combinations of tRNA\textsubscript{Phe} halves can be charged to 40–90% of the acceptance of the intact tRNAs [5]. From an extrapolation of the melting data (fig. 2) to the conditions of the assay system one may conclude that the halves should be recombined in the incubation mixture. This makes unlikely one possible reason for the low acceptance, i.e. incomplete recombination of the halves. Homologous and heterologous combinations of half molecules are interesting also with respect to the three-dimensional structure of tRNA itself. It is not known whether Mg\textsuperscript{2+}, which is probably essential in biologically active tRNA, only stabilizes the tRNA structure as it is present in the absence of Mg\textsuperscript{2+} or leads to a drastically different structure. Therefore up to now considerations of tRNA structure had to be based on nucleotide interactions without specifying the role of Mg\textsuperscript{2+}. A comparison of measured and calculated $\Delta H$-values [6] strongly supports the assumption that in a Mg\textsuperscript{2+}-free solution the tRNA\textsubscript{Phe} \texttext{Yeast} structure is mainly determined by those base pairs which are written in the cloverleaf model. There is an indication for stacking of a few additional bases in the loops [6]. Very little energy, however, is left for the formation of additional base pairs between the remaining single stranded regions. These observations are pertinent to the construction of threedimensional models of tRNA.

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References

