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, $tRNA^{Phe}_{yeast}$ and $tRNA^{Phe}_{wheat}$, were combined [5]. In the preceding paper the mechanism of the helixcoil transitions in half molecules of $tRNA^{Phe}_{yeast}$ is described [6]. In the present paper, the results of thermodynamic and kinetic measurements on the half molecules of $tRNA^{Phe}_{yeast}$ and $tRNA^{Phe}_{wheat}$ 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 $tRNA^{Phe}_{yeast}$ halves [6] the half molecules from $tRNA^{Phe}_{wheat}$ 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

$tRNA^{Phe}_{yeast}$, $tRNA^{Phe}_{wheat}$ and the acid conversion products $tRNA^{Phe}_{yeast}$ (HCl) and $tRNA^{Phe}_{wheat}$ (HCl) were the same preparations as previously described [5]. The splitting of $tRNA^{Phe}$ (HCl) with aniline into half molecules was carried out under standard conditions [2]. 450 A_{260} units of a mixture of half molecules of $tRNA^{Phe}_{yeast}$ were separated at room temperature on a DEAE-Sephadex A-25 column (1.0 × 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 $tRNA^{Phe}_{wheat}$ 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 $tRNA^{Phe}_{yeast}$ (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

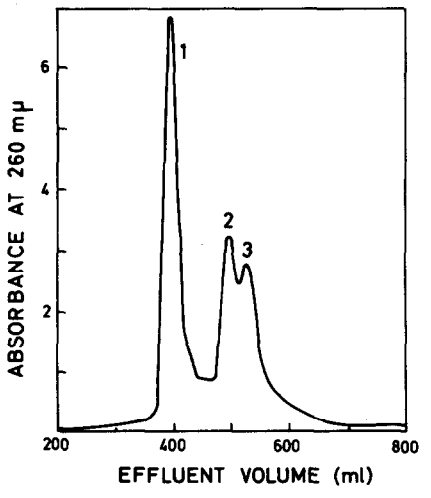

Fig. 1. Chromatographic separation of half molecules of $tRNA^{Phe}_{yeast}$, see Methods.

from peak 2 only on columns of high resolving power, also contains pure pG-half. Peaks 2 and 3 have the same mobilities in disc electrophoresis under previously described conditions [2]. On recombination with peak 1 the recovery of acceptor activity was identical. The appearance of the pG-half in two peaks is probably due to a conversion of its 3′-terminal ribose to several products, which are also observed on aniline treatment of the oligonucleotide GmpApAp-pApψ [2,8].

Fig. 2 shows differential melting curves in the presence of Mg^{++} of separate and combined half molecules of $tRNA^{Phe}_{yeast}$ and $tRNA^{Phe}_{wheat}$ as well as the melting curves of the intact tRNAs. In both, the homologous and the heterologous combinations, the contribution of the halves and of the recombination of the halves can be seen. As the basis for the kinetic studies the melting curve of one heterologous combination was measured in the absence of Mg^{++} (fig. 3). The kinetics in the recombination peak are qualita-

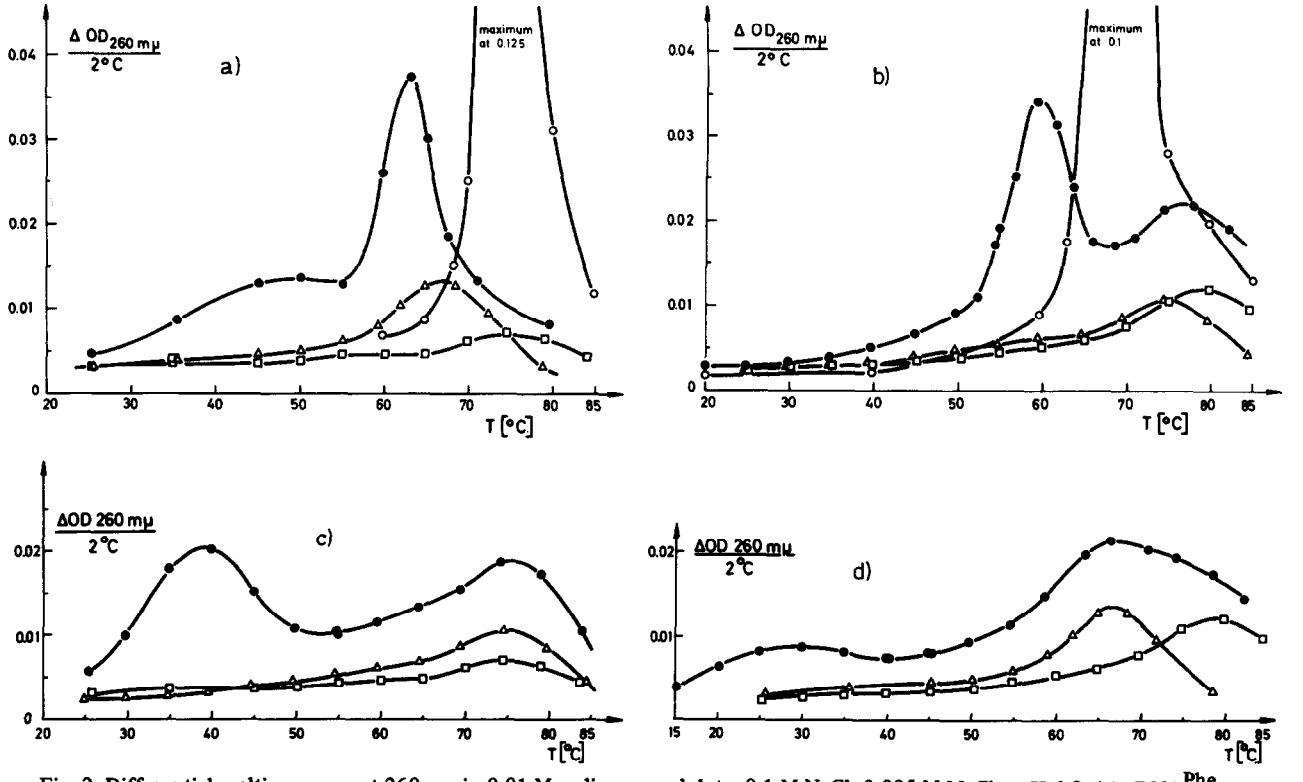

Fig. 2. Differential melting curves at 260 mμ in 0.01 M sodium cacodylate, 0.1 M NaCl, 0.005 M $MgCl_2$, pH 6.8; (a) $tRNA^{Phe}_{yeast}$ intact and half molecules, (b) $tRNA^{Phe}_{wheat}$ intact and half molecules, (c) CCA-half from $tRNA^{Phe}_{wheat}$ and pG-half from $tRNA^{Phe}_{yeast}$ separate and recombined, (d) CCA-half from $tRNA^{Phe}_{yeast}$ and pG-half from $tRNA^{Phe}_{wheat}$ separate and recombined. △–△ CCA-half; □–□ pG-half; ●–● 1:1 mixture (by A_{260} units) of CCA- and pG-half; ○–○ intact $tRNA^{Phe}$.

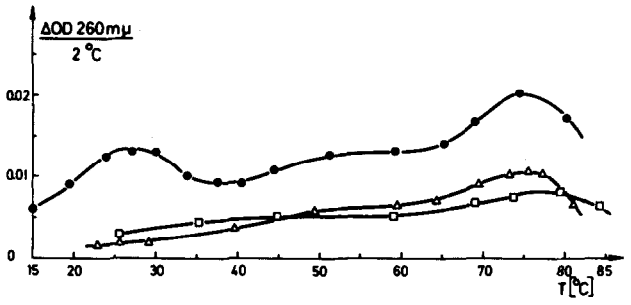

Fig. 3. Differential melting curves at 260 mμ in 0.01 M sodium cacodylate, 0.5 M NaCl, pH 6.8; △–△ CCA-half of $tRNA^{Phe}_{wheat}$, □–□ pG-half of $tRNA^{Phe}_{yeast}$, ●–● 1:1 mixture (by A_{260} units) of these half molecules.

tively the same as in the homologous combination of the $tRNA^{Phe}_{yeast}$ halves [6]. The following values for reaction enthalpy and activation enthalpies were found: $\Delta H = (-60 \pm 5)$kcal/mole, $\Delta E_R = (8 \pm 5)$ kcal/mole, $\Delta E_D = (68 \pm 5)$kcal/mole.

4. Discussion

The melting curves (figs. 2 and 3) can be interpreted on the basis of the known sequences and cloverleaf models of $tRNA^{Phe}_{yeast}$ [9] and $tRNA^{Phe}_{wheat}$ [10] and the cloverleaf models of the heterologous combinations of half molecules [5]. As in the $tRNA^{Phe}_{yeast}$ halves [6] all double helical regions of the models can be identified with certain elements of the melting curves.

The shoulder between 30° and 60° in the melting curve of the $tRNA^{Phe}_{wheat}$ (fig. 3), which is absent in the corresponding curve of the $tRNA^{Phe}_{yeast}$ half (fig. 1 in [6]), appears to be quite interesting. Temperature jump experiments in this temperature range reveal three distinguishable effects, the first one faster than 5 μsec, the second between 30 and 50 μsec and the third between 0.5 and 500 msec depending on temperature. The relaxation time between 30 and 50 μsec indicates the cooperative melting of extra base pairs in addition to the base pairs given by the cloverleaf model. Such pairs are possible between complementary bases in the free ends of this fragment (fig. 4). The relaxation time between 0.5 and 500 msec suggests an equilibrium between two competitive double helical structures which are kinetically coupled through an open chain as an intermediate which is present only in negligible concentrations at these temperatures. Dimer formation between identical halves can be ruled out because the relaxation times are independent of concentration.

The recombination of the CCA-half of $tRNA^{Phe}_{wheat}$ with the pG-half of $tRNA^{Phe}_{yeast}$ proceeds at temperatures where the additional base pairs are still present. Therefore, one has to assume a pre-equilibrium (fig. 4) prior to the recombination. This assumption is supported by a consideration of the activation enthalpies. While ΔE_R is negative for the recombination of the $tRNA^{Phe}_{yeast}$ halves [6], a positive ΔE_R is found in the present heterologous combination. The dissociation of the additional base pairs of the CCA-half of $tRNA^{Phe}_{wheat}$ requires a positive ΔH which overcompensates the negative ΔE of the recombination process

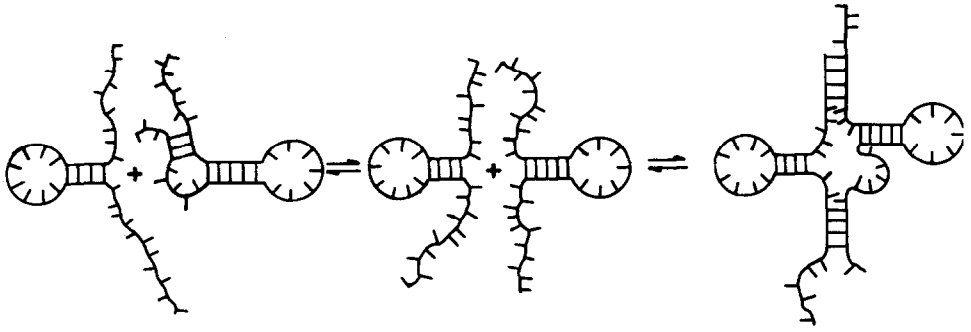

Fig. 4. Simplified scheme for association and dissociation of the CCA-half of $tRNA^{Phe}_{wheat}$ and the pG-half of $tRNA^{Phe}_{yeast}$, see text.

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 $tRNA^{Phe}_{wheat}$ and the pG-half of $tRNA^{Phe}_{yeast}$ can be obtained from the activation enthalpy of dissociation ΔE_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 $tRNA^{Phe}_{yeast}$ 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 $tRNA^{Phe}_{wheat}$ versus CCA-half of $tRNA^{Phe}_{yeast}$, 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^{++} 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^{++} the pG-half of $tRNA^{Phe}_{yeast}$ and the CCA-half of $tRNA^{Phe}_{wheat}$ 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^{++} with higher NaCl concentrations. Mg^{++} is not absolutely required for the combination of $tRNA^{Phe}$ halves ([6] and fig. 3) as stated for the halves of $tRNA^{Ala}_{yeast}$ II [4].

Under the conditions of the standard amino acid acceptance assay the various combinations of $tRNA^{Phe}$ 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 threedimensional structure of tRNA itself. It is not known whether Mg^{++}, which is probably essential in biologically active tRNA, only stabilizes the tRNA structure as it is present in the absence of Mg^{++} 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^{++}. A comparison of measured and calculated ΔH-values [6] strongly supports the assumption that in a Mg^{++}-free solution the $tRNA^{Phe}_{yeast}$ 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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