COOPERATIVE HELIX-COIL TRANSITIONS IN HALF MOLECULES OF PHENYLALANINE SPECIFIC tRNA FROM YEAST

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1. Introduction

The mechanism of helix-coil transitions in synthetic homo-oligonucleotides has been elucidated by recent kinetic studies [1]. It was demonstrated that the rate determining step in the formation of short double helices is a nucleation process of three base pairs. An appropriate kinetic description is given by an "all or none" process in which the only molecules in measurable concentration are fully base paired or single stranded. Related studies have been carried out with oligo d(A-T) [2]. The thermodynamics of the melting of a natural nucleic acid, $\mathrm{tRNA}^{\mathrm{Ala}}_{\mathrm{yeast}}$, have been studied recently [3]. Evidence for double stranded regions in isolated tRNA fragments was reported in [4,5]. In the present paper the thermodynamic and kinetic behavior of half molecules from $\mathrm{tRNA}^{\mathrm{Phe}}_{\mathrm{yeast}}$ in the absence of magnesium is described. It was found that the double stranded structure of the halves is maintained and melts cooperatively. Similarly, the bimolecular recombination of the halves proceeds in a cooperative manner. From the negative apparent activation enthalpy of these reactions it is concluded that the formation of two or three base pairs is rate-limiting. The kinetic and thermodynamic data strongly support the clover-leaf model.

2. Materials and methods

The separation of the $\mathrm{tRNA}^{\mathrm{Phe}}$ halves is described in the accompanying paper [6]. All experiments were made in the absence of magnesium. The sample preparation and the recording of the melting curves were as in [3]. Kinetic experiments were carried out with two temperature-jump techniques which cover the time ranges between 5 μsec and 1 sec [7] and longer than 5 sec [8].

3. Results and discussion

3.1. *Helix-coil transitions of the $\mathrm{tRNA}^{\mathrm{Phe}}$ halves*

Fig. 1 shows the differential UV-temperature profiles of the CCA- and pG-halves of $\mathrm{tRNA}^{\mathrm{Phe}}$. Both curves exhibit a very broad transition at low temperature (10–40°C) and a narrower one at higher temperature. The high temperature transitions are homogeneous as judged by the difference spectrum ratios [3].

The kinetic investigation of the CCA-half reveals a fast relaxation time (< 5 μsec) in the low temperature transition, and an additional single relaxation time, between 20 and 100 μsec depending on tem-

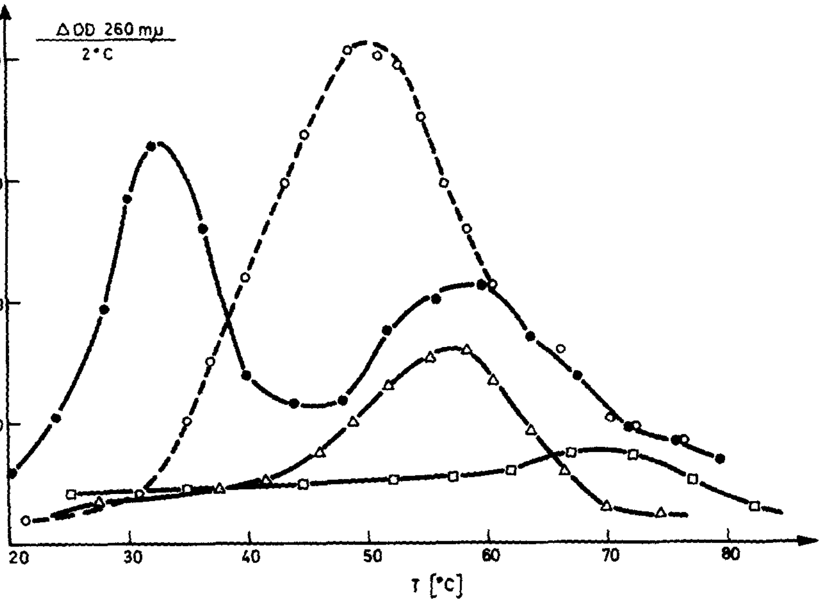

Fig. 1. Differential melting curves at 260 mμ in 0.01 M sodium cacodylate, 0.1 M NaCl, pH 6.8; ○–○–○: untreated $\mathrm{tRNA}^{\mathrm{Phe}}$, OD (20°C, 260 mμ) = 2.08; ●–●–●: 1:1 mixture of the CCA- and pG-halves (by optical density), OD(20°C) = 2.08; △–△–△: CCA-half, OD(20°C) = 1.04; □–□–□: pG-half, OD(20°C) = 1.04.

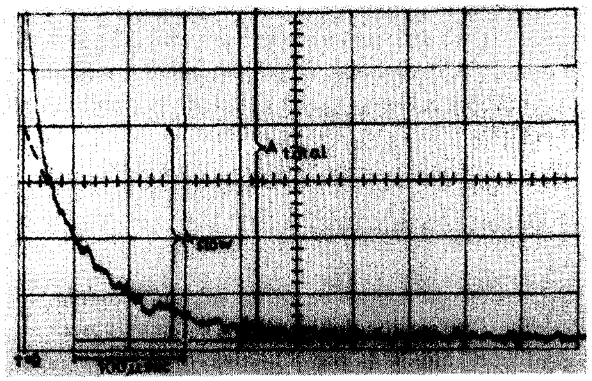

Fig. 2. Typical oscillogram of a temperature-jump relaxation curve for helix-coil transition in the CCA-half; the fast contribution to the total amplitude has been partially compensated.

perature, in the high temperature transition (fig. 2). The fast time is attributed to the melting of single stranded stacked conformations [1,9,10]. The fact that only one slow relaxation time was observed leads to the assumption of a simple two-state model for the slow process, i.e. either fully base-paired or single stranded states. Since the melting kinetics are not affected by 10-fold higher concentrations of the halves, the rate-determining step is a monomolecular process. The kinetics of the pG-fragment are essentially the same.

The kinetic measurements can be used for a refinement of the equilibrium data: The differential melting curves (fig. 1) are multiplied by the temperature-dependent quotients $A_{\mathrm{slow}}/A_{\mathrm{total}}$ (fig. 2), where the amplitude A_{slow} corresponds to double strand melting and A_{total} in addition comprises the single strand unstacking. Integration of the corrected differential melting curve leads to the values of K given in the van 't Hoff plot in fig. 3. The same figure shows an Arrhenius plot of

$$k_{\mathrm{D}} = \frac{1}{\tau(1+K)}$$

and

$$k_{\mathrm{R}} = \frac{K}{\tau(1+K)};$$

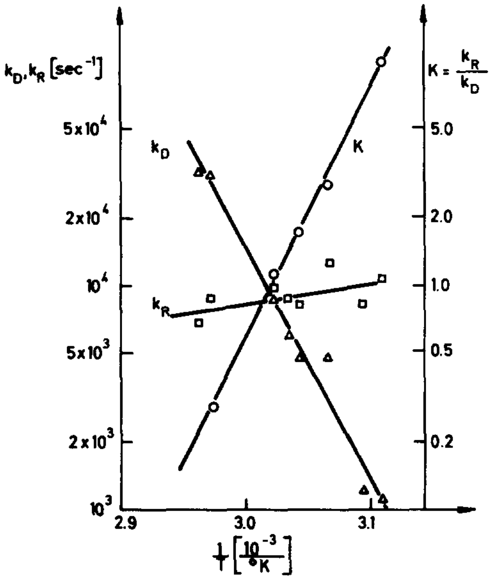

Fig. 3. Temperature dependence of the equilibrium constant and the rate constants for the helix-coil transition in the CCA-half.

($1/\tau = k_R + k_D$; $k_R/k_D = K$; τ, relaxation time; k_R and k_D, rate constants of recombination and dissociation, resp.). The parameters calculated from the plots (fig. 3) are given in table 1. The negative value of the apparent activation enthalpy ΔE_R demonstrates that the measured rate constant k_R does not represent a single elementary process. In the oligo A:oligo U system, a ΔE_R of -9 kcal/mole was found, and the following interpretation was given [1]: A rapid pre-equilibrium between one or two base pairs is followed by the rate-limiting formation of the next base pair, and finally by the fast "zippering up" of the rest of the helix. The values of ΔE_R in table 1 are in agreement with this hypothesis and indicate a nucleus of two or three base pairs. The values of ΔH are discussed below.

3.2. *Recombination of the pG- and CCA-halves*

The melting curve of the mixed halves (fig 1) above 40°C is a superposition of the melting curves of the separated half molecules. An additional peak appears below 40°C. The total hyperchromicity of the mixed halves and of the untreated tRNA^{Phe} is equal within the limits of error. If the additional peak represents the recombination of the two halves, the T_m value belonging to this bimolecular reaction should be concentration-dependent according to

$$\frac{1}{T_m} = \frac{\Delta S}{\Delta H} + \frac{R}{\Delta H} \ln \frac{c_0}{2},$$

where c_0 is the total concentration of one half. Lowering the concentration by a factor of 4 resulted in a decrease of the T_m-value of 3 ± 0.5°C.

A more detailed study of the mechanism of recombination was undertaken by carrying out two to four degree temperature jumps between 24° and 32°C. Approximately 10% of the relaxation effect is fast (< 5 μsec) in the maximum of the peak. 85% can be described by a single relaxation process in the range of seconds, as can be seen in fig. 4. This demonstrates that only one cooperative process accounts for 95% of the slow effect.

Table 1

T_m, ΔH, ΔE_R are the experimental transition-midpoint, reaction enthalpy and activation enthalpy for helix formation, resp. obtained under the conditions of fig. 1. ΔH_{Cl} is calculated from the clover leaf model [11] for the $T\psi C$-stem (CCA-half), the hU-stem (pG-half) and the combined acceptor- and anticodon-stems (recombined halves). These enthalpies were calculated as the sum of nearest neighbor interactions in the respective stems. The nearest neighbor enthalpies for the combinations of G:C with G:C, A:U with A:U and A:U with G:C which were used (cf. the three columns following ΔH_{Cl}), are simple approximations from the literature [3,9,12].

Reactants	T_m	$-\Delta H$	$-\Delta H_{Cl}$	$-\Delta H\begin{bmatrix}GC\\GC\end{bmatrix}$	$-\Delta H\begin{bmatrix}GC\\AU\end{bmatrix}$	$-\Delta H\begin{bmatrix}AU\\AU\end{bmatrix}$	ΔE_R
CCA-half	58 ± 2	55 ± 5	40 ± 10	11	10	8	− 5 ± 5
pG-half	70 ± 2	40 ± 10	35 ± 10	13	11	9	−15 ± 10
Recombined halves	32.5 ± 0.5	95 ± 10	70 ± 20	7	7	6	−13 ± 5

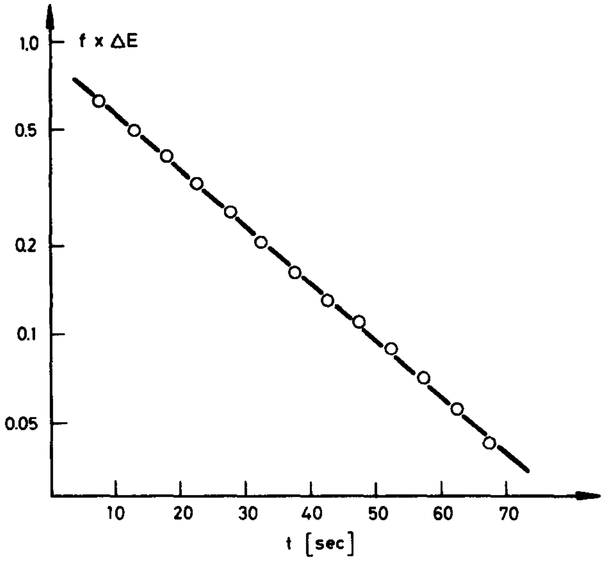

Fig. 4. Semilogarithmic plot of the absorbance change of the mixed halves versus time; final temperature 29.5°C; the total absorbance change was normalized to 1; OD(20°C, 260 mμ) = 0.9.

If the rate-limiting step in the recombination of the two halves is a bimolecular process according to

$$A+B \underset{k_D}{\overset{k_R}{\rightleftharpoons}} AB$$

one obtains

$$\frac{1}{\tau} = k_R(c_A+c_B)+k_D$$

or, eliminating the equilibrium concentration of reactants c_A and c_B :

$$\frac{1}{\tau^2} = 4k_R k_D c_o + k_D^2 \ .$$

Fig. 5.shows a plot of $1/\tau^2$ against c_0 at two different temperatures; the concentration dependence is characteristic for a bimolecular reaction. A combination of the kinetic with the equilibrium data according to the law of mass action

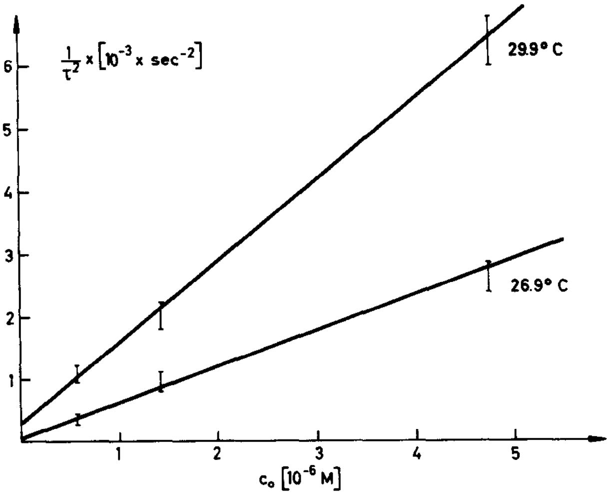

Fig. 5. Concentration dependence of $1/\tau^2$ for the recombination of the mixed halves (25 OD at 260 mμ are taken as 1 mg/ml).

$$\frac{c_{AB}}{c_A c_B} = \frac{c_o - c_A}{c_A^2} = \frac{k_R}{k_D} = K$$

gave the following values:
$k_R = (4 \pm 0.4) \times 10^4$ M^{-1} sec^{-1}, $k_D = (3 \pm 0.3) \times 10^{-3}$ sec^{-1} at 26.9°C; $k_R = (2.8 \pm 0.3) \times 10^4$ M^{-1} sec^{-1}, $k_D = (1.2 \pm 0.1) \times 10^{-2}$ sec^{-1} at 29.9°C. The absolute values of k_R are smaller by a factor of 20 than the corresponding values for the oligo A:oligo U system [1]. This may be due to the presence of GC pairs.

The correction of the equilibrium data for fast effects is performed as described above. A more extended temperature dependence of k_R and k_D was measured at only one concentration. The results are shown in van't Hoff and Arrhenius plots (fig. 6). Activation and reaction enthalpies are summarized in table 1, where theoretical values based on the clover leaf model are also listed. The experimental values of ΔH for all three reactions are larger than the calculated ones by about 20%. The same difference already appeared with tRNA$^{\mathrm{Ala}}_{\mathrm{yeast}}$ [3]. This general deviation indicates a contribution of the loops to the reaction enthalpies, possibly by additional stacking of the bases in the loops.

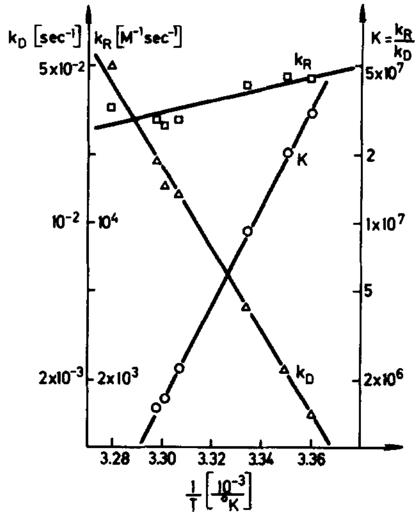

Fig. 6. Temperature dependence of the equilibrium constant and the rate constants for the recombination of the halves.

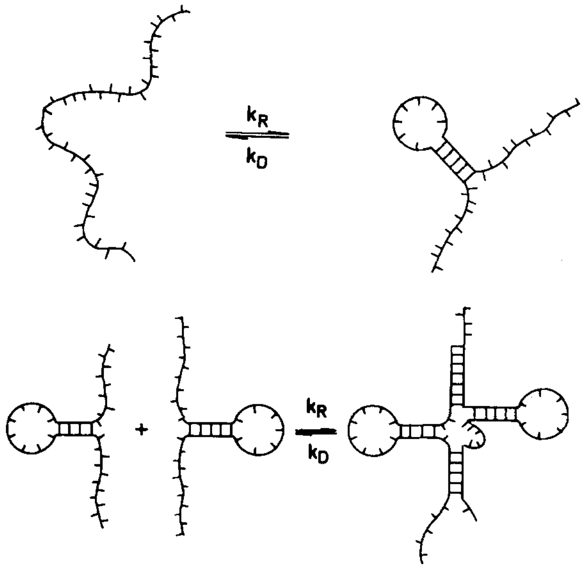

Fig. 7. Simplified reaction schemes for cooperative helix-coil transitions in $\mathrm{tRNA}^{\mathrm{Phe}}_{\mathrm{yeast}}$ halves.

From the thermodynamic and kinetic data it can be concluded that the helix-coil transitions in separate $\mathrm{tRNA}^{\mathrm{Phe}}_{\mathrm{yeast}}$ halves are cooperative processes, in which only fully base-paired and single stranded molecules occur in measurable concentrations. The recombination of the half molecules occurs in a single cooperative process (fig. 7).

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