THERMODYNAMIC PROPERTIES OF THE THREE CONFORMATIONAL TRANSITIONS OF ALANINE SPECIFIC TRANSFER RNA FROM YEAST

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SUMMARY

The melting behavior of alanine specific tRNA was studied by a modified differential absorption technique. It was found that the melting proceeds in three resolvable steps, 1, 2, 3. Steps 1 and 3 were interpreted as single processes, step 2 as two superimposed transitions. The corresponding reaction enthalpies were determined to be $\Delta H(1)=40\pm10$, $\Delta H(2)=2\cdot(65\pm10)$ and $\Delta H(3)=70\pm10$ kcal/mole, and are discussed on the basis of the "clover-leaf" model. Only the ionic strength dependence of steps 2 and 3 agrees with other double-stranded nucleic acids. Step 1 produces a smaller change in sedimentation coefficient than step 2.

The mechanism of double strand formation of oligonucleotides has been investigated recently by kinetic methods (11). In order to extend this work to the kinetics of conformational transitions of specific transfer RNA molecules it is useful to know their thermodynamic parameters. Therefore, the melting behavior of alanine specific transfer RNA from yeast ($tRNA_{ala}$) has been studied in detail by a modified differential absorption technique. The data are interpreted in terms of four two-state transitions characterized by transition midpoints (T_m) and reaction enthalpies (ΔH).

Materials

The purification of $tRNA_{ala}$ from crude baker's yeast tRNA (Boehringer) was carried out by countercurrent distribution (2). The purity was judged to be 90% on the basis of amino acid accep-

tor activity (3). Before use, the samples were dialyzed twice against 5 mM EDTA and twice against triple-distilled water, dried in vacuo and dissolved in buffer solution. The Magnesium ion concentration of this solution was less than one molecule Mg^{2+} per tRNA molecule, as checked by atomic absorption.

Methods

The melting behavior of $tRNA_{ala}$ was studied by ultraviolet absorption spectra in a manner similar to that described by Felsenfeld (4) and Fresco (5). However, instead of measuring the absolute absorption spectrum at different temperatures, optical density differences, $\Delta OD(\lambda)$, between two identical solutions at two different temperatures were determined as a function of wavelength. The temperature difference, ΔT, was adjusted to a value between one and two degrees centigrade, depending upon the slope of the differentiated melting curve. The two solutions were observed at the same temperature every five degrees, in order to correct for background absorbancy changes. The $\frac{\Delta OD(260\ m\mu)}{\Delta T}$ values and the difference spectrum ratios $\frac{\Delta OD(260\ m\mu)}{\Delta OD(240\ m\mu)}$ and $\frac{\Delta OD(260\ m\mu)}{\Delta OD(280\ m\mu)}$ were plotted against temperature (Figs. 1, 2). These ratios are of diagnostic aid in defining single melting processes, as demonstrated in Figure 2, where two overlapping melting processes are resolved by observing the change in the ratios as a function of temperature.

Sedimentation coefficients were determined in a Spinco model E analytical ultracentrifuge equipped with a high temperature unit modified for scanning.

Results and Discussion

The melting behavior of $tRNA_{ala}$ in a buffer containing o.o1 M Sodium cacodylate and o.1 M NaCl at pH 6.8 is shown in Figure 1.

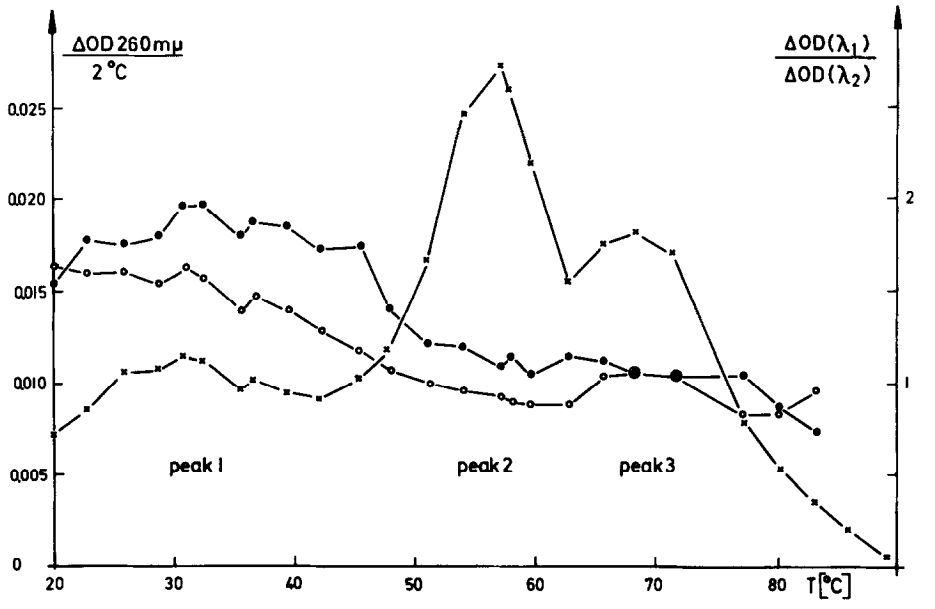

Figure 1: Differentiated melting curve of $tRNA_{ala}$ in o.o1 M Sodium-cacodylate, o.1 M NaCl, pH 6.8; ΔOD(26o mμ)/2°C: x-x-x, ΔOD(26o mμ)/ΔOD(28o mμ): O-O-O, ΔOD(26o mμ)/ΔOD(24o mμ): .-.-.; the optical density at 2o°C was 1.776 OD.

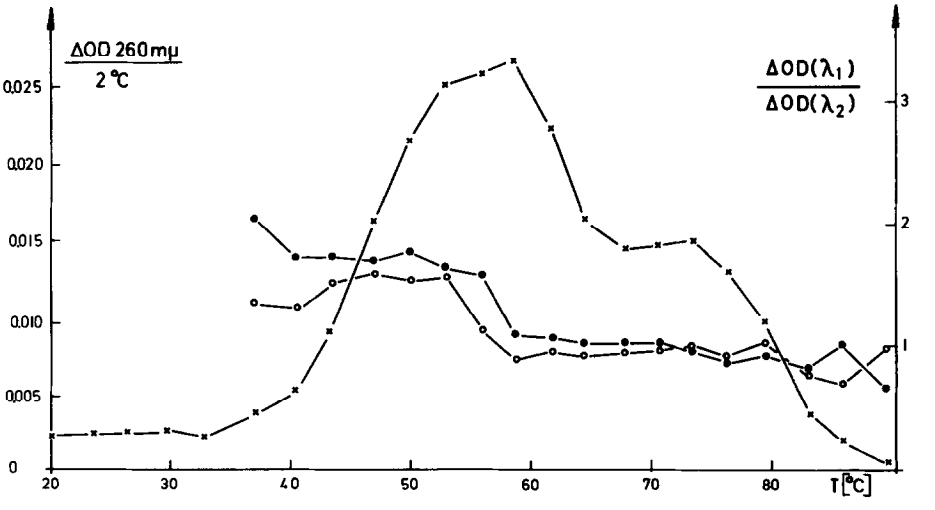

Figure 2: Differentiated melting curve of $tRNA_{ala}$ in o.o1 M Sodium-cacodylate, o.1 M NaCl, o.3 mM $MgCl_2$, pH 6.8; ΔOD(26o mμ)/2°C: x-x-x, ΔOD(26o mμ)/ΔOD(28o mμ): O-O-O, ΔOD(26o mμ)/ΔOD(24o mμ): .-.-.; the optical density at 2o°C was 1.51o OD.

This curve shows three steps, instead of two as observed by Fresco (5) under similar conditions. Our results cannot be interpreted quantitatively in terms of A-U and G-C content of the melting nucleotide structures according to Fresco, as will be shown in a more detailed paper (3). The difference spectrum ratios are constant over the width of each peak, but change between peaks 1 and 2. This indicates that the nature of the melting process remains the same within each peak, which in turn supports a two state model $A \rightleftharpoons B$ for each transition. This finding is in agreement with a theoretical treatment of the clover leaf model given by Kallenbach (6).

The reaction enthalpy, ΔH, for each transition can be calculated for such a two state model from the following expression:

$$(d\theta/dT)_{T_m} = (\partial\theta/\partial \ln K)_{T_m} \cdot (\partial \ln K/\partial T)_{T_m} = 1/4 \Delta H/RT^2$$

where $\theta = c_B/(c_A+c_B)$ and c_A, c_B = concentration of species A or B. The values for $d\theta/dT$ are derived from the melting curves in Figure 1, and the calculated values for ΔH are given in Table 1. The contributions from single strand melting and any coupling between the three different processes are neglected (a justification for this approximation will be given later (3)). From the same figure it is observed that the difference spectrum ratios are the same in peaks 2 and 3, indicating that analogous processes take place during these transitions. Although the ΔH values of the two peaks are the same, the total absorbancy change of peak 2 is about twice that of peak 3. Therefore, peak 2 is interpreted to represent two very similar, uncoupled transitions. One thus obtains a reaction enthalpy of $\Delta H \approx 240$ kcal/mole for the entire melting process. The interpretation of the three melting steps as four all or none transitions is the simplest possible

Table 1

Reaction enthalpies for the three melting steps of $tRNA_{ala}$ derived from Figure 1

peak	1	2	3
$\Delta H \frac{kcal}{mole}$	40±10	2·(65±10)	70±10
T_m °C	30	55	70

Table 2

Sedimentation coefficient $S^o_{20,w}$ of $tRNA_{ala}$ in 0.01 M Sodium cacodylate, 0.1 M NaCl at pH 6.8

T °C	20	35	60
$S^o_{20,w}$	4.0±0.05	4.0±0.1	3.3±0.1

one. However, it cannot be excluded that more than four transitions are hidden under the three peaks, if one assumes a correspondingly narrower temperature range for each transition. This leads to a drastic increase in total ΔH and thus to disagreement with the clover leaf model (cf. later discussion). Large deviations from the all or none model would produce a similar disagreement.

In order to correlate these results with the clover leaf model, one has to estimate the number of base pairs melting in peaks 1, 2 and 3. Below 70°C the melting of double helical regions into completely unstacked single strands proceeds through stacked,

single stranded intermediates. Therefore, one has to consider a certain contribution to ΔH from single strand stacking. One obtains a ΔH of about 13 kcal/mole·basepair for the dissociation of a G-C double strand and a complete unstacking of the bases (7). Using the single strand melting data of Brahms et al. (8), one estimates values for ΔH of 13±2, 1o±2 and 7±2 kcal/mole at the T_m values of peaks 3, 2 and 1, respectively. Dividing the ΔH values from Table 1 by the corresponding ΔH values per mole base pair, and assuming that the total ΔH is produced by double strand melting, one obtains 5±2, 2·(6±2) and 5±2 base pairs melting in peaks 3, 2 and 1, respectively. These figures, although slightly too large, agree within experimental error with the clover leaf model for $tRNA_{ala}$.

The ionic strength dependence of the three T_m values is described in Figure 3. The dependence of T_{m2} and T_{m3} on the salt concentration agrees well with similar data on a large variety of

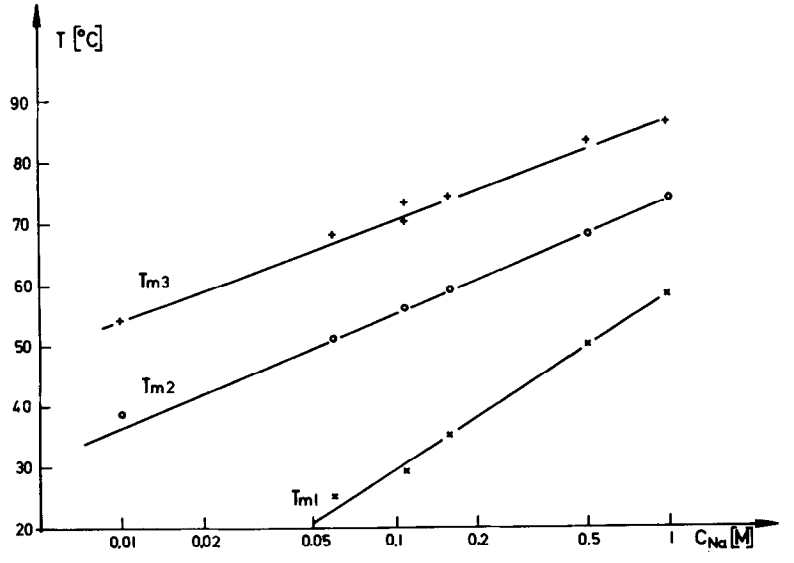

Figure 3: Ionic strength dependence of the T_m-values of $tRNA_{ala}$ in o.o1 M Na^+-cacodylate, pH 6.8.

double stranded poly- and oligonucleotides (9,10,11). The value of T_{m1}, however, depends much more strongly on the Sodium ion concentration, indicating a higher ratio of electrostatic repulsion to stabilizing forces. This effect may be due to the presence of:

a) single nucleotides looped out of the double helix as a consequence of mismatching of noncomplementary base pairs (acceptor branch), and/or
b) triple helices and/or
c) tertiary structure.

Unfolding of tertiary structure in the temperature range of the first transition has been suggested by Fresco et al. (12), who found large changes in the viscosity and sedimentation coefficients of unfractionated tRNA at these temperatures. The sedimentation behavior of $tRNA_{ala}$ in a buffer consisting of o.o1 M Sodium cacodylate and o.1 M NaCl at pH 6.8 was investigated. The results are given in Table 2.

The data demonstrate that in the range of transition 1 the sedimentation coefficient does not change appreciably, whereas a major change in sedimentation is observed during transition 2. If one assumes that unfolding of tertiary structure lowers the sedimentation coefficient (12), one has to conclude that no such unfolding occurs during the first transition and that the overall structure of the molecule undergoes major changes only at higher temperatures. Further studies on other specific tRNA's are under way in order to clarify these points.

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