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A COMPARATIVE CALORIMETRIC STUDY ON tRNA UNFOLDING

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The heat effects involved in thermal unfolding of five tRNAs with different primary structures have been determined by direct differential scanning microcalorimetry. The overall molar values of the transition enthalpy (ΔH_t) are 1150 kJ/mol for $tRNA_2^{Lys}$ (yeast), 1250 kJ/mol for $tRNA^{Phe}$ (yeast), 1350 kJ/mol for $tRNA_1^{Val}$ (yeast), 1490 kJ/mol for $tRNA_1^{Val}$ (*E. coli*) and 1630 kJ/mol for $tRNA^{Tyr}$ (*E. coli*). The tRNAs differ in their melting behaviour as can be shown by a comparison of the calorimetric curves. The calorimetrically measured ΔH_t values are about 350 kJ/mol higher than the transition enthalpy values for the cloverleaf arrangement, which were estimated using the known parameters for G·C and A·U base pairs.

1. Introduction

The results from X-ray investigations on crystallised $tRNA^{Phe}$ [1,2] and $tRNA^{fMet}$ [3,4] give detailed information on the three-dimensional structure of a tRNA in the solid state. But for the understanding of the function of biomolecules their behaviour in aqueous solution is of particular interest. Therefore, detailed information on the thermodynamic parameters of stabilisation of the structure is required as a basis for the discussion of relations between structure and function.

Adiabatic scanning microcalorimetry is a useful method to get data for the enthalpy change ΔH, which is needed to unfold the compact native conformation of the tRNA to random coil. To distinguish between the enthalpy of stabilisation for the tertiary and secondary structure of tRNA, it is necessary to derive an approximate value of the stabilisation enthalpy for the secondary structure from the known numbers of the different base-pairs related to the cloverleaf model. The difference between the calorimetric ΔH-values and the values estimated for the secondary structure may then be assigned to an additional contribution due to the unfolding of the tertiary structure. From X-ray investigations it can be seen that besides base–base interactions also sugar–base and phosphate–base intramolecular hydrogen bonding interactions are involved to stabilise the tertiary structure in the solid state. Whether these interactions are relevant in solution is not clear. Furthermore, the spatial structure given by X-ray investigations does not provide the information which is necessary to calculate an enthalpy change involved in a helix–random coil transition of tRNA. Therefore these thermodynamic data must be determined by means of calorimetric measurements.

Surprisingly the published calorimetric studies show similar values of the transition enthalpy (ΔH_t) for tRNAs having a different primary structure [5–7]. Therefore, it was suggested that the ΔH_t of tRNA is controlled by a universal tertiary structure [6]. This assumption is in accordance with the results of NMR and Raman studies [8,9]. Nevertheless, there were some remaining uncertainties [6], which gave reason for a re-examination of the system. Hence, an extensive calorimetric study employing 5 tRNAs with different primary structure was performed and a method based on phosphorus determination was used for the evaluation of the tRNA concentrations.

2. Materials and methods

tRNA (yeast) and $tRNA^{Val}$ (*E. coli*) were purchased from Boehringer Mannheim. $tRNA^{Phe}$ was purified by column chromatography on benzoylated DEAE-cellulose using the procedure described by Schneider et al. [10]. $tRNA_1^{Val}$ (yeast) was purified as described by Kryukov et al. [11] by RPC [12] as last step. $tRNA_2^{Lys}$ (yeast) was purified by a modification method [13] and RPC as last step (for the detailed procedure, see ref. [14]). $tRNA^{Tyr}$ (*E. coli*) was kindly provided by Dr. G. Kraus, Hannover. The purity of the tRNAs was checked by reversed phase chromatography and the activity of the various species was assayed by aminoacylation. CCA-repairing was performed as described by Sternbach et al. [15]. All tRNAs tested had activities higher than 1450 pmol of amino acid per unit of optical density at 260 nm. Chemicals used were of reagent grade quality, the water was quartz bidistilled.

The concentrations of tRNA solutions were determined by an optical phosphorus analysis [16], which was performed with aliquots of the solution after all organic material had been destroyed by evaporation with $HClO_4$.

For measurements on magnesium-free solutions magnesium was removed according to Levy et al. [17]. In the studies without Mg^{2+} the solvent contained 0.5 mmol EDTA/l. The solutions used for the calorimetric measurements were prepared by dissolving the dry tRNA samples in the appropriate buffer solutions and dialyzing them at 4°C against 100-fold larger volume with three changes of the buffer solution.

Calorimetric experiments were carried out in a scanning microcalorimeter DASM-1 M [18] at a heating of 1 K min^{-1}. The transition enthalpies were calculated from the area between the calorimetric curves and the baseline as can be seen elsewhere [9].

3. Results

As can be shown by a comparison of the calorimetric curves (fig. 1), $tRNA^{Phe}$ (yeast), $tRNA_1^{Val}$ (yeast), $tRNA_1^{Lys}$ (yeast, $tRNA_2^{Lys}$ (yeast), $tRNA_1^{Val}$ (*E. coli*) and $tRNA^{Tyr}$ (*E. coli*) differ in melting behaviour. The transition occurs at a higher temperature for $tRNA^{Tyr}$ (rich in G · C base pairs) compared with the other tRNAs.

There are also differences in the ΔH_t-values which range from 1150 ± 100 kJ/mol for $tRNA_2^{Lys}$ up to 1630 ± 100 kJ/mol for $tRNA^{Tyr}$ (cf. table 1).

In principle the measured ΔH values as obtained at different 'melting' temperatures should be corrected for a common temperature using precise C_p values which may be derived from carefully recorded calorimetric transition curves, so that loss of meaning for molecular biology might be avoided. Actually, however, the very small difference in partial molar heat capacities of the solute associated with the denaturation process of the dissolved tRNA does not admit an application of the well-known basic thermodynamic equations as proposed by Benzinger in a previous paper [20].

The information on the influence of changes of the buffer is not yet very clear.

At present, significant differences of ΔH values in the different buffers are lost in the scatter of values. These differences should be discussed in relationship to the differences in the primary and secondary structure of the tRNAs as given by the cloverleaf model. To see whether there is a relationship between the number of hydrogen bonds in the secondary structure and the transition enthalpies the ΔH_t-values are plotted against the number of hydrogen bonds in the secondary structure of the tRNAs, taking three hydrogen bonds for a GC base pair and two hydrogen bonds for an A · U or G · U base pair, respectively (see fig. 2).

The enthalpy of denaturation is increased with increasing number of hydrogen bonds in the secondary structure. A value of 25 kJ/mol of hydrogen bonds can be estimated (cf. section 4).

If the average value of the interaction energy per base pair is known, it is possible to evaluate the approximate enthalpy of transition for the secondary structure given by the cloverleaf model. Unfortunately we do not know the values of all interaction energies between the different bases in tRNA. As a result of measurements on poly A · U Neumann and Ackermann [21] and Filimonov and Privalov [7] have reported an interaction enthalpy of about 40 kJ/mol of base pair for A · U base

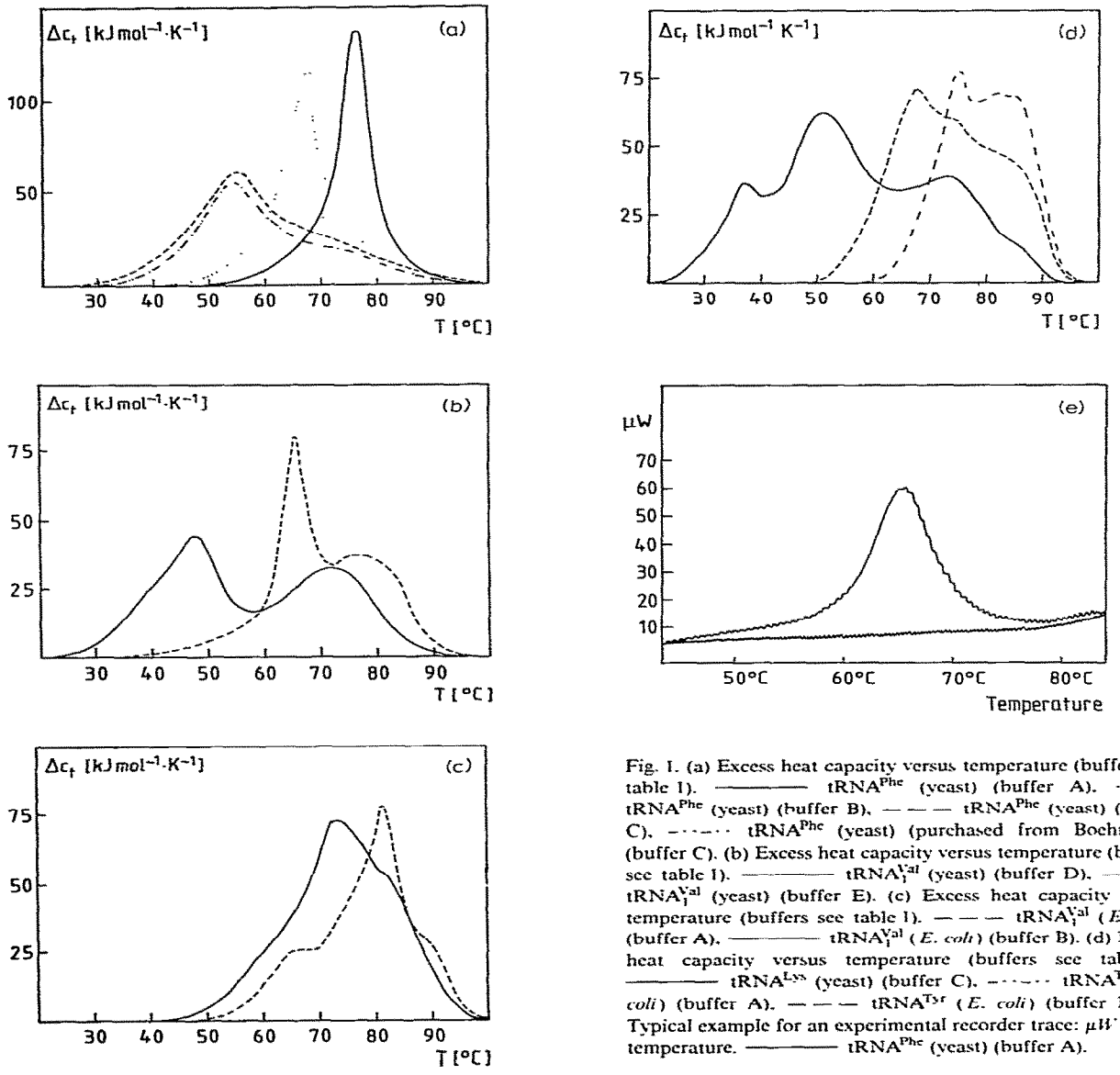

Fig. 1. (a) Excess heat capacity versus temperature (buffers see table 1). ———— $tRNA^{Phe}$ (yeast) (buffer A). ····· $tRNA^{Phe}$ (yeast) (buffer B). — — — $tRNA^{Phe}$ (yeast) (buffer C). -·-·-· $tRNA^{Phe}$ (yeast) (purchased from Boehringer) (buffer C). (b) Excess heat capacity versus temperature (buffers see table 1). ———— $tRNA_1^{Val}$ (yeast) (buffer D). — — — $tRNA_1^{Val}$ (yeast) (buffer E). (c) Excess heat capacity versus temperature (buffers see table 1). — — — $tRNA_1^{Val}$ (*E. coli*) (buffer A). ———— $tRNA_1^{Val}$ (*E. coli*) (buffer B). (d) Excess heat capacity versus temperature (buffers see table 1). ———— $tRNA^{Lys}$ (yeast) (buffer C). -·-·-· $tRNA^{Tyr}$ (*E. coli*) (buffer A). — — — $tRNA^{Tyr}$ (*E. coli*) (buffer B). (e) Typical example for an experimental recorder trace: μW versus temperature. ———— $tRNA^{Phe}$ (yeast) (buffer A).

pairing. For G · C base pairing, Gralla and Crothers [22] have measured a value of 54 kJ/mol of base pair by indirect methods, whereas Privalov et al. [18] assumed a value of 60 kJ/mol of base pair. We used an averaged value of 57 kJ/mol of G · C base pair for our calculations. A · Ψ and G · U base pairs are not very often found, therefore their contribution to ΔH_t is not so important and the estimated values of 40 and 30 kJ/mol of base pair can be used without correction. The

Table 1

Transition enthalpy (ΔH_t) values involved in the helix-random coil transition of tRNA [a]

Sample	ΔH_t (kJ/mol)	$C_{t\,max}$ (°C)	Buffer [a]	Figure
$tRNA^{Phe}$ (yeast)	1190±100	76	A	1a
$tRNA^{Phe}$ (yeast)	1200±100	66.5	B	1a
$tRNA^{Phe}$ (yeast)	1290±100	53.5	C	1a
$tRNA^{Phe}$ (yeast) [c]	1350±100	55	C	1a
$tRNA_1^{Val}$ (yeast)	1300±100	48/72	D	1b
$tRNA_1^{Val}$ (yeast)	1340±100	65	E	1b
$tRNA_1^{Val}$ (*E. coli*)	1510±100	82	A	1c
$tRNA_1^{Val}$ (*E. coli*)	1480±100	72	B	1c
$tRNA_2^{Lys}$ (yeast)	1150±100	51	C	1d
$tRNA^{Tyr}$ (*E. coli*)	1620±100	77/87	A	1d
$tRNA^{Tyr}$ (*E. coli*)	1640±100	68	B	1d

[a] For the calorimetric experiments 1 ml solution of 20–40 nmol tRNA/ml buffer was used. The exact concentrations of tRNA were calculated on the basis of phosphorus determination.

[b] Buffers:
A: 10 mmol Cacodylate/l (pH 7.0; 2 mmol $MgCl_2$/l).
B: 10 mmol Cacodylate/l (pH 7.0; 150 mmol NaCl/l; 2 mmol $MgCl_2$/l).
C: 5 mmol Citrate/l (pH 7.0; 150 mmol NaCl/l; 1 mmol EDTA/l).
D: 5 mmol Citrate/l (pH 7.0; 150 mmol NaCl/l; 1 mmol $MgCl_2$/l).
E: 5 mmol Citrate/l (pH 7.0; 150 mmol NaCl/l; 10 mmol $MgCl_2$/l).

[c] Purchased from Boehringer Mannheim.

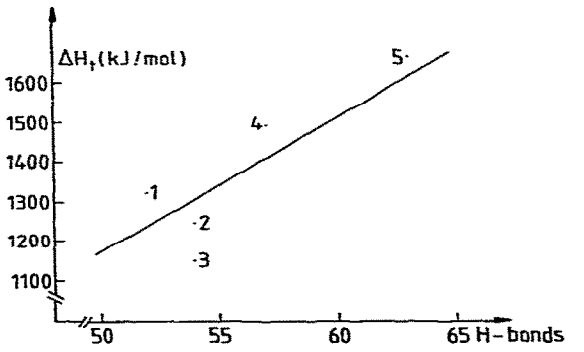

Fig. 2. ΔH_t versus number of hydrogen bonds. 1. $tRNA_1^{Val}$ (yeast), 2. $tRNA^{Phe}$ (yeast), 3. $tRNA_2^{Lys}$ (yeast), 4. $tRNA_1^{Val}$ (*E. coli*), 5. $tRNA^{Tyr}$ (*E. coli*).

Table 2

The averaged values of calorimetric enthalpy changes and the calculated values for the interaction in the secondary structure and their differences

	$\Delta H_{calor.}$ (kJ/mol)	$\Delta H_{calc.}$ (kJ/mol)	$\Delta H_{calor.} - \Delta H_{calc.}$ (kJ/mol)
$tRNA^{Phe}$ (yeast)	1250	1034	216
$tRNA_1^{Val}$ (yeast)	1350	984	366
$tRNA_1^{Val}$ (*E. coli*)	1490	1085	405
$tRNA_2^{Lys}$ (yeast)	1150	1024	126
$tRNA^{Tyr}$ (*E. coli*)	1630	1169	461

averaged calorimetric enthalpy changes and the calculated values for the interaction in the secondary structure and their differences are given in table 2. It can be seen that the calculated values are by 20–30% smaller than the calorimetrically measured enthalpies of transition.

4. Discussion

A critical review on molar UV absorbance of tRNA [23] shows that the reported values of A_{260}/base differ remarkably. For instance, values of 1.72 nmol tRNA/A_{260} unit [24] and 2.2 nmol tRNA/A_{260} unit [25] are reported. Furthermore, the extinction coefficients depend strongly on temperature and salt conditions.

The differences between the reported values of extinction coefficients show that the determination of tRNA concentrations by UV measurements cannot be used to calculate molar transition enthalpies of different tRNAs, because the expected differences in the molar transition enthalpies are of the same order of magnitude as the uncertainties of the measured concentrations. The concentrations in our investigation are evaluated by a phosphorus determination. The uncertainty by this method is less than ±2%. Probably the uncertainty of the optical method used for the determination of tRNA concentrations is responsible for the difference between the values of the transition enthalpy of $tRNA_1^{Val}$ (yeast) (1549 kJ/mol) [18] and 1350 kJ/mol (this work)).

The calorimetric curves (excess heat capacity versus temperature, fig. 1) show a very different

unfolding behaviour for the tRNAs investigated in this study. This is somewhat surprising because X-ray investigations and NMR studies on 14 different tRNAs [26] lead to the conclusion that all tRNAs have a similar three-dimensional structure. But this must not be a contradiction, because the unfolding is directed by intramolecular interactions and not simply by the geometry of the three-dimensional arrangement.

The unfolding of tRNA is a result of several sequential transitions and not a single two-state transition as can be seen from fig. 1. In principle, the observed calorimetric curve can be interpreted as a superposition of single elementary components corresponding to two-state transitions with the assumption that each transition is independent [18]. This approximation is somewhat arbitrary, because it is not quite clear how many different transitions occur. Furthermore, the heat capacity versus temperature curve as obtained by the calorimetric experiment is only exact within experimental error. The more 'gaussian' curves [18] are used for the approximation the better the fit. But the number of these curves must not correspond to real transitions of the system. Therefore further results from other investigations such as NMR and differential unfolding UV experiments are needed to give a definite interpretation of the calorimetric 'melting' curves, so that a correlation between these curves and the regions of transition of the tRNA molecule can be obtained. It can be seen from fig. 2 that there is a definite relationship between the number of H-bonds in the secondary structure given by the cloverleaf model and the ΔH_t values.

The experimental value of about 25 kJ/mol of hydrogen bonds is much higher than the estimated values which can be derived from thermodynamic parameters evaluated from calorimetric studies on solutions of model compounds. Although the calorimetric data shown in fig. 2 must still be regarded as preliminary results of limited precision, there is no doubt that a large part of the total transition enthalpy is associated with the denaturation of the tertiary structure.

If the number of H-bonds is derived from the cloverleaf model, the discussion of error limits is reduced to what can be said about validity of the secondary structure model. It should be mentioned, however, that the cloverleaf model has been checked for different specific tRNAs by IR spectroscopic measurements [27]. The results of spectroscopic studies of this kind show clearly that an approximate uncertainty of ± 1 base pair (i.e. ± 3 hydrogen bonds) must be taken into account.

All isolated tRNAs can be arranged in the cloverleaf structure, if the primary structure is known. Therefore one can take the cloverleaf arrangement as a model for the interactions in the secondary structure. The estimated ΔH_t values for the cloverleaf arrangement of the different tRNAs ranging from 948 kJ/mol to 1169 kJ/mol (see table 2) are much smaller than the calorimetrically measured transition enthalpies, concerning the unfolding of the compact native conformation to random coil. The difference of about 400 kJ/mol between the approximate value for the secondary structure and the calorimetrically measured value must be assigned to the additional interactions in the tertiary structure. This difference may be even higher, because the enthalpy values used for calculation by increments may be too large. Furthermore, the fact that each first base pair of a continuous stack does not contribute to the stacking enthalpy [28] is not taken into account. In addition, the base pairs beside a loop have a smaller contribution.

The enthalpy difference of 400 kJ/mol is equal to the total stabilisation enthalpy of 7 G · C or 10 A · U base pairs. But this hypothetical number of additional base pairs cannot be realized within the pattern of the primary structure of the tRNAs.

In the following section other examples of possible interactions will be discussed:

NMR studies of tRNA in solution show two to four base pairs in addition to those required by the cloverleaf model [8]. By X-ray investigation there are found many factors which may contribute to the stability of the tertiary structure of tRNA. Besides the additional base pairs, hydrogen bonds between base and backbone and between backbone and backbone are detected [1].

Some of these additional interactions as found for the solid state may be relevant for the structure in aqueous solution. NMR studies give evidence for interaction of the 2′ OH hydrogen to the 3′

phosphoester oxygen via one molecule of H_2O [29]. Further stabilisation of the tRNA can result from stacking of short tRNA regions which are not involved in base pairing.

Approximately one third of the transition enthalpy of the investigated tRNAs in aqueous solution must be contributed by these interactions. This information on the stabilisation of the a tertiary structure is also important for the discussion of larger RNA molecules. Therefore the information obtained by the calorimetric measurements gives a basis for investigations on the structures of 5 S-RNA and viroids, for which a great deal of research by use of physico-chemical methods is still needed.

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