dria it is closely associated with malonyl-CoA decarboxylase and thus may share its submitochondrial localization. The effectiveness of externally added CoA-SH on malonate activation by intact mitochondria suggests that no significant barriers are involved.

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A Calorimetric Study of the Thermal Transitions of Three Specific Transfer Ribonucleic Acids†

John F. Brandts,* William M. Jackson, and Thomas Yao-Chung Ting

ABSTRACT: Using differential heat capacity calorimetry, the thermal transitions of $tRNA^{Val}_{E.\ coli}$, $tRNA^{fMet}_{E.\ coli}$, and $tRNA^{Phe}_{yeast}$ have been examined, the latter one in greater detail due to the availability of larger amounts of pure material. The two species from *Escherichia coli* each show two well-separable thermal transitions in the calorimeter, a thermodynamically small transition at low temperature and a much larger one at elevated temperature. Neither of these transitions appear to be associated with changes in molecular weight. Only a single transition is seen for $tRNA^{Phe}_{yeast}$ and this is apparently the analog of the high-temperature transition seen for the other two species. Study of the $tRNA^{Phe}_{yeast}$ transition as a function of Mg^{2+} concentration reveals that there are very large increases in both the calorimetric enthalpy and heat capacity change as the transition temperature is raised by increasing the concentration of the divalent cation. The van't Hoff enthalpy changes have also been estimated and it is found that the ratio $\Delta H_{cal}/\Delta H_{VH}$ has a value of the order of 1.5. It is thereby concluded that the $tRNA^{Phe}_{yeast}$ thermal transition is not cooperative enough to be accurately approximated by an all-or-none (*i.e.*, two-state) model, but that it exhibits substantially more cooperativity than would be expected if the four cloverleaf helices melted independently. The results appear to be very consistent with the recently determined crystal structure of Kim *et al.* (*Science 179,* 285 (1973)), which reveals but two continuous helices with an indication of some interaction between them.

The thermal transitions of various tRNAs have been frequently investigated by spectrophotometric methods. The melting curves have generally (*e.g.*, Riesner *et. al.*, 1969) appeared to be biphasic or multiphasic in nature with the implication that different structural regions of the tRNA molecules melt out independently or semi-independently. In a particularly detailed investigation, Cole *et al.* (1972) studied the transitions of the Phe-, Val-, Tyr-, and fMet-tRNA from *Escherichia coli* and concluded that each of these show several different unfold-

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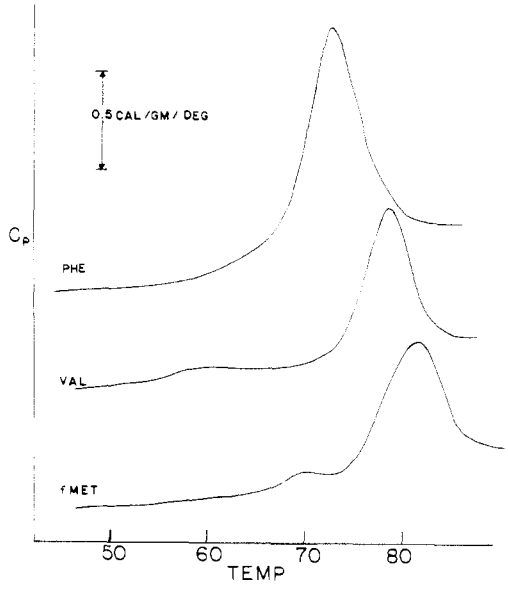

FIGURE 1: Calorimetric scanning curves showing the denaturation of $tRNA^{Phe}_{yeast}$, $tRNA^{Val}_{E.\ coli}$, and $tRNA^{fMet}_{E.\ coli}$ (respectively, from top to bottom). All solutions are 0.01 M Tris buffer (pH 7.0) with 2.5 $\times 10^{-4}$ M $MgCl_2$ added. The heat capacity values are plotted on a relative scale. Systematic thermal noise which was present in the original data has been averaged out in this reproduction of the data, as discussed in the Experimental Section. The concentrations of tRNA was about 0.12% in each experiment.

ing and refolding transitions which may be induced by temperature or salt variations. They postulated the existence of three folded states (the cloverleaf or a close variant, the "native" form with additional tertiary structure, and an extended form which has substantially different base pairing from the other two forms) in addition to the unfolded or coil form. It was postulated that these four structural forms exist in equilibrium with different species predominating in different regions of the salt–temperature plane.

In contrast to the above picture, Levy *et al.* (1972) and Levy and Biltonen (1972) have studied the thermal transition of $tRNA^{Phe}$ from yeast and have been led to a different conclusion from most other investigators. Using spectrophotometry and mixing calorimetry, they have shown that their data are consistent with the idea that only a single folded state and a single unfolded state exist in equilibrium throughout the range of conditions which they used. Their data were analyzed in terms of a simple two-state model (Lumry *et al.*, 1966).

One possible explanation for this basic disagreement is that different tRNAs unfold by different mechanisms. Thus, $tRNA^{Phe}_{yeast}$ is not one of the tRNAs which was studied by Cole *et al.* (1972) and in other cases where the melting of $tRNA^{Phe}_{yeast}$ was studied the melting profile appeared to be less complicated than for other tRNAs which have been examined. It is well known that calorimetric data are capable of distinguishing a two-state transition from one that is more complicated since, in the latter case, the enthalpy change obtained directly from calorimetry (ΔH_{cal}) is expected to be larger than the apparent enthalpy change obtained by analysis of the shape of the transition curve (the so-called van't Hoff heat change, ΔH_{VH}). For a two-state transition, of course, the two will be equal (Lumry *et al.*, 1966).

This paper presents calorimetric results obtained on the transition of three purified tRNAs; Val and fMet from *E. coli* and Phe from yeast. These results show that significant differences do exist in the mechanism of unfolding for different tRNAs. They also permit certain qualitative conclusions about the degree of cooperativity involved in the transition of one of these, $tRNA^{Phe}_{yeast}$.

Experimental Section

Materials. The $tRNA^{Val}$ and $tRNA^{fMet}$ (*E. coli* K-12, M07) samples were provided by Oak Ridge Laboratory, and the purities which were determined prior to shipping were 82 and 97%, respectively, by amino acid accepting capacity (Kelmers *et al.*, 1965). The samples were stored at −20° for 2 years prior to use in the calorimeter. The $tRNA^{Phe}$ (Brewer's yeast) was purchased from Boehringer-Mannheim and had a biological activity of 1154 pmol of phenylalanine accepted/A_{260} unit of total accepting capacity for all 21 amino acids. This latter tRNA was generally used within about 1 week of receipt.

The usual buffer solution (0.01 M Tris (pH 7.0)) was prepared from Ultrapure Biological Grade Tris (Schwarz Bioresearch Inc.) and double distilled water. All other reagents were of the highest grade available.

Preparation of Solutions. The tRNA solutions were prepared at a concentration of about 0.12% for calorimetric study. The solutions were dialyzed against the appropriate buffer solution for at least 12 hr with three to five changes of buffer. Concentrations were determined gravimetrically by drying to constant weight at 110°. Extinction coefficients ($E_{258}^{1\%}$) calculated from dry-weight concentrations were 23.0 (Val), 23.4 (fMet), and 20.8 (Phe). The high values for the former two were presumably due to significant amounts of denaturation which occurred during the long storage time (see text).

Calorimetry. Calorimetric measurements were made with a differential heat capacity calorimeter (Jackson and Brandts, 1970; Jackson, 1970) which utilized twin 5-ml platinum cells and a heating rate of 18°/hr. Because of the low concentrations of tRNA (*ca.* 0.1%), absolute values of the specific heat capacities of tRNA showed some scatter (±25%) so that all data are plotted on a relative scale. Enthalpies and heat capacities were obtained from base line corrected calorimetric data in the manner previously described (Jackson and Brandts, 1970). In transposing the raw data for publication, the systematic short-term noise has been averaged out by eye. This thermal noise corresponds to fluctuations about the average C_P value of *ca.* 0.05 cal g^{-1} deg^{-1} in Figure 3, for example, and had an average period of *ca.* 10 sec.

Results

Results in 0.00025 M Mg^{2+}. Shown in Figure 1 are the calorimetric scans for $tRNA^{fMet}_{E.\ coli}$, $tRNA^{Val}_{E.\ coli}$, and $tRNA^{Phe}_{yeast}$ in 0.01 M Tris buffer (pH 7.0) and a $MgCl_2$ concentration of 2.5 $\times 10^{-4}$ M. Only small amounts of the first two species were available to us so that but a single calorimetric experiment was possible. The data themselves were of good quality and the reversibility on heating through the transitions a second time was fair (50–60%). However, these samples were supplied to us by Oak Ridge National Laboratories over 2 years prior to their use. Although they initially assayed at high purity (amino acid acceptor activity of 97% for fMet and 82% for Val), Cole *et al.* (1972) have reported that the activity of these Oak Ridge samples are significantly lower than the initial values after long storage times. Their particular samples assayed at only 55% (Val) and 75% (fMet) immediately prior to use. Although we did not check the activity of our samples before use, we assume that a similar deterioration occurred. This is in fact obvious from the calorimetric data since the apparent ΔH values for the transitions of the two *E. coli* tRNAs are considerably smaller than for $tRNA^{Phe}_{yeast}$. The latter sample was not stored before use and also exhibited higher reversibility, *ca.*

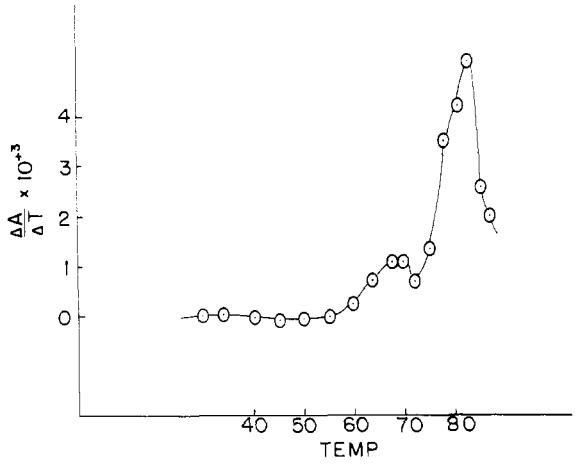

FIGURE 2: The temperature derivative of absorbance (335 nm) for $tRNA^{fMet}_{E.\ coli}$ under solution conditions identical with those of Figure 1. The total absorbance of the solution at 335 nm (1-cm cell) was 0.126 at low temperature.

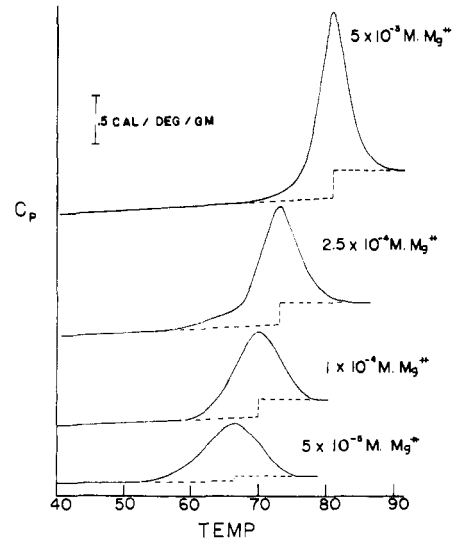

FIGURE 3: Calorimetric scanning curves showing the denaturation of $tRNA^{Phe}_{yeast}$ at four different concentrations of $MgCl_2$, as indicated. The dashed lines indicate the extrapolated base lines which were used in estimating ΔH_{cal}, ΔC_P, and ΔH_{VH}. The buffer solution was identical with that in Figure 1.

90%. Therefore, we cannot make valid estimates of ΔH for Oak Ridge samples due to the fact that we do not know the concentration of the tRNA which actually participates in the thermal transitions since, presumably, the inactive material is already "denatured" at low temperature. It appears that the active material which remains in these samples does behave normally (Cole *et al.*, 1972) so that the *shapes* of the transition curves should give some useful information.

There is one very apparent difference in the three transition curves shown in Figure 1. In addition to the major heat capacity transition which occurs above 70°, $tRNA^{Val}_{E.\ coli}$ and $tRNA^{fMet}_{E.\ coli}$ each have a second broader transition which occurs at lower temperature and appears to be independent of the major transition. For $tRNA^{fMet}_{E.\ coli}$, this small transition is centered near 70°, while for $tRNA^{Val}_{E.\ coli}$ it is centered closer to 60° and is considerably broader. The small transition was also largely reversible on the second heating. The low-temperature transition was not seen for $tRNA^{Phe}_{yeast}$ on either the first or second heating.

Under these identical conditions, there is also a significant difference in the temperature at which the midpoint of the major transition occurs. These melting temperatures (T_m) are 73° for $tRNA^{Phe}_{yeast}$, 78.5° for $tRNA^{Val}_{E.\ coli}$ and 82° for $tRNA^{fMet}_{E.\ coli}$. It seems likely that these differences are a reflection of a differing proportion of G·C base pairs in the secondary structure of these tRNAs. The cloverleaf models show that 12 of 21 base pairs (57%) are G·C for $tRNA^{Phe}_{yeast}$, 15 of 21 (71%) for $tRNA^{Val}_{E.\ coli}$, and 17 of 20 (85%) for $tRNA^{fMet}_{E.\ coli}$.

It is not known why the smaller transition is not seen for $tRNA^{Phe}_{yeast}$, although this observation is in agreement with spectrophotometric data (Romer *et al.*, 1970; Wintermeyer *et al.*, 1969) which show only a single transition for the above tRNA, whereas many other tRNAs exhibit multiple spectrophotometric transitions (Romer *et al.*, 1970; Cole *et al.*, 1972). It is possible that this small transition is superimposed on the large one for $tRNA^{Phe}_{yeast}$ and obscured because of this. This possibility will be explored later.

It is known that tRNA exhibits a tendency to dimerize and that this is favored by high Mg^{2+} concentrations. The results of Millar and Steiner (1966) on unfractionated *E. coli* tRNA (25°) suggest that at our concentrations of *ca.* 0.1%, dimerization does not become significant until Mg^{2+} concentrations near 10^{-2} M are reached, or about 50 times higher than the concentrations used here. Also, we have examined the $tRNA^{fMet}_{E.\ coli}$ transition spectrophotometrically at 335 nm where the single thiouridine base absorbs. The concentration used for the spectrophotometric study was about 20 times lower than for the calorimetric curve shown in Figure 1. In order to compare these results directly with the heat capacity data, we have plotted the temperature derivative of A_{335} in Figure 2. These data also show two transitions—a small one at low temperature and a larger transition at high temperature. The apparent midpoints of these spectrophotometric transitions occur within 1° of the temperatures where the calorimetric transitions are seen. This demonstrates that neither of the two transitions for $tRNA^{fMet}_{E.\ coli}$ show a significant dependence on concentration and rules out the possibility that dimerization or other association processes are significantly influencing the calorimetric data. It seems very likely that this is also true for the calorimetric data on the other two tRNAs shown in Figure 1.

Dependence of the $tRNA^{Phe}_{yeast}$ Transition on Mg^{2+}. The thermal transition of $tRNA^{Phe}_{yeast}$ has been examined as a function of Mg^{2+} concentration in 0.01 M Tris buffer (pH 7.0). The calorimetric scans at four concentrations of Mg^{2+} from 5×10^{-5} M to 5×10^{-3} M are shown in Figure 3. The reversibility on the second heating varied from 65% at the highest Mg^{2+} concentration to better than 95% at the lowest Mg^{2+} concentration. The values of ΔH_{cal} and ΔC_P were estimated at T_m using the extrapolated base lines shown in Figure 3 and the method previously described (Jackson and Brandts, 1970). These values are tabulated in Table I along with the value of T_m. The experimental uncertainties are estimated to be about 10% in ΔH and about 1500 cal mol^{-1} deg^{-1} in ΔC_P. These errors are mostly due to uncertainties in extrapolating the base lines into the transition region.

Increasing the concentration of Mg^{2+} can be seen to have several effects on the thermal transition. As the salt concentration is increased, the transitions show the expected shift in T_m to higher temperatures. In addition, there are very significant changes in thermodynamic properties which accompany this increase in stability. Raising the Mg^{2+} concentration causes (a) an increase in the total area (*i.e.*, ΔH_{cal}) of the transition, (b) an increase in the ΔC_P of denaturation, and (c) an increase

TABLE I: Thermodynamic Parameters Estimated for the Thermal Denaturation Reaction of Phenylalanine tRNA from Yeast, under Various Conditions.[a]

$[Mg^{2+}]$ (M)	T_m (°C)	ΔH_{cal}	ΔC_P	ΔH_{VH}	$\Delta H_{cal}/\Delta H_{VH}$
5×10^{-5}	66.5	140	1500	98	1.43
1×10^{-4}	70	156	4500	112	1.39
2.5×10^{-4}	73	216	5800	128	1.69
5×10^{-3}	80.5	248	7000	170	1.46
2×10^{-4}	68	175	1200	127	1.38

[a] Enthalpy units are kcal/mol of tRNA, and heat capacity units are cal deg^{-1} mol of $tRNA^{-1}$. All samples except the last one listed are in 0.01 M Tris (pH 7.0). The last one is in 0.005 M phosphate (pH 7.2) with 0.1 M NaCl. The calorimetric estimates of ΔH and ΔC_P have not been corrected for the fact that the tRNA sample assayed as only 97.5% pure, rather than 100%. Were this correction made, it would increase the estimates of ΔH_{cal} and ΔC_P by 2.5% while leaving ΔH_{VH} unchanged. The ratio $\Delta H_{cal}/\Delta H_{VH}$ would thereby be increased by *ca.* 0.04.

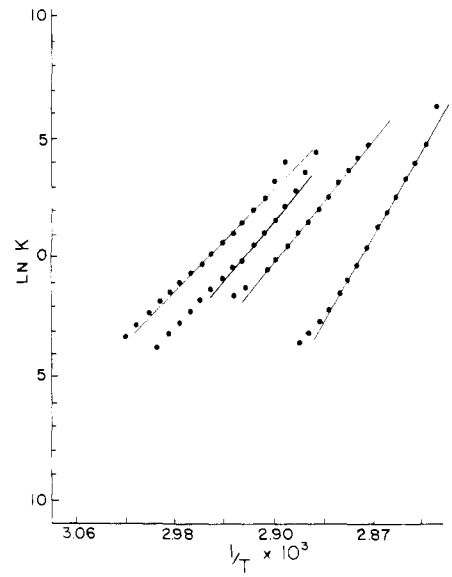

FIGURE 4: The logarithm of the apparent two-state equilibrium constant *vs.* reciprocal temperature, calculated from the four calorimetric scans on $tRNA^{Phe}_{yeast}$ shown in Figure 3. An estimate of ΔH_{VH} was obtained in each case by taking the slope to these curves at the thermal midpoint (*i.e.*, where ln *k* is zero).

in the sharpness as judged by the shorter temperature span over which the transition occurs. Though the transition temperature varies only over a temperature range of 14° (*i.e.*, from 66.5 to 80.5°), the value of ΔH_{cal} increases by more than 75% from 140 kcal/mol (5×10^{-5} M Mg^{2+}) to 248 kcal/mol (5×10^{-3} M Mg^{2+}). The calorimetric estimates of ΔC_P show even larger relative variations, increasing from 1500 cal mol^{-1} deg^{-1} at the low Mg^{2+} concentration to 7000 cal deg^{-1} mol^{-1} at the highest concentration. These ΔC_P values are indeed very large and of the same order of magnitude as have been observed for the denaturation of globular proteins of equivalent molecular weight (*e.g.*, ΔC_P for chymotrypsinogen denaturation is *ca.* 3000 cal mol^{-1} deg^{-1} at 50°). However, ΔC_P for protein denaturation does not show such large variations in magnitude as the transition temperature is changed by varying the pH (Jackson and Brandts, 1970).

Using the assumption that the denaturation occurs as a two-state process, the apparent enthalpy change, ΔH_{VH}, can also be obtained from the calorimetric data of Figure 3, using the method of Jackson and Brandts (1970). The van't Hoff plots of the data at the four different Mg^{2+} concentrations are shown in Figure 4. As might be expected, the data generate curves that show increasing slope toward the high temperature end of the transition, consistent qualitatively with the positive ΔC_P. The slopes were taken at the thermal midpoints of each transition to determine ΔH_{VH} with indicated errors of the order of 10%. These values, as well as the corresponding estimates of ΔH_{cal} are plotted as a function of T_m in Figure 5. The two-state enthalpy change, ΔH_{VH}, increases by about 80% in going from the lowest to the highest Mg^{2+} concentration and in that sense is similar to the trend noted above for ΔH_{cal}. However, the absolute values of ΔH_{cal} are systematically larger than the corresponding two-state estimates.

We will define the ratio $\Delta H_{cal}/\Delta H_{VH}$ as the *cooperativity index*. For a transition with perfect cooperativity, a two-state transition, the cooperativity index will be 1.0. For the unfolding of a structure with *n identical and independent* "subunits" the cooperativity index will of course be equal to the actual number of subunits, *n* (Lumry *et al.*, 1966; Tanford, 1968). For more complicated transitions with nonidentical and semiindependent structural regions, the cooperativity index cannot be so precisely defined and we will consider it to be a measure of the *effective* number of independent structural units. Any interdependence of structural regions, *i.e.*, mutual stabilization, will generally tend to reduce the cooperativity index to a value which is less than the number of structural regions which participate in the transition. The values of this parameter are tabulated in Table I. It is seen that the cooperativity index has a value of 1.4–1.7 and that this shows no systematic variations as a function of Mg^{2+} concentration.

In addition to the above studies in Tris buffer, the $tRNA^{Phe}$ transition was also examined in a phosphate buffer (0.005 M phosphate (pH 7.2)–0.005 M NaCl–0.001 M $MgCl_2$) which was more similar to that used by Levy *et al.* The results are shown in Table I and Figure 6 (solid line). The results are very comparable to those previously noted for the Tris systems. At a T_m of 68° the calorimetric ΔH was found to be 175 kcal/mol and this is 38% larger than ΔH_{VH} obtained from the same data. The deviations of the experimental data from the behavior expected if the $tRNA^{Phe}$ transition were in fact a two-state process can be better visualized by comparing the experimental (solid) and calculated (dashed) curves in Figure 6. The calculated curve is the behavior expected for a two-state transition which has exactly the same T_m, ΔH, and ΔC_p which were determined experimentally for $tRNA^{Phe}$ under these conditions. The most significant differences in the two curves are seen in the low temperature half of the transition since the experimental curve leaves the base line at significantly lower temperatures. Similar plots of experimental and calculated curves under other conditions (Figure 3) have this same feature. This might be a reflection of the early melting transition which was observed to occur as a separate process for Val- and fMet-tRNA, although other interpretations seem equally likely.

Discussion

In the case of $tRNA^{Phe}$ from yeast, only a single calorimetric transition was discernible over a 100-fold variation in magnesium ion concentration. At the qualitative level, this observation is in agreement with other studies (Romer *et al.*, 1969; Wintermeyer *et al.*, 1969; Levy *et al.*, 1972) where only a sin-

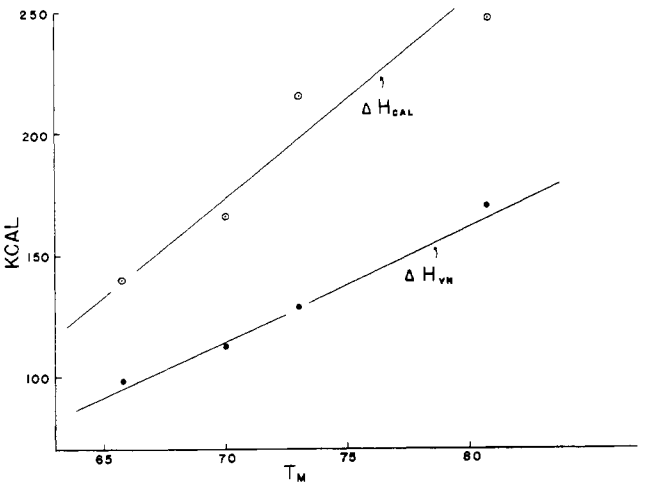

FIGURE 5: The variation of ΔH_{cal} and ΔH_{VH} as a function of the thermal midpoint of the transition (T_m), obtained from the data of Figure 3.

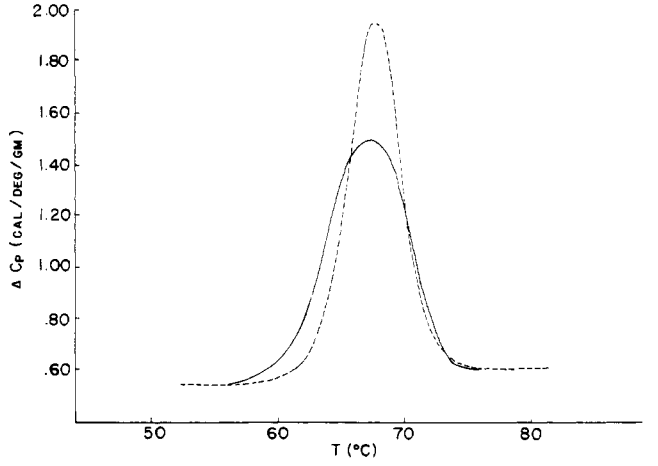

FIGURE 6: The experimental (—) calorimetric curve showing the denaturation of $tRNA^{Phe}_{yeast}$ in 0.005 M phosphate buffer (pH 7.2)–0.1 M NaCl–0.0002 M $MgCl_2$. A calculated curve (- - -) is also shown. This corresponds to the calorimetric curve which would be observed for a transition with precisely the same ΔH_{cal} and ΔC_P as were found for the solid curve, but with the additional assumption that the transition is of the all-or-none type.

gle transition was resolved from A_{260} measurements. However, on the basis of results obtained with half-molecules of $tRNA^{Phe}$, Romer *et al.* (1970) have suggested that there are actually five separate transitions involved in the $tRNA^{Phe}$ denaturation.

Our studies on $tRNA^{Phe}$ do show quite clearly that the single calorimetric transition which is observable must be complex. The cooperativity index, $\Delta H_{cal}/\Delta H_{VH}$, is of the order of 1.5 and this is by itself sufficient grounds for rejecting an all-or-none mechanism (Lumry *et al.*, 1966). We disagree then with the conclusion of Levy *et al.* (1972). On the other hand, we also feel that the cooperativity index is much too small to be consistent with a mechanism which involves as many as five separate transitions (Romer *et al.*, 1970) if these transitions are regarded as being at all independent. For a transition involving *only two* independent and identical steps, a ratio of 2.0 is expected (Lumry *et al.*, 1966; Tanford, 1968). Even though the $tRNA^{Phe}$ transition is not an all-or-none transition, it is nevertheless a highly cooperative process and not at all what would be expected for the independent unfolding of several different helical sections of approximately the same stability and size. That this is true can be further seen by comparing our experimental results on $tRNA^{Phe}$ (Figure 3) with the calculations of De Lisi (1973), for example. Assuming that each of the four helical branches melts out independently, the calculated transition curve for $tRNA^{Phe}$ is spread out over a temperature range of some 40° and is much more broadened on the high-temperature side of the midpoint (θ *vs.* T). The calorimetric transitions, on the other hand, are considerably narrower (*ca.* 20° in breadth) and slightly broader on the low-temperature end. This shows that the assumption of independent unfolding of the four cloverleaf helical segments is not at all realistic for $tRNA^{Phe}$. The structure of the crystalline form (Kim *et al.*, 1973) does in fact suggest an interdependence of cloverleaf stems. In this structure, the CCA and TψC helices are colinear and juxtaposed to form one continuous double helix. The dihydrouridine stem and the anticodon stem likewise form a single continuous helix. These two helices are then oriented at right angles to one another. This would suggest that a good starting point for attempting to understand $tRNA^{Phe}$ unfolding would be two, rather than four, semi-independent structural regions. It then becomes easy to visualize how a small amount of mutual stabilization between these two regions might give rise to a transition with a cooperativity ratio of *ca.* 1.5.

Perhaps the most unusual feature of the $tRNA^{Phe}$ transition is its large sensitivity to Mg^{2+} concentration. In view of the fact that the cooperativity index does not change as salt is added, it would appear that the thermodynamic mechanism of unfolding is not drastically altered. The predominant effect of Mg^{2+}, according to our data, is that it causes systematic increases in ΔC_P of denaturation which is, of course, also reflected in ΔH as the transition temperature is changed. This is an enormously large effect, since ΔC_p changes from only 1500 cal mol^{-1} deg^{-1} to 7000 cal mol^{-1} deg^{-1} as the Mg^{2+} concentration is increased from 5×10^{-5} to 5×10^{-3} M. Although large changes in heat capacity have previously been observed for polynucleotide reactions (Bunville *et al.*, 1965; Rawitscher *et al.*, 1963; Krakauer and Sturtevant, 1968), the strong sensitivity to Mg^{2+} had not previously been seen. The cause of this sensitivity is not known. Levy *et al.* (1972) have reported that there are no significant enthalpy effects associated with the binding of Mg^{2+} to either native or denatured $tRNA^{Phe}$ but their study was restricted to a single temperature (25°) so that significantly large heat capacities of binding are consistent with their data. Large increases in heat capacity during protein denaturation have been attributed to the exposure of hydrophobic groups to water and this same factor is certainly present to some extent in tRNA transitions. However, the large sensitivity to Mg^{2+} would seem to argue against a simple interpretation exclusively in terms of solvation effects. Our results are *consistent* with the idea that the addition of Mg^{2+} causes the structure of native tRNA to "tighten up" in the sense that it becomes less susceptible to small structural variations as the temperature is increased below the transition region; thereby decreasing the heat capacity of the native form, and increasing ΔC_P. Previously observed changes in circular dichroism (Blum *et al.*, 1972), hypochromism (Goldstein *et al.*, 1972), and proton exchange (Englander and Englander, 1965) upon the addition of Mg^{2+} are consistent with this suggestion.

Due to the heat capacity effects mentioned above, the calorimetric enthalpy change varies from 140 (5×10^{-5} M Mg^{2+}) to 248 kcal/mol (5×10^{-2} M Mg^{2+}). Assuming that the cloverleaf model with 20 base pairs is realistic (and forgetting about any possible increases in pairing as Mg^{2+} is increased), this would suggest a ΔH of 7–12.4 kcal/mol of base pairs. In view of the strong dependence on Mg^{2+} and temperature, it becomes

difficult to compare this with previous estimates. Using the method of Gralla and Crothers (1973), one can predict a total ΔH of *ca.* 140 kcal for disruption of the cloverleaf of $tRNA^{Phe}$. This estimate pertains to 1 M NaCl and temperature effects on ΔH were not considered by them. Krakauer and Sturtevant (1968) have shown experimentally that disruption of the poly(A + U) double helix leads to a ΔH of 8.4 kcal/mol of base pairs in high Na^+ and at 68°. Others have obtained comparable estimates (Neumann and Ackermann, 1967; Rawitscher *et al.*, 1963; Ross and Scruggs, 1965) from calorimetry. Thus, these independent estimates are experimentally consistent with what was observed in this study and also consistent with the cloverleaf structure or some variant of this which involves tertiary structure (such as the recently reported crystalline structure of $tRNA^{Phe}$ by Kim *et al.*, 1973). However, the ability of thermodynamic data to discriminate between various structural models is not very great under the best of circumstances and is further complicated in this case by the large effects of Mg^{2+} which suggest that perhaps multiple forms of the native molecule could exist.

In summary then, the most important conclusion from this work is that the thermal transition of $tRNA^{Phe}_{yeast}$ does not take place as a single thermodynamic step nor does it take place with as many as four independent steps. The value of the cooperativity index is consistent with the unfolding of two *semi-independent* structural regions and these can tentatively be identified with the two continuous helical regions which have already been shown to exist in the crystalline structure of $tRNA^{Phe}_{yeast}$.

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