Dube, S. K., and Marcker, K. A. (1969), *Eur. J. Biochem. 8*, 256.
Dube, S. K., Marcker, K. A., Clark, B. F. C., and Cory, S. (1968), *Nature (London) 218*, 232.
Eisinger, J., Feuer, B., and Yamane, T. (1971), *Nature (London), New Biol. 231*, 126.
Felsenfeld, G., and Cantoni, G. L. (1964), *Proc. Nat. Acad. Sci. U. S. 51*, 818.
Felsenfeld, G., and Sandeen, G. (1962), *J. Mol. Biol. 5*, 587.
Fresco, J. R., Adams, A., Ascione, R., Henley, D., and Lindahl, T. (1966), *Cold Spring Harbor Symp. Quant. Biol. 31*, 527.
Fresco, J. R., Klotz, L. C., and Richards, E. G. (1963), *Cold Spring Harbor Symp. Quant. Biol. 28*, 83.
Gartland, W. J., and Sueoka, N. (1966), *Proc. Nat. Acad. Sci. U. S. 55*, 948.
Goodman, H. M., Abelson, J., Landy, A., Brenner, S., and Smith, J. D. (1968), *Nature (London) 217*, 1019.
Gralla, J., and Crothers, D. M. (1972), *J. Mol. Biol.* (in press).
Hoskinson, R. M., and Khorana, H. G. (1965), *J. Biol. Chem. 240*, 2129.
Ishida, T., and Sueoka, N. (1968), *J. Biol. Chem. 243*, 5329.
Lindahl, T., Adams, A., and Fresco, J. R. (1966), *Proc. Nat. Acad. Sci. U. S. 55*, 941.
Nishimura, S., Harada, F., Narushima, U., and Seno, T. (1967), *Biochim. Biophys. Acta 142*, 133.
Pörschke, D., and Eigen, M. (1971), *J. Mol. Biol. 62*, 361.
RajBhandary, U. L., Chang, S. H., Gross, H. J., Harada, F., Kimura, F., and Nishimura, S. (1969), *Fed. Proc., Fed. Amer. Soc. Exp. Biol. 28*, 409.
Reeves, R. H., Cantor, C. R., and Chambers, R. W. (1970), *Biochemistry 9*, 3993.
Riesner, D., Römer, R., and Maass, G. (1969), *Biochem. Biophys. Res. Commun. 35*, 369.
Römer, R., Riesner, D., Coutts, S. M., and Maass, G. (1970a), *Eur. J. Biochem. 15*, 77.
Römer, R., Riesner, D., and Maass, G. (1970b), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 10*, 352.
Seno, T., Kobayashi, M., and Nishimura, S. (1969), *Biochim. Biophys. Acta 174*, 71.
Strittmatter, P. (1964), *in* Rapid Mixing and Sampling Techniques in Biochemistry, Chance, B., Eisenhardt, R., Gibson, Q., and Lonberg-Holm, K., Ed., New York, N. Y., Academic Press, p 76.
Uhlenbeck, O. C., Baller, J., and Doty, P. (1970), *Nature (London) 225*, 508.
Yang, S. K. (1972), Thesis, Yale University.
Yang, S. K., Söll, D. G., and Crothers, D. M. (1972), *Biochemistry 11*, 2311.

Conformational Changes of Transfer Ribonucleic Acid. Relaxation Kinetics of the Early Melting Transition of Methionine Transfer Ribonucleic Acid (*Escherichia coli*)†

P. E. Cole and D. M. Crothers*

ABSTRACT: We report temperature-jump studies of the early melting transition of $tRNA^{fMet}$ (*Escherichia coli*). There are two measurable relaxation times τ, both independent of concentration and visible at 266 and 335 nm. The temperature dependence of the τ values establishes apparent activation energies, and implies that the process of structure formation has negligible activation energy, while dissociation of structure requires energy. We also measured the thermal difference spectrum for both relaxation effects, and found that each of these is apparently less G·C rich than the total tRNA melting. These results allow a general comparison of the rate of regenerating the "native" structure (zone I of the tRNA phase diagram), either from the low-salt (zone III) form, or from the zone II form (the product of the early melting transition). The conversion from the low-salt form is several orders of magnitude slower, and has a much larger activation energy. We conclude that zone II and zone III represent different conformations. Further consideration of all the factors leads to the conclusion that zone II is a structure with no noncloverleaf bonding, but in which the dihydrouridine helix of the cloverleaf may be melted. The kinetic results imply that the tertiary structure of $tRNA^{fMet}$ contains at least two regions of interaction whose melting is not obligatorily coupled. We present a simple hypothesis to interpret the results further, in which there are two interaction regions whose melting is virtually independent. With this hypothesis, we find that (in 0.17 M Na^+) region 1 is formed in approximately 2–3 msec, and has a dissociation heat of about 22 kcal/mole, while region 2 is formed in about 7 msec, and has a dissociation heat of 51 kcal/mole. The melting of region 2 may include opening of the dihydrouridine helix, but all other interactions involve tertiary structure.

The most interesting current questions about tRNA structure involve the nature of the tertiary folding (Arnott, 1971). In the previous paper (Cole *et al.*, 1972) we used equilibrium and kinetic measurements to develope a conformational phase diagram, in which it was proposed that the low-temperature melting process seen at high Na^+ concentration

† From the Department of Chemistry, Yale University, New Haven, Connecticut. *Received June 9, 1972.* This is the second paper in a series; the preceding paper was by Cole *et al.* (1972). This work was supported by a grant (GM 12589) from the National Institutes of Health. D. M. C. holds a Career Development award (GM 19978) from the same source. P. E. C. was supported by NIH Predoctoral Fellowship GM 41629.

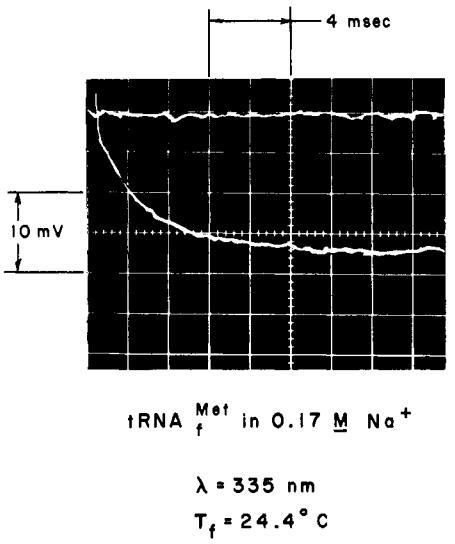


FIGURE 1: Observed relaxation signal for the melting of tRNA^{fMet} in 0.17 M Na^+ at 335 nm and a final temperature of 24.4°. The horizontal trace at the top of the picture indicates the absorbance before the temperature jump. The T-jump size is 3.4°. Total light intensity 5 V.

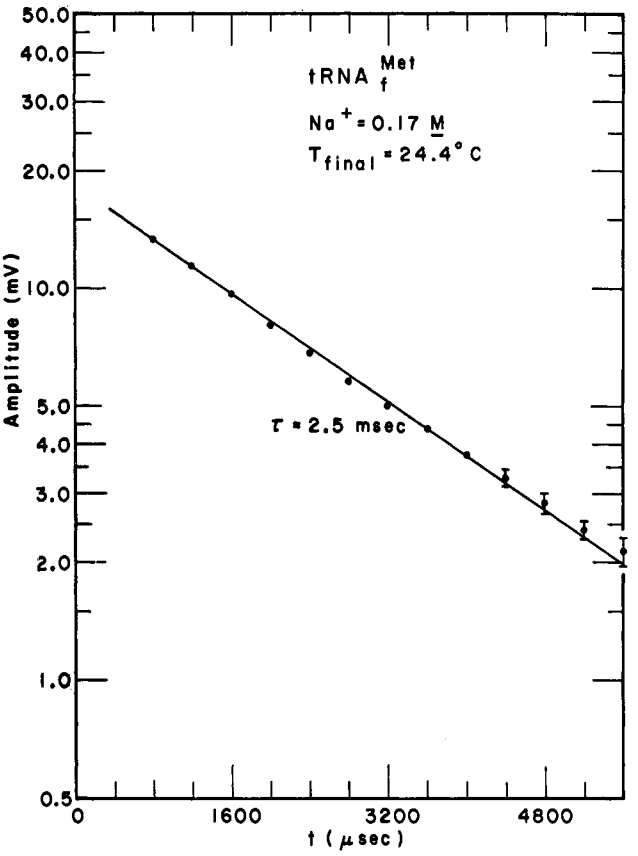


FIGURE 2: Semi-logarithmic plot of the relaxation effect shown in Figure 1. The relaxation time is 2.5 msec.

represents loss of tertiary structure. This conclusion was tentative and involved consideration of the following features. 1. Most of the absorbance change at 335 nm (the 4-thiouridine absorbance band, a nucleotide not bonded in the cloverleaf structure), but only a fraction of the change at 260 nm, occurs in the first transition. 2. Thermal difference spectra imply melting of a structure less G·C rich (assuming standard pairs) than the base pair composition of the cloverleaf. 3. The transition midpoint is more sensitive to Na^+ concentration than is the case for double-helical DNA or RNA. 4. The transition occurs under conditions where theory (DeLisi and Crothers, 1971; Gralla and Crothers, 1972) clearly predicts that the hairpin helices of the cloverleaf, excepting the dihydrouridine helix, should be stable. These factors taken together suggest that the early melting transition involves loss of tertiary structure, with possible simultaneous melting of the dihydrouridine arm.

Because of the intrinsic interest of tRNA tertiary structure, it is clearly appropriate to investigate further this particular transition. We report here relaxation kinetic studies of the early melting transition of tRNA^{fMet} (0.17 M Na^+, no Mg^{2+}), designed to elucidate the mechanism of the folding process. The results also provide further support for the equilibrium conformational phase diagram proposed previously (Cole *et al.*, 1972).

Materials and Methods

Materials. tRNA^{fMet} samples were those described previously (Cole *et al.*, 1972). Relaxation experiments were carried out in a buffer (pH 7) containing 0.1 M phosphate, 10 mM sodium cacodylate, and 1 mM EDTA, Na^+ concentration 0.173 M.

Temperature-Jump Measurements. The instrument used was a modified version (Crothers, 1971) of that marketed by Messanlagen (Göttingen, Germany). The cell was constructed of Kel-F, with solid gold electrodes, and had a volume of approximately 1.5 ml. Temperatures in the cell were monitored with a thermistor inserted into the upper (ground potential) electrode. The entire T-jump cell was enclosed in a cylindrical thermostatted jacket, and the whole assembly was insulated to reduce heat loss and minimize thermal gradients in the cell. The thermal differential between bath and cell was less than 2°.

In experiments where the relaxation amplitude was measured as a function of wavelength, an interference filter (254-

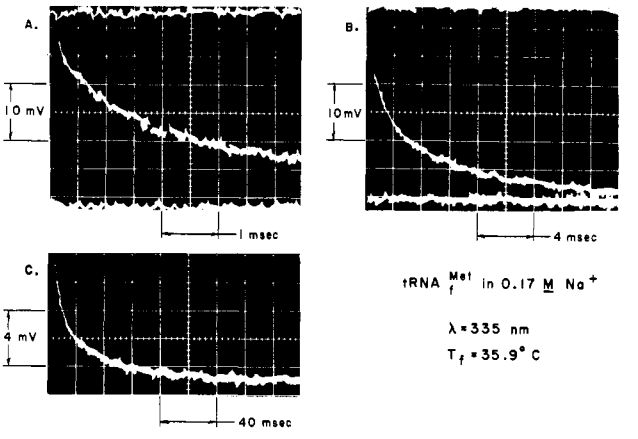


FIGURE 3: Oscilloscope traces illustrating the relaxation effects observed in various time ranges for tRNA^{fMet} melting in 0.17 M Na^+ at 335 nm and a final temperature of 35.9°. The upper horizontal line of traces A and B indicates the absorbance before the T jump. The lower horizontal line seen in traces A and B is a second trigger signal which corresponds to the final equilibrium absorbance value after completion of kinetic effects. Trace C is expanded on a 2-mV/cm sensitivity and thus the absorbance before the T jump is not shown. Total light intensity 5 V.

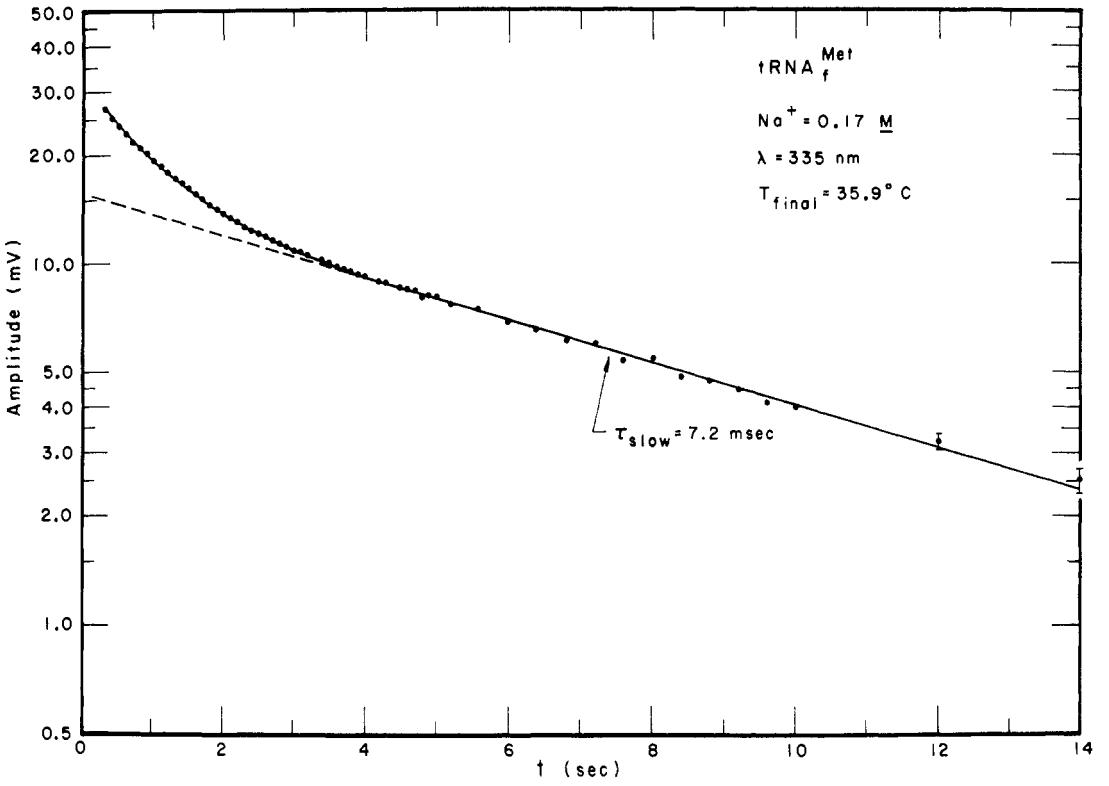


FIGURE 4: Semi-logarithmic plot of the overall relaxation amplitude *vs.* time for the data of Figure 3. The slower part of the overall effect can be fit to a straight line with a relaxation time $\tau_{slow} = 7.2$ msec.

nm transmission maximum, 28-nm width at half-height) was placed in the beam to reduce the serious problem of visible stray light transmission by the instrument's monochromator (Bausch and Lomb high intensity grating). In these same experiments a 1000-channel transient recorder (Biomation Model 802) was used to improve data collection.

tRNA samples studied at 266 nm generally contained about 1.4 A_{260}/ml; for 335-nm measurements, solutions had an absorbance at 335 of approximately 0.28, equivalent to 12 A_{260}/ml.

Since ultraviolet (uv) irradiation inactivates tRNA, and 335-nm light induces a photodimerization of 4-thiouridine with cytidine (Favre *et al.*, 1971), precautions were taken to avoid photochemical damage. Yang (1972) calibrated the inactivation in the temperature-jump instrument, and found 20% loss of activity after exposure at 266 nm for 30 min. Therefore all exposures were kept below this value. Photodimerization at 335 nm is more problematic, since it will not affect the activity assay (Yaniv *et al.*, 1971). We judge our exposures to be much less than required for half-conversion to the photodimer (Favre *et al.*, 1971). Frequent comparison of the behavior of exposed samples with fresh samples was also used to guard against artifacts arising from photomodification. We used borohydride reduction (Favre *et al.*, 1971) to measure the extent of photodimerization in the fresh sample as received from Oak Ridge, and found that 15–20% of the 4-thiouridine residues had been cross-linked.

Results

There Are Two Measurable Relaxation Times. The relaxation kinetics of the first melting transition exhibited by $tRNA^{fMet}$ in 0.17 M Na^+ (no Mg^{2+}) was examined at both 335 and 266 nm (see Cole *et al.*, 1972, for equilibrium melting curves). Typical data taken at 335 nm and low temperature (below 25°) are illustrated by the oscilloscope trace in Figure 1; no slower optical changes were observed. Figure 2 shows a semi-logarithmic plot of the data in Figure 1. The linear relation implies a single relaxation time at this temperature. Extrapolation of the line to $t = 0$ shows that the single exponential decay accounts for the entire observed optical change at 335 nm. For reasons that will become apparent, the relaxation time associated with this effect will be designated τ_{fast}.

As the cell temperature is raised so that the final value is greater than 25°, an additional, slower, kinetic effect becomes evident. As an example of this behavior, the traces of Figure 3 show the relaxation effects in various time ranges at 35.7°. Figure 4 is a semi-logarithmic plot of the overall relaxation amplitude, and Figure 5 demonstrates the resolution of the faster part of the curve into a second exponential decay. The slower of these two effects is τ_{slow}, and the faster is a continuation of τ_{fast} seen at lower temperatures. These two effects account for at least 90% of the total absorbance change at 335 nm. There is apparently a contribution of a few per cent from a very fast relaxation.

At final temperatures above 47°, the major relaxation was associated with τ_{slow}. No additional slow effects appeared, and τ_{fast} became too fast and too small in amplitude to measure.

The Temperature Dependence of τ Establishes Apparent Activation Energies. Figure 6 shows a plot of log τ against temperature. For both τ_{fast} and τ_{slow} there are two linear regions, with slope nearly zero at lower temperature, and

much larger at higher temperature. For a simple first-order conversion

$$A \underset{k_1}{\overset{k_{-1}}{\rightleftharpoons}} B$$

$$1/\tau = k_1 + k_{-1} \quad (1)$$

and τ will be dominated by k_1 or k_{-1}, whichever is larger, at the extremes of the transition. For example, at low temperature, $1/\tau$ would be approximately equal to the rate constant k_1 for forming the ordered structure, and at higher temperature it would equal the rate constant k_{-1} for its dissociation. Therefore the linear regions of a plot like Figure 6 can be used to calculate apparent activation energies for structure formation and dissociation. (This simple argument must be examined more carefully for the temperature region where two relaxation times are observed; see Discussion section.) The Arrhenius activation energy E_{act} is defined by

$$\partial \ln k/\partial(1/T) = -E_{act}/RT \quad (2)$$

Hence Figure 6 implies that *the process of structure formation has negligible apparent activation energy, while dissociation of structure requires energy.*

The Same Relaxation Effects Appear at Lower Concentration and Other Wavelengths. We found the observed relaxation behavior to be virtually the same at all concentrations and wavelengths studied. For example, Figure 7 shows measurements at 266 nm, and about 10 times lower tRNA concentration than for the data in Figure 6. The dotted lines in Figure 7 represent the best fit to the data of Figure 6. The only significant deviation is for τ_{fast} above 38°, where the measurements at 266 nm yield slightly slower relaxation times than those at 335 nm. On this basis we consider it possible that in this temperature region τ_{fast} is actually a composite effect of two closely spaced relaxation times, with different relative amplitudes at 266 nm and 335 nm. Judged by the lack of concentration dependence at other temperatures, however, all relaxations appear to be first-order processes.

Each Relaxation Effect Has a Thermal Difference Spectrum Less G·C Rich Than That of the Total tRNA Melting. In the previous paper (Cole *et al.*, 1972) we showed thermal difference spectra for the whole process observed in the first transition, compared with difference spectra for the second transition. On the basis of spectral shape, and assuming standard base pairs, we concluded that the structure that melts in the first transition is less G·C rich than that which melts in the second transition. Figure 8 shows that the same conclusion holds for both of the measured kinetic components in the first transition. Each spectrum was measured over a temperature range where the contribution from the other effect was unimportant. The data of Fresco *et al.* (1963) suggest that the fast process is 38% A·U pairs and the slow process is 45% A·U pairs. (The hazards of such a calculation are great, but the numbers are useful to give a general idea of the base composition implied if the structures melting in Figure 8 were RNA double helices.)

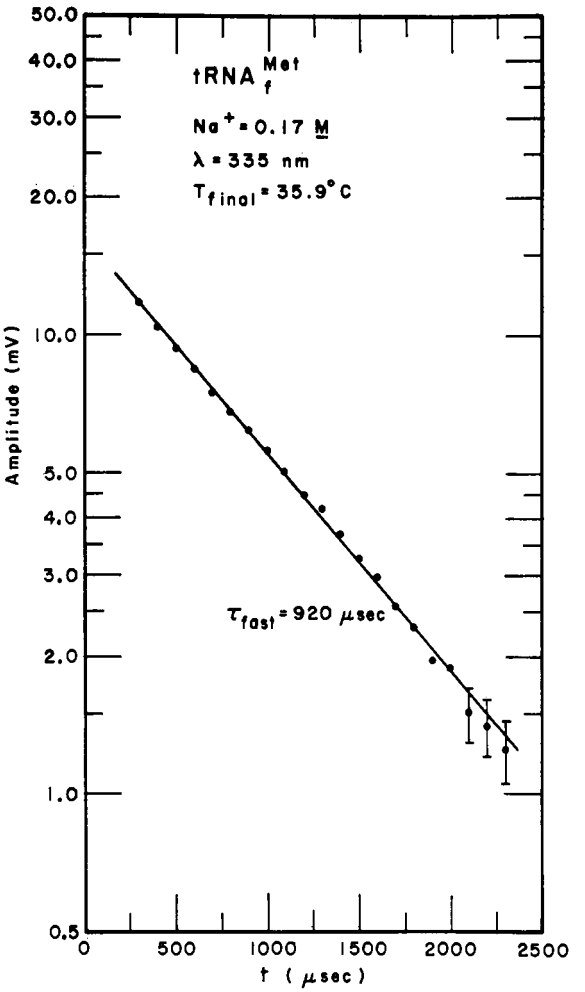


FIGURE 5: Semi-logarithmic plot of the faster relaxation effect observed for tRNA^{fMet} melting in 0.17 M Na^+ at 35.9° and 335 nm. The amplitudes plotted here correspond to the differences between the observed points and the straight line of Figure 4 extrapolated over the faster part of the curve. τ_{fast} is 920 μsec.

Discussion

General Implications. The kinetic results have certain general implications whose appreciation does not require discussion of the detailed kinetic mechanism. First, there is the clear contrast between these observations and the kinetics of conversion of tRNA^{fMet} to the "native" form (zone I of the phase diagram, Cole *et al.*, 1972) following salt jump from the low-salt form (zone III of the phase diagram). The salt-jump conversion is orders of magnitude slower than the rates studied here, and has an activation energy of 61 kcal/mole (Cole *et al.*, 1972). This may be compared with the rates for the analogous process, studied in this paper, in which the "native" structure is reformed from the material which is the product of the early melting transition, or zone II of the phase diagram. According to Figure 6, this reformation occurs in a few milliseconds, and there is no evidence for activation energy in the structure formation process. The conclusion seems inescapable that *zone II and zone III represent different conformations.*

The lack of any indication of activation energy for converting from zone II to the "native" form (zone I) provides important information concerning the conformation in zone II. Dissociation of nucleic acid secondary structure usually requires energy, which appears in the activation energy associated with the corresponding rate constant (Pörschke and Eigen, 1971; Craig *et al.*, 1971). Assuming that the "native" structure is based on a cloverleaf bonding pattern, the large activation energy (and slow rate) of the salt-jump conversion implies that noncloverleaf bonding must be broken in the

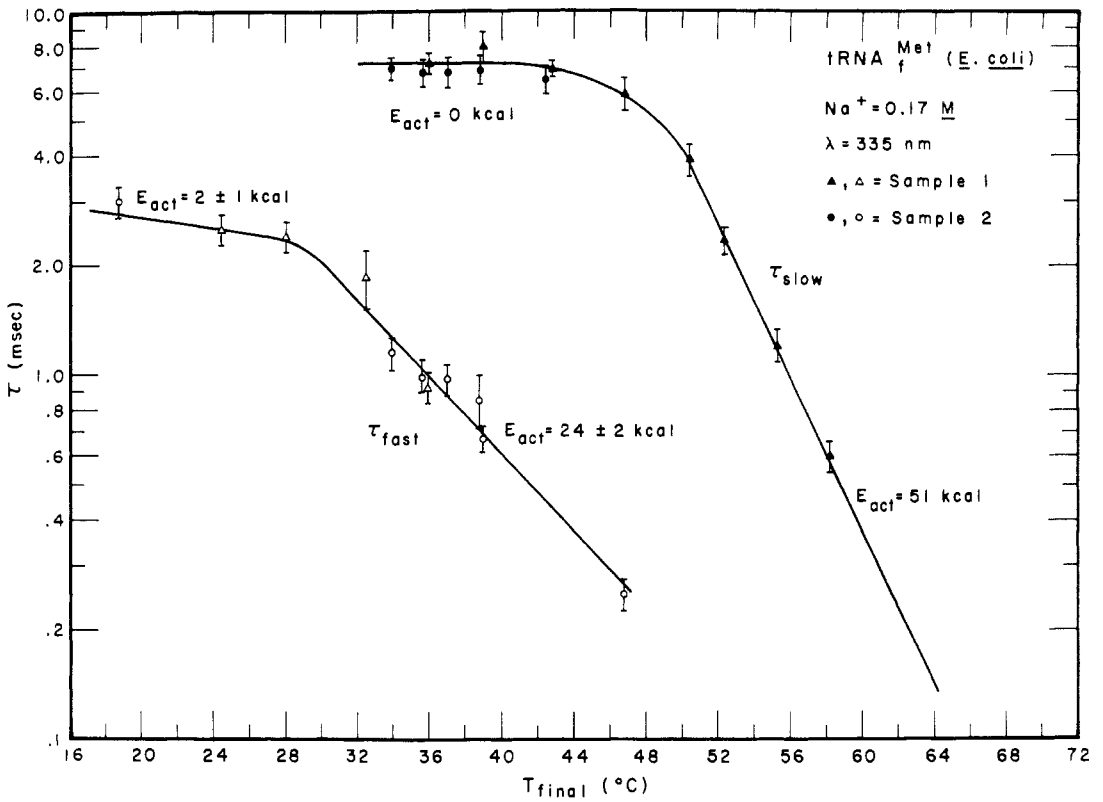


FIGURE 6: Temperature dependence of the two relaxation times, τ_{fast} and τ_{slow}, observed for the first melting transition of $tRNA^{fMet}$ in 0.17 M Na^+ at 335 nm. T_{final} is the temperature after the T jump.

conversion. Correspondingly, the lack of evidence for activation energy (and the fast kinetics) for the conversion of form II to form I ("native") argues that no noncloverleaf bonding must be broken in this case. Hence, *form II is a cloverleaf, or partly melted cloverleaf, for $tRNA^{fMet}$.*

The only condition on this conclusion is that one recognize the possibility that a zero activation energy could result from accidental cancellation of negative and positive contributions to the apparent activation energy. In this connection we note that noncloverleaf bonding in zone II can be demonstrated for $tRNA^{Tyr}$ on the basis of positive activation energy for the II → I conversion (Yang and Crothers, 1972). The reaction is, as would be expected if it is impeded by the necessity to break "wrong" structure, slower than for $tRNA^{fMet}$. Furthermore, the noncloverleaf bonding has a plausible explanation in terms of sequence complementarity not present in $tRNA^{fMet}$. Hence the existence of a contrasting case reinforces the strength of the conclusion that zone II contains no important noncloverleaf bonding for $tRNA^{fMet}$.

A final general conclusion that our kinetic results permit is that the early melting transition is not an all-or-none process, since two relaxations are observed. There must be at least one intermediate, and hence *there are at least two regions of the tRNA structure that melt in this temperature range, and their unbonding is not obligatorily coupled.*

What Melts in the Early Transition? This question is central to a detailed understanding of the process, and can be partially answered now. We consider that the possibilities are the four helix arms of the cloverleaf, plus the tertiary structure of unknown nature, and proceed to eliminate as many of these as possible. The time scale of the relaxation processes (several milliseconds) is two to three orders of magnitude slower than that observed for melting of simple model hairpin helices (Coutts, 1971; Gralla and Crothers, 1972). Therefore neither of the two relaxation signals can represent exclusively melting of any of the three hairpin helices of the cloverleaf. Theory clearly predicts (DeLisi and Crothers, 1971; Gralla and Crothers, 1972) that the anticodon helix and the TψC helix should be stable under these conditions, and should melt in the second transition. Therefore we exclude these two hairpin helices as contributors to the early transition. The acceptor stem may be eliminated by the following argument. Only the slower relaxation has sufficient optical change or activation energy that it could possibly arise from melting of the stem. However, that helix is all G·C pairs in $tRNA^{fMet}$, whereas the thermal difference spectrum implies more than 40% A·U pairs. Therefore the spectroscopic equivalent of at least 3 A·U pairs from some other source would need to melt simultaneously with the stem in order to account for the thermal difference spectrum. This, however, should supply a total dissociation heat (Gralla and Crothers,[1] 1972) in excess of the 51 kcal/mole measured for τ_{slow}. We conclude that the acceptor stem does not melt in the early transition. We are left by this process of elimination with the dihydrouridine helix and the tertiary structure. Since neither relaxation can be simply melting of a hairpin helix, loss of tertiary structure must be involved in each relaxation. Consequently, *the tertiary structure of $tRNA^{fMet}$ can melt in at least two steps.*

We consider it possible, and even likely, that the dihydrouridine helix melts simultaneously with tertiary structure in τ_{slow}, accounting for the surprisingly large dissociation heat associated with that process. *By this interpretation, the product of the early transition of $tRNA^{fMet}$ is a cloverleaf in which the dihydrouridine helix may have melted.*

What is the Detailed Kinetic Mechanism of the Early Transition? The answer to this question remains debatable. We

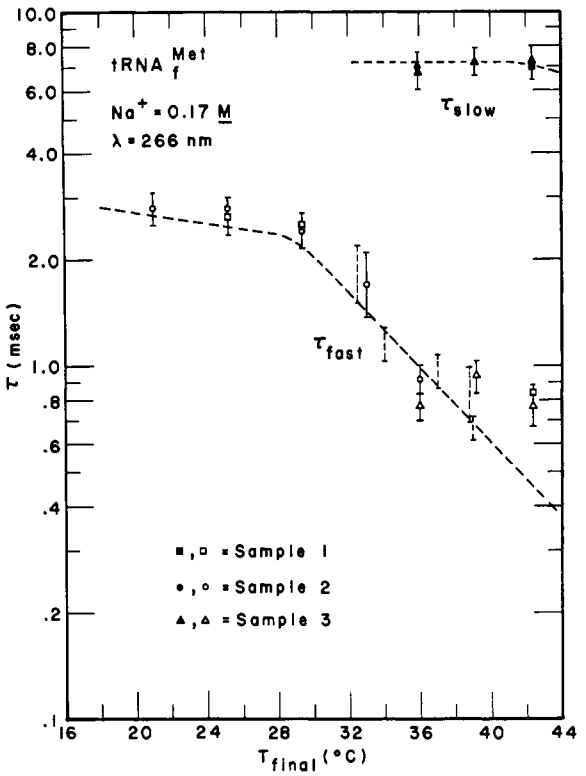


FIGURE 7: Temperature variation at 266 nm of the two relaxation times observed during the first melting transition of tRNA^{fMet} in 0.17 M Na^+. The dotted lines represent the best fit to the data of Figure 6.

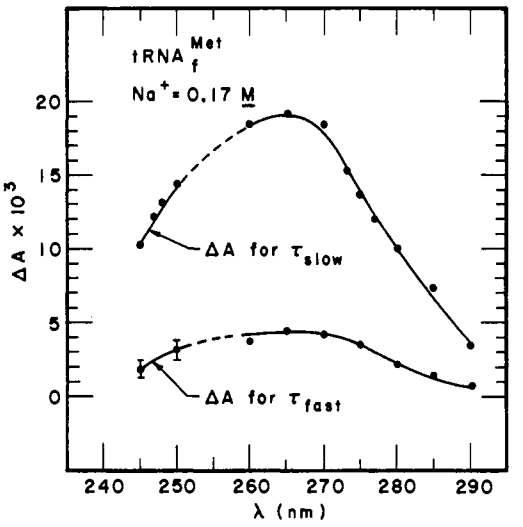
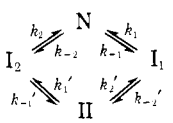


FIGURE 8: Thermal difference spectra of the two relaxation processes observed during the first melting transition of tRNA^{fMet} in 0.17 M Na^+. The total amplitude ΔA for each process is determined from semilogarithmic analysis of the oscilloscope trace. The T-jump size for all jumps was 10°. For τ_{fast} the relaxation was 2.8 ± 0.3 msec at all wavelengths and T_{final} was 24.4°. τ_{slow} was 4.8 ± 0.3 msec at all wavelengths with T_{final} = 47.7°.

know that there must be at least three conformational states in order to account for two relaxation times. These could be arranged in a linear or a cyclic mechanism, and we see no way to distinguish these unambiguously with our present data. If one takes seriously the suggestion in Figure 7 that the fast relaxation is actually a composite of two closely spaced relaxations at intermediate temperature, then four conformational states are required. The mechanistic possibilities then become even more numerous; these are considered in detail by Cole (1972).

Fortunately, the different mechanisms do not differ so greatly in the general physical picture they provide. In all cases the conclusion remains intact that there are at least two regions of the tRNA tertiary structure whose melting can be separated. The mechanisms differ in the order in which the regions can or must melt. We present here one mechanism which seems to us a reasonable hypothesis of how the tertiary structure melts. There are two main regions in the tertiary structural interactions, each of which can be broken while the other remains intact. This is formally a four-state, cyclic mechanism



(3)

where N is the "native" or low-temperature form, and II is the product of the low-temperature transition. The rate constants k_1 and k_1' (or k_2 and k_2') refer to formation of the bonds in region 1 (or region 2) and the constants k_{-1} and k_{-1}' (or k_{-2} and k_{-2}') refer to corresponding reactions that break the ordered structure.

Suppose the rate constants $k_2 + k_{-2}$ and $k_2' + k_{-2}'$ are much smaller than $k_1 + k_{-1}$ and $k_1' + k_{-1}'$. Then there will be two fast relaxations:

$$1/\tau_1 = k_1 + k_{-1}$$
$$1/\tau_2 = k_1' + k_{-1}' \quad (4)$$

which correspond simply to the reactions of structural region 1, and one slow relaxation

$$1/\tau_3 = k_2\left(\frac{K_1'}{1 + K_1'}\right) + k_2'\left(\frac{1}{1 + K_1'}\right) + k_{-2}\left(\frac{K_1}{1 + K_1}\right) + k_{-2}'\left(\frac{1}{1 + K_1}\right) \quad (5)$$

where

$$K_1 = k_1/k_{-1}$$
$$K_1' = k_1'/k_{-1}' \quad (6)$$

The relaxation τ_3 corresponds to the reactions of structural region 2, with each rate constant multiplied by a weighting factor involving an equilibrium constant for a fast reaction.

An interesting, and possibly very important, limit of this mechanism is the case where melting of each region has no effect on the kinetic parameters for the other structural region, or $k_1 = k_1'$, $k_{-1} = k_{-1}'$, $k_2 = k_2'$, and $k_{-2} = k_{-2}'$. In this case there would be a single fast relaxation

$$1/\tau_{fast} = k_1 + k_{-1} \quad (7)$$

and a single slow relaxation

$$1/\tau_{slow} = k_2 + k_{-2} \quad (8)$$

In other words, in this limit, the melting of the two regions is completely independent. An advantage of this limiting case is that it explains in a natural way the indication in Figure 7 that the fast relaxation is degenerate. If the limit is only approximated, then the fast relaxation would be a composite, at intermediate temperatures, of two nearly equal relaxation times. Needless to say, the validity of this hypothetical mechanism will be greatly strengthened if the anticipated resolution of the three-dimensional structure of tRNA reveals two regions of interaction in the tertiary structure, with independent melting of each region sterically possible.

Meaning of the Activation Parameters. In the arguments developed above, we have made considerable use of the activation energy for particular processes. At low temperatures there is only a single relaxation, and the simple argument is valid that $1/\tau_{fast}$ is approximately the rate constant for forming the "native" structure from a partly melted intermediate. Hence the activation energy for this process is nearly zero (2 kcal/mole). Similarly, at high temperatures only τ_{slow} contributes appreciably, and the same argument gives 51 kcal/mole as the activation energy for dissociating the structure corresponding to τ_{slow}. However, at intermediate temperatures there are two relaxations, and the meaning of the activation energies is not unambiguous. For example, in mechanism 3, the way the two fast relaxations τ_1 and τ_2 (eq 4) are weighted in τ_{fast} would change with temperature, and would influence the apparent activation energy. Similarly, the variation of τ_3 (eq 5) with temperature would be influenced by K_1 and K_1' as well as by the kinetic parameters. It is possible that an accidental cancellation of enthalpy and activation energy terms could lead to zero apparent activation energy. If so, this accident would have to be widespread, since we have found (unpublished observations) that several other tRNAs show relaxation times that are independent of temperature when they first appear in the melting process.

In contrast, the limit of independent melting of two structural regions (eq 7 and 8) gives unambiguous meaning to the activation parameters, since the relaxations are uncoupled and each can be considered by the simple argument for a first-order process. This has the general advantage that no accidental and widespread cancellation of energy terms is required, only a general similarity of the mechanism of tertiary structure formation by tRNA. In particular, this would mean that many tRNAs, including $tRNA^{fMet}$, would show no appreciable noncloverleaf bonding above the temperature where the native tertiary structure melts (at high Na^+ concentration).

In summary, the activation parameters derived from the temperature region where both relaxations appear are, in principle, ambiguous in their meaning. However, if the limiting mechanism of independent melting is correct, this ambiguity is removed. In that case, the activation energy for formation of both structural regions is nearly zero, and the dissociation activation energy is 24 kcal/mole for region 1 and 51 kcal/mole for region 2. The corresponding equilibrium enthalpy changes are 22 kcal/mole for region 1 and 51 kcal/mole for region 2. (Recall that region 2 may include melting of the dihydrouridine helix.)

Possible Functional Significance. One feature of these results that seems to us of possible importance for tRNA function is the conclusion that the tertiary structure can be broken in steps. This suggests that tRNA could show several tertiary structural changes in the course of its role in protein synthesis. For example, at one stage, region 1 of the tertiary structure could be broken by provision of specific interactions, allowing a certain geometric flexibility to the tRNA molecule. At another reaction step, region 2 might be broken and a different flexibility allowed. This would permit the various functional centers of tRNA to adapt to movement of ribosomes, messenger, and other components of the protein synthesis system.

References

Arnott, S. A. (1971), *Progr. Biophys. Mol. Biol. 22*, 181.

Cole, P. E. (1972), Ph.D. Thesis, Yale University.

Cole, P. E., Yang, S. K., and Crothers, D. M. (1972), *Biochemistry 11*, 113.

Coutts, S. (1971), *Biochim. Biophys. Acta 232*, 94.

Craig, M. E., Crothers, D. M., and Doty, P. (1971), *J. Mol. Biol. 62*, 383.

Crothers, D. M. (1971), *Proc. Nucl. Acid Res. 2*, 369.

DeLisi, C., and Crothers, D. M. (1971), *Proc. Nat. Acad. Sci. U. S. 68*, 2682.

Favre, A., Michelson, A. M., and Yaniv, M. (1971), *J. Mol. Biol. 58*, 367.

Fresco, J. R., Klotz, L. C., and Richards, E. G. (1963), *Cold Spring Harbor Symp. Quant. Biol. 28*, 83.

Gralla, J., and Crothers, D. M. (1972), *J. Mol. Biol.* (in press).

Pörschke, D., and Eigen, M. (1971), *J. Mol. Biol. 62*, 361

Yang, S. K. (1972), Ph.D. Thesis, Yale University.

Yang, S. K., and Crothers, D. M. (1972), *Biochemistry 11*, 4375.

Yaniv, M., Chestier, A., Gros, F., and Favre, A. (1971), *J. Mol. Biol. 58*, 381.