Strickland, J. E., & Hellman, A. (1977) *Anal. Biochem. 82*, 342.
Tamaoki, T., Miura, K., Lin, T., & Banks, P. (1976) *Onco-Developmental Gene Expression* (Fishman, W. H., & Sell, S., Eds.) pp 115–122, Acadamic Press, New York.
Theilen, G. H., Kawakami, T. G., Rush, J. D., & Munn, R. J. (1969) *Nature (London) 222*, 589.
Van Beveren, C., & Goulian, M. (1979) *Cancer Res. 39*, 2532.
Yogo, Y., & Wimmer, E. (1972) *Proc. Natl. Acad. Sci. U.S.A. 69*, 1877.

Multistep Mechanism of Codon Recognition by Transfer Ribonucleic Acid†

Damian Labuda*,‡ and Dietmar Pörschke*

ABSTRACT: The mechanism of codon recognition by tRNA is investigated in the system $tRNA^{Phe}$ + UUC by temperature-jump measurements using the Wye base fluorescence as a label. In 0.4 M Na^+ and 5 mM Mg^{2+} a two-step reaction is observed and described quantitatively; UUC is shown to bind preferentially to one of two conformations on the anticodon loop. In 0.1 M Na^+ and 10 mM Mg^{2+} an additional relaxation effect is observed, which indicates a codon-induced conformation change leading to an association of tRNA molecules. The codon-induced tRNA association is demonstrated independently by equilibrium sedimentation. The present results suggest a more active role of tRNA during translation than anticipated.

The main step in the translation of a genetic message is the recognition of codons by adaptor molecules, the tRNAs. Compared to the codons, the adaptors are large and complex structures consisting of ~80 nucleotide residues and yet using only 3 of them for *direct* interaction with the codon. There are several reasons for the rather high complexity of tRNA molecules. tRNAs have to provide recognition sites during numerous biological activities in which they participate (Clark, 1977). The following question arises, however. (1) Is tRNA a passive structure, which exposes a variety of sites for interaction with different components of the cell, or (2) does tRNA participate more actively in the decoding process? In the second case the whole tRNA structure could modulate the interaction between codon and anticodon triplets and, conversely, the interaction between codon and anticodon could trigger some response in the remote parts of the molecule.

In recent years much conflicting evidence has been presented concerning different conformations of tRNAs and their function during translation. Two structural transitions are mainly under discussion.

The anticodon loop of tRNA may convert from the 3′-stacked conformation, found in crystals [for review, cf. Rich & RajBhandary (1976) and Clark (1977)], to the 5′-stacked conformation. This transition, proposed by Fuller & Hodgson (1967), may be important during protein biosynthesis (Woese, 1970). Recently, a relaxation process observed in $tRNA^{Phe}$ has been assigned to the conversion between the 3′- and 5′-stacked conformations (Urbanke & Maass, 1978).

The second transition is the unfolding of the TΨC loop induced by the codon–anticodon interaction (Ofengang & Henes, 1969; Shimuzu et al., 1969). The TΨC loop is usually hidden due to tertiary interactions (Rich & RajBhandary, 1976). According to oligonucleotide binding data, the TΨCG sequence becomes exposed upon complex formation between codon and anticodon (Möller et al., 1979). Evidence for a codon-induced rearrangement of tRNA structure has also been obtained from chemical modification experiments (Wagner & Garrett, 1979). Other measurements using different techniques did not give any evidence for codon-induced unfolding of tRNA (Yoon et al., 1975; Geerdes et al., 1978; Grosjean et al., 1976; Davanloo et al., 1979; Geerdes, 1979).

In the present investigation the binding of UUC to its cognate $tRNA^{Phe}$ is studied by temperature-jump spectroscopy and equilibrium centrifugation. The fluorescence of the Wye base, a native label located adjacent to the 3′ side of the anticodon triplet (RajBhandary & Chang, 1968), is used to follow the reaction. The results indicate a four-step mechanism of codon recognition, involving two conformational changes and dimerization of tRNA. It shows that tRNA is not a passive adaptor but actively participates in the process of codon reading.

Materials and Methods

$tRNA^{Phe}$ from yeast and ApApA were purchased from Boehringer, Mannheim. The tRNA specific acceptor activity was 1.1 nmol/A_{260} unit. Highly pure $tRNA^{Phe}$ samples (specific activity of 1.5 nmol/A_{260} unit) were kindly given by Drs. M. Sprinzl and H. Faulhammer from Max-Planck-Institut für experimentelle Medizin, Göttingen. The codon oligonucleotide UpUpC was synthesized as described by Sprinzl et al. (1976). $tRNA^{Phe}$ samples were dissolved in buffer AB (10 mM Tris–cacodylate, 400 mM $NaClO_4$, and 5 mM $Mg(ClO_4)_2$, pH 7.1) or in buffer ABT (50 mM Tris–cacodylate, 100 mM $NaClO_4$, and 10 mM $Mg(ClO_4)_2$, pH 7.2) and annealed at 65 °C for 5 min as described by Grosjean et al. (1976). For measurements of Mg^{2+} concentration (c_{Mg}) dependence, the c_{Mg} was varied in ABT buffer as indicated. For c_{Mg} = 0, 0.5 mM Na_3EDTA was added instead of Mg^{2+}–salt. The $tRNA^{Phe}$ concentration (c_{tRNA}) was calculated on the basis of its acceptor activity. The concentrations of UpUpC (c_{UUC})

† From the Max-Planck-Institut für biophysikalische Chemie, 3400 Göttingen-Nikolausberg, West Germany. *Received December 11, 1979.*
‡ On leave from Międzyuczelniany Instytut Biochemii, Sekcja Biochemii, Uniwersytet im. Adama Mickiewicza, 61-701 Poznań, Fredry 10, Poland.

0006-2960/80/0419-3799$01.00/0

were calculated by using $\epsilon(260) = 2.7 \times 10^4$ M^{-1} cm^{-1} (Yoon et al., 1975).

All temperature-jump experiments were performed with the fluorescence–temperature-jump apparatus described previously (Rigler et al., 1974; Rabl, 1976). The excitation light at λ_{ex} = 312 nm was obtained from a Hanovia 200-W Xe-Hg lamp using a double monochromator (two Schoeffel GM 250). The emitted light was detected by two photomultipliers after passage through Schott GG 385 cutoff filters. The cell volume was 1.0 mL, with an optical path of 0.7 cm. The size of the temperature jump was 3.2 or 7.0 °C. The temperature before the perturbation (measured by a thermistor inside the upper electrode) was kept constant at 0.2 °C. The measured signal was stored in a transient recorder (Datalab 905) and then transferred via a cassette to a Univac 1108 where the relaxation times and amplitudes were analyzed with the "Discrete Program" of Provencher (1976). The data obtained from relaxation curves, which required a fit with three exponentials, were confirmed independently by using a fitting procedure based upon nonlinear analysis by a Simplex method, reducing the number of nonlinear variables (G. Striker, unpublished results). The reported values of the relaxation times (τ) and relative relaxation amplitudes (AMP) are mean values obtained by averaging the results of 4–12 temperature-jump measurements. The relative amplitude is defined as the ratio of the measured amplitude and the total fluorescence signal at the temperature after perturbation.

Equilibrium ultracentrifugation measurements were done with a Spinco Model E analytical ultracentrifuge equipped with ultraviolet optics, a photoelectric scanner, an electronic multiplexer, and an electronic speed control. All runs were made in a 12-mm Epon double-sector cell at a concentration of 25 μM tRNA. The solutions were scanned at 310 nm.

Results

Relaxation Experiments in Buffer AB. When a temperature jump is applied to a solution of $tRNA^{Phe}$ in buffer AB or ABT, the fluorescence of the Wye base is observed to decrease considerably. The main part of this effect corresponds to the usual decrease of the fluorescence quantum yield with increasing temperature and is too fast to be resolved by temperature-jump equipment. However, some of the fluorescence decrease is associated with a time constant around 1 ms. This relaxation effect has been characterized previously (Urbanke & Maass, 1978) and is attributed to an intramolecular transition in the anticodon loop of $tRNA^{Phe}$.

$$tRNA_F \underset{k_-^A}{\overset{k_+^A}{\rightleftharpoons}} tRNA_H \qquad (1)$$

For simplicity, we will refer to this as transition A, from its origin in the anticodon loop. We have studied the transition A both in the commercial and in the highly purified $tRNA^{Phe}$ sample; the relaxation times and the relative amplitudes were identical. Thus, any contaminations present in the commercial sample do not influence the fluorescence relaxation. Figure 1A shows the relaxation observed for $tRNA^{Phe}$ in buffer AB. Very similar effects were found for the other solution conditions used in the present investigations.

When the codon UUC was added to the $tRNA^{Phe}$ solution, using buffer AB, an additional relaxation effect appeared. Its amplitude is opposite to that found for transition A and its time constant is around 100 μs at $c_{UUC} \approx 1$ mM. The relaxation rate increases with increasing c_{UUC}, and thus the process is assigned to the bimolecular step of UUC association with $tRNA^{Phe}$. According to the bimolecular nature, we call this process B. Relaxation times and relative amplitudes of process B are presented in Figure 2 as a function of c_{UUC}. All available evidence implies that transition A, observed also in the absence of UUC, corresponds to a conformation change of the anticodon loop and thus should be directly coupled to the UUC binding at the anticodon. This expectation is confirmed by our experiments. Both AMP_A and $1/\tau_A$ (cf. Figure 2) depend upon the UUC concentration. The simplest model

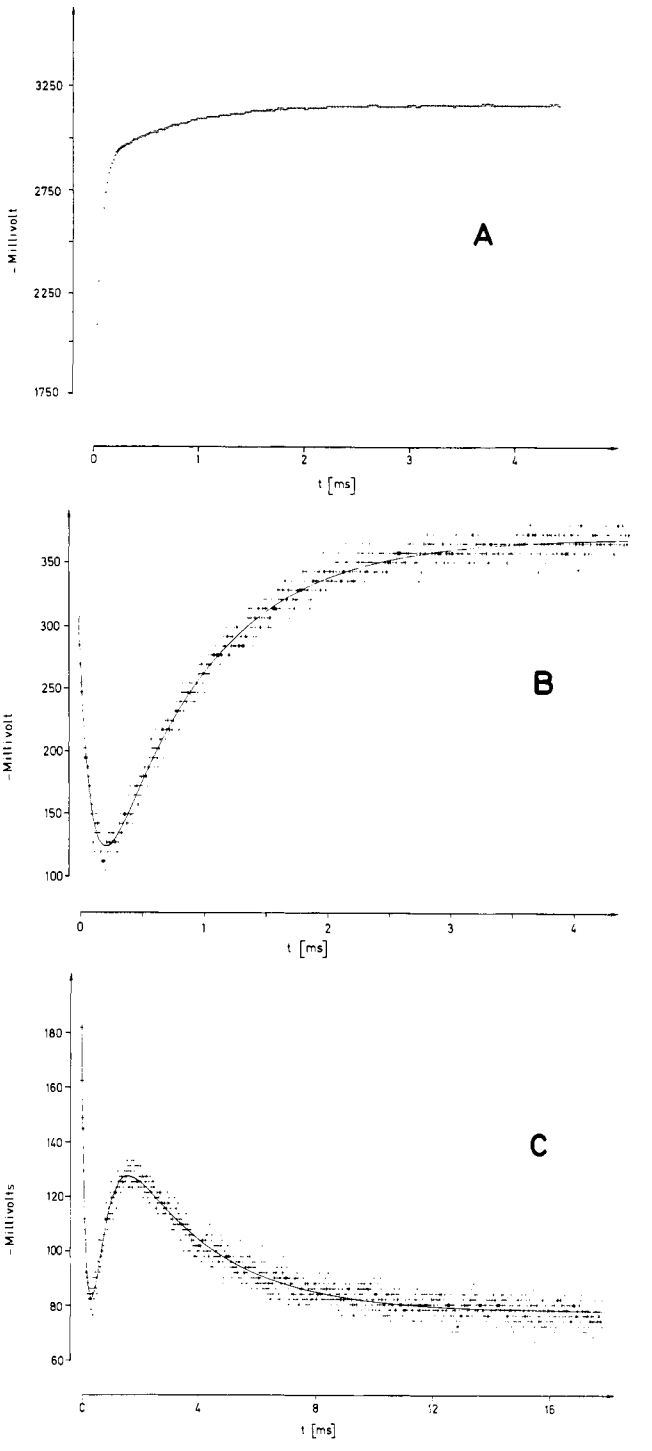

FIGURE 1: Fluorescence-detected temperature-jump experiments with $tRNA^{Phe}$ (yeast). Final temperature was 7.2 °C. (A) 10 μM $tRNA^{Phe}$ in the absence of UUC, in buffer AB. (B) 10 μM $tRNA^{Phe}$ in the presence of 0.8 mM UUC, in buffer AB. (C) 10 μM $tRNA^{Phe}$ in the presence of 0.1 mM UUC, in buffer ABT. Solid line in (B) and (C) is a calculated relaxation curve representing the best fit of the experimental points according to the fitting program (Provencher, 1976). In (B) and (C) the fast signal corresponding to the unspecific quenching of the fluorescence was removed by an electronic zero suppress circuit (Rabl, 1976).

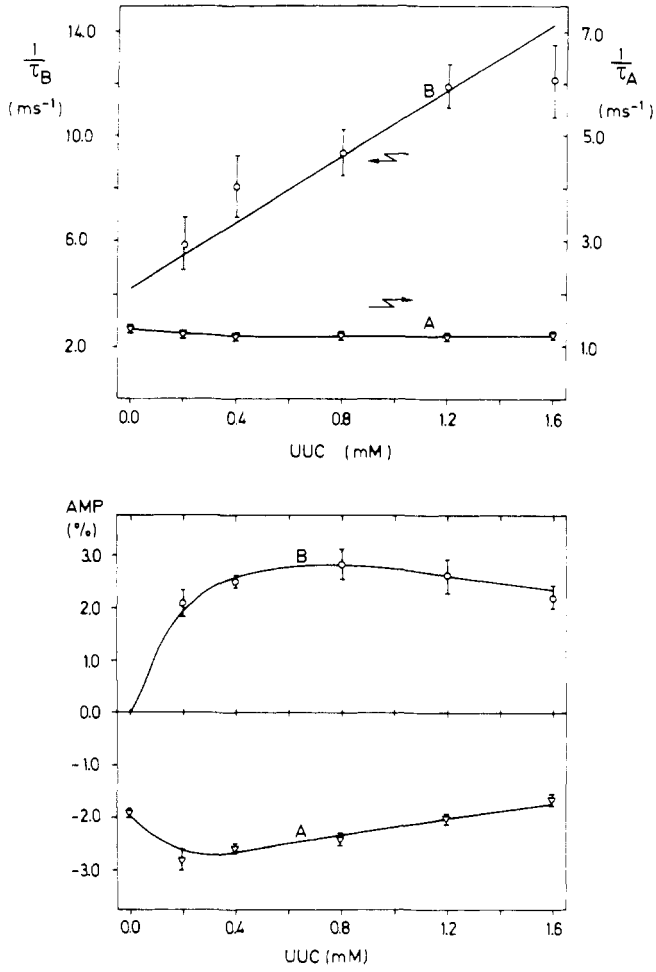

FIGURE 2: c_{UUC} dependence of reciprocal relaxation time ($1/\tau$) and relative relaxation amplitude (AMP) measured in buffer AB. Final temperature was 3.4 °C. Lines connect calculated points on the basis of parameters summarized in Table I.

describing these data assumes the binding of the codon to a single anticodon conformation according to

$$tRNA_H + UUC \underset{k_-^B}{\overset{k_+^B}{\rightleftharpoons}} C \qquad (2)$$

The amplitudes and time constants observed in buffer AB were fitted according to the coupled reactions 1 and 2 by using a program that was developed especially for this purpose (unpublished results). The quality of the fit, illustrated in Figure 2, demonstrates that the coupled mechanism (eq 1 + 2) provides a correct description of UUC binding to $tRNA^{Phe}$ under the conditions of buffer AB. The data obtained from the fitting procedure are compiled in Table I.

As usual in kinetics, we cannot absolutely prove that our assignment of the mechanism is correct. However, we can state with certainty that the mechanism presented is the simplest one consistent with the experimental data. The accuracy of the parameters given in Table I is quite different for the various categories. Some of them, like the rate constant k_+^B, can be defined with relatively high accuracy. Other sets of parameters show strong mutual coupling. This is observed for the ΔH values and the relative quantum yields. This uncertainty could partially be reduced by using the information obtained at the two different temperatures. However, a more accurate determination of the ΔH values requires more measurements. The error limits given in Table I are estimated by fitting the data keeping one parameter fixed and evaluating the sum of errors as a function of the "fixed" parameter.

Table 1

(a) Thermodynamic and Kinetic Parameters of UUC Binding to $tRNA^{Phe}$ in Buffer AB

step	temp (°C)	k_+^a	k_-^a	K^a	ΔH (kcal/mol)
B	3.4	(5.0 ± 1.0) × 10^6	2600 ± 500	1900 ± 450	−9.8 ± 5.0
B	7.2	(6.2 ± 1.0) × 10^6	4200 ± 800	1500 ± 350	−9.8 ± 5.0
A	3.4	740 ± 150	93 ± 25	8.0 ± 2.5	−3.9 ± 2.0
A	7.2	1160 ± 250	145 ± 40	8.0 ± 2.5	−3.9 ± 2.0

(b) Quantum Yields of $tRNA_F$ and $tRNA_H$ Relative to That of Complex C

temp (°C)	q_F/q_C	q_H/q_C
3.4	0.03 ± 0.03	1.26 ± 0.15
7.2	0.01 ± 0.01	1.28 ± 0.15

[a] Dimensions: k_+^A (s^{-1}), k_+^B (M^{-1} s^{-1}); k_-^A and k_-^B (s^{-1}); K^A (-) and K^B (M^{-1}).

Relaxation Experiments in Buffer ABT. Codon binding to $tRNA^{Phe}$ has been studied previously by various methods (Yoon et al., 1975; Geerdes et al., 1978; Geerdes, 1979; Eisinger et al., 1971; Pongs & Rheinwald, 1973). Using the dialysis technique, Gassen and co-workers obtained evidence for codon-induced unfolding of the tRNA (Schwarz et al., 1976; Schwarz & Gassen, 1977) in the presence of 30S ribosomes. The unfolding was only observed at a characteristic ion composition, especially with respect to Mg^{2+}. Recently, unfolding of the TΨC loop in $tRNA^{Lys}$ induced by its codon AAA was observed also in the absence of 30S ribosomes. Again the reaction required a characteristic ion composition (Möller et al., 1979). Thus, experiments were also conducted under these conditions (buffer ABT). An example of the relaxation observed is given in Figure 1C, showing the appearance of a separate third process. This process is slower than that associated with the transitions A and B. Its amplitude is opposite that of transition A and in the same direction as that of process B. Since it is probably associated with unfolding of tRNA tertiary structure, we will call this process T. The dependence of relative amplitudes and time constants upon c_{UUC} for the three relaxation processes observed in buffer ABT is given in Figure 3.

Since the appearance of transition T is very sensitive to the ion composition and in particular to the Mg^{2+} level, amplitudes and time constants were studied as a function of c_{Mg}. These experiments were performed at constant c_{UUC} (0.2 mM) and c_{tRNA} (10 μM) in a buffer containing 50 mM Tris–cacodylate and 100 mM $NaClO_4$ (pH 7.2). In close correspondence to the data reported in the literature (Möller et al., 1979; Schwarz et al., 1976; Schwarz & Gassen, 1977), the T process is observed only at relatively high c_{Mg}. In order to derive the extent of process T as a function of c_{Mg}, the amplitudes found for the T process have to be normalized with respect to the extent of UUC binding, which will increase itself with increasing c_{Mg}. This correction was performed in an approximate way by presenting the amplitude observed for process T relative to that observed for transition B (cf. Figure 4). The data presented in Figure 4 support the assignment of process T to the unfolding reaction observed by Gassen and co-workers (Möller et al., 1979; Schwarz et al., 1976; Schwarz & Gassen, 1977). Moreover, the behavior of $1/\tau_T$ as a function of c_{UUC} supports the assignment of process T to an intramolecular conversion of complexed $tRNA^{Phe}$. Thus, the reaction scheme for the binding of UUC to $tRNA^{Phe}$ under the conditions of buffer ABT seems to be

$$tRNA_H + UCC \overset{B}{\rightleftharpoons} C_1 \overset{T}{\rightleftharpoons} C_2 \qquad (3)$$

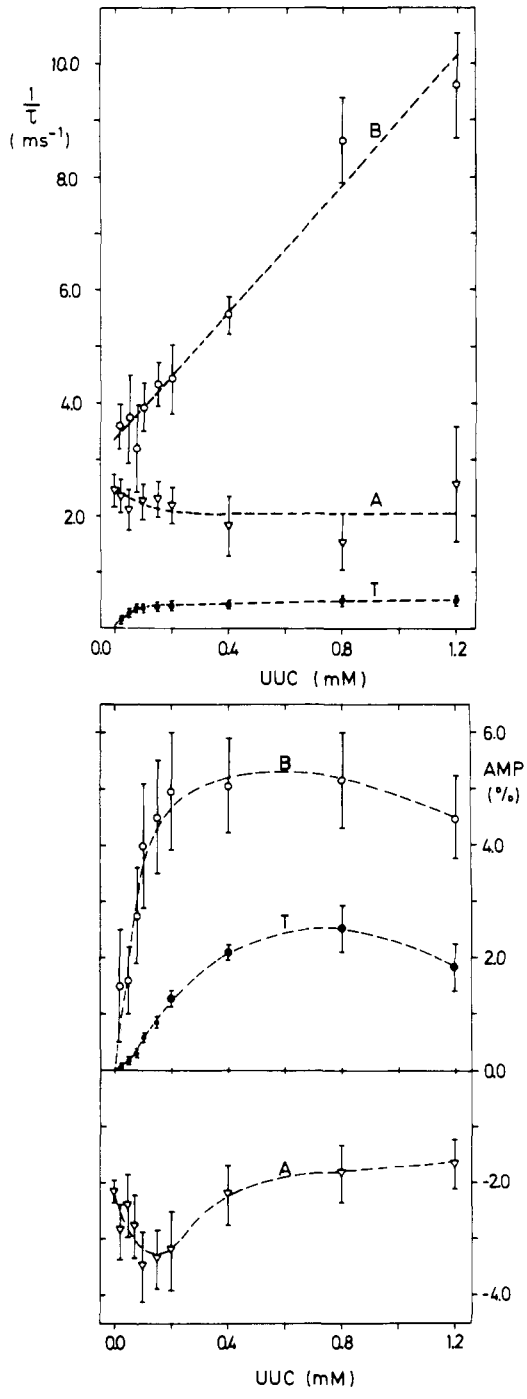

FIGURE 3: c_{UUC} dependence of reciprocal relaxation time ($1/\tau$) and amplitude (AMP) measured in buffer ABT. Final temperature was 7.2 °C. Dashed lines show the apparent behavior of reciprocal relaxation times and amplitudes.

According to this scheme, the relaxation time and the *relative* amplitude associated with process T should not depend upon c_{tRNA}, when c_{UUC} is constant and much higher than c_{tRNA}. As shown in Figure 5, the expected result is obtained for the relaxation time but not for the relative amplitude. The relative amplitude increases with increasing c_{tRNA} and thus indicates an association reaction of tRNA molecules. Usually an association reaction is also reflected in a concentration dependence of the corresponding relaxation time constant. The absence of this concentration dependence may be explained by the following model. We assume that the observed association reaction is a dimerization of tRNA species C_2. If this dimerization is a fast reaction, the time constant of the observed relaxation process will be mainly determined by the transition T from species C_1 to C_2. Thus, in accordance with the experimental data, the relaxation time constant is independent of c_{tRNA}, whereas the amplitude increases with c_{tRNA} as long as c_{tRNA} is below $1/K_{assn}$, where K_{assn} is the association constant for dimerization. The complete reaction scheme is

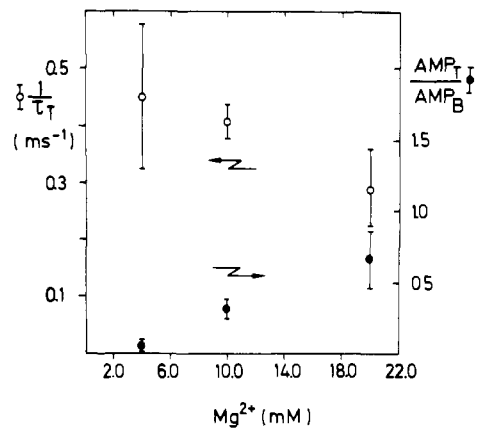

FIGURE 4: c_{Mg} dependence of reciprocal relaxation time ($1/\tau_T$) associated with process T and of the ratio of the relaxation amplitude (AMP_T) to the amplitude of the bimolecular process (AMP_B); in 50 mM Tris-cacodylate and 100 mM $NaClO_4$, pH 7.2; for 10 μM $tRNA^{Phe}$ in the presence of 0.2 mM UUC. Final temperature was 7.2 °C.

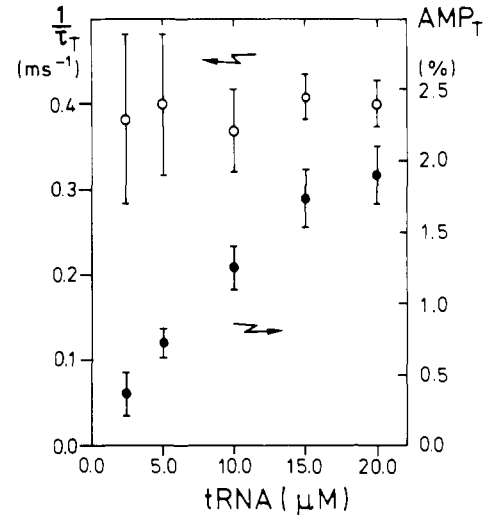

FIGURE 5: c_{tRNA} dependence of reciprocal relaxation time ($1/\tau_T$) and amplitude (AMP_T) associated with process T in the presence of 0.2 mM UUC. Final temperature was 7.2 °C.

$$tRNA_F \overset{A}{\rightleftharpoons} tRNA_H$$

$$tRNA_H + UCC \overset{B}{\rightleftharpoons} C_1 \overset{T}{\rightleftharpoons} C_2$$

$$C_2 + C_2 \overset{D}{\rightleftharpoons} D \qquad (4)$$

This scheme has four independent reaction steps, and consequently it should be possible to observe four separate relaxation processes. Thus, the observation of only three relaxation processes may be considered as an argument against the validity of scheme 4. However, the observation of only three processes may be easily explained as follows. According to the dependence of AMP_T upon c_{tRNA}, it is possible that the transition T itself is not associated with a measurable change of fluorescence. The transition T is mainly indicated by the subsequent dimerization D, and the observed slow relaxation process is due to coupling the reactions T and D. If the dimerization reaction D is associated with a relatively small

ΔH and furthermore is a relatively fast reaction as proposed above, the relaxation directly associated with process D is expected to be hidden in the fast and large unspecific fluorescence quench preceding the observed relaxation process.

We also have to consider the following reaction scheme, which does not involve the intramolecular T transition:

$$\mathrm{tRNA_F} \overset{A}{\rightleftharpoons} \mathrm{tRNA_H}$$

$$\mathrm{tRNA_H} + \mathrm{UCC} \overset{B}{\rightleftharpoons} \mathrm{C}$$

$$\mathrm{C} + \mathrm{C} \overset{D}{\rightleftharpoons} \mathrm{D} \qquad (5)$$

In this case the observed number of relaxation processes directly corresponds to the number of reaction steps. The reaction D is expected to be mainly reflected by the relaxation process T and the rather low dependence of τ_T upon c_{tRNA} is explained by a relatively large dissociation term k_-^D compared to the recombination term $4k_+^D \times c_C$. At present it seems to be difficult, however, to find a reasonable set of parameters for a quantitative representation of the experimental data according to this model. The concentration dependence of AMP_T upon c_{tRNA} suggests a relatively high equilibrium constant ($\geq 2 \times 10^4$ M^{-1}) for the tRNA dimerization. From this number and from the observed relaxation time constants, we have to conclude that the rate constant for dimer formation is also relatively high ($\geq 8 \times 10^6$ M^{-1} s^{-1}). Thus, we should find a measurable concentration dependence of τ_T upon c_{tRNA}. Since we did not find this effect in our experiments, we conclude that reaction scheme 5 is not likely to provide a reasonable quantitative description of the experimental data. A final decision about the validity of reaction schemes 4 and 5 will be possible by additional measurements and quantitative data fitting. This work is in progress. At present reaction scheme 4 should only be taken as a working hypothesis, which explains our present results qualitatively and will be useful as a basis for further experimental and theoretical work.

Test for the Influence of Anions. The experiments described above have been performed in buffers containing perchlorate as anions. Since perchlorates are known as "chaotropic" reagents, we have tested for any particular influence of perchlorate on the reactions found in the present investigation. For this purpose, perchlorate was exchanged against sulfate both in buffer AB and ABT, keeping the Na^+ concentration constant in both cases. Temperature-jump experiments using 10 μM $tRNA^{Phe}$ + 0.8 mM UUC in these buffers showed relaxation phenomena closely corresponding to those observed in the presence of perchlorate. Thus, any influence due to the chaotropic nature of perchlorate is relatively small under the conditions of the present experiments.

Equilibrium Sedimentation Experiments. The relaxation experiments demonstrate an association reaction of $tRNA^{Phe}$ molecules induced by UUC binding under the conditions of buffer ABT. This conclusion can be tested independently by centrifugation experiments. Figure 6 shows the result of an equilibrium centrifugation experiment performed with $tRNA^{Phe}$ samples in the absence and in the presence of 0.5 mM UUC as well as with a control sample containing 0.5 mM AAA. The slope of the plot of ln c vs. r^2 (where c is the c_{tRNA} at the distance r from the rotor axis) is directly related to the apparent molecular weight (M_{app}) of the sample. The experiments were done essentially as described by Yang et al. (1972). M_{app} values calculated for the sample of $tRNA^{Phe}$ alone and for the control sample are $(24.2 \pm 1.0) \times 10^3$ and $(25.0 \pm 1.0) \times 10^3$, respectively. The plot obtained for $tRNA^{Phe}$ in the presence of 0.5 mM UUC shows a small deviation from linearity (cf. Figure 6), which indicates the

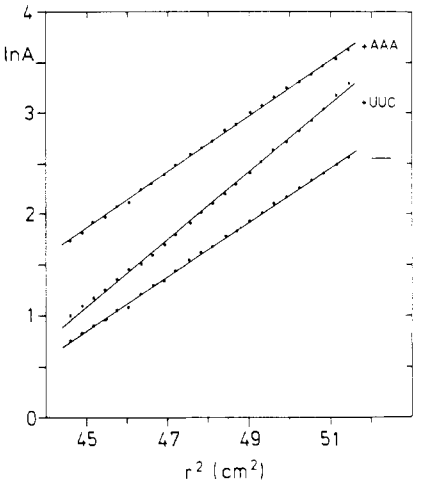

FIGURE 6: Plot of ln A ($A \equiv c_{tRNA}$, measured as optical density at 310 nm) vs. r^2 (squared distance from the rotor axis) for $tRNA^{Phe}$ alone and in the presence of 0.5 mM UUC or 0.5 mM AAA in buffer ABT, measured at 5 °C and 9990 rpm. In all samples c_{tRNA} = 25 μM. For clarity, the plot of tRNA + UUC and tRNA + AAA samples was shifted on the y axis by addition of ln A = 0.5 and ln A = 1.0, respectively, to all experimental points.

sedimentation of more than one species. When the experimental data are approximated by a straight line, the resulting apparent molecular weight is higher by 24% than that of $tRNA^{Phe}$ in the absence of UUC. Thus, the codon binding promotes an association of tRNA molecules.

Discussion

Comparison to Previous Investigations. The kinetics of UUC binding to $tRNA^{Phe}$ (yeast) has been analyzed previously by the same technique under almost identical solvent conditions and yet only a single relaxation process was observed (Yoon et al., 1975). Our separation and quantitation of up to three relaxation processes were rendered possible by the development of a sensitive fluorescence temperature-jump instrument (Rigler et al., 1974) and in particular thanks to further improvements in optics and electronics by C. R. Rabl. The rate constant derived previously (Yoon et al., 1975) for the UUC binding to $tRNA^{Phe}$ ($k = 2 \times 10^5$ M^{-1} s^{-1}) is rather unusual, since it is an order of magnitude lower than those found for complementary oligonucleotides (Pörschke, 1977). Thus, the values obtained in the present investigation for the buffer AB (a preliminary fit of the data for buffer ABT yielded similar values) are more satisfactory, since they do not deviate from "conventional" rates of oligomer double-helix formation. In this connection, the rate constant derived by Yoon et al. (1975) for UUCA ($k = 2 \times 10^7$ M^{-1} s^{-1}) is quite revealing: it is very close to the conventional ones (Pörschke, 1977) and apparently its measurement was not perturbed by any additional, unresolved relaxation process. It must be concluded that UUCA binding to $tRNA^{Phe}$ does not induce any conformation change similar to that induced by UUC. Further evidence for this conclusion comes from NMR measurements, which demonstrate more extensive changes in the proton and phosphorus resonances of $tRNA^{Phe}$ upon binding of UUC than of UUCA (Geerdes, 1979).

The presence of two distinct conformations of the $tRNA^{Phe}$ anticodon loop was first demonstrated by relaxation measurements (Urbanke & Maass, 1978). These results are confirmed by the present investigation. Moreover, independent evidence for the presence of two conformations is obtained by lifetime and polarization measurements of the Wye base fluorescence (Ehrlich et al., 1980; H. Grosjean, private communication). These data are interpreted in terms of a stacked

and an unstacked conformation of the Wye base (Ehrlich et al., 1979), which may correspond to the F and H forms of $tRNA^{Phe}$ (cf. eq 1) associated with low and high fluorescence quantum yields, respectively (cf. Table I). Recent relaxation measurements with ethidium derivatives of $tRNA^{Phe}$ provided evidence for the existence of three conformations (Ehrenberg et al., 1979). The equilibrium between these conformations was found to be strongly dependent upon c_{Mg}.

The relaxation data obtained in buffer ABT suggest a relatively high equilibrium constant for the association of $tRNA^{Phe}$ in the presence of UUC, since the corresponding effect is observed already at a c_{tRNA} of 2.5 μM (cf. Figure 5). Association of $tRNA^{Phe}$ was previously noticed at millimolar c_{tRNA} (Geerdes, 1979). At present we cannot exclude the possibility that base pairing between the tRNA anticodon and the open TΨC sequence of another tRNA molecule is the driving force for dimerization. However, this particular mechanism is feasible only in the case of $tRNA^{Phe}$. Preliminary results indicate that there is also dimerization in $tRNA^{Lys}$ from *Escherichia coli* induced by the presence of its codon oligomer AAA. The effect is smaller than that observed in $tRNA^{Phe}$ but nevertheless suggests that the association of tRNAs induced by codons is a general phenomenon.

Tentative Assignment of Reaction Steps to Structure Changes. Process A is an intramolecular transition, which is observed already in $tRNA^{Phe}$ alone. Since the transition is indicated by the fluorescence of the Wye base located in the anticodon loop and is influenced by codon binding, it must be associated with a conformation change of the anticodon loop. The relatively long relaxation time (~1 ms) associated with this transition and its absence in the hexanucleotide G_m-A-A-Y-A-ΨP (Urbanke & Maass, 1978) show that it is not just a simple stacking reaction of the Wye base. It is therefore very plausible to follow the arguments of our precedents (Urbanke & Maass, 1978) and assign tentatively transition A with a conversion between the 3′- and 5′-stacked conformations of the anticodon loop proposed by Fuller & Hodgson (1967). According to our results, UUC preferentially binds to the conformer H, which is likely to be the 3′-stacked conformation, because it is highly fluorescent and predominates in solution (Rich & RajBhandary, 1976; Langlois et al., 1975).

Process T is induced by codon binding in a very specific ionic environment. The absence of process T in buffer AB and its presence in buffer ABT can certainly not be explained on the basis of the ionic strength and indicate that the details of the ion composition, for instance the ratio of Mg^{2+} and Na^+ concentrations, are more important (Labuda et al., 1977). The dependence of the process T upon the ion composition is the best indication of its nature. In a series of investigations using equilibrium dialysis and spectrophotometric titrations, Gassen and co-workers (Möller et al., 1979; Schwarz et al., 1976; Schwarz & Gassen, 1977) provided evidence that codon binding to tRNA induces unfolding of TΨC loop. The unfolding reaction described by Gassen and co-workers exhibits the same Mg^{2+} dependence as that of the transition T observed in the present investigation. Thus, it is very likely that the transition T corresponds to the unfolding of the TΨC loop. Apparently the base pairs formed by the TΨC loop are crucial for the overall shape of the tRNA molecules. It may be expected that the dissociation of these base pairs leads to a change in the arrangement of the cloverleaf arms. This change may then be responsible for the association of tRNA molecules.

Biological Implications. The reaction steps observed in the present investigation may be directly involved in the translation process at the ribosome and could be important for a high fidelity of the codon recognition. Because of space limitations, we cannot discuss these aspects in any detail. Probably the most important result of the present investigation is the codon-induced tRNA association. The binding of two tRNA molecules close to each other at the ribosome implies an intimate contact between these rather large molecules. This can be achieved only if the repulsive force between the tRNAs e.g., by the negatively charged phosphates, is not too large. The present results even demonstrate the existence of attraction between tRNAs when a particular conformation is induced by the recognition of a cognate codon. This reaction may have been extremely important during an early stage of evolution, when the translation of a message was not yet supported by ribosomes. The affinity between two adjacent tRNAs bound to a message may increase the lifetime of that complex and make the synthesis of peptide bonds more probable.

The observation of a rather complex reaction scheme for the binding of a cognate codon to a tRNA in solution demonstrates a more active role of tRNA molecules in translation than anticipated. The present results support the view that tRNAs are nucleic acid molecules with characteristics similar to enzymes. Furthermore, the present results demonstrate that the different functional states of tRNA do not require the ribosome for their expression but can be characterized in solution by using relatively simple model systems.

Acknowledgments

We are indebted to R. Clegg and H. Grosjean for critical comments on the manuscript. We would like to thank R. Austin, R. Clegg, D. Hellwig, M. Jung, C. R. Rabl, G. Striker, and R. Weise for their help during measurements and analysis of relaxation curves. We are also indebted to H. Faulhammer and M. Sprinzl for samples of highly purified $tRNA^{Phe}$.

References

Clark, B. F. C. (1977) *Prog. Nucleic Acid Res. Mol. Biol. 20*, 1–19.

Davanloo, O., Sprinzl, M., & Cramer F. (1979) *Biochemistry 18*, 3189–3199.

Ehrenberg, M., Rigler, R., & Wintermeyer, M. (1979) *Biochemistry 18*, 4588–4599.

Ehrlich, R., Lefevre, J. F., & Remy, P. (1980) *Eur. J. Biochem. 103*, 145–153.

Eisinger, J., Feuer, B., & Yamane, T. (1971) *Nature (London) 231*, 126–128.

Fuller, W., & Hodgson, A. (1967) *Nature (London) 215*, 817–821.

Geerdes, H. A. M. (1979) Ph.D. Thesis, University of Nijmwegen.

Geerdes, H. A. M., Van Boom, J. H., & Hilbers, G. W. (1978) *FEBS Lett. 88*, 27–32.

Grosjean, H. J., Söll, D. G., & Crothers, D. M. (1976) *J. Mol. Biol. 103*, 499–519.

Labuda, D., Haertlé, T., & Augustyniak, J. (1977) *Eur. J. Biochem. 79*, 293–301.

Langlois, R., Kim, S. M., & Cantor, R. Ch. (1975) *Biochemistry 14*, 2554–2558.

Möller, A., Wild, U., Riesner, D., & Gassen, H. G. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 3266–3270.

Ofengang, J., & Henes, C. (1969) *J. Biol. Chem. 244*, 6241–6253.

Pongs, O., & Rheinwald, E. (1973) *Biochem. Biophys. Res. Commun. 50*, 357–367.

Pörschke, D. (1977) in *Chemical Relaxation in Molecular Biology* (Pecht, I., & Rigler, R., Eds.) pp 191–218, Springer-Verlag, West Berlin.

Provencher, S. W. (1976) *Biophys. J. 16*, 27–41.
Rabl, C. R. (1976) *NATO Adv. Study Inst. Ser., Ser. C 50*, 77–82.
RajBhandary, U. L., & Chang, S. M. (1968) *J. Biol. Chem. 243*, 598–608.
Rich, A., & RajBhandary, U. L. (1976) *Annu. Rev. Biochem. 45*, 805–860.
Rigler, R., Rabl, C. R., & Jovin, T. M. (1974) *Rev. Sci. Instrum. 45*, 580–588.
Schwarz, U., & Gassen, H. G. (1977) *FEBS Lett. 78*, 267–270.
Schwarz, V., Menzel, M. M., & Gassen, H. G. (1976) *Biochemistry 15*, 2484–2490.
Shimuzu, N., Hayashi, H., & Miura, K.-J. (1969) *J. Biochem. (Tokyo) 67*, 373–387.
Sprinzl, M., Wagner, T., Lorenz, S., & Erdmann, V. (1976) *Biochemistry 15*, 3031–3039.
Urbanke, C., & Maass, G. (1978) *Nucleic Acids Res. 5*, 1551–1560.
Wagner, R., & Garrett, R. A. (1979) *Eur. J. Biochem. 97*, 615–621.
Woese, C. (1970) *Nature (London) 226*, 817–820.
Yang, S. K., Söll, D. G., & Crothers, D. M. (1972) *Biochemistry 11*, 2311–2320.
Yoon, K., Turner, D. H., & Tinoco, J., Jr. (1975) *J. Mol. Biol. 99*, 507–518.

Structure–Volume Relationships: Singular Volume Effects Produced by Cupric Ion–Globular Protein Interaction†

Sam Katz,* Roberta G. Shinaberry, Evelyn L. Heck, and William Squire

ABSTRACT: The nature of the volume isotherms produced by the coordination of Cu(II) with ovalbumin and bovine serum albumin differs substantially from the adsorption isotherms produced by these systems. Whereas there was increased binding of Cu(II) associated with a pH increase from pH 5.3 to pH 7.4, the volume isotherms for these systems did not exhibit this type of pH dependence. The volume changes were determined at 30.0 ± 0.001 °C with microdilatometers which could be read to 0.01 μL. The binding isotherms for ovalbumin at pH 5.3 and 7.4 and for bovine serum albumin at pH 5.3 was resolved by a Scatchard plot to yield the appropriate thermodynamic parameters. An algorithm was derived to calculate the distribution of the individual PM_i complexes, i.e., $PM_{i-1} + M \rightleftharpoons (K_i)\ PM_i$ where i equals 1, 2, 3, ..., n moles of cation, M, bound per mole of protein, P, for the above systems. The volume isotherms were then resolved in terms of the constituent ΔV_i terms, i.e., the volume change produced by the formation of the individual PM_i complexes. These values were verified by an independent graphical differentiation procedure. The coordination of Cu(II) to BSA at pH 7.4 produced a cooperative adsorption isotherm which was not amenable to a Scatchard analysis. The resultant anomalous volume isotherm was resolved into a component related to Cu(II)–site interaction and a negative volume effect attributable to a conformational change induced by complex formation. This structural transition which occurs at physiological pH may constitute a control mechanism for regulating the serum level of Cu(II) and possibly other divalent ions.

The conversion of an apometalloenzyme from the inactive to active form by combination with a specific metal ion effector is considered to be a consequence of a structural transition engendered by the coordination process. The mechanism of this conversion is a matter of conjecture. Insight with respect to the mechanism of this activation process can be gained from dilatometric studies which may provide information pertinent to the type of ligands involved, the change of hydration states of the reactants, and the possible occurrence of conformational changes. A necessary prerequisite is to establish a frame of reference, namely, by determining the volume effects produced by nonspecific binding, i.e., the ΔV[1] resulting from metal ion interaction with a protein. This consideration prompted this dilatometric study of the coordination of Cu(II) with BSA and ovalbumin as a function of pH. To interpret these volume effects, we have determined the volume changes produced by complexing of Cu(II) to specific ligands and to the peptide backbone.

Ovalbumin, M_r 45 040, and BSA, M_r 66 210, were selected because they are proteins with isoionic points in the region of pH 5 (Katz & Ellinger, 1963). These water-soluble proteins differ in several respects: ovalbumin contains about two phosphoserine groups and the N-terminal amino residue is blocked by an acetyl group (Marshall & Neuberger, 1972). Dilatometric titration of this protein by H^+ and OH^- produced volume isotherms which did not exhibit any demonstrable structural transitions in the pH region of 2–11 (Katz & Miller, 1971a). BSA, on the other hand, upon dilatometric titration gave evidence for at least three structural transitions as a

† From the Department of Biochemistry (S.K. and R.G.S.), the Department of Anesthesiology (E.L.H.), and the Department of Aerospace Engineering (W.S.), West Virginia University, Morgantown, West Virginia 26506. *Received January 28, 1980.* This research was supported in part by grants provided by the National Science Foundation (PCM 77-07708) and by the West Virginia Affliliate of the American Heart Association.

[1] Abbreviations used: ΔV, volume change; BSA, bovine serum albumin; ΔV_{exp}, experimentally determined volume change; ΔV_{23}, volume change produced by binding of solute, component 3, to protein, component 2; $(Cu_0)/(\text{protein})$, the number of moles of cupric ion added per mole of protein; ν, mean number of moles of cation bound per mole of protein as determined experimentally; n, total number of binding sites in a protein; pI, isoelectric pH; z^+ and z^-, anionic and cationic charge; S, siemens, a unit of conductivity.

0006-2960/80/0419-3805$01.00/0