Codon-Induced Association of the Isolated Anticodon Loop of tRNA^{Phe}

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Received April 7, 1986; Revised Manuscript Received June 12, 1986

ABSTRACT: The binding of the codon UUC to the isolated anticodon loop of tRNA^{Phe} (yeast) has been studied as a model of codon recognition by a simple adaptor. Fluorescence titrations demonstrate that UUC binds to the isolated anticodon loop with an equilibrium constant of 1.4×10^3 M^{-1} (at 7.2 °C). Equilibrium sedimentation curves reveal that UUC binding induces association of anticodon loops beyond the dimer stage. A set of complete sedimentation curves obtained for various reactant concentrations was analyzed according to a model with an infinite number of subsequent association steps for UUC–anticodon loop complexes and with equal affinity for each step. The coupling of association and sedimentation was considered quantitatively, and the information resulting from conservation of mass was used by integration. According to this procedure, the experimental data can be described by an isodesmic association constant of 8×10^3 M^{-1} with satisfactory accuracy. Temperature-jump relaxation detected by fluorescence measurements provides independent evidence for codon-induced association of the anticodon loop. The data are consistent with the following mechanism: UUC preferentially binds to one of two loop conformations with a rate constant of 4.5×10^6 M^{-1} s^{-1}; the UUC–anticodon loop complex undergoes association with a rate constant of 6.5×10^6 M^{-1} s^{-1}. The reactions observed for the isolated anticodon loop are surprisingly similar to those observed previously for the complete tRNA, suggesting that simple hairpin loops are appropriate adaptors for a translation process at an early stage of evolution; the codon-induced association of the hairpin loop should be very useful to facilitate the transfer of cognate amino acids during translation.

The translation of the genetic message by the ribosomal complex involves a large number of coupled reactions, which are hardly understood in sufficient detail. A first approach for an investigation of the translation process is an analysis of separate reaction steps. According to this simple procedure, it has been shown recently that the key step of translation—the decoding reaction by tRNA adaptors—cannot be regarded as a "simple" base-pairing process, even in the absence of ribosomes. Temperature-jump experiments demonstrated that the recognition of the cognate codon UUC by tRNA^{Phe} (yeast) involves at least three reaction steps (Labuda & Porschke, 1980; Labuda et al., 1984): (1) a transition between two conformations of the anticodon loop; (2) preferential binding of the codon to one of these conformations; and (3) dimerization of codon–tRNA complexes. The codon-induced dimerization of tRNA molecules could also be demonstrated independently by NMR spectroscopy for tRNA^{Phe} (yeast) (Geerdes et al., 1980; Clore et al., 1984) and by analytical ultracentrifugation for various cases including tRNA^{Phe} (yeast), tRNA^{Phe} (*Escherichia coli*), tRNA^{Lys}, and tRNA^{fMet} (Porschke & Labuda, 1982; Labuda & Porschke, 1983). This special property of tRNA molecules appears to be very attractive for the translation process, since close contact of tRNA adaptors induced by cognate codons should facilitate the peptidyl-transfer reaction. The codon-induced dimerization of tRNA also is a remarkable example for a directed functional complexity of a relatively small RNA molecule. This special reaction also raises some questions, e.g., for the nature of the interactions and for the parts of the tRNA molecule involved in the interactions. A simple approach to these questions is a further reduction of molecular complexity and an analysis of codon binding to an isolated anticodon loop. An appropriate candidate for such investigations is the anticodon loop of tRNA^{Phe} owing to the presence of the Wye base, which is very useful as a natural fluorescent label. We have studied the binding of the UUC codon to a pentadecamer prepared from the anticodon loop of tRNA^{Phe} by equilibrium titrations, equilibrium sedimentation, and temperature-jump experiments. The results demonstrate a surprising analogy of the reactions observed for the isolated anticodon loop with those characterized previously for the complete tRNA molecule.

Materials and Methods

The pentadecamer 5′-r(C-A-G-A-C_m-U-G_m-A-A-Y-A-Ψ-m^5C-U-G) and UUC were prepared as described by Bujalowski et al. (1986) and Labuda and Porschke (1980), respectively. Fluorescence titrations, equilibrium sedimentation, and fluorescence-detected temperature-jump experiments were performed as described by Labuda and Porschke (1980), Porschke and Labuda (1982), and Bujalowski et al. (1986). The standard buffer used for all measurements (unless specified otherwise) contained 100 mM $NaClO_4$, 50 mM tris(hydroxymethyl)aminomethane–cacodylate (Tris–cacodylate), pH 7.1, and 10 mM $Mg(ClO_4)_2$. The error bars given in the figures have been estimated from the deviations observed upon repetition of the experiments.

Results and Discussion

UUC Binding Constant from Wye Base Fluorescence Quenching. As a basis for all further evaluations, a reliable value for the binding constant of UUC to the pentadecamer is required. For this purpose, the fluorescence intensity of the Wye base, located adjacent to the anticodon, has been measured as a function of the UUC concentration. Addition of UUC in the millimolar concentration range induces a clear decrease of the fluorescence intensity. The experimental data

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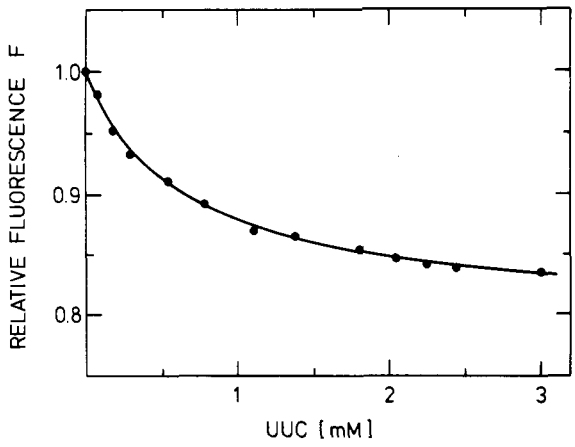

FIGURE 1: Relative fluorescence intensity (F) of the pentadecamer as a function of UUC concentration (standard buffer, 7.2 °C, 4.25 μM pentadecamer). The continuous line shows a least-squares fit according to a single-step binding mechanism providing an equilibrium constant $K_T = 1.4 \times 10^3$ M^{-1} and a fluorescence intensity of the complex relative to the free pentadecamer of 0.8.

(cf. Figure 1) can be fitted with high accuracy by a simple binding reaction:

$$\text{anticodon} + \text{UUC} \leftrightarrow \text{complex} \qquad (1)$$

with an association constant $K_T = 1.4 \times 10^3$ M^{-1} and a quantum yield of the complex relative to that of the free anticodon loop of 0.8 (standard buffer, 7.2 °C). Since the association constant has been determined from measurements at a very low pentadecamer concentration ($\sim$4 μM), its value is not affected by the process induced by codon binding, which is described below.

Codon-Induced Association of the Anticodon Loop from Equilibrium Sedimentation. As described previously for the case of tRNA^{Phe} (yeast) (Porschke & Labuda, 1982), equilibrium sedimentation profiles for the isolated anticodon loop have been determined in the long-wavelength tail of the spectrum, where only modified bases of the loop contribute to the absorbance and the absorbance of UUC remains negligible. Furthermore, it has been demonstrated that UUC binding to the anticodon loop only leads to minor changes of this absorbance. Because of these facts, the analysis of experimental data is simplified considerably.

Equilibrium sedimentation curves clearly show a UUC-induced increase of the apparent molecular weight of the anticodon loop, which is much higher than expected for simple binding of UUC. These data demonstrate a codon-induced association of the anticodon loop, in analogy to the previously described codon-induced association of tRNA molecules. Thus, we have attempted to fit the equilibrium sedimentation data by the model used previously for the case of tRNA (Porschke & Labuda, 1982). However, the model with a codon-induced dimerization fails to fit the data obtained for the isolated anticodon loop with sufficient accuracy. The deviations clearly indicate the presence of higher aggregates than the dimer, and thus the model has to be extended.

Although the experimental data unambiguously demonstrate that the model should include aggregates higher than the dimer, there are various ways to extend the model, none of which can be falsified by the present set of experimental data. For example, the experimental data may be described by an association scheme including dimers and trimers of the codon–anticodon loop complex. However, if a trimer is present, it is very likely that there are also still higher aggregates. For this reason, we decided to use an "isodesmic" reaction scheme with unlimited association of codon–anticodon loop complexes and equal contributions of free energy for each subsequent reaction step (for an exact definition, cf. eq 3 and 4 below). This model seems to be appropriate for the present system and also does not have more than a single adjustable parameter, which is a clear advantage for the evaluation.

Theoretical Basis. The first step in the sequence of reactions is binding of the codon triplet to the anticodon loop according to

$$T + L \leftrightarrow C \qquad (2)$$

with an equilibrium constant $K_T = c_C/c_T c_L$ where c_C, c_T, and c_L are the concentrations of the complex, triplet, and loop, respectively. The complex (C) undergoes association according to

$$C + C \leftrightarrow C_2$$
$$C_2 + C \leftrightarrow C_3 \qquad (3)$$

in an infinite series of reactions, which are all characterized by the same equilibrium constant:

$$K_c = c_{C_2}/c_C c_C = c_{C_3}/c_{C_2} c_C = \ldots \qquad (4)$$

In addition to these equilibrium conditions, the concentration of each species in the sedimentation equilibrium is subjected to a further condition describing the gradient in the centrifuge force field. This may be described conveniently by chemical potentials, e.g., for the loop:

$$\mu_L^T = \mu_L^0 + RT \ln c_L - \tfrac{1}{2} M_L (1 - \nu_L \rho) r^2 \omega^2 \qquad (5)$$

where μ_L^T and μ_L^0 are the total and zero chemical potential, respectively, c_L is the concentration at the distance r from the rotor center, ω is the angular velocity, M_L is the molecular weight, ν_L is the partial specific volume of L, and ρ is the density of the solvent. At equilibrium, the potential μ_L^T is constant over the cell ($d\mu_L^T/dr = 0$) and

$$\frac{d \ln c_L}{dr^2} = \frac{M_L(1 - \nu_L \rho)\omega^2}{2RT} = \xi_L \qquad (6)$$

Corresponding relations can be given for all other species, but each of them need not be considered explicitly because the system would then be overdetermined. In the case of the present experiments, the triplet (T) is in large excess with respect to the anticodon loop. Thus, c_T is not affected by complex formation, and the distribution of c_T along the centrifuge cell is given by

$$c_T(r) = c_T^0 \xi_T (r_b^2 - r_m^2) \left(\frac{e^{\xi_T r^2}}{e^{\xi_T r_0^2} - e^{\xi_T r_m^2}} \right) \qquad (7)$$

where c_T^0 is the concentration at homogeneous distribution and r_b and r_m are the radii of a sector-shaped cell at the bottom and at the meniscus, respectively. According to eq 5, the concentration of the loop (L) as a function of the radius r is given by

$$c_L(r) = e^{(\mu_L^T - \mu_L^0 + \xi_L)/RT} \qquad (8)$$

By use of eq 7 and 8 together with the appropriate laws of mass action, the concentration of each species can be calculated as a function of the radius r. The infinite series of associating species can be described conveniently as a sum of the loop molarity for each species:

$$\sum i c_i(r) = c_C(r) + 2c_{C_2}(r) + 3c_{C_3}(r) + \ldots =$$
$$c_C(r) + 2K_c c_C^2(r) + 3K_c^2 c_C^3(r) \ldots = c_C(r)/[1 - K_c c_C(r)] \qquad (9)$$

According to these relations, the experimental data may be fitted by a simple numerical procedure: starting from an estimated value for the term $\mu_L^T - \mu_L^0$, the concentrations of all species may be calculated as a function of the radius and

converted to a sedimentation profile by an absorbance coefficient, which is determined experimentally. The deviation between calculated and experimental data may then be minimized by application of a standard least-squares fitting routine, which provides optimal values for the binding constant K_c and the term $\mu_L^T - \mu_L^0$.

This procedure does not use any additional information resulting from the conservation of mass. The total concentration of species contributing to the sedimentation profile may be determined at the start of the ultracentrifuge run, when the species are still equally distributed. The sum obtained by this measurement should correspond to the integral of the profile obtained under sedimentation equilibrium. The integral of the loop molarity is calculated according to

$$(r_b^2 - r_m^2)c_L^t = \int_{r_m}^{r_b}[c_L(r) + c_C(r) + 2c_{C_2}(r) + 3c_{C_3}(r) + ...]2r\,dr = \frac{1}{\xi_L}e^{\mu_L^T-\mu_L^0}(e^{\xi_L r_b^2} - e^{\xi_L r_m^2}) + \frac{1}{K_c\xi_C}\left\{\frac{1}{1 - K_c e^{\mu_C^T-\mu_C^0+\xi_C r_b^2}} - \frac{1}{1 - K_c e^{\mu_C^T-\mu_C^0+\xi_C r_m^2}}\right\} \quad (10)$$

where c_L^t is the total loop molarity at homogeneous distribution. Using this relation together with equations given above, the term $\mu_L^T - \mu_L^0$ may be calculated as a root of a third-order polynomial, and thus only a single parameter K_c remains to be determined from the experimental data.

Evaluation. A set of experimental data obtained at various concentrations of the anticodon loop and of UUC, shown in Figure 2, provides a binding constant $K_c = 8 \times 10^3$ M^{-1}. Similar values were obtained from other data sets. The experimental data were also fitted according to a cooperative isodesmic model, where the first step of association in the infinite series of reactions is described by a binding constant, which is different from the constants for all subsequent steps. These fits demonstrate, however, that the binding constant for the first step is equivalent to that for the subsequent steps within the limits of experimental accuracy.

When the equilibrium sedimentation curves for the pentadecamer in the presence of UUC were fitted by the "simple" codon-induced dimerization model (Porschke & Labuda, 1982), the error sum was higher by usually 50–100% than that found for the isodesmic model. The equilibrium constant for codon-induced dimerization evaluated according to the simple model is 2×10^4 M^{-1}.

Reaction Mechanism from Fluorescence-Detected Temperature-Jump Experiments. The temperature-jump technique proved to be very useful for an analysis of the reactions involved in codon binding to $tRNA^{Phe}$ (yeast). Thus, a similar analysis has been performed for the binding of UUC to the isolated anticodon loop. As described separately (Bujalowski et al., 1986), temperature-jump experiments using the fluorescence of the Wye base demonstrate the existence of conformation changes in the anticodon loop and probably also in the helix stem, which are coupled to the binding of Mg^{2+} ions. The present measurements were performed at relatively high Mg^{2+} concentrations, where one of the transitions is driven completely to one side corresponding to one conformational state. A second transition appearing at higher Mg^{2+} concentration is still reflected by a process which has been assigned to a conversion between an inner- and an outer-sphere complex of Mg^{2+} in the anticodon loop.

When the codon triplet is added to the system, an additional "fast" relaxation effect is observed, which is attributed to the binding reaction of UUC to the anticodon. This effect has been identified over a broad range of UUC concentrations. Since the UUC concentration (0.15–2 mM) was much higher than that of the pentadecamer (3.4 μM), the binding parameters were evaluated directly from the exactly linear dependence of the reciprocal time constant upon the UUC concentration according to

$$1/\tau = k^+[UUC] + k^- \quad (11)$$

providing an association rate constant $k^+ = 5.7 \times 10^6$ M^{-1} s^{-1}, a dissociation rate constant $k^- = 4.4 \times 10^3$ s^{-1}, and a stability constant $k^+/k^- = 1300$ M^{-1} (standard buffer, 7.2 °C). Both the binding constant and the association rate constant are similar to those obtained previously for the case of the complete $tRNA^{Phe}$ (Labuda et al., 1984). The association rate constant is also consistent with standard rate constants observed for the formation of short double helices (Porschke, 1977).

Variation of the anticodon loop concentration at a constant excess of UUC demonstrates the existence of a third relaxation effect. Since the time constant of the third effect is rather close to that observed in the absence of UUC, it cannot be easily separated. However, its existence can be clearly demonstrated by an increase of the amplitude with increasing anticodon loop concentration at a constant excess of UUC. This result provides independent evidence for a codon-induced association of the anticodon loop. Although the existence of three independent relaxation effects can be clearly demonstrated and can also be assigned unambiguously, the quantitative analysis is relatively difficult due to the appearance of the time constants in a very narrow range.

Since the separation of relaxation processes with similar time constants is notoriously difficult, we had to avoid the intermediate step of exponential fitting and use a procedure with a direct fit of the model to the experimental data. For this procedure, a reaction model is developed according to the observed relaxation effects. The relaxation time constants are

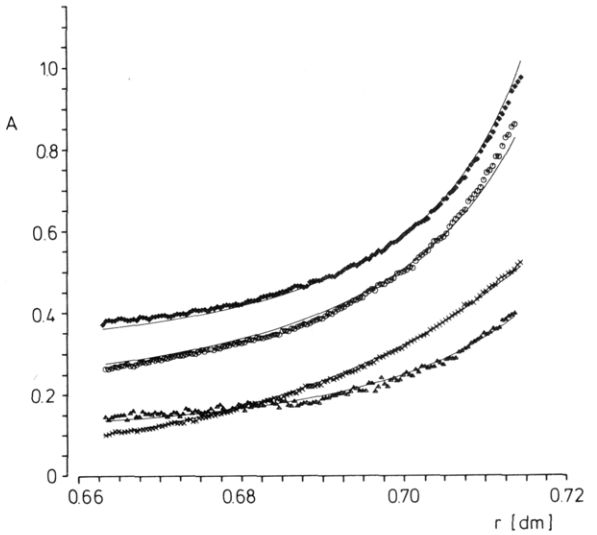

FIGURE 2: Absorbance (A) of the pentadecamer at 313 nm as a function of the radius r at sedimentation equilibrium for various pentadecamer and UUC concentrations, respectively: (×) 65.5 μM, 0 mM; (△) 30.6 μM, 1 mM; (○) 65.6 μM, 0.5 mM; (♦) 65.5 μM, 1 mM. The continuous lines represent a joint least-squares fit according to the isodesmic model with an association constant of 8×10^3 M^{-1}. The conservation of mass was considered via integration; individual base-line corrections were considered: (×) 0.001, (△) 0.010, (○) 0.007, and (◊) −0.006 absorbance units; standard buffer, 7 °C, 20 000 rpm, partial specific volume 0.534; binding constant of UUC to the haipin loop 1400 M^{-1}; the data (△, ○, and ♦) are shifted on the absorbance scale by 0.1, 0.2, and 0.3 units, respectively.

calculated for this model in a general form from a given set of rate constants for all combinations of reactant concentrations used in the relaxation measurements. Then the amplitudes for each relaxation curve are evaluated on the basis of the calculated time constants by linear regression, and the sum of squared deviations between calculated and experimental curves is determined. Application of a nonlinear fit routine for the rate constants (together with linear fitting of amplitudes at each iteration step) finally leads to a best fit of the model to the experimental data. This procedure provides rate constants for the reaction model, which are evaluated on the basis of all relaxation curves used in the fitting process, together with a large set of amplitudes, which are evaluated for each relaxation curve separately.

Since the relaxation effects observed for UUC binding to the anticodon loop are similar to those observed previously for UUC binding to the complete tRNA^{Phe} (Labuda & Porschke, 1980; Labuda et al., 1984), we used an analogous reaction model. The transition between two conformations of the anticodon loop is described by

$$A \underset{k_\alpha^-}{\overset{k_\alpha^+}{\rightleftharpoons}} B \qquad (12)$$

UUC may bind to both conformations, leading to a cyclic reaction scheme. However, as shown for the case of the complete tRNA, one of the binding reactions is dominant, and the other one may be neglected for simplicity. Using the same simplification in the case of the isolated anticodon loop, UUC binding is described by

$$B + UUC \underset{k_\beta^-}{\overset{k_\beta^+}{\rightleftharpoons}} C \qquad (13)$$

As shown by equilibrium sedimentation and also by temperature-jump amplitudes, the complex C undergoes association:

$$C + C \underset{k_\delta^-}{\overset{k_\delta^+}{\rightleftharpoons}} D \qquad (14)$$

Although the equilibrium sedimentation curves demonstrate the existence of aggregates higher than dimers, these higher aggregates have not been included for the evaluation of kinetic parameters, in order to keep the complexity of the model at a level that can be managed relatively easily. The coupled reactions 12–14 are reflected by three independent relaxation processes with reciprocal time constants that have been calculated as the roots of a third-order polynomial (Eigen & deMaeyer, 1963).

The parameters of this reaction mechanism have been determined by "global" fitting as described above, using the equilibrium parameters obtained independently from fluorescence titrations and analytical ultracentrifugation. As shown in Figure 3, the relaxation curves can be represented by the three-step mechanism with high accuracy, whereas a two-step mechanism involving reactions 12 and 13 did not provide a sufficiently accurate fit of the experimental data. Global fitting according to the three-step mechanism leads to well-defined values for the rate constants of reactions 13 and 14, whereas the parameters of reaction 12 cannot be defined unambiguously. The problem with the anticodon loop transition results from the fact that its relaxation is associated with a relatively small amplitude compared to those observed in the same time range for the association of UUC–anticodon loop complexes.

Since the amplitudes are evaluated by the present global fit procedure without direct relation to the reaction model, the quality of the fit should be tested by a simulation of the amplitudes on the basis of the reaction model. For this purpose, we used an improved version of the program "Simula" (Avery, 1982; unpublished results). As shown in Figure 4, the amplitudes obtained from the global fit given in Figure 3 can be represented with reasonable accuracy. The sets of optical and thermodynamic parameters used for the description of amplitudes are coupled to each other and thus cannot be determined, unless a reference value is available. We used a value of −63 kJ/mol for the enthalpy change associated with UUC binding to the anticodon as a reference, in analogy to the value for UUC binding to the anticodon of the complete tRNA^{Phe}. The parameters resulting from a trial and error fitting procedure using the program Simula are given in the legend to Figure 4.

Binding of the Pentadecamer to tRNA^{Glu}. The decoding properties of the isolated anticodon loop can also be compared to those of the complete tRNA^{Phe} by relatively simple binding experiments using the complementary tRNA^{Glu}. As shown in Figure 5, the fluorescence of the pentadecamer is strongly quenched by addition of tRNA^{Glu}. Evaluation of the titration curves according to a single-step binding mechanism provides binding constants in the range from 2.9×10^7 M^{-1} at 1.7 °C to 1.5×10^6 M^{-1} at 25.2 °C. According to a van't Hoff plot (cf. Figure 5), the complex formation is associated with an enthalpy change of −96 kJ·mol^{-1} and an entropy change of −208 J deg^{-1}. Temperature-jump measurements with fluorescence detection revealed a single relaxation process. The concentration dependence of its time constant can be used for an independent determination of binding constants between the pentadecamer and tRNA^{Glu}. The parameters obtained by this procedure are consistent with those evaluated from the equilibrium titrations. The rate constants of association are 3.3×10^6 M^{-1} s^{-1} at 7.2 °C and 4.0×10^6 M^{-1} s^{-1} at 10.2 °C; the corresponding binding constants are 1.6×10^7 and 9.4×10^6 M^{-1}, respectively. These rate constants as well as the binding constants are similar to those obtained for the complex formation between tRNA^{Phe} and tRNA^{Glu} (Grosjean et al., 1976; Labuda et al., 1982).

Conclusions

The anticodon loop studied in the present investigation involves less than 20% of the residues of the parent tRNA. Since the anticodon itself and the loop structure are preserved in the pentadecamer, it is not surprising that the thermodynamic and kinetic parameters of the codon binding reaction are very similar to those observed for the complete tRNA (cf. Table I). However, the existence of a codon-induced association reaction has hardly been expected for the isolated anticodon loop.

Since tRNA molecules do not show any strong affinity for self-association in the absence of codons, the codon-induced dimerization reaction has been taken as evidence for a codon-induced change of the tRNA structure. The close correlation of the experimental conditions with respect to the ion composition required for codon-induced association and for codon-induced changes of the tRNA structure observed independently (Gassen, 1980) has been used as further evidence for this interpretation. The extent of codon-induced changes of the tRNA structure is still under discussion. According to Gassen and co-workers (Schwarz et al., 1976; Möller et al., 1979; Gassen, 1980), codon binding leads to unfolding of the TΨCG loop from its tertiary interactions found in the crystal structure. The results obtained in the present investigation demonstrate that the main part of the free energy for the codon-induced association results from interactions between nucleotides from the anticodon loop and stem domain of codon–tRNA complexes. Nevertheless, a rearrangement of the

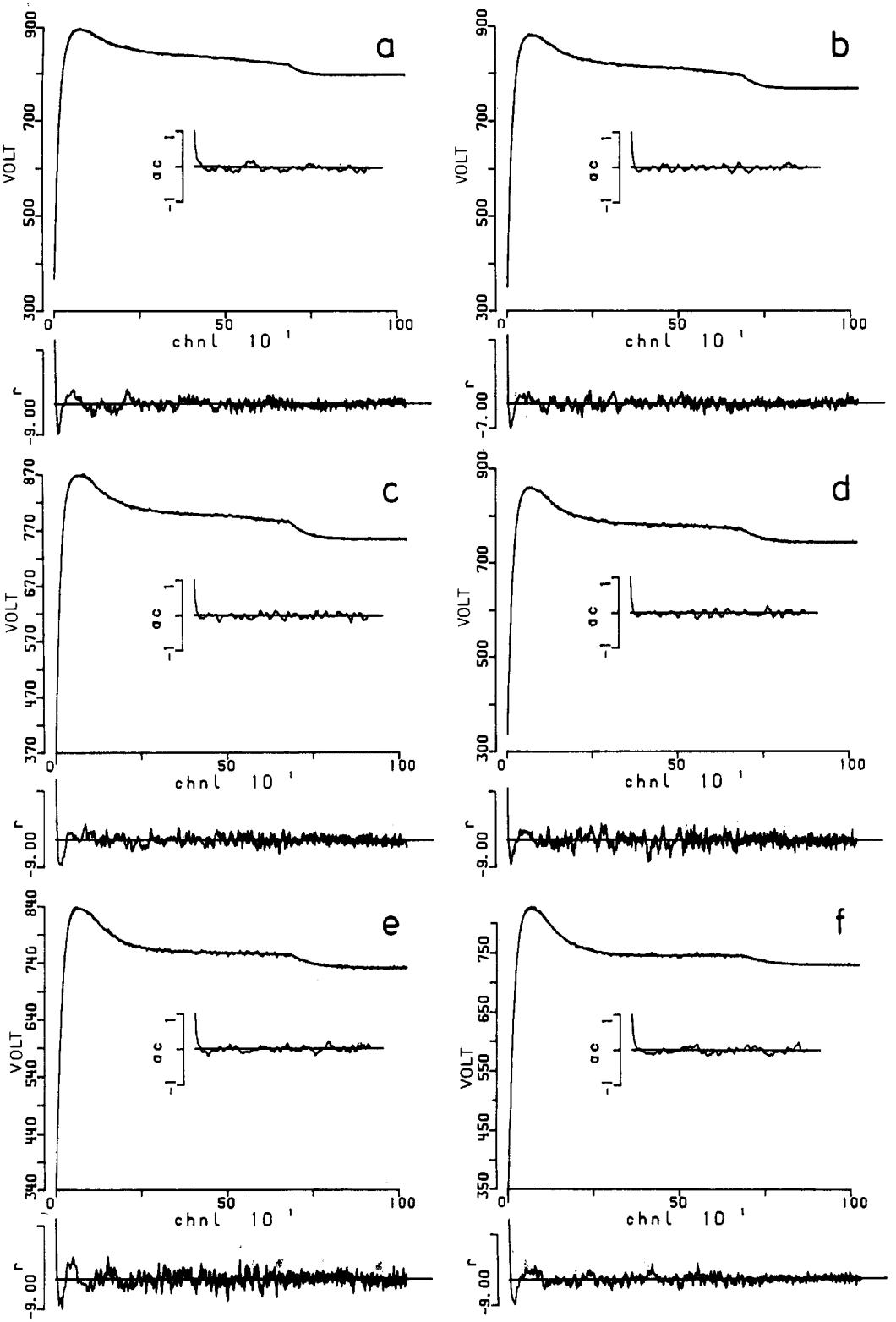

FIGURE 3: Global fit of relaxation curves obtained at various pentadecamer concentrations and at a constant UUC concentration of 2 mM (standard buffer, 7.2 °C). The pentadecamer concentrations are (a) 112.8, (b) 72.2, (c) 43.3, (d) 26.0, (e) 15.5, and (f) 9.35 μM. The time intervals are 0.5, 1, 2.5, and 25 μs between channels 1–102, 102–512, 512–684, and 684–1024, respectively. In addition to the experimental and the fitted curves, which are virtually indistinguishable, plots of the deviations (r) and the autocorrelation of the deviations (ac) are also shown for each curve.

structure may be necessary in the case of complete tRNA molecules in order to enable close contact between two codon–tRNA complexes over their whole length from the anticodon to the CCA end.

Although the main part of the interactions leading to the codon-induced association of tRNA can be localized more closely owing to the present results, the exact nature of these interactions remains to be established. Since it is hard to imagine that the helix stems strongly interact with each other, the seven residues of the loop appear to be more appropriate candidates for attractive contacts. Among these residues, the Wye base may form particularly strong contacts. Studies of the fluorescence-detected circular dichroism (Yoon et al., 1975) as well as measurements of fluorescence lifetimes and

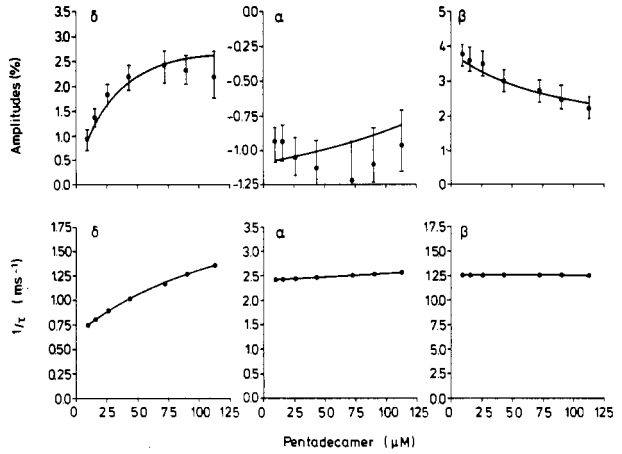

FIGURE 4: Reciprocal relaxation times ($1/\tau$) and relative fluorescence amplitudes of the processes α, β, and δ as a function of the pentadecamer concentration at a constant UUC concentration of 2 mM (standard buffer, 7.2 °C). The data are taken from the global fit (cf. Figure 3), and thus the $1/\tau$ values are in exact agreement with the model and the rate constants given in the legend to Figure 3, whereas the amplitudes had to be fitted by adjusting enthalpy changes (ΔH_i) and relative quantum yields (ϕ_i) ($\Delta H_\alpha = -9.8$ kJ/M, $\Delta H_\beta = -63$ kJ/M, $\Delta H_\delta = -1.4$ kJ/M, $\phi_A = 0.63$, $\phi_B = 1.39$, $\phi_C = 1.00$, $\phi_D = 1.53$).

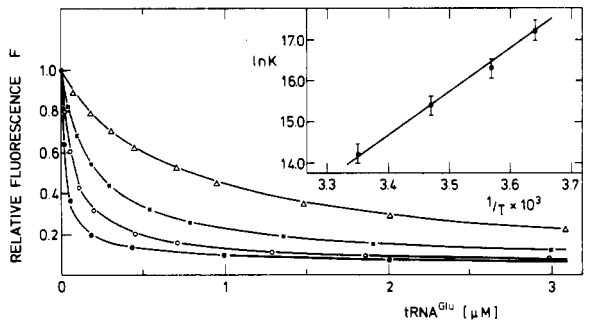

FIGURE 5: Fluorescence intensity of the pentadecamer in relative units as a function of the concentration of tRNA^{Glu} at different temperatures: (●) 1.7, (○) 7.2, (×) 15.2, and (Δ) 25.2 °C. The lines represent a least-squares fit according to a single-step binding model. The insert shows a van't Hoff plot of the binding constants: enthalpy change, −96 kJ mol^{-1}; entropy change, −208 J deg^{-1} (standard buffer; pentadecamer concentration 0.87 μM; limit decrease of fluorescence 94.3, 94.4, 93.7, and 94.3% at 1.7, 7.2, 15.2, and 25.2 °C, respectively).

Table I: Parameters for the Anticodon Loop Transition ($k_\alpha^+ + k_\alpha^-$), UUC Binding (k_β^+, K_β), and UUC-Induced Association (k_δ^+, K_δ) for Complete tRNA^{Phe} from Labuda et al. (1984) and for the Pentadecamer (Standard Buffer, 7.2 °C)[a]

parameter	tRNA^{Phe}	pentadecamer
$k_\alpha^+ + k_\alpha^-$ (s^{-1})	2×10^3	2.8×10^3
k_β^+ (M^{-1} s^{-1})	4.8×10^6	4.5×10^6
K_β (M^{-1})	1550	1400
k_δ^+ (M^{-1} s^{-1})	6.1×10^6	6.4×10^6
K_δ (M^{-1})	2×10^4	8×10^3

[a] The anticodon loop transition is characterized only by its reciprocal relaxation time, $1/\tau_\alpha = k_\alpha^+ + k_\alpha^-$, because a reliable evaluation of the individual rate constants was not possible for the pentadecamer; the parameter K_δ given for tRNA^{Phe} was evaluated from relaxation data according to a dimerization model, whereas K_δ for the pentadecamer is from ultracentrifuge data according to an isodesmic model; the parameters are defined by eq 12–14 with $K_\beta = k_\beta^+/k_\beta^-$ and $K_\delta = k_\delta^+/k_\delta^-$.

anisotropies (Rigler et al., 1985) suggest that codon binding leads to an increased mobility of the Wye base. Probably codon binding leads to a change in the environment of the Wye base which enables contact of the Wye base to an adjacent anticodon loop and thus promotes formation of aggregates. However, this interpretation is not supported by results obtained from NMR studies (Clore et al., 1984) which indicate that the Wye base is involved in a single-stranded stacked structure both in the free anticodon loop and in its complex with UUC.

From recent measurements of UUC binding to the isolated anticodon loop of tRNA^{Phe} (yeast) by NMR spectroscopy (Clore et al., 1984), it has been concluded that the stability of the pentadecamer–UUC complex is approximately an order of magnitude greater than that of the tRNA^{Phe}–UUC complex. According to our present results obtained by two independent methods (fluorescence and relaxation spectrometry), the affinities of UUC to the pentadecamer and to the complete tRNA^{Phe} are very similar. We have also tested the base-pairing affinity of the anticodon in the pentadecamer by binding experiments with tRNA^{Glu} and verified by these measurements that the base pairing of the anticodon is very similar in the isolated anticodon loop and the complete tRNA^{Phe}. Probably the difference between the present results and those reported previously is partly due to the fact that in the present investigation the binding constants have been determined at a very low pentadecamer concentration of about 4 μM, whereas the NMR measurements required concentrations in the millimolar range. As shown by the present results, high concentrations of pentadecamer and UUC promote the formation of aggregates. The existence of these aggregates has not been detected by NMR spectroscopy. Formation of aggregates induced by codon binding clearly leads to an increase of an apparent binding constant, which is evaluated without consideration of these aggregates.

It is very likely that the codon-induced dimerization of tRNA molecules is not only an interesting example of a complex reaction mechanism but also an important step in the ribosomal translation process. Obviously, tRNA molecules have to come very close to each other during reading of the genetic message. When a cognate codon has been recognized, the approach of two adjacent tRNA molecules must be close enough to facilitate the transfer of the amino acid to the growing polypeptide chain. For this purpose, the codon-induced association of tRNA appears to be optimal, since it is induced only upon recognition of a cognate codon and thus contributes to the fidelity of translation.

In the case of complete tRNA molecules, the codon-induced association apparently does not exceed the dimer stage (Porschke & Labuda, 1982; Labuda & Porschke, 1983), whereas the existence of higher aggregates can be clearly demonstrated for codon–anticodon loop complexes. The difference may be explained by repulsion between parts of the complete tRNA structure, e.g., the TΨCG loop or the dihydro-U loop, which may prevent addition of a third codon–tRNA complex to the aggregate. This interpretation remains speculation, however, until more information on the structure of the complex becomes available, for example, by cross-linking experiments.

Loop structures of ribonucleic acids are known to be very useful for adaptor function because of their remarkably high affinity for sequences, which are complementary to the loop (Eisinger et al., 1970; Uhlenbeck et al., 1970; Högenauer, 1970; Grosjean et al., 1976). It has also been demonstrated that the isolated anticodon loop of tRNA^{Phe} (yeast) strongly binds to poly(U)-programmed 30S ribosomes (Rose et al., 1983). The present demonstration of a codon-induced association of the isolated anticodon loop provides further evidence that relatively simple hairpin loops can be very useful as adaptors for an early translation process.

Registry No. 5′-r(C-A-G-A-C$_m$-U-G$_m$-A-A-Y-A-Y-m^{5}C-U-G), 103932-94-1; UUC, 2791-46-0.

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Half-Life of Synovial Cell Collagenase mRNA Is Modulated by Phorbol Myristate Acetate but Not by *all-trans*-Retinoic Acid or Dexamethasone†

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Received May 14, 1986; Revised Manuscript Received July 2, 1986

ABSTRACT: As part of our studies on the mechanisms controlling the synthesis of the neutral proteinase collagenase by rabbit synovial cells, we used a cDNA clone to measure total collagenase mRNA levels and to determine mRNA half-life. Phorbol myristate acetate was used to induce collagenase synthesis while *all-trans*-retinoic acid and dexamethasone were used to inhibit it. Cells stimulated with phorbol myristate acetate contained substantial amounts of collagenase mRNA, but cells treated with *all-trans*-retinoic acid or dexamethasone contained decreased amounts of collagenase mRNA which correlated well with levels of collagenase protein. Studies on mRNA half-life showed that the $t_{1/2}$ for total poly(A+) RNA was about 25 h, while that of collagenase varied from as short as 12 h to as long as 36 h. The half-life was not affected by treatment with *all-trans*-retinoic acid or dexamethasone but was affected by the level of induction of collagenase mRNA: the greater the amount of collagenase mRNA induced, the longer the $t_{1/2}$. We conclude that (1) our data are consistent with the hypothesis that retinoic acid and dexamethasone act at the level of transcription to decrease collagenase production and (2) the increased level of collagenase mRNA resulting from stimulation with phorbol esters is, in part, due to increased stability of the induced collagenase mRNA.

The metalloproteinase collagenase (EC 3.4.24.7) is the only enzyme capable of initiating breakdown of the interstitial collagens, types I, II, and III, at a neutral pH [for reviews, see Harris et al., (1984) and Harris (1985)]. Nowhere is the result of the action of collagenase more apparent than in the connective tissue disease rheumatoid arthritis, where production of collagenase by the synovial cells that line the joint culminates in extensive joint destruction and crippling (Harris, 1985). Previous work from this and other laboratories has, therefore, been concerned with mechanisms regulating the induction and inhibition of collagenase synthesis in synovial cells. Using a model system of monolayer cultures of rabbit synovial fibroblasts, we have shown that collagenase synthesis can be stimulated with a variety of agents, such as poly(ethylene glycol) which causes giant cell formation, phorbol myristate acetate (PMA), crystals of monosodium urate monohydrate, or the monocyte/macrophage product interleukin 1 (Brinckerhoff & Harris, 1978; Brinckerhoff et al., 1979; McMillan et al., 1981; Mizell et al., 1981; Dayer et al., 1986).

Addition of a collagenase inducer results in an increase in the level of collagenase mRNA within the cell by 5 h, measured by cell-free translation and Northern blot hybridization

† Supported by grants from the USPHS (AM-26599), from the National and New Hampshire Chapters of the Arthritis Foundation, from Hoffmann-La Roche, Inc., and from The RGK Foundation (Austin, TX).
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0006-2960/86/0425-6378$01.50/0