translocation from the A to the P site of the ribosome (Woese, 1970).

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References

Adler, A., Grossman, L., & Fasman, G. D. (1969) *Biochemistry 8*, 3846-3859.
Bernasconi, C. F. (1976) *Relaxation Kinetics*, Academic Press, New York.
Danchin, A., & Guéron, M. (1970) *J. Chem. Phys. 53*, 3599-3609.
Eigen, M. (1963) *Pure Appl. Chem. 6*, 97-115.
Eigen, M., & deMaeyer, L. (1963) *Tech. Org. Chem., 8*.
Eigen, M., & Maass, G. (1966) *Z. Phys. Chem. (Wiesbaden) 49*, 163-177.
Fuller, W., & Hodgson, A. (1967) *Nature (London) 215*, 817-821.
Gassen, H. G. (1980) *Prog. Nucleic Acid Res. Mol. Biol. 24*, 57-86.
Grosjean, H. J., Söll, D. G., & Crothers, D. M. (1976) *J. Mol. Biol. 103*, 449-519.
Hingerty, B., Brown, R. S., & Jack, A. (1978) *J. Mol. Biol. 124*, 523-534.
Holbrook, S. R., Sussman, J. L., Warrant, R. W., Church, G. M., & Kim, s. H. (1977) *Nucleic Acids Res. 4*, 2811-2820.
Jack, A., Ladner, J. E., Rhodes, D., Brown, R. S., & Klug, A. (1977) *J. Mol. Biol. 111*, 315-328.
Janssens de Varebeke, P., Labuda, D., & Augustyniak, J. (1978) *Biochem. Biophys. Res. Commun. 81*, 734-742.
Labuda, D., & Augustyniak, J. (1977) *Eur. J. Biochem. 79*, 303-307.
Labuda, D., & Pörschke, D. (1980) *Biochemistry 19*, 3799-3805.
Labuda, D., Haertlé, T., & Augustyniak, J. (1977) *Eur. J. Biochem. 79*, 293-301.
Pörschke, D. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70*, 2683-2686.
Pörschke, D. (1979) *Nucleic Acids Res. 6*, 833-898.
Quigley, G. J., Teeter, M. M., & Rich, A. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 64-68.
Reddy, R. P., Schweizer, M. P., & Chheda, G. B. (1979) *FEBS Lett. 106*, 63-66.
Riesner, D., Maass, G., Thiebe, R., Philippsen, P., & Zachau, H. G. (1973) *Eur. J. Biochem. 36*, 76-88.
Rigler, R., Rabl, C. R., & Jovin, T. M. (1974) *Rev. Sci. Instrum. 45*, 580-588.
Robinson, B., & Zimmerman, T. P. (1971) *J. Biol. Chem. 246*, 110-117.
Römer, R., & Hach, R. (1975) *Eur. J. Biochem. 55*, 271-284.
Schimmel, R. R., & Redfield, A. G. (1980) *Annu. Rev. Biophys. Bioeng. 9*, 181-121.
Stein, A., & Crothers, D. M. (1976) *Biochemistry 15*, 160-168.
Szer, W., & Ochoa, S. (1964) *J. Mol. Biol. 8*, 823-834.
Teeter, M. M., Quigley, G. J., & Rich, A. (1980) in *Nucleic Acid-Metal Ion Interactions* (Spiro, T. G., Ed.) pp 147-177, Wiley, New York.
Thedford, R., & Straus, D. B. (1974) *Biochemistry 13*, 535-542.
Urbanke, C., & Maass, G. (1978) *Nucleic Acids Res. 5*, 1551-1560.
Woese, C. (1970) *Nature (London) 226*, 817.

Codon-Induced Transfer Ribonucleic Acid Association: Quantitative Analysis by Sedimentation Equilibrium†

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ABSTRACT: It is shown by measurements of the sedimentation equilibrium that binding of the codon UUC to $tRNA^{Phe}$ from yeast induces an association of tRNA molecules. Sedimentation measurements at different concentrations demonstrate that the tRNA-codon complexes form dimers. The sedimentation profiles are analyzed quantitatively in terms of a simple monomer-dimer model as well as a model which considers the sedimentation of four species (tRNA, tRNA·UUC, $(tRNA·UUC)_2$, and UUC) separately. The information resulting from the conservation of mass relation is used directly in the determination of equilibrium constants via integration of the sedimentation profiles. Using this procedure, we determine the equilibrium constants for dimerization of the $tRNA^{Phe}$·UUC complex, $K_D = 8.6 \times 10^4$ M^{-1}, and for the binding of UUC to $tRNA^{Phe}$, $K_L = 1800$ M^{-1} (5 °C). The large free energy for dimerization of the tRNA-codon complex suggests that interactions between adjacent tRNAs are important for the ribosomal translation process.

During translation of the messenger RNA at the ribosome, tRNA molecules bind at adjacent trinucleotide codons and thus come very close to each other. Since tRNA molecules are rather large, it seems to be almost inevitable that they make direct contacts with each other at the ribosome binding sites. These contacts may be necessary for the peptidyl transfer reaction. If the contacts are favorable, it should be possible to provide evidence for them by experiments in free solution. Actually recent relaxation experiments (Labuda & Porschke, 1980) showed an association of $tRNA^{Phe}$, which was induced by binding of the codon UUC. In the present contribution, this reaction is analyzed quantitatively by measurements of the sedimentation equilibrium in an analytical ultracentrifuge.

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Materials and Methods

$tRNA^{Phe}$ (yeast) was purchased from Boehringer Mannheim. The codon UpUpC was prepared as described by Sprinzl et al. (1976). All measurements were performed in a buffer containing 50 mM Tris–cacodylate, pH 7, 100 mM $NaClO_4$, and 10 mM $Mg(ClO_4)_2$. The $tRNA^{Phe}$ samples were annealed in this buffer at 65 °C for 5 min as described by Grosjean et al. (1976).

The sedimentation equilibrium was measured with a Spinco Model E analytical ultracentrifuge equipped with ultraviolet optics, photoelectric scanner, multiplexer, and electronic speed control. All runs were made with a 12-mm charcoal-filled-epon double-sector cell at 5 °C. The solutions were scanned at 313.2 or 302.2 nm (mercury lines). At 313.2 nm, the absorbance is only due to the $tRNA^{Phe}$ (modified bases), and UUC does not contribute to the absorbance. A small contribution of UUC to the absorbance at 302.2 nm was compensated by using appropriate UUC solutions in the reference sector. At the beginning of a run, the solution was scanned just after arriving at the operation speed (usually 10000 rpm) to read the absorbance of the homogeneously distributed sample. Sedimentation equilibrium was usually attained after 3 days. The sedimentation was considered to be in equilibrium when scans separated by time intervals of 6 h did not change anymore. The base line was determined by complete sedimentation of the tRNA by acceleration to 40 000 rpm for 8 h and a subsequent scan at the operation speed.

Densities were measured by a DMA 60 density meter together with a DMA 601-W measuring cell produced by Anton Paar KG, Graz (Austria). In this instrument, the density is determined by vibration measurements with an accuracy of 10^{-6}. Owing to the high accuracy, partial volumes could be determined for the relatively dilute solutions used in the present investigation.

Theory

Model with Two Sedimenting Components. The sedimentation equilibrium of a single species A in an ideal solution is described by (cf. textbooks on biophysical chemistry, e.g., Eisenberg & Crothers, 1979)

$$\mu_A^T = \mu_A^0 + RT \ln c_A - \frac{1}{2}M_A(1 - v_A\rho)r^2\omega^2 \qquad (1)$$

where μ_A^T and μ_A^0 are the total and the zero chemical potential, respectively, c_A is the concentration at the distance r from the rotor center, ω is the angular velocity, M_A is the molecular weight, v_A is the partial specific volume of A, and ρ is the density of the solvent. At equilibrium, the potential μ_T^A is constant over the cell ($d\mu_A^T/dr = 0$) and thus

$$\frac{d \ln c_A}{dr^2} = \frac{M_A(1 - v_A\rho)\omega^2}{2RT} \qquad (2)$$

When the species A forms a dimer according to

$$A + A \rightleftarrows D \qquad (3)$$

the concentration profile of the dimer must follow a sedimentation equation corresponding to that given above for the monomer:

$$\mu_D^T = \mu_D^0 + RT \ln c_D - \frac{1}{2}M_D(1 - v_D\rho)r^2\omega^2 \qquad (4)$$

The condition of chemical equilibrium requires that at each point of the gradient

$$2(\mu_A^0 + RT \ln c_A) = \mu_D^0 + RT \ln c_D \qquad (5)$$

It is not necessary to include each of these equations explicitly in the calculation (Adams & Fujita, 1963), since the system is overdetermined by eq 1, 4, and 5. The combination of eq 1 and 5 or eq 4 and 5 is sufficient.

In the present investigation, the experimental data are analyzed in terms of the above equations by using numerical procedures. The most simple one is as follows: We define a starting value for the term $\mu_A^T - \mu_A^0$ and calculate the concentration profile of A along the centrifuge cell by using eq 1. The molecular weight M_A required for this calculation is determined by an independent equilibrium centrifugation under conditions without association of A ($tRNA^{Phe}$ in the absence of UUC). The concentration profile of the dimer D can be calculated from that of the monomer A by using eq 5 in the form of the law of mass action $K_D = c_D/(c_A)^2$. For this calculation, we introduce a starting value K_D. The calculated concentration profile is then compared with the experimental data. Using a standard least-squares routine, we determine values for the term $\mu_A^T - \mu_A^0$ and the stability constant K_D, which provide an optimal representation of the experimental data. In our experience, this procedure always leads to well-defined error minima and thus also to well-defined parameters $\mu_A^T - \mu_A^0$ and K_D.

The procedure described above does not use the independent information resulting from the conservation of mass. The total concentration $c_t = c_A + 2c_D$ can be determined in absence of sedimentation and must correspond to the integral of the concentration profiles at sedimentation equilibrium. For a sector-shaped cell with a radius r_b at the bottom and r_m at the meniscus, this condition is

$$(r_b^2 - r_m^2)c_t = \int_{r_m}^{r_b} c_A(r)2r\,dr + 2\int_{r_m}^{r_b} c_D(r)2r\,dr \qquad (6)$$

The details of the calculation will not be given here. The term $\mu_A^T - \mu_A^0$ can be obtained as the solution of a quadratic equation:

$$\mu_A^T - \mu_A^0 = RT \ln \left\{ -\frac{1}{2K_D(e^{\xi_A r_b^2} + e^{\xi_A r_b^2})} + \left[\frac{1}{4K_D^2(e^{\xi_A r_b^2} + e^{\xi_A r_m^2})^2} + \frac{\xi_A(r_b^2 - r_m^2)c_t}{K_D(e^{2\xi_A r_b^2} - e^{2\xi_A r_m^2})} \right]^{1/2} \right\} \qquad (7)$$

with

$$\xi_A = M_A(1 - v_A\rho)\omega^2/(2RT) \qquad (8)$$

and by using the relation $M_D = 2M_A$ as well as $\mu_D^T - \mu_D^0 = RT \ln K_D + 2(\mu_A^T - \mu_A^0)$ which follow from the equations given above.

Equation 7 can be used to define $\mu_A^T - \mu_A^0$ such that only K_D has to be fitted to the experimental data. In some cases it may be useful, however, to fit both $\mu_A^T - \mu_A^0$ and K_D to the experimental data as described above, since errors in the determination of c_t, r_b, and r_m may reduce the quality of the fit considerably. In such cases, eq 6 or 7 should be used to determine the deviation from the ideal mass conservation.

Model with Four Sedimenting Components. The model described in the above section does not consider the fact that the codon binding by itself will lead to some change in the sedimentation profile. In the present section, UUC binding is considered explicitly according to

$$A + L \rightleftarrows C \qquad K_L = c_C/(c_A c_L)$$
$$2C \rightleftarrows D \qquad K_D = c_D/c_C^2 \qquad (9)$$

Since the UUC ligand represented by L is in large excess with respect to the total tRNA, the concentration c_L is not changed

by the complex formation. The distribution of c_L along the centrifuge cell is given by

$$c_L(r) = c_L^0 \xi_L (r_b^2 - r_m^2) \left(\frac{e^{\xi_L r^2}}{e^{\xi_L r_b^2} - e^{\xi_L r_m^2}} \right) \quad (10)$$

where c_L^0 is the concentration at homogeneous distribution and ξ_L is defined in analogy to eq 8.

The numerical procedure for fitting of the experimental data can be made analogous to that described above. In the present case, however, two equilibrium constants are required. Again the term $\mu_A^T - \mu_A^0$ may be fitted to the experimental data or determined according to the conservation of the tRNA mass.

In the present case, the conservation of mass is given by

$$(r_b^2 - r_m^2)c_t = \int_{r_m}^{r_b} c_A(r) 2r\, dr + \int_{r_m}^{r_b} c_C(r) 2r\, dr + 2\int_{r_m}^{r_b} c_D(r) 2r\, dr \quad (11)$$

The term $\mu_A^T - \mu_A^0$ is again the solution of a quadratic equation:

$$\mu_A^T - \mu_A^0 = RT \ln \left[-\frac{\sigma_A + K_L c_L^0 \gamma \sigma_C}{4K_D (K_L c_L^0 \gamma)^2 \sigma_D} \right] + \left[\left(\frac{\sigma_A + K_L c_L^0 \gamma \sigma_C}{4K_D (K_L c_L^0 \gamma)^2 \sigma_D} \right)^2 + \frac{(r_b^2 - r_m^2)c_t}{2K_D (K c_L^0 \gamma)^2 \sigma_D} \right]^{1/2} \quad (12)$$

with $\sigma_i = (e^{\xi_i r_b^2} - e^{\xi_i r_m^2})/\xi_i$ (i = A, C, D, and L); the definition of ξ_i is analogous to that of ξ_A in eq 8 and $\gamma = (r_b^2 - r_m^2)/\sigma_L$.

Results

Sedimentation Measurements. The association of tRNA^{Phe} induced by UUC binding can be analyzed relatively easily owing to various favorable circumstances. First of all, tRNA^{Phe} does not form any aggregates in the absence of UUC under the conditions of the present experiments. The molecular weight of tRNA^{Phe} determined by equilibrium sedimentation does not depend upon the tRNA concentration. The value found in the present investigation (M_r = 24 200) is consistent with the value calculated according to the sequence. The ligand UUC does not absorb at a wavelength of 313.2 nm used for scanning of most sedimentation profiles. A small absorbance of UUC observed in scans at 302.2 nm could be compensated by using an appropriate UUC solution in the reference sector of the centrifuge cell. Moreover we demonstrated by spectrophotometric titration that the binding of UUC does not change the absorbance of tRNA^{Phe} in the wavelength range around 310 nm by more than 1–2%. From this result we may also conclude that the association of tRNA^{Phe} does not lead to any significant change in the absorbance around 310 nm.

Sedimentation profiles of tRNA^{Phe} were recorded at various UUC concentrations. The addition of UUC induced a clear increase of the apparent molecular weight. Since the increase in molecular weight did not exceed values around 70%, even at relatively large concentrations, the association apparently does not exceed the dimer stage to any significant extent. Thus the quantitative analysis is restricted to models involving a dimerization of tRNA. In the present case, two different models of dimerization are conceivable: (1) Dimerization may be restricted to tRNA molecules, which form a complex with UUC; (2) a dimer is formed between a tRNA·UUC complex and a tRNA molecule without UUC.

A distinction is possible on the basis of the UUC concentration dependence. According to the first model, the degree of tRNA association will show a continuous increase with increasing UUC concentration, whereas the second model predicts a maximum of tRNA association at half-saturation of the tRNA with UUC. Equilibrium sedimentation experiments showed a continuous increase of the apparent molecular weight for UUC concentrtions from 0.2 to 2.5 mM. From independent relaxation measurements (amplitudes and time constants; cf. Labuda & Porschke, 1980), we know that the equilibrium constant for UUC binding is around 2000 M^{-1} under the conditions of the centrifuge experiments. Thus the apparent molecular weight increases continuously in a range from ~29% to ~83% degree of UUC binding, providing clear evidence in favor of the first model.

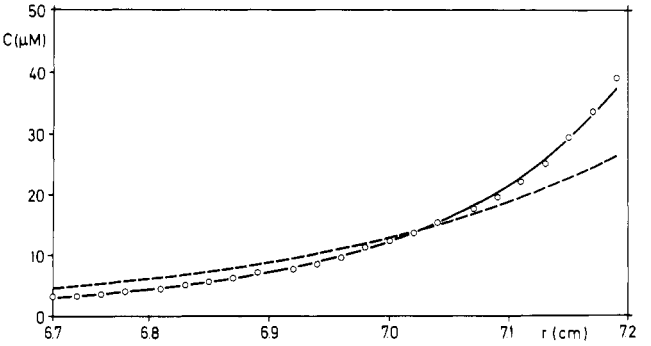

FIGURE 1: Sedimentation profile of tRNA^{Phe} in the presence of 2.5 mM UUC. The solid line shows a least-squares fit of the data according to the model with two sedimenting species: K_{app} = 6.4 × 10^4 M^{-1}. The broken line shows a least-squares fit with K_{app} = 0 for comparison.

Table I: Equilibrium Constants from Sedimentation Profiles (for tRNA Dimerization K_D and for UUC Binding K_L)[a]

UUC (mM)	K_{app} (mM^{-1})	K_L (mM^{-1})	K_D (mM^{-1})
0.2	8.8	2.2	86
1.0	44.0	2.3	89
		2.6	75
2.5	64.0		

[a] Each pair of K_L and K_D is calculated from a pair of K_{app} values. The combinations are indicated by the symbols between the K_{app} and the K_L column. Estimated accuracies: K_{app} ±15%; K_L and K_D ±30%.

A quantitative analysis of the sedimentation profiles in terms of the model with two sedimenting species (cf. Theory and Figure 1) yields a set of apparent equilibrium constants K_{app}, which has to be corrected for the different degrees of UUC binding. Any combination of two K_{app} measured at different UUC concentrations may be used for calculation of the "intrinsic" association constants for dimerization K_D and UUC binding K_L. These values are compiled in Table I.

The parameters given in Table I show that the model with two sedimenting components provides a satisfactory and consistent representation of the experimental data. However, the parameters obtained from this model may be subjected to some systematic error, since some details of the reaction have not been considered in the model. In order to test for any systematic error, the model with four sedimenting species described under Theory was also used. In this case, three sedimentation profiles measured at three different UUC concentrations were fitted together in order to determine K_D and K_L. The fit is shown in Figure 2 with K_D = 8.6 × 10^4 M^{-1} and K_L = 1800 M^{-1}. A comparison of these parameters with those given in Table I shows that the error introduced by using the simple model is relatively small.

Further sedimentation experiments were performed at constant UUC concentration (1.75 mM) and various tRNA

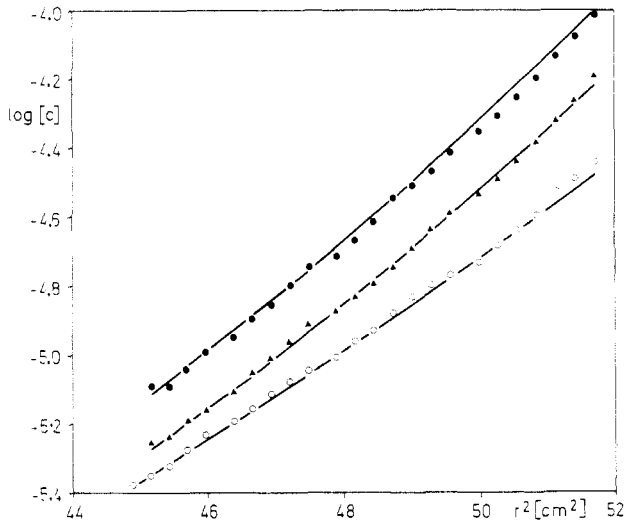

FIGURE 2: Logarithm of the tRNA concentration as a function of the square of the radius for three different UUC concentrations: 0.2 (○), 1 (▲), and 2.5 mM (●). The data for 1 and 2.5 mM UUC are shifted by 0.2 and 0.4 unit, respectively, on the y axis. The solid lines represent a joint least-squares fit of the three data sets according to the model with four sedimenting species: $K_D = 8.6 \times 10^4$ m^{-1} and $K_L = 1800$ M^{-1} (estimated accuracy ±20%).

concentrations. The parameters obtained from these experiments were in close agreement with those given above, providing further evidence in favor of the reaction scheme used for the interpretation of the sedimentation data.

Density Measurements. It is known from various investigations that conformation changes in nucleic acids are usually not accompanied by large volume changes. This has been demonstrated for example for the double helix coil transition (Gunter & Gunter, 1972; Hawley & Macleod, 1974). In the present case, the nature of the interactions leading to tRNA association are not yet known. Thus it cannot be excluded a priori that the formation of tRNA dimers is associated with some volume change, for instance, by an alteration in the hydration state of ions, which are known to be important for the reaction. For this reason we have measured the partial specific volumes of tRNA^{Phe}, of UUC, and of their mixture under conditions of extensive association. We obtained the following results for the partial volumes: v(tRNA^{Phe}) = 0.534 and v(UUC) = 0.551. The partial volume of tRNA^{Phe} + UUC in a solution containing 56.8 μM tRNA^{Phe} and 1.75 mM UUC was exactly equivalent to that expected for an ideal mixture. As shown in the previous section, approximately 85% of the tRNA molecules are in the dimer form under these conditions. Thus the tRNA association is not accompanied by any substantial volume change.

Discussion

The quantitative analysis of chemical equilibria by equilibrium sedimentation offers several advantages. The main advantage is of course the direct information about the molecular weight of the species involved in the reaction. Another advantage is the fact that for a single solution within a centrifuge cell information can be displayed for a relatively wide concentration range. The evaluation of quantitative parameters from complex sedimentation profiles, which has been a problem in the past, can be performed easily with the aid of computers. This procedure provides more accurate information than those used previously via apparent molecular weights.

The efficiency of the present approach may be tested by comparison with other methods. A direct comparison is possible with respect to the equilibrium constant K_L for UUC binding to tRNA^{Phe}. Independent relaxation measurements (Labuda & Pörschke, 1980; D. Labuda and D. Pörschke, unpublished results) provided K_L values closely corresponding to the present values.

The equilibrium constant K_D for the codon-induced tRNA dimerization is surprisingly high. At present we do not have any clear information about the nature of the interactions between the codon–tRNA complexes. Relaxation measurements demonstrated that the dimerization depends in a very special manner upon the ion composition of the solution (Labuda & Porschke, 1980). Mg^{2+} concentrations above 1 mM are necessary for the dimerization. The required Mg^{2+} concentration depends, however, on the level of the monovalent ions. The Mg^{2+} requirement for the dimerization is very similar to that observed for the biological function of the tRNA at the ribosome. This similarity suggests that the reactions observed in the present investigation with tRNA in free solution has some relevance for the biological function. For example, the dimerization may be very useful for the peptidyl transfer reaction. However, further investigations are necessary to substantiate the phenomena observed in the case of tRNA^{Phe} for other tRNAs.

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References

Adams, E. T., & Fujita, H. (1963) in *Ultracentrifugal Analysis* (Williams, J. W., Ed.) Academic Press, New York.

Eisenberg, D., & Crothers, D. (1979) *Physical Chemistry with Applications to the Life Sciences*, Benjamin Cummings Publishing Co., London.

Grosjean, H., Söll, D. G., & Crothers, D. M. (1976) *J. Mol. Biol. 103*, 449–519.

Gunter, T. E., & Gunter, K. K. (1972) *Biopolymers 11*, 667.

Hawley, S. A., & Macleod, R. M. (1974) *Biopolymers 13*, 1417–1426.

Labuda, D., & Pörschke, D. (1980) *Biochemistry 19*, 3799–3805.

Sprinzl, M., Wagner, T., Lorenz, S., & Erdmann, V. (1976) *Biochemistry 15*, 3013–3039.