Thermodynamic Studies of the Reversible Association of *Escherichia coli* Ribosomal Subunits†

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ABSTRACT: The kinetics of association of *Escherichia coli* 30S and 50S ribosomal subunits have been carried out as a function of temperature after a magnesium jump from 1.5 to 3 mM. Turbidimetric recordings combined with a stopped-flow apparatus were used to follow the kinetics. The data show that the rates of formation and dissociation of the 70S particles at 3 mM Mg^{2+} and +25 °C were, respectively: $k_2 = 10^5\ M^{-1}\ s^{-1}$, $k_1 = 4.5 \times 10^{-3}\ s^{-1}$; lowering the temperature decreases the rate constants with activation energies equal to $E_2 = 7.5$ kcal/mol, $E_1 = 26.5$ kcal/mol and enhances the association equilibrium towards the 70S species with an enthalpy change ($\Delta H°_{assoc} = -19.9$ kcal/mol) dominant over the entropy change ($\Delta S°_{assoc} = -33$ cal/(deg mol)). These thermodynamic parameters were compared to those obtained from studies on the interactions of codon–anticodon in yeast phenylalanine transfer RNA as well as of ribooligonucleotides. The kinetic and thermodynamic data are shown to be consistent with 16S–23S RNA interaction.

Physico-chemical studies of reversible association of *E. coli* ribosomal subunits indicating that the ribosomes are in dynamic equilibrium with their subunits have been reported by several authors (Spirin et al., 1970; Spirin and Lishnevskaya, 1971; Infante et al., 1971). Quantitative data using ^{3}H-labeled subunit exchange with nonlabeled 70S ribosomes and light-scattering techniques (Page et al., 1967; Zitomer et al., 1972) have only been obtained recently. Moreover, it was realized that ribosomes could be separated in two types, type A or "tight" couples (Van Diggelen et al., 1973; Van Diggelen and Bosch, 1973; Noll and Noll, 1976), which are pressure-resistant, and type B or "loose" couples, which are not.

Using the light-scattering technique, Debey et al. (1975) showed that the association–dissociation equilibrium curves of A-type ribosomes as a function of Mg^{2+} concentrations differ widely from that of the B type.

Temperature dependence of equilibrium parameters of both types of ribosomes also differs widely, but lowering the temperature decreases the half-saturation parameter $[Mg^{2+}]_{1/2}$ for both types.

Kinetics of association and dissociation of *E. coli* 30S and 50S subunits have been performed by Wishnia et al. (1975) using a stopped-flow apparatus combined with light-scattering technique. Their results show that A-type subunits appear to fit in the simple scheme of second-order reaction governed by the rate of collision of the particles over a wide range of Mg^{2+} and ribosome concentrations.

Quantitative results, obtained by static as well as dynamic studies, indicate that the primary effect of Mg^{2+}, or of any other di- or multivalent cations binding to ribosomes, is to decrease the contribution of electrostatic repulsion to the free energy of activation. Ribosomal RNAs are polyanions and develop a strong electrostatic potential: the interactions of divalent cations, such as Mg^{2+}, with ribosomal substructures possibly modulate this electrostatic potential according to the polyelectrolyte theory (Kliber et al., 1976). But the electrostatic forces are certainly not the only ones involved in ribosome stability: attracting forces, such as hydrogen bonding and apolar bonding (hydrophobic bonds), may be very important at short distance (Walters et al., 1970; Spirin and Lishnevskaya, 1971).

The exact nature of the interactions governing the association equilibrium 30 S + 50 S ⇌ 70 S is still unknown. We have analyzed the temperature effect on this equilibrium in terms of free energy, heat and entropy of activation, as well as the enthalpy and entropy changes of the reaction. These thermodynamic parameters can be compared to those previously reported for ribooligonucleotides (Borer et al., 1974) and codon–anticodon interactions (Yoon et al., 1975). The latter interaction appears to be of the same order of magnitude as the ribosomal subunit association, suggesting that its mechanism, as already postulated (Van Duin et al., 1975, 1976; Branlant et al., 1976), could involve an interaction between the RNA chains of each subunits.

Materials and Methods

Preparation of A-Type Ribosomes. E. coli ribosomes were prepared by an alumina grinding; the extract was then centrifuged at low and high speed and washed twice in high concentration salt buffer (1.5 M NH_4Cl, 10 mM magnesium acetate, 20 mM Tris[1]-HCl, pH 7.5, 7 mM β-mercaptoethanol), according to the procedure of Dondon et al. (1974). A-type ribosomes were then purified on a 10–30% sucrose gradient by centrifugation for 17 h at 31 000 rpm in a Beckman T14 zonal rotor, in the presence of 5 mM $Mg(OAc)_2$, 60 mM NH_4Cl, 10 mM Tris-HCl (pH 7.5). The 70S fractions were collected and brought to 7 mM β-mercaptoethanol and 10 mM $Mg(OAc)_2$ before concentration by further centrifugation for 24 h at 25 000 rpm in rotor 30 (Spinco centrifuge). The 70S pellet was then rehomogenized in buffer, 10 mM Tris-HCl (pH

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[1] Abbreviation used: Tris, tris(hydroxymethyl)aminomethane.

7.5), 100 mM NH_4Cl, 10 mM $Mg(OAc)_2$, 7 mM β-mercaptoethanol, and stored frozen at −90 °C. Before use, each ribosomal stock solution was reactivated by heating for 15 min at 37 °C and then centrifuged for 45 min at 14 000*g* at 5 °C to eliminate dust or precipitates and kept at 0 °C until use, no longer than 20 h. The differences between the A- and B-type ribosomes are as follows. At a given concentration of monovalent cations, the interval of Mg^{2+} concentration between 10 and 90% association is narrower and the half-saturation is lower for the A type (2.5–3 mM Mg^{2+}) than for the B type (5–9 mM Mg^{2+}). Furthermore, in contrast to B-type ribosomes, when A-type ribosomes have been exposed to 1 mM Mg^{2+} they show no hysteresis effect in their reassociation curve, and the double-logarithmic plot of the equilibrium constant vs. Mg^{2+} concentration is linear only in the central part of the curve. A-type 70S ribosomes are pressure resistant at 5 mM $MgCl_2$. The A-type ribosomes were washed at high salt concentration in order to remove initiation factors as well as peptidyl- and aminoacyl-tRNA. They have thus a $[Mg^{2+}]_{1/2}$ slightly higher than the "tight" ribosomes of Noll and Noll (1976).

Chemicals and Solutions. Magnesium acetate was from Carlo Erba; ammonium chloride was from Prolabo; β-mercaptoethanol was from Merck-Schuchardt; Tris-HCl and cacodylic acid were from Sigma. The ribosome concentrations were estimated by measuring absorbancy at 260 nm (for a 1-cm light path) using the molar extinction coefficient $\epsilon_{70} = 4.15 \times 10^7$. Stock ribosomal solution ($\simeq$355 A_{260} units/mL) was diluted just before use to a final concentration of 5 A_{260} units/mL for static measurements and to an initial concentration of 16 to 22 A_{260} units/mL for stopped-flow measurements. For both types of experiments, the solutions were adjusted to the following composition: *buffer A* is composed of 50 mM cacodylate, pH 7, 60 mM NH_4Cl, 7 mM β-mercaptoethanol; *buffer B* is composed of 10 mM Tris-HCl, pH 7.5, 50 mM NH_4Cl, 7 mM β-mercaptoethanol. (1) Buffer B was used solely for kinetic experiments at +25 °C in order to compare our results with those of Wishnia et al. (1975) under the same conditions. (2) All the other experiments used buffer A, the enthalpy of ionization in aqueous solution being quite smaller ($\Delta H_A \simeq 3$ kcal/mol) for this buffer than for buffer B ($\Delta H_B \simeq 11.4$ kcal/mol).

Light-Scattering and Turbidimetric Techniques. Equilibrium Measurements. Light-scattering measurements were carried out in a Jobin-Yvon "Bearn" fluorescence spectrophotometer. Both the excitation and emission monochromators were set to 436 nm and the slits were set to 6-nm band-pass; a 4-mL fluorescence cuvette was used; the temperature of the cuvette was regulated by a Haak K_{41} cryostat to ±0.1 °C by means of water circulating through the cuvette holder.

In a typical experiment, all solutions were previously filtered through nitrocellulose filters (Millipore, Type GS, 0.22-μm pore size); 2 mL of buffer A containing 0.8 mM Mg^{2+} was placed in the cuvette and the intensity of light scattering at 90° was measured after temperature equilibration; 40 μL of concentrated 70S ribosomes (in buffer A containing 10 mM Mg^{2+}) was diluted and mixed by magnetic stirring in the cuvette; the final intensity of light scattered by the dissociated ribosomes at 1 mM Mg^{2+} was measured; finally the reassociation of 70S ribosomes at increasing Mg^{2+} concentration was followed at various temperatures by the relative light-scattering increase after each addition of 0.5 mM of Mg^{2+} mixed by magnetic stirring; concentration of magnesium acetate varied from 1 to 6 mM, and 70S ribosome concentration from 5 to 10 A_{260} units/mL (120–240 nM). The time of each experiment varied from 20 to 40 min. The exposure time of the ribosomes to 1 mM Mg^{2+} varied from 20 s at high temperature to 3 or 5 min at lower temperature (<15 °C); we verified that, under these conditions, the ribosomes are not inactivated, as shown by the reversibility curve of Debey (1975). The major error in those experiments came from pipetting and from the interference of dust particles light scattering. Ribosomal and buffer A solutions were placed in the cuvette by means of a 50-μL Hamilton syringe and a 1000-μL H. E. Pedersen pipette, respectively; aliquots of 2 μL of a concentrated solution of magnesium acetate (0.5 M) were added in 2 mL of ribosome solution, using a Hamilton syringe 701 N; the relative error of the syringe was 1%, but because of the steepness of the Mg^{2+} titration curve, the total error was about ±5% of the amount of 70S particles for each addition of magnesium acetate.

Static measurements were also carried out in a spectrophotometer Aminco DW_2 using the turbidimetric method; the apparent absorbancy at 310 nm of ribosomes dissociated at different magnesium concentrations was determined as shown by Debey (1975) in order to calibrate the stopped-flow apparatus.

Kinetic Measurements. Kinetic measurements were carried out in a low-temperature stopped-flow apparatus built in this laboratory (Hui Bon Hoa and Douzou, 1973). Recently, a reference beam was added to the absorbancy beam; both beams were modulated with light choppers of distinct frequency before falling onto the same photomultiplier. This procedure allows accurate measurements of small absorption changes and slow kinetics. The dead time of the apparatus was 10 ms. The absorbancy flow cell was a modified 2-cm path cell in order to reduce the volume ($\simeq$60 μL) and to increase the apparent absorbancy (as compared to the normal 1-cm path cell). The flow cell and injection syringes were thermostated to 0.1 °C from a cryostat described elsewhere (Hui Bon Hoa, 1973).

In a typical experiment, stock solution of ribosome freshly activated and centrifuged was diluted in 4 mL of buffer, containing 1.5 mM magnesium acetate, to an initial ribosomal concentration of 16 to 22 A_{260} units/mL (384–528 nM). The dissociated ribosomes were kept at 0 °C for less than 1 h; 1.7 mL of this ribosomal solution was used to fill one of the injection syringes and then mixed with solutions contained in the second injection syringe and composed of buffer with successively 0, 1.5, 4.5, and 10.5 mM magnesium acetate: the first and last experiments were used to calibrate the turbidity level of 0 and 100% 70S ribosomal solution on the stopped-flow; the second experiment was used to calibrate the kinetic baseline at every temperature at 1.5 mM final concentration of magnesium acetate; and the third experiment was the kinetic run corresponding to change of concentration of the 70S species after a magnesium jump from 1.5 to 3 mM. Each series of experiments was run several times; between two series, the flow cell was washed four times with the new solutions. The total turbidity change at $\lambda = 310$ nm did not exceed $\Delta OD = 3.7 \times 10^{-2}$ when the dissociated ribosomes became fully associated at 6 mM Mg^{2+}; the precision of the determination of each point of the kinetic curve was about ±5%.

Analysis of Data. Light scattering at 90° by ribosomal solutions at high dilution (as well as their turbidity) is proportional to the weight-average molecular weight ($\overline{M}_w$) of the species in the solution (Wishnia, 1975; Godefroy-Colburn, 1975). Thus, for a constant ribosomal concentration, the intensity of light scattering at 90° varies linearly with the concentration of the 70S species; the ratio (x) of the relative intensity of the light scattering ($I_y - I_0$) at selected y mM Mg^{2+} to the relative intensity of light scattering ($I_{100} - I_0$) at 6 mM

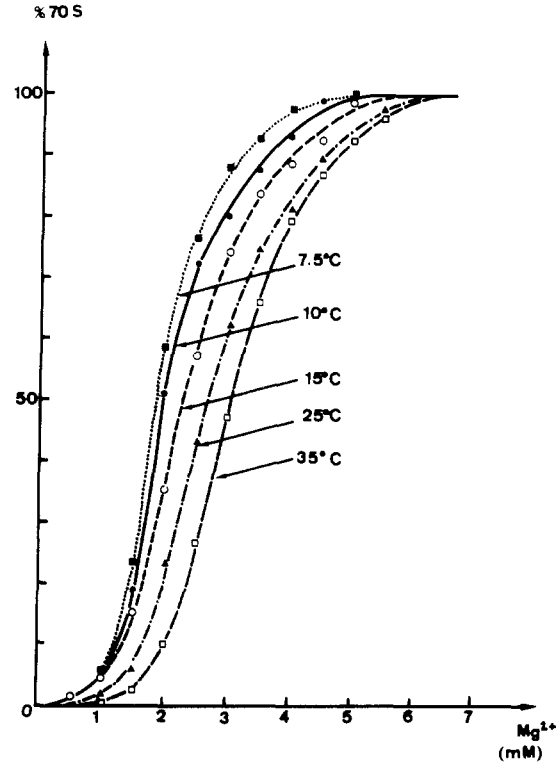

FIGURE 1: Association equilibria of A-type ribosomes as a function of Mg^{2+}, at different temperatures. Mg^{2+} concentration was as indicated. Buffer A was used. Final ribosome concentration was A_{260} = 7.1 units/mL for curves at 7.5, 10, and 15 °C; A_{260} = 8.4 units/mL for curves at +25 and 35 °C.

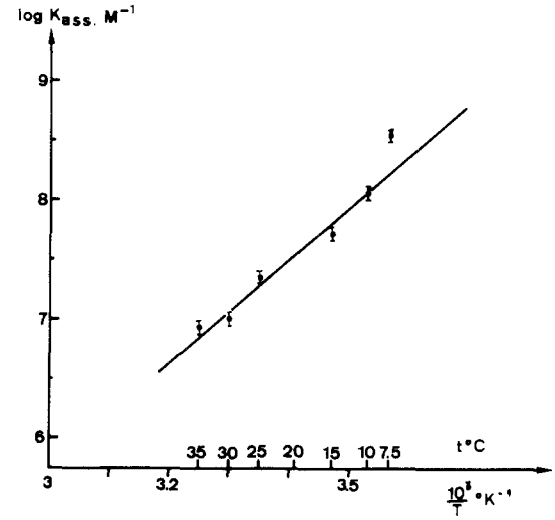

FIGURE 2: van't Hoff plot of log K_{assoc} vs. $1/T$. Mg^{2+} concentration was 3 mM. The association equilibrium constant, K_{assoc}, was calculated from Figure 1 at 3 mM Mg^{2+}.

Mg^{2+}, where the ribosomes are 100% 70S, gives the percent of 70S present in y mM Mg^{2+}. This procedure, similar to that of Debey (1975), was used for determining the percentage of 70S species as a function of magnesium and temperature. The apparent dissociation constant (K_{dissoc}) of the 70S ribosome into subunits at any Mg^{2+} concentration is given by

$$K_{dissoc} = \frac{1}{K_{assoc}} = \frac{(1-x)^2}{x} C$$

where x is the percent of 70S species present in the solution and C the total molar concentration of ribosomes (the 30S and 50S free subunit concentrations are equal).

Kinetic curves obtained by the stopped-flow apparatus were analyzed according to second-order kinetics with the help of a computer program for which we are very much indebted to Dr. Wishnia. By this procedure, each curve yields the apparent forward and backward rates of reaction, k_1 and k_2

$$70\,S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} 30\,S + 50\,S$$

and the apparent equilibrium constant

$$K_{dissoc} = \frac{1}{K_{assoc}} = \frac{k_1}{k_2}$$

The scale factor of the stopped-flow was obtained as described by Wishnia, either directly by determining the turbidity of the solution corresponding to total dissociation and association, or by the values of $\overline{M}_w$ expected for initial and final Mg^{2+} concentration, as extrapolated from the static curve of Figure 1. The weight-average molecular weight ($\overline{M}_w$) used was 1.4×10^6 for 0% association and 2.6×10^6 for 100% association. The total error of the turbidimetric method for the determination of the kinetic parameters k_2, k_1 was about ±10 to 15%.

Results

Equilibirum. Figure 1 shows the magnesium titrations of 70S ribosomes in buffer A, at five different temperatures. As can be seen in these curves, the $[Mg^{2+}]_{1/2}$ at +25 °C is equal to 2.7 mM. We checked that the log plot of the apparent association equilibrium constant, K_{assoc} = [70 S]/[30 S][50 S], as a function of log[Mg^{2+}], was only linear between 1.5 and 4 mM Mg^{2+} with a slope n equal to 7, showing that the ribosomal preparation used consisted, indeed, of A-type ribosomes (Debey, 1975). Furthermore, a decrease in temperature from +35 °C to 7.5 °C results in a decrease of the values of $[Mg^{2+}]_{1/2}$ from 3.1 to 1.9 mM and a decrease of n from 7.5 to 6, indicating that low temperatures favor association of the 70S species. These results are in agreement with those of Debey et al. (1975) and Spirin et al. (1971).

For a fixed magnesium concentration (for example, 3 mM Mg^{2+}), and at each temperature, the apparent equilibrium association constant K_{assoc} was calculated, as explained under Materials and Methods, using the fraction of 70S species given by the curves of Figure 1. A van't Hoff plot of log K_{assoc} vs. $1/T$ (Figure 2) enables the calculation of the standard enthalpy and entropy changes for the reaction written in the direction of association. The thermodynamic constants are summarized in Table I: a decrease in temperature increases the apparent equilibrium association constant (K_{assoc}); the standard enthalpy change of this reaction calculated from the plot of Figure 2 is $\Delta H°_{assoc}$ = −19.9 kcal/mole and the standard entropy change is $\Delta S°_{assoc}$ = −33 cal/(deg mol); $T\Delta S°_{assoc}$ was calculated to be −9.8 kcal/mol, indicating that the enthalpy change is the dominant term in the association reaction of ribosomal subunits.

Kinetics. The association kinetics of the ribosomal subunits, after a magnesium jump from 1.5 to 3 mM at +25 °C, is shown in Figure 3. The apparent rate constant of association is $k_2 = 4.8 \times 10^5\ M^{-1}\ s^{-1}$. The apparent equilibrium dissociation constant (K_{dissoc}) is equal to 89×10^{-9} M. The apparent rate constant of dissociation (k_1) was calculated from k_2 and K_{dissoc} ($= k_1/k_2$) and was found to be $k_1 = 43 \times 10^{-3}\ s^{-1}$. These results were compared to those of Wishnia et al. (1975) under

TABLE I: Kinetic and Thermodynamic Parameters of Association of A-Type Ribosome.[a]

t (°C)	35	30	25	15	10	7.5
$K_{assoc} \times 10^{-7}$ (M^{-1})	0.87	1	2.2	6.7	11.8	37
$\Delta G°_{assoc}$ (kcal/mol)	−9.8	−9.7	−10	−10.3	−10.5	−11
$\Delta H°_{assoc}$ (kcal/mol)			−19.9			
$\Delta S°_{assoc}$ (cal/(deg mol))			−33			
$k_2 \times 10^{-5}$ (M^{-1} s^{-1})		1.4	1	0.7	0.66	
$\Delta H^{\ddagger}_2$ (kcal/mol)			+7			
$\Delta S^{\ddagger}_2$ (cal/(deg mol))			−12			
$k_1 \times 10^{3}$ (s^{-1})		14.4	4.5	1	0.56	
$\Delta H^{\ddagger}_1$ (kcal/mol)			+26			
$\Delta S^{\ddagger}_1$ (cal/(deg mol))			+18			

[a] The Mg^{2+} concentration was 3 mM in buffer A.

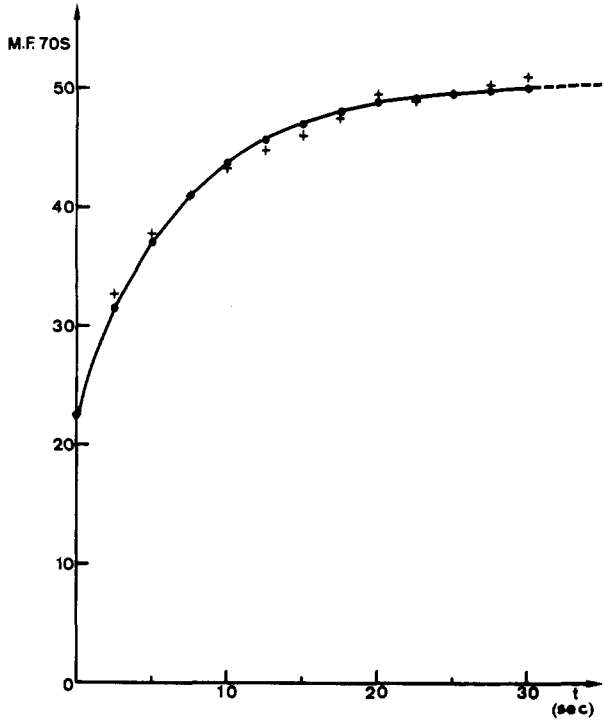

FIGURE 3: Kinetics of association of ribosomal subunits. Experiments were performed at 3 mM magnesium concentration, +25 °C, and in buffer B. Jump is 1.5 to 3 mM Mg^{2+}. Final ribosome concentration A_{260} = 7.51 units/mL. The curve is cut off at 40 s to emphasize the changes in relative rates, and values below 1 s have been suppressed for clarity. (+) Experimental values; (-●-) theoretical curve.

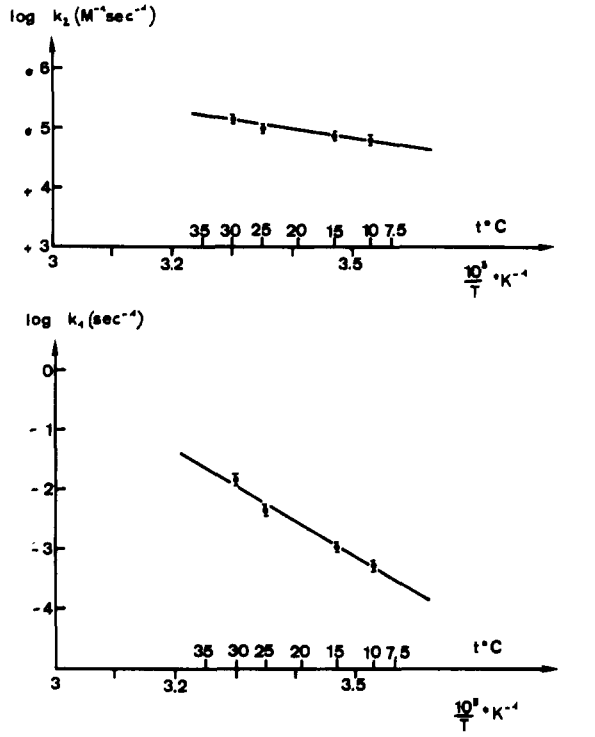

FIGURE 4: Arrhenius plots of kinetics of association and dissociation parameters of A-type ribosomes at fixed magnesium concentration (3 mM) and in buffer A.

the same buffer and ionic strength conditions, buffer B at +25 °C, in order to check our preparation. They found k_2 = 6.3 or 4.3×10^5 M^{-1} s^{-1}, $k_1 = 2.5 \times 10^{-3}$ s^{-1}, and $K_{dissoc} = 4 \times 10^{-9}$ M. The apparent rate constants of association (k_2) are similar for both our and their experiments, but the apparent rate constant of dissociation (k_1) is greater for the ribosomal preparation used in this study.

Kinetic studies were then performed as a function of temperature, using the same procedure, except that buffer A was used. This buffer was preferred to buffer B, as indicated under Materials and Methods. Apparent rate constants and equilibrium constant were obtained under these conditions for four different temperatures after a magnesium jump from 1.5 to 3 mM. The average of three runs are given in Table I. We note that at +25 °C, the values of the apparent rate constants of association ($k_2 = 10^5$ M^{-1} s^{-1}) and dissociation ($k_1 = 4.5 \times 10^{-3}$ s^{-1}) are smaller than those obtained in buffer B; the apparent equilibrium dissociation constant ($K_{dissoc} = 4.5 \times 10^{-8}$ M) is also smaller, indicating that more ribosomal subunits are associated in buffer A than in buffer B, but comparison of these results is actually difficult because of the strong differences between the two buffers (nature and pH of the buffer as well as concentration of NH_4Cl and total ionic strength). Systematic experiments are needed to understand the individual effect of these parameters on the subunit association.

Temperature effect of the apparent rate constants is shown in Table I: both rate constants of association and dissociation decrease when temperature decreases, but the decrease of k_1 is much more drastic.

The activation parameters for the reaction were obtain from the temperature dependence of the rate constants. Arrhenius plots for k_2 and k_1 (Figure 4) give the activation energies of association (E_2 = 7.5 ± 1 kcal/mol) and dissociation (E_1 = 26.5 ± 3 kcal/mol). The enthalpy of activation is given by

$\Delta H^{\ddagger} = E_a - RT$ and the entropy of activation is calculated from the equation

$$k = \frac{RT}{Nh} \exp(-\Delta H^{\ddagger}/RT) \exp(\Delta S^{\ddagger}/R)$$

where R is the gas constant, N is Avogadro's number, h is Planck's constant, and T is the absolute temperature. The calculated activation enthalpy and entropy (Table I) are equal to $\Delta H^{\ddagger}_2 = 7$ kcal/mol, $\Delta S^{\ddagger}_2 = -12$ cal/(deg mol) for the association; $\Delta H^{\ddagger}_2 = 26$ kcal/mol, $\Delta S^{\ddagger}_1 = 18$ cal/(deg mol) for the dissociation. The free energies of activation for association and dissociation depend very little on temperature and are, respectively, equal to $\Delta F^{\ddagger}_2 \simeq 10.5$ kcal/mol, and $\Delta F^{\ddagger}_1 \simeq 20.5$ kcal/mol. These results show that the reactions of association and dissociation at 3 mM Mg^{2+} are controlled by the enthalpy terms $T\Delta S^{\ddagger}_2 = -3.6$ kcal/mol and $T\Delta S^{\ddagger}_1 = +5.5$ kcal/mol (for $T = 298$ K) and smaller than $\Delta H^{\ddagger}_2$ and $\Delta H^{\ddagger}_1$. The interaction between 30S and 50S subunits yielding 70 S occurs with an energy barrier of 7 kcal/mol at 3 mM Mg^{2+} and with little loss of entropy; in contrast, the dissociation of 70S species into 30S and 50S subunits occurs with a higher energy barrier of 26 kcal/mol and a small increase of entropy.

Discussion

In the present work, we have defined an apparent equilibrium association constant at fixed magnesium concentration according to the following relation

$$K_{assoc}^{app} = \frac{k_2^{app}}{k_1^{app}} = \frac{[70\text{ S-}Mg^{2+}]}{[30\text{ S-}Mg^{2+}][50\text{ S-}Mg^{2+}]}$$

We included in the equilibrium the magnesium bound to the free subunits as well as to the associated ones, since Choi and Carr (1967) have shown that the magnesium binding mechanism with ribosomal particles was primarily an electrostatic interaction of the cations with the phosphate groups of RNA. They also showed that this binding was essentially identical (in terms of molar ratio of Mg^{2+} to RNA phosphate) for each individual 30S, 50S, and 70S ribosome within the limits of experimental error; but no information has been reported about the temperature effect on such an equilibrium.

We therefore verified that the temperature effect on the association of subunits does not involve a change in affinity of RNA phosphate groups for Mg^{2+}. This latter equilibrium can be estimated from the results of Clarke (1954) giving the standard enthalpy of ionization of some magnesium phosphates ($\Delta H^{\circ}_{dissoc} \simeq -2$ kcal/mol); the value obtained is small and of opposite sign as compared to the one for ribosome subunit association.

Our results show that the enthalpy change of the ribosomal equilibrium written in the direction of association is negative and has a high value ($\Delta H^{\circ}_{assoc} = -19.9$ kcal/mol), indicating that the process is exothermic with a small contribution of entropy ($\Delta S^{\circ}_{assoc} = -33$ cal/(deg mol)). This had also been observed by Noll and Noll (1976) for "tight" couples and by Zitomer and Flaks (1972) for "loose" couples. Our results confirm the finding of Noll and Noll that tight couples exhibit less negative ΔH°_{assoc} and ΔS°_{assoc} than the loose couples ($\Delta H^{\circ}_{assoc} \simeq -38$ kcal/mol, $\Delta S^{\circ}_{assoc} \simeq -90$ cal/(deg mol) for loose couples at $[Mg^{2+}]_{1/2} = 6$ mM and $\Delta H^{\circ}_{assoc} \simeq -10$ kcal/mol, $\Delta S^{\circ}_{assoc} \simeq +5.5$ cal/(deg mol) at 2 mM Mg^{2+} for the tight couples). This indicates that tight couples probably undergo less conformation change upon association than loose couples, or have a smaller difference in amount of bound water between the free and associated subunits.

The thermodynamic data we obtained can be discussed in terms of enthalpy and entropy energies developed by the different types of forces which may contribute to the interaction between the subunits.

Apolar interactions are generally endothermic processes and the apolar bonds are stabilized largely by entropy effects (Kauzmann, 1959; Butler, 1937). This situation is quite different for the interactions between ribosomal subunits where the reactions are exothermic, controlled by the standard enthalpy effect and a loss of entropy. The activation enthalpy of dissociation of the 70S complex is positive and has a more important value than the one developed for breaking hydrophobic bonds. The activation entropy is also positive for the dissociation and is negative for the association of 70S couples. These activation entropy changes are not favorable for the formation of apolar bonds. Other arguments cast doubt on the significant participation of hydrophobic interactions. The effects of temperature and nonpolar substances on ribosomal subunit interaction are opposite to those on hydrophobic bonds: lowering the temperature strengthens the interaction between subunits, as shown by our results and those of Debey (1975), while it decreases the stability of hydrophobic bonds. Addition of a nonpolar substance strongly stabilizes 50S–30S couples (Spirin and Lishnevskaya, 1971), while it weakens the hydrophobic bonds (Kauzmann, 1959).

Recently, considerable evidence indicates that the binding of mRNA by bacterial ribosomes involves hydrogen-bond interactions between nucleotide sequences at the 3′ end of the 16S ribosomal RNA and complementary sequences in the mRNA (Van Duin et al., 1975; Schine, 1974; Steitz, 1975). Moreover, IF_3 bound to the 30S ribosomal subunit is also in the immediate neighborhood of the 3′ region of the 16S RNA and its associated proteins such as S_1 (Van Duin et al., 1975; Van Duin, 1976). In addition, sequence data for the 23S rRNA reveal the presence of two complementary sequences between nucleotides near the 3′- and 5′-terminal sequence of this rRNA and sequences contained in the 3′-OH end of the 16S rRNA (Ehresmann, 1975; Fellner, 1970; Branlant et al., 1976). It has been argued (Kurland, 1974; Branlant et al., 1976) that base pairing between the RNAs of each ribosomal subunits could provide a convenient way of forming a 70S couple.

Are the thermodynamic results on ribosomal subunit association consistent with the above model? To check this, we compared our data with those reported by Borer (1974) on ribooligonucleotide interactions as well as with those of Yoon et al. (1975) on codon–anticodon interaction on yeast phenylalanine transfer RNA. The equilibria enthalpies for this last interaction, $\Delta H^{\circ}_{assoc} = -14$ kcal/mol (UUC-G_mAA), -19 kcal/mol (UUCA-UG_mAA), are quite close to the one we obtained (-19.9 kcal/mol) for the association of ribosomal subunits. These values are also quite close to those predicted from the studies by Borer (1974) on ribooligonucleotide interaction: the enthalpy change for the formation of the second base pair was from -6 to -15 kcal/mol. The equilibrium entropy changes for subunit association ($\Delta S^{\circ}_{assoc} = -33$ cal/(deg mol)) and for codon–anticodon interaction of (ΔS°_{assoc} of UUC-G_mAA $= -36$ cal/(deg mol)) are also quite consistent. It is however smaller than those predicted by Yoon et al. (1975) from the studies of Borer (1974): -16 to -35 cal/(deg mol) for the second base pair.

For codon–anticodon association, rate constants are 2×10^5 M^{-1} s^{-1} for U-U-C, and 2×10^7 M^{-1} s^{-1} for U-U-C-A; the corresponding apparent activation enthalpy is positive, 3 and 2 kcal/mol, respectively. The rate constants for the dissociation process are 330 s^{-1} for U-U-C, and 1700 s^{-1} for U-U-C-A with positive apparent enthalpies of activation of 17 and 21

kcal/mol, respectively.

These results agree with those obtained by Pörschke (1973) on the kinetics of helix-coil transition with oligomers containing G-C pairs. The enthalpies of activation for the association and dissociation of ribosomal subunits are of the same sign and order of magnitude than the results reported above. Some small differences exist due to experimental conditions: for example, the enthalpy of activation $\Delta H^{\ddagger}_2 = +7$ kcal/mol for 70S couples was obtained at a final concentration of magnesium of 3 mM, instead of 10 mM for the experiments of codon-anticodon interaction. Under our conditions, the electrostatic repulsions from charged phosphates of the nucleic acid backbone remain important and probably act by increasing the energy barrier which decreases the rate constant of the 70S complex formation (Wishnia, 1975).

The kinetics and thermodynamic data on ribosome association are therefore consistent with a 16S-23S RNA interaction through hydrogen-bonded base pairs reinforcing the model postulated by Van Duin (1976). It would be premature at this time to speculate on the number of base pairs involved, since the exact mechanism of these interactions is complicated by the presence of ribosomal proteins.

Acknowledgments

The authors are very much indebted to Dr. P. Douzou who initiated this work and to Dr. A. Wishnia for the help of a computer program; we are most grateful to Dr. B. Cooperman and A. Danchin for critical comments and helpful discussion and to H. Costinesco for skillful assistance in reviewing the manuscript.

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