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Magnesium Dependence of the Association Kinetics of *Escherichia coli* Ribosomal Subunits†

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ABSTRACT: The magnesium dependence of the *Escherichia coli* ribosomal subunits association has been investigated by the stopped-flow technique using isolated 30S and 50S particles depleted of polyamines and any initiation factor. Binding of the fluorescent probe bis(8-anilino-1-naphthalenesulfonate) to the ribosomal proteins occurs through biphasic kinetics. A dark reaction corresponding to a very rapid, reversible complexation of the dye molecule is followed by a slow photochemical reaction that gives rise to irreversible addition of the probe. Only the 30S subparticle exhibits a magnesium-dependent conformational change from the kinetic analysis of the dark reaction. The 70S formation kinetics are limited by a conformational change of the 30S subunit if this particle is depleted of Mg^{2+} (1 mM Mg^{2+}/50 mM K^+), while its activated structure is restored by incubation with 8 mM Mg^{2+}/50 mM K^+. No rate-limiting conformation rearrangement of the 50S subunit could ever be evidenced. The Mg^{2+} dependence of the association kinetics of preactivated ribosomal particles is satisfactorily explained by electrostatic effects and/or formation of salt bridges, in agreement with the results of Wishnia and co-workers (Wishnia, A., Boussert, A., Graffe, M., Dessen, P., and Grunberg-Manago, M. (1975), *J. Mol. Biol. 93,* 499). Equilibrium studies indicate that the ribosomal preparations we used are of B type, according to Debey et al. (Debey, P., Hui Bon Hoa, G., Douzou, P., Godefroy-Colburn, T., Graffe, M., and Grunberg-Manago, M. (1975), *Biochemistry 14,* 1553). The addition of spermidine results in a drastic fall of the need of Mg^{2+} for association, but it does not allow conversion of B-type particles into A-type ones at 25 °C. In addition to that, some 30S-bound spermidine appears to be involved directly in the coupling reaction.

Repeated association and dissociation of the ribosomal particles is essential in the living cell (Kaempfer et al., 1968). As a matter of fact, the initiation of protein synthesis in *Escherichia coli* requires dissociation of resting 70S ribosomes into 30S and 50S subunits that, after binding of several factors to the 30S particle, reassociate to yield activated ribosomes (Nomura and Lowry, 1967). In vitro a variety of factors, such as monovalent (destabilizing) and divalent (stabilizing) cations, polyamines, antibiotics, and nonionic agents, are involved in the coupling of ribosomal subunits (Spirin and Lishnevskaya, 1971). The magnesium ion dependence of the association reaction, known for a long time (Chao and Schachman, 1956; Chao, 1957; Tissières et al., 1959), obeys a true equilibrium as shown by Zitomer and Flaks (1972).

However, artifacts arise from the heterogeneity of the ribosome preparations. Two types of 30S/50S couples have been discriminated from their resistance to hydrostatic pressure in centrifugation experiments (Van Diggelen and Bosch, 1973; Van Diggelen et al., 1973; Noll et al., 1973). These two types show marked differences in their magnesium-dependent association equilibrium (Debey et al., 1975). No rate-limiting step expected from a conformational change was observed in the association kinetics of those subunits (A type) that exhibit the most sharp Mg^{2+} dependence for coupling (Wishnia et al., 1975), while 70S formation from ribosomal subunits with a loose Mg^{2+} dependence for association (B type) was suggested to involve some rearrangement of the conformational state of the subparticles (Zitomer and Flaks, 1972; Wolfe et al., 1973; Pochon and Ekert, 1973). Limited changes in the topography of the ribosomal subunits upon dissociation of the 70S complex have been evidenced unambiguously from a variety of methods, namely, hydrogen-exchange rate studies (Huang and Cantor, 1972), binding of dye molecules (Sherman and Simpson, 1969; Chang, 1973), iodination (Litman et al., 1974), and nonenzymatic binding of cofactors (Zamir et al., 1974). In other words, both the association and dissociation reactions can involve conformational changes within quite a number of ribosomal proteins rather than simple joining of rigid association-specific interfaces. It is by no means certain that such conformational rearrangements (possibly related to enzymatic activities of the ribosomal proteins) appear in the association kinetics of isolated subunits, since activation of the ribosomal particles proceeds through several intermediate stages (Zamir

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et al., 1974). For example, the primary effect of the magnesium ion in the association kinetics of A-type subunits is to decrease electrostatic repulsion between 30S and 50S particles (Wishnia et al., 1975). Observation of a (first-order) rate-limiting step in the kinetics of 30S/50S coupling should thus agree with a rather large conformational alteration of one or both subunits (Zamir et al., 1974). The importance of these structural changes should depend essentially on the way the ribosomes have been prepared. In particular, reversible inactivation follows the removal of certain specific cations (Zamir et al., 1971). We are reporting here on the Mg^{2+} dependence of the association kinetics (and equilibria) of extensively washed ribosomal particles from *E. coli* that, although depleted of polyamines and any initiation factor, exhibit a rather high activity for polyphenylalanine synthesis through standard assays. Spermidine was reintroduced to the system in order to know whether the ribosomes had been damaged upon purification. We also measured the kinetics for the binding of the fluorescent probe bis(8-anilino-1-naphthalenesulfonate),[1] since the noncovalent association of this dye reflects the conformational state of the ribosomal particles (Pochon and Ekert, 1973) and affects neither 30S/50S coupling nor polypeptide synthesis (Pochon et al., 1974).

Experimental Procedure

Buffers. Buffer I: 50 mM Tris-HCl, 20 mM $MgCl_2$, 50 mM KCl, pH 7.8. Buffer II: 50 mM Tris-HCl, 1 mM $MgCl_2$, 50 mM KCl, pH 7.8. Buffer III: 10 mM Tris-HCl, 1 mM $MgCl_2$, 100 mM KCl, pH 7.4.

70S Ribosomes. 70S ribosomes have been prepared from midlogarithmic MRE 600 *E. coli* cells according to Kurland (1966), then suspended in buffer I at a concentration of about 60 mg/ml, and stored at liquid nitrogen temperature. The samples used for the experiments reported in this paper were free of peptidyl-tRNA, polyamines, and mRNA fragments.

Ribosomal Subunits. 70S ribosomes were dissociated by suspending them in buffer III, and the 30S and 50S subunits were isolated by zonal centrifugation in a Ti 14 rotor according to Eikenberry et al. (1970). Isolated subunits were recovered by sedimentation for 16 h at 50 000 rpm in a Ti 60 rotor after the Mg^{2+} concentration was raised to 20 mM. 30S subunits were 99% pure; 50S subparticles were 97% pure only, due to partial contamination by 30S subunits. Free subunits were resuspended in buffer II at a concentration of about 40 mg/ml, stored in liquid nitrogen, and used a few days after their preparation. Further dilutions were made with buffer II adjusted to the desired $MgCl_2$ concentration. Each experimental series has been performed by using the same sample of ribosomal subparticles in order to prevent systematic errors in the relative rate measurements. 70S ribosomes were obtained by mixing equimolar amounts of the isolated subunits. The ribosomal subunits concentrations were measured spectrophotometrically at 258 nm, using values of 1.0×10^7, 2.7×10^7, and 3.9×10^7 M^{-1} cm^{-1} for the molar extinction coefficients of the 30S, 50S, and 70S particles, respectively.

Analytical Sucrose Gradients. All SW 41 gradients (40 000 rpm for 4 h at 5 °C) were 7–38% sucrose in buffer II adjusted to the appropriate $MgCl_2$ concentration.

Two-Dimensional Gel Electrophoresis. Isolated ribosomal subunits were disrupted by LiCl-urea treatment (Traut et al., 1971). The protein fractions were analyzed by two-dimensional gel electrophoresis following the method of Kaltschmidt and Wittmann (1970). The rRNA fraction was treated with phenol for sucrose gradient analysis. Protein extraction was also carried out by the acetic acid method (Waller and Harris, 1961): the dialysate obtained from the supernatant fraction was free of spermidine, as evidenced by the dansyl method (Cohen et al., 1969).

Stopped-Flow Experiments. The kinetic measurements have been performed on a Durrum Instruments stopped-flow spectrophotometer, using a rather low syringe drive rate in order to minimize hydrodynamic troubles upon mixing (mixing dead time: 4 ms). Transmittance measurements were carried out at 315 nm through a 2-cm light path length cuvette. The detection of fluorescence emission and of scattered light was performed using a photomultiplier disposed close by the optical cuvette with convenient shut-off filters, at 90° from the incident beam. All the solutions were 50 mM with respect to both Tris-HCl buffer and KCl (buffer II) in order to prevent any concentration jump (specially of KCl) that could perturb the kinetics investigated as a function of the concentration in magnesium ion or in bis-ANS. Those conditions are similar to those used by Zitomer and Flaks (1972) but different from those of Wishnia et al. (1975) who used NH_4Cl in lieu of KCl with a lower Tris-HCl concentration. On investigations as a function of the Mg^{2+} concentration, magnesium was added as $MgCl_2$ up to 300 mM. No pH change was observed under these conditions. All the experiments have been performed at controlled temperature (25 °C). Every sample of ribosomal subunits was allowed to stand in buffer II adjusted to the desired Mg^{2+} concentration for at least 60 min at 25 °C or 15 min at 37 °C before it could be used in kinetic experiments.

Let us consider an exponential time-course evolution of the absorbance:

$$\ln [(A_e - A)/(A_e - A_0)] = -k_i t \quad (1)$$

where A_e, A_0, and A are the absorbance values measured at the end of the reaction, at the mixing time, and at any reaction time, respectively. The initial reaction rate can be calculated as:

$$v_i = k_i C_e \quad (2)$$

where C_e is the end-reaction concentration of the product formed. Now, let us consider a second-order reaction where starting concentrations of each reactant are the same; a hyperbolic variation of absorbance is expected:

$$1/(A_e - A) = \alpha t + 1/(A_e - A_0) \quad (3)$$

The initial rate of such a second-order reaction can be calculated through eq 2, provided the initial first-order slope k_i is calculated as follows:

$$\lim (e^{k_i t})_{t \to 0} = 1 + k_i t \quad (4)$$

$$\Rightarrow k_i = \alpha(A_e - A_0) = 1/t_{1/2} \quad (5)$$

As a matter of fact, k_i could not be estimated accurately from log plots in case of a hyperbolic absorbance variation.

Results and Discussion

Functional Assays. The two-dimensional polyacrylamide electrophoretic patterns were similar to those published by Kaltschmidt and Wittman (1970), although small quantitative differences cannot be excluded. On the other hand, the 16S, 23S, and 5S rRNA molecules were demonstrated to be undamaged by sucrose gradient centrifugation analysis.

The poly(U)-directed synthesis of poly(phenylalanine) was

[1] Abbreviations used are: ANS, 8-anilino-1-naphthalenesulfonate; poly(U), poly(uridylic acid); Tris, 2-amino-2-hydroxymethyl-1,3-propanediol.

carried out essentially according to Nirenberg (1963), using a chromatographically fractionated poly(U) having a molecular weight of about 100 000. The ribosomes incorporated 60 mol of phenylalanine/mol of ribosome under conditions of limiting ribosome. Washed 70S particles had the same activity in poly(phenylalanine) synthesis as those reconstituted from subunits isolated by sucrose gradient centrifugation in buffer III.

Binding of Bis-ANS to the Ribosomal Subunits. Binding of bis-ANS to specific sites of still debated structure (Reeves et al., 1974; Kosower and Dodiuk, 1974; Kosower et al., 1975; Seliskar and Brand, 1971; Kosower and Tanizawa, 1972; Turner and Brand, 1968) in ribosomal proteins results in a 200-fold enhancement of the fluorescence quantum yield of the dye as compared with its free form (Pochon and Ekert, 1973; Rosen and Weber, 1969). The association kinetics of bis-ANS to purified 30S and 50S subunits preequilibrated with two selected magnesium concentrations (either buffer II, or buffer II plus 20 mM $MgCl_2$) have been investigated as a function of the dye concentration, using the stopped-flow apparatus operated in the light-emission mode. The excitation wavelength was 400 nm; observation through a shut-off filter (1% T at 424 nm, 85% T at 480 nm) allowed integration of the major part of the fluorescence band of bis-ANS.

The time-course evolution of the fluorescence intensity observed upon mixing suspensions (0.2–0.4 μM) of 30S or 50S ribosomal subunits with bis-ANS is essentially dependent upon the dye concentration (Figure 1). At the lower bis-ANS concentrations used, the fluorescence intensity increases through biphasic kinetics. The slower phase disappears as the bis-ANS concentration is raised, in such a way that the kinetics appear monophasic for a dye concentration of 1.5 μM. At higher bis-ANS amounts, a slow decrease of the fluorescence intensity happens after a maximum value has been attained rapidly through an exponential phase (Figure 1). These two steps do not follow the same dependence upon the initial conditions (nature of the ribosomal subunit, concentration of Mg^{2+}, and of bis-ANS). Therefore, the overall reaction must be regarded as the superimposition of two distinct mechanisms.

Using a conventional spectrofluorometer, it could be evidenced that the light irradiation (400 nm) of a suspension of 30S or 50S subunits (0.1 μM) in buffer II containing bis-ANS (5 μM) results in a drastic decay of the fluorescence intensity, up to 50%, with respect to that of a reference sample kept in the dark. No other modification of the fluorescence yield was observed upon addition of bis-ANS to the irradiated sample, while addition of bis-ANS free buffer simply resulted in a fluorescence decrease proportional to the dilution factor. Thus, bis-ANS binds irreversibly to ribosomal subparticles through a photochemical reaction, the rate of which agrees with that of the slow phase evidenced from stopped-flow experiments ($t_{1/2}$ = 20–50 s, depending upon the conditions). Since long enough light irradiation of a mixture of bis-ANS and 50S subunit results in a constant fluorescence intensity as far as the concentration ratio [bis-ANS]/[50S] is higher than 6–7, the photolytic step involves only a limited number of amino acid residues. The sites that control the dark reaction are involved in the photochemical reaction, since this reaction results in a serious decrease of the fluorescence quantum yield of the dye as compared with its reversibly bound form. Since the irreversibility of the photochemical step makes it unsuited to the study of any magnesium-induced conformational change of the ribosomal subparticles, the following discussion will concern only the analysis of the dark, reversible reaction.

At bis-ANS concentrations lower than 2 μM, the photolytic

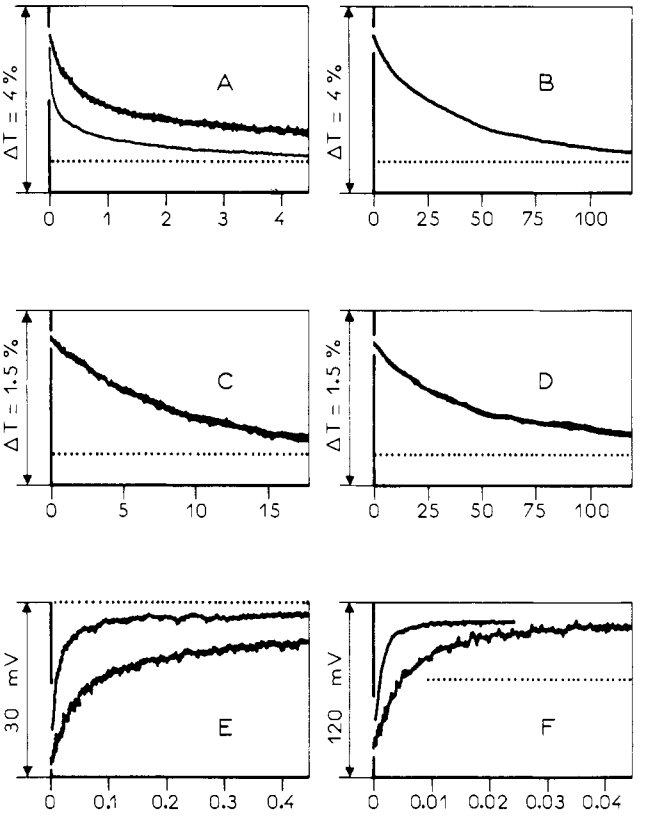


FIGURE 1: Oscilloscope recordings of the transmittance variations (315 nm) upon ribosomal subunits association (A–D) and of the changes of the fluorescence intensity upon binding of bis-ANS to the 50S subparticle (E and F). The dotted lines correspond to the position of the oscilloscope trace at the end of the reactions. Time (X axis) is given as seconds; for double-trace oscillograms, the larger sweep time is tenfold that indicated. Association experiments: isolated subunits were preequilibrated with 30 mM (A) or 150 mM (C) Mg^{2+}, or an equimolar suspension of 30S and 50S subunits in buffer II was mixed with an equal volume of 60 mM (B) or 300 mM (D) of $MgCl_2$ in buffer II; each subunit was 0.5 μM before mixing. Experiments with bis-ANS: the final concentration of the 50S subunit was 0.12 μM; Mg^{2+} was 1 mM (buffer II); bis-ANS was 0.5 μM (E) or 5.0 μM (F).

step cannot be clearly distinguished from the rapid kinetic phase, but it is well differentiated at higher dye concentrations (Figure 1). Then the variations of the fluorescence intensity with respect to the maximum transitory value in the course of the first step obey an exponential function for several half-reaction times. Extrapolation of the first-order log plots to the zero-reaction time (4 ± 0.2 ms) thus gives a precise determination of the total fluorescence change ΔI_0 involved in the fast step. The reciprocal of ΔI_0 and of the total bis-ANS concentration $[\text{bis-ANS}]_0$ fit linearly with each other (Figure 2). Such a behavior reveals that the binding of bis-ANS to the proteins of the 30S or 50S ribosomal particle obeys an equilibrium reaction with indistinguishable formation constants for the various binding sites:

$$\text{subunit} + \text{bis-ANS} \underset{k_-}{\overset{k_+}{\rightleftharpoons}} (\text{subunit-bis-ANS})^*$$

Under the conditions used, the amount of bis-ANS involved in the formation of the fluorescent complex (subunit–bis-ANS)* is negligibly low relatively to the total bis-ANS concentration. So, the mathematical treatment of the above equilibrium can be made simply by assuming that the fluorescence quantum yield of the dye is not significantly dependent upon the site to which it is bound:

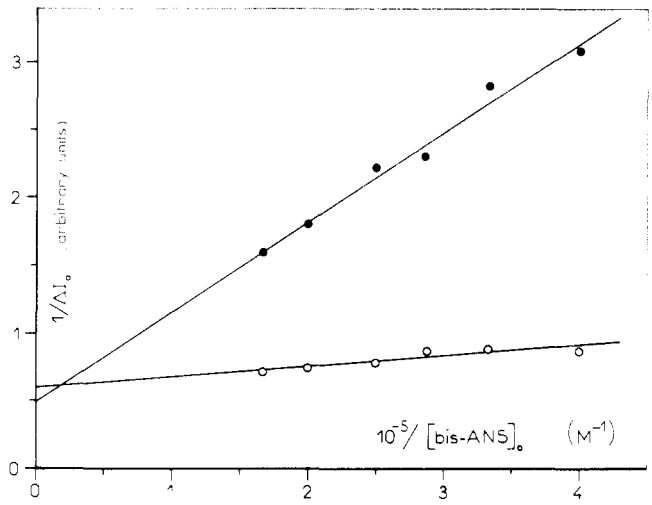


FIGURE 2: Benesi-Hildebrand double-reciprocal plot (least-squares calculation) of the fluorescence intensity change involved in the first step, ΔI_0, vs. the overall bis-ANS concentration, [bis-ANS]$_0$, for the 30S subunit (0.15 μM) at a magnesium ion concentration of 1 mM (○) or 20 mM (●).

$$\frac{1}{\Delta I_0} = \frac{\alpha}{\bar{n}[S]_0}\left[1 + \frac{1}{K[\text{bis-ANS}]_0}\right] \qquad (6)$$

where α is an arbitrary constant, $\bar{n}$ the number of binding sites of the ribosomal subunit considered (which can be deduced from the ΔI_0 obtained at infinite concentration of bis-ANS), and $[S]_0$ the whole concentration of the ribosomal particle.

The equilibrium constant K for the complexation of bis-ANS to the 30S subunit exhibits a remarkable magnesium dependence, while the number $\bar{n}$ of binding sites, as deduced from the ordinate intercept of the Benesi-Hildebrand plots, is not appreciably varying (Figure 2). From the comparison of previous titrations (Pochon and Ekert, 1973) with the present data, the 30S particle in any conformational state appears to have two equivalent binding sites for bis-ANS. The first-order slope k exhibits a strictly linear dependence with respect to the bis-ANS concentration, as expected from a bimolecular reaction under conditions of pseudo-first-order. The value of the rate constant k_+ can be calculated as the ratio $k/[\text{bis-ANS}]_0$. It appears from the values of k_+ and k_- that the magnesium-induced conformational change of the 30S subunit results in better accessibility and smaller bonding strength of the specific binding sites (Table I).

On the contrary, the conformational state of the 50S particle should be regarded as invariable under the conditions used, since neither the equilibrium and rate constants nor the number of binding sites ($\bar{n} = 4$) are dependent on the Mg^{2+} concentration used (Table I).

So, the kinetic analysis of the binding of bis-ANS to ribosomal subunits preequilibrated with Mg^{2+} does evidence the stability of the 50S particle, while free 30S subunits exhibit a marked magnesium-dependent conformational change. Such a conformational rearrangement could correspond to a change in the location of the reversible bis-ANS binding sites, or simply to a change in their affinity toward the dye. This question cannot be settled out using bis-ANS because, when applying a Mg^{2+} concentration jump to subunits equilibrated with bis-ANS, slight changes in the fluorescence intensity are overcome by the photolytic process. The kinetic study of such conformational changes appears worthy to be investigated directly through the association of free ribosomal subunits.

Magnesium Dependence of the Association Equilibrium of Ribosomal Subunits. The mobility and the sedimentation profiles of 30S or 50S isolated subunits in analytical sucrose gradients were found independent of Mg^{2+} ion over the 1-200 mM concentration range: the possibility for a self-aggregation of isolated subunits under the conditions used is ruled out therefrom. The absence of aggregates of isolated subparticles, as well as of 70S couple, has also been evidenced directly by the measurement of the relaxation time for the depolarization of the triplet-triplet transition of a dye molecule covalently bound to the related particles (Lavalette, D., personal communication).

TABLE I: Equilibrium and Rate Constants for the Reversible Complexation of Bis-ANS to the Ribosomal Subunits.

	$[Mg^{2+}]$ (mM)	K (M^{-1})	k_+ ($M^{-1}\,s^{-1}$)	k_- (s^{-1})
30 S	1	7.92×10^5	1.9×10^7	24
	20	0.75×10^5	3.4×10^7	450
50 S	1 or 20	3.70×10^5	5.8×10^7	157

The kinetic experiments have been carried out either by mixing equimolar suspensions of isolated 30S and 50S subparticles preequilibrated with buffer II adjusted to the appropriate concentration of $MgCl_2$, or by mixing with buffer II containing increasing amounts of $MgCl_2$ a suspension of both 30S and 50S subunits resting in buffer II. The initial concentration of each ribosomal subunit was kept constant (0.25 μM after mixing). The association degree can be measured by the absorbance variation ΔA_{315} from the mixing dead time ($t = 4 \pm 0.5$ ms, where the dissociation is still complete) to the end-reaction equilibrium (Figure 3). The association extent increases with the magnesium ion concentration. It reaches completion for a Mg^{2+} concentration of about 30 mM, as evidenced from analytical sucrose gradient centrifugation. As a matter of fact, the sedimentation profiles are free of any trace of dissociated particles at 30 mM of Mg^{2+}. The amount of $MgCl_2$ required for half-association is about 11 mM (Figure 3).

In addition to this well-known association equilibrium, reversible dissociation occurs at Mg^{2+} concentrations higher than 60 mM. The concentration of $MgCl_2$ required for half-dissociation is about 155 mM (Figure 3). That such a decrease of ΔA_{315} actually reflects a Mg^{2+}-dependent dissociation equilibrium has been evidenced by a lot of observations. (1) The association degree at any Mg^{2+} concentration does not depend on preequilibration of the ribosomal subunits with $MgCl_2$, but only on the conditions achieved after mixing, as expected from any equilibrium reaction. (2) The centrifugation of equimolar suspensions of 30S and 50S subunits in sucrose gradients at Mg^{2+} concentrations ranging over 1-200 mM exhibits the same association-dissociation pattern as turbidimetric measurements in the stopped-flow. (3) Upon dilution of Mg^{2+}, reversibility is directly related to the dilution factor. (4) The pH remained unchanged at all the Mg^{2+} concentrations used, while the kinetic analysis indicates that the various equilibria cannot be explained simply by ionic strength effects from the added $MgCl_2$. (5) No absorbance change was observed at any Mg^{2+} concentration for the isolated 30S or 50S subunits, which indicates the lack of self-association of these subparticles to yield aggregates under the conditions used, in agreement with the results reported above.

On the other hand, the stopped-flow measurements are not vitiated by the transient hydrodynamic shocks inherent in this

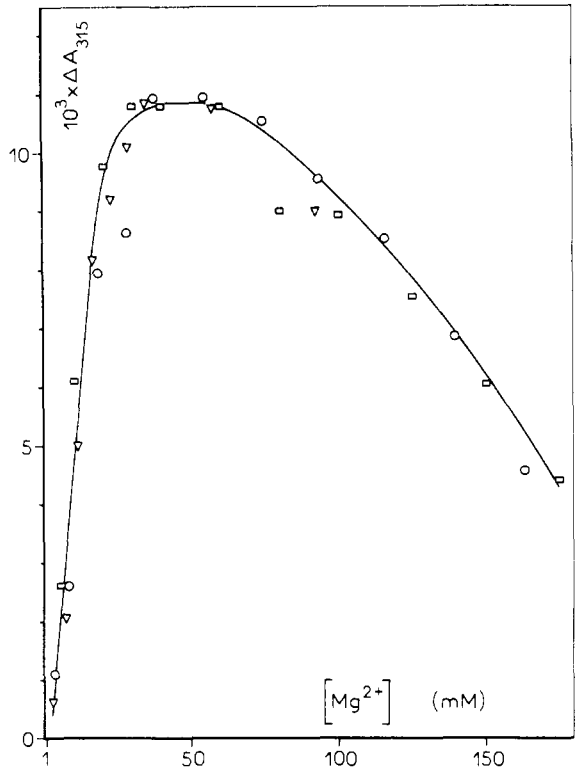


FIGURE 3: Magnesium-dependence of the equilibrium of 70S couple formation from equimolar (0.25 μM) 30S and 50S subunits. In stopped-flow experiments (○, □), the yield of association is related to the value of the absorbance change ΔA_{315} between mixing and the infinite reaction time; the subparticles were incubated either with 1 mM of Mg^{2+} (□) or directly with the final Mg^{2+} concentration (○). Light-scattering measurements (▽) were performed under similar conditions; an arbitrary scale proportional to ΔA_{315} was used for the scattered light intensity.

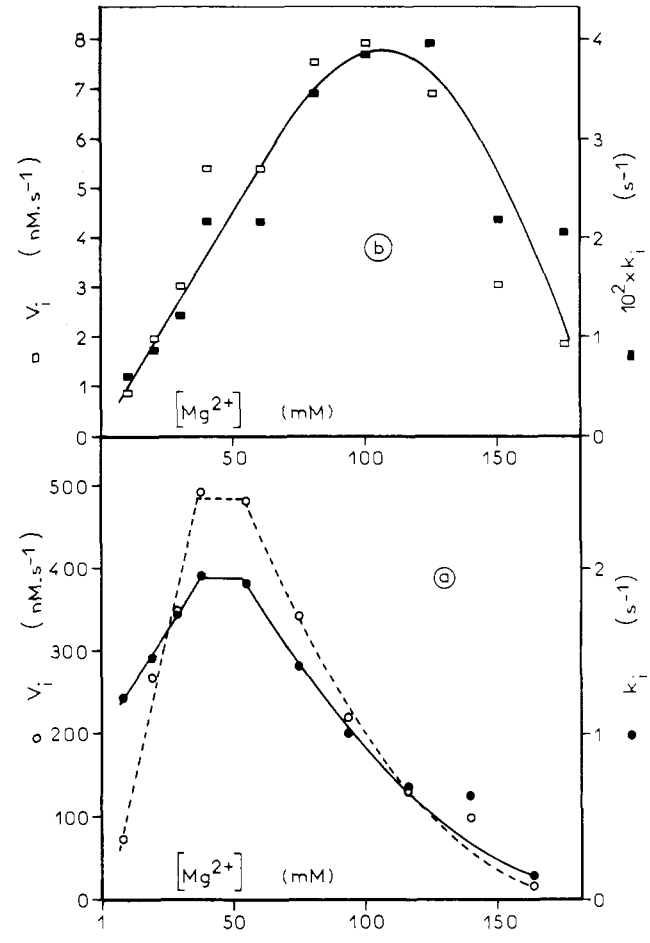


FIGURE 4: Magnesium dependence of the initial rate v_i (light dots) and of the initial slope k_i (black dots) for association of 30S and 50S subunits. (a) Ribosomal subparticles incubated separately with the final Mg^{2+} concentration. (b) Application of a concentration jump of Mg^{2+} to a fully dissociated suspension of ribosomal subunits in buffer II. All conditions are as in Figure 3. k_i was calculated according to 1-5.

technique, since light-scattering measurements performed on a conventional spectrofluorometer, as well as analytical sucrose gradients at several Mg^{2+} concentrations, correlate closely the results obtained from stopped-flow experiments. Moreover, the association kinetics were found independent of the syringe drive rate.

Magnesium Dependence of the Association Kinetics of Ribosomal Subunits. (a) The kinetic experiments can be performed by mixing equimolar suspensions of isolated subunits, both equilibrated separately with the same $MgCl_2$ concentration. Under these conditions, the equation that describes the time dependence of the 315-nm absorbance depends on the Mg^{2+} concentration and, although the kinetics can exhibit a biphasic character (Figure 1), it cannot be regarded as the superimposition of two exponential terms.

As the Mg^{2+} concentration is ranging from 8 to 55 mM, the absorbance changes obey the hyperbolic equation (3) for at least one-half reaction time and a roughly exponential function at long reaction times. The relative importance of the latter decreases somewhat as the Mg^{2+} concentration is raised; it vanishes nearly completely at 40–55 mM of $MgCl_2$, i.e., under conditions allowing both a complete association at equilibrium and a maximum value for the initial rate of 70S formation (Figure 3 and 4a). Therefore, the second order (with respect to time) of the rapid phase reflects the bimolecular association of this part of the subunits pool that is in an "activated" state directly suited to association. Deviation from strict second order, as observed at long reaction times, should correspond essentially to backward dissociation (first order) of the 70S couple:

$$30\,S^* + 50\,S^* \rightleftharpoons 70\,S \qquad (7)$$

although the existence of a small yield of subparticles with a Mg^{2+} dependence of activation particularly loose (from a strictly kinetic point of view) cannot be excluded.

The hyperbolic phase vanishes gradually as the Mg^{2+} concentration is increased beyond 60 mM, then an increasing part of the overall absorbance variation occurs through an exponential phase having a nearly constant half-reaction time ($t_{1/2} \simeq 6.5$ s), in such a way that the whole reaction obeys pseudo-first-order at Mg^{2+} amounts higher than 150 mM. This change in the apparent order of the association reaction agrees with an inhibitory saturation of one or both ribosomal subunits by the Mg^{2+} ion, i.e., the exponential step is expected to correspond to an equilibrium displacement (in favor of the 70S couple) which is kinetically limited by the steady-state concentration of unsaturated subparticles, as indicated by the invariability of the half-reaction time related to the exponential phase.

The initial slope k_i, calculated according to the eq 5 for best fit with the experimental data, exhibits a magnesium dependence similar to that for the initial association rate, except at the lower Mg^{2+} concentrations used (Figure 4a). The conclusions that can be drawn from this behavior will be detailed later in the discussion.

TABLE II: Initial Rates of Association of 30S and 50S Subunits Preequilibrated Asymmetrically with the Magnesium Ion.[a]

	I				II			
	a	b	c	d	a	b	c	d
30 S: $[Mg^{2+}]_{pre}$ (mM)	40	1	80	1	150	1	300	1
50 S: $[Mg^{2+}]_{pre}$ (mM)	40	1	1	80	150	1	1	300
$[Mg^{2+}]_f$ (mM)	40	40	40	40	150	150	150	150
v_i (nM s^{-1})	244	4.2	222	4.3	30.3	3.4	31.2	3.1

[a] The magnesium concentrations used for preequilibration of subunits and those obtained after mixing are indicated as $[Mg^{2+}]_{pre}$ and $[Mg^{2+}]_f$, respectively. 30S and 50S subparticles were 0.5 μM before mixing.

(b) The association kinetics depend not only upon the Mg^{2+} concentration at the mixing time, but also on the way the ribosomal subunits have been preequilibrated with $MgCl_2$ (Figure 1). In particular, a concentration jump of Mg^{2+} can be applied to an equimolar suspension of 30S and 50S subunits resting in buffer II, where dissociation is complete. The resulting association is much slower than the one measured by preequilibration of both isolated subparticles with the final magnesium ion concentration (Figure 4b). Such a kinetic effect agrees with the existence of a rate-limiting conformational change of one or both subunits, the activation of which is restored by Mg^{2+}.

The time dependence of the absorbance changes is strictly exponential at Mg^{2+} concentrations lower than 40 mM and higher than 150 mM, as expected from a rate-limiting conformational change. In the intermediate range of Mg^{2+} concentration, however, the kinetics are biphasic and can be analyzed roughly as the superimposition of two exponential terms with relative indices of $\frac{3}{1}$. The relative importance of the rapid phase never exceeds 30% of the overall absorbance variation and the kinetics remain essentially pseudo-first-order over the Mg^{2+} concentration range investigated, since the half-reaction time ($t_{1/2} \simeq 60$ s) does not depend appreciably upon the Mg^{2+} concentration used. The Mg^{2+} dependence of the apparent order indicates that activation of 70% of the subparticles proceeds from a fully resting state, while a 30% yield of subunits had recovered a *partly* activated structure before the Mg^{2+} concentration jump was applied. Given the relative importance and the slope of the kinetic phases corresponding to these two classes of subparticles, the contribution from the more active ones to the value of v_i is nearly equal to that from the looser ones at, say, 100 mM of Mg^{2+}. Therefore, the decrease of v_i at very high concentration in the magnesium ion could issue from the partly activated subunits only, and should correspond to the same saturation effect as already observed with preequilibrated subunits.

(c) Finally, isolated ribosomal subunits can be preequilibrated with Mg^{2+} in an asymmetric manner. Using a final Mg^{2+} concentration of 40 mM corresponding to complete association at equilibrium and to maximum initial association rate, it is clearly evidenced from the values of v_i under the four possible initial conditions (Table II) that the rate-limiting conformational change required to permit association concerns only the 30S subparticle, while activation of the 50S subunit does not depend appreciably on Mg^{2+} over the 1–40 mM concentration range.

On the other hand, the reversible inhibition of the subparticles at very high Mg^{2+} concentration should be regarded as a shielding due to chelation of magnesium ions, possibly to some association-specific interface, rather than as a secondary conformational change. As a matter of fact, only the former process could be fast enough to account for the nearly equal values of v_i in the experiments (IIa) and (IIc) on the one hand, and (IIb) and (IId) on the other hand (Table II).

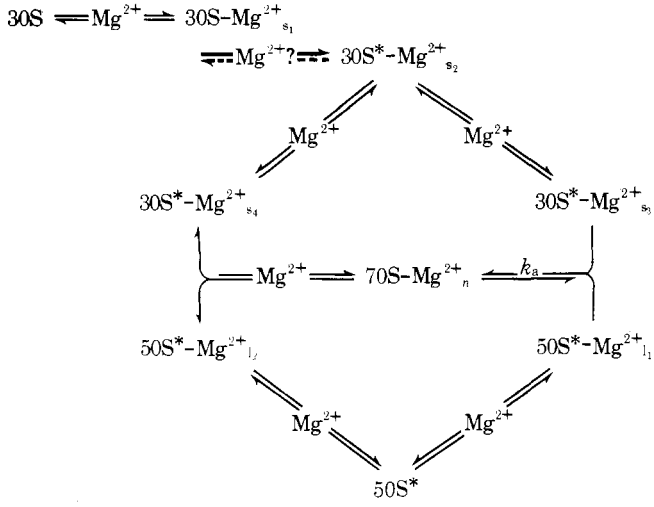
(d) One decisive observation is the dissimilarity between the Mg^{2+} dependence of the initial rate of association v_i and that of the initial slope k_i for subparticles preequilibrated separately with Mg^{2+} amounts lower than 40 mM (Figure 4a). For instance, a Mg^{2+} concentration of 19 mM is required to achieve a value of v_i equal to the half-part of its highest possible value, viz., $v_{i,max}$, while $k_i/k_{i,max} \simeq 0.76$ and the association degree at equilibrium is about 83% at the same Mg^{2+} concentration. Keeping in mind that $k_i = 1/t_{1/2}$ in case of a hyperbolic (second-order) absorbance variation, the value of the ratio $k_i/k_{i,max}$ is related to the yield of subunits that are in this state immediately suitable (i.e., without rate-limiting conformational change prior) to formation of the 70S couple. Now, k_i does not go to zero with $[Mg^{2+}]$ under the conditions used (Figure 4a). As a consequence, the conformational change of the 30S subunit that appears rate limiting by lack of preequilibration of this subparticle with the final Mg^{2+} concentration should not require a too high concentration in the magnesium ion to reach completion after a long enough incubation time, otherwise k_i would depend on $[Mg^{2+}]$ in a roughly proportional way, as does v_i up to 40 mM of Mg^{2+}. In other words, the conformational change of the 30S subunit seems to require a critical level of Mg^{2+}, lower than 8 mM from our experiments. The increase of k_i when going from 8 to 40 mM of Mg^{2+} thus results from another route than a conformational change. A diffusion-controlled equilibrated binding of Mg^{2+} to chelation sites located or not located in the association-specific interface of the subparticles appears as the more probable possibility in light of the work of Debey et al. (1975) and Wishnia et al. (1975) who established that the effect of the magnesium ion is essentially to decrease the coulombic repulsion between negatively charged subunits having the fully active conformation, and possibly to allow the formation of salt bridges. Then, any 30S/50S contact would not result in tight association as long as the chelation sites are not filled up with Mg^{2+} to a sufficient extent, i.e., the association rate should be controlled by the steady-state yield of these subparticles that have incorporated the average number of magnesium ions required to permit effective cancelling of electrostatic repulsion and possible formation of salt bridges. The association rate constant k_a of the fully activated 30S* and 50S* subparticles (Scheme I) should thus be calculated by:

$$k_a = v_{i,max}/[30S]_{total} \cdot [50S]_{total} \quad (8)$$

k_a is 7.8×10^6 M^{-1} s^{-1} under the conditions related to the Figure 4a.

The decrease of k_i at Mg^{2+} concentrations higher than 60 mM (Figure 4a) originates in chelation of magnesium ions to

SCHEME I: Magnesium-Dependent Association of Washed Ribosomal Subunits in the Absence of Spermidine.[a]



[a] The ribosomal subunits are under several possible states, depending on $[Mg^{2+}]$. Those at 1 mM of Mg^{2+} are 30S and 50S*. The species that have the conformation required to allow couple formation (k_a) are shown by an asterisk. Each subunit can incorporate magnesium ions through rapid, equilibrated chelation. Tight association is assumed to require chelation of an average number $n = (s_3 + l_1)$ of magnesium ions in order to permit cancelling of electrostatic repulsion and/or formation of salt bridges. Further chelation of Mg^{2+} (s_4, l_2) results in reversible 70S splitting. The rate-limiting conformational change of the 30S subunit is shown by bold arrows.

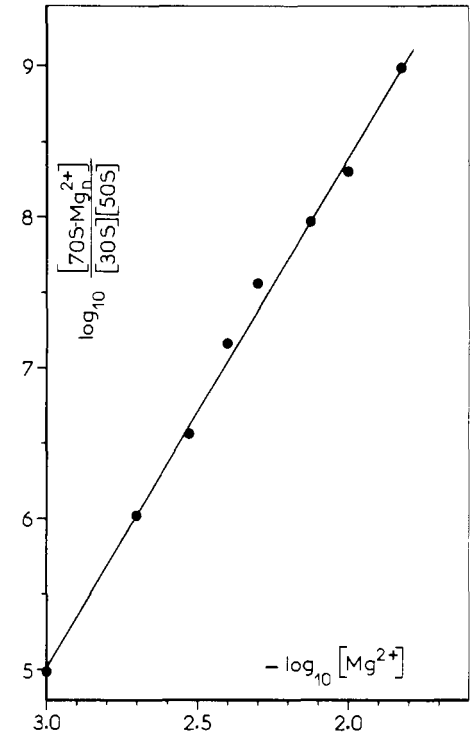


FIGURE 5: Log-log plot of the association equilibrium vs. the Mg^{2+} concentration (eq 10 in text) after addition of 1 mM spermidine to washed ribosomal subparticles. Final ribosome concentration: 0.5 μM. Buffer: 50 mM Tris/50 mM KCl, pH 7.8. Observation wavelength: 315 nm (differential absorption spectroscopy, 5-cm light path length cuvette).

one or both subunits, as evidenced from the kinetic analysis (Table II). In that case, coulombic repulsion between positively charged subunits is more than likely the rate-limiting factor. However, both the time dependence of the absorbance variations at high Mg^{2+} concentration and the difference in the Mg^{2+} dependence of k_i (Figure 4a) and of the association yield at equilibrium (Figure 3) suggest that some inhibitory magnesium ions are directly bound to the association-specific interface of the subparticles.

(e) Upon application of a Mg^{2+} concentration jump to a suspension of fully dissociated ribosomal subunits ($[Mg^{2+}]$ = 1 mM), the association kinetics are essentially limited by the conformational change of the 30S subunit, as discussed previously. That the initial slope k_i is roughly proportional to $[Mg^{2+}]$ over the 10–80 mM range could be explained by assuming that 30S activation is initiated from rapid chelation of Mg^{2+} to this subparticle (Scheme I), since 8 mM Mg^{2+} is more than enough to allow complete activation after a long enough incubation time. On the other hand, the decrease of k_i at Mg^{2+} amounts higher than 100 mM (Figure 4b) would hardly be taken into account by other processes than partial shielding of the association interface and/or electrostatic repulsion between positively charged subunits.

Effects of Spermidine on the Association of Ribosomal Subunits. The Mg^{2+} concentration required to achieve half-association of the washed ribosomal preparations we used, namely $[Mg^{2+}]_{1/2} \simeq 11$ mM (Figure 3), is about the same as that reported by Wolfe et al. (1973) but twice as large as the value obtained by Zitomer and Flaks (1972). Better still, the samples prepared by Debey et al. (1975) and Wishnia et al. (1975) (A-type subunits) displayed a very sharp Mg^{2+} dependence for couple formation, nearly fourfold lower than ours, and no conformational change of the 30S subunit upon 70S formation was observed by these authors. Such a heterogeneity should result from differences in the methods used for ribosome purification. In particular, the ribosomes we prepared were free of polyamines, which could result in a partly irreversible loss of the native conformation and/or composition of the rRNA-ribosomal protein complexes. Depletion of the ammonium ion can also inactivate subunits (Zamir et al., 1971) but the Mg^{2+} concentration was never lowered to such a degree that unfolding of the proteins could happen (White et al., 1972). Since the presence of polyamines is generally regarded as essential in the in vivo state of the ribosomes (Cohen, 1971), it appeared of value to study how the 30S/50S association is altered by introduction of spermidine to our system. The temperature and buffer conditions were the same as used for the experiments reported in the preceding paragraphs.

The magnesium dependence of the association equilibrium has been studied by differential absorption spectroscopy (315 nm), starting from equimolar dissociated subparticles (0.5 μM each) suspended in 50 mM Tris–50 mM KCl–0.5 mM $MgCl_2$–1 mM spermidine buffer (pH 7.8), then adding $MgCl_2$ in one cuvette. The plot of the log of the ratio [70S]/[30S][50S] vs. the log of $[Mg^{2+}]$ is linear over the 1–16 mM range of Mg^{2+} concentration (Figure 5); therefore, the association fits the scheme proposed by Zitomer and Flaks (1972):

$$30\,S + 50\,S + n Mg^{2+} \overset{k_a}{\rightleftharpoons} 70S\text{-}Mg^{2+}_n \qquad (9)$$

$$\log \frac{[70S\text{-}Mg^{2+}_n]}{[30S][50S]} = \log K_a + n \log [Mg^{2+}] \qquad (10)$$

The values obtained from the log–log plot are $[Mg^{2+}]_{1/2} \simeq 2.9$ mM, $n \simeq 3.4$, $K_a \simeq 1.35 \times 10^{15}$ $M^{-4.4}$. In the absence of spermidine (Figure 3), the association equilibrium also obeyed equation 10, with the following parameters: $[Mg^{2+}]_{1/2} \simeq 10.7$ mM, $n \simeq 3.6$, and $K_a \simeq 6.4 \times 10^{14}$ $M^{-4.6}$. The presence of spermidine thus results in an unexpectedly drastic fall in the values of $[Mg^{2+}]_{1/2}$. However, the value of $[Mg^{2+}]_{1/2}$ depends on the overall ribosome concentration. Given the values of n

TABLE III: Spermidine Dependence of the Initial Rates of Association of 30S and 50S Ribosomal Subunits.[a]

	I					II			
	a	b	c	d	e	a	b	c	d
30S: $[Mg^{2+}]_{pre}$ (mM)	1	1	1	1	1	20	20	20	20
$[spermidine]_{pre}$ (mM)	0	0	0	2	1	0	0	2	1
50S: $[Mg^{2+}]_{pre}$ (mM)	40	40	40	40	40	20	20	20	20
$[spermidine]_{pre}$ (mM)	0	0	2	0	1	0	2	0	1
$[spermidine]_f$ (mM)	0	1	1	1	1	0	1	1	1
v_i ($nM\ s^{-1}$)	2.1	3.3	3.3	448	397	384	456	655	501

[a] The subscripts "pre" and "f" refer to the concentrations used for preequilibration of dissociated subparticles and to those obtained after mixing, respectively. $[Mg^{2+}]_f$ was constant (20 mM). 30S and 50S subunits were 0.5 μM before mixing.

and K_a, $[Mg^{2+}]_{1/2} \simeq 3.6$ mM for a ribosome concentration of 0.25 μM and 4.6 mM for a ribosome concentration of 0.11 μM (4.3 A_{260} units/ml). Both the excellent linearity of the log–log plot (Figure 5) and the value of K_a indicate that the spermidine-reactivated subparticles are essentially B-type ones, according to Debey et al. (1975). On the other hand, backward dissociation, together with some aggregation, occur at Mg^{2+} concentrations higher than 100 mM. The amount of $MgCl_2$ required to achieve half-dissociation is nearly the same as measured in the absence of spermidine (Figure 3). Thus, binding of spermidine should not alter too seriously the Mg^{2+} affinity of certain chelation sites, although such a titration is made difficult by the existence of the poorly reversible aggregation.

The kinetic analysis was made by asymmetric preequilibration of dissociated subparticles with Mg^{2+} and spermidine using a constant value (20 mM) of the final Mg^{2+} concentration (Table III). (1) Upon application of a magnesium and spermidine concentration jump to the 30S subunit initially resting at 1 mM of Mg^{2+}, the rate-limiting conformational change of this subunit is accelerated by the polyamine (expt Ib). However, no other modification of v_i occurs on preequilibration of the 50S subparticle with spermidine (expt Ic). (2) No rate-limiting conformational change of the 30S subunit is observed when this subparticle is incubated with both Mg^{2+} and spermidine: restoration of the activated structure of the 30S subunit is complete at 1 mM of Mg^{2+} if spermidine is present (expt Id and Ie). On the other hand, preequilibration of the 30S subunit with spermidine always results in a strictly hyperbolic (second-order) time dependence of the absorbance variation. (3) The higher the concentration of Mg^{2+} and spermidine used for preequilibration of the 30S subunit, the larger the value of the initial rate of association. In other words, both spermidine and the magnesium ion operate in a cooperative way to alter the structure of the 30S subparticle, probably through a slowly reversible binding of spermidine to this subunit. As a matter of fact, direct involvement of the spermidine in the association step itself should be considered in order to explain the differences in the values of v_i for the experiments Id and Ie on the one hand, and IIb, IIc, and IId, on the other hand (Table III), since association is always complete under these conditions, i.e., since the value of v_i is strictly proportional to the initial slope k_i (eq 1–5).

Conclusions

The complexation of bis-ANS to dissociated ribosomal subunits involves two mechanisms. The first one corresponds to a fully reversible binding of the dye molecule to specific protein sites at the ribosomal surface. The kinetic analysis shows the existence of a marked, magnesium-dependent conformational change of the 30S subunit. That the 30S subparticle undergoes a conformational change upon depletion (to 1 mM) of Mg^{2+} has also been shown by Schechter et al. (1975) using the covalently bound probe *N*-(3-pyrene)maleimide. In addition to the reversible, dark reaction that does not affect 30S/50S coupling nor poly(phenylalanine) synthesis (Pochon et al., 1974), the light irradiation of bis-ANS–ribosomal subparticles mixtures results in an apparently irreversible bonding of a limited number of dye molecules to the ribosomal subunits. As far as the photochemical reactivity of bis-ANS can be compared to that of the parent compound ANS (Kosower et al., 1975), the observed photolysis could agree either with a photodynamical process (through an oxygen-sensitive triplet state), or to another mechanism involving singlet or triplet excited states of the dye. The extreme affinity of bis-ANS for the protein used on synthesis from ANS (Rosen and Wever, 1969) could result from a photochemical reaction similar to that we observed.

The 70S formation kinetics are controlled by a conformational change of the 30S subunit if this subparticle is preequilibrated with 1 mM Mg^{2+}–50 mM K^+, while the activated structure is recovered after incubation with 8 mM Mg^{2+}–50 mM K^+. According to Zamir et al. (1971), depletion of NH_4^+ inactivates both subunits and depletion of Mg^{2+} inactivates more specifically the 30S particle. Inactivation is reversible upon introduction of the depleted cation. However, interrelation between cations has been observed, e.g., K^+ and Ca^{2+} can serve as substitutes for NH_4^+ and Mg^{2+}, respectively. Inactivation corresponds to conformational changes rather than to the loss of ribosomal components (Zamir et al., 1974). As a matter of fact, ab initio reconstitution of the 30S subunit from isolated rRNA and ribosomal proteins exhibits too high energy requirements (Wireman and Sypherd, 1975) and would hardly explain the Mg^{2+} dependence of the rate-limiting process we measured (Figure 4b). No conformational change of the 50S subunit appears from the association kinetics we performed in the absence of the ammonium ion (Table II). Hence, K^+ (50 mM) allows complete activation of the 50S particle at 1 mM of Mg^{2+}, in good agreement with the results of Zamir et al. (1971). The restoration of the active conformation of the 30S subunit, on the other hand, is strictly dependent on the concentration of Mg^{2+}. (a) At 1 mM Mg^{2+}–50 mM K^+, a 30% yield of 30S particle is under a partly active conformation. (b) Activation is complete after incubation at 8 mM Mg^{2+}–50 mM K^+, then (c) the kinetic effects observed upon preequilibration with higher Mg^{2+} amounts agree with rapid chelation of magnesium ions that would permit cancelling of electrostatic

repulsion, according to Wishnia et al. (1975). Coulombic effects also account for the reversible uncoupling observed upon saturation by the magnesium ion ($[Mg^{2+}] > 60$ mM).

Reactivation of ribosomal subunits by restoration of the depleted cation(s) is a strongly temperature-dependent process (Zamir et al., 1971). In the experiments we performed, preequilibration of the ribosomal subunits with any solution of $MgCl_2$ was made at 25 °C for at least 60 min. Preequilibration at 37 °C for 15 min, followed by ~15 min at 25 °C, was also made at various concentrations of Mg^{2+}. No difference in the association kinetics was observed using either method. The Mg^{2+}-dependent reactivation of the ribosomal samples we used thus appears rather fast as compared with the values reported by Zamir et al. (1971). In fact, the activation kinetics of the 30S subunit can be measured directly by application of a concentration jump of Mg^{2+} (Figure 4b): the reactivation rate depends on the final concentration of Mg^{2+}, and activation is half over in about 30 s at 50 mM of Mg^{2+} (25 °C).

Introduction of 1 mM of spermidine results in a threefold fall of the need of Mg^{2+} for half-association, $[Mg^{2+}]_{1/2}$, i.e., it promotes the chelation of these magnesium ions that are involved in 30S/50S coupling. The Mg^{2+} dependence of the association equilibrium reveals that spermidine-treated ribosomal particles are essentially B-type ones (Figure 5; Debey et al., 1975). Spermidine does not alter seriously the state of activated B-type subparticles, since neither the stability constant K_a of the 70S couple nor the average number n of magnesium ions involved in association depend significantly on the presence of spermidine. From a strictly kinetic point of view, complete activation of our B-type subunits is achieved by spermidine at 1 mM of Mg^{2+}, and some polyamine appears to be involved directly in the joining of the ribosomal interfaces (Table III). The rate constant k_a related to the association of activated particles (Scheme I) depends somewhat on the ribosomal sample used and on the presence of spermidine: it is ranging between 4×10^{-6} M^{-1} s^{-1} (Table II) and 10×10^6 M^{-1} s^{-1} (Table III), but k_a was found to be higher than 20×10^6 M^{-1} s^{-1} for A-type ribosomes (Wishnia et al., 1975). Therefore, A-type couples can be distinguished from B type by higher values of both the equilibrium constant K_a and the rate constant k_a of association. Such differences agree quite well with minor (but irreversible) changes of the conformation of the association interfaces. The part taken by Mg^{2+} in the association reaction is not strictly identical for the two types of subunits (Debey et al., 1975), although n does not depend markedly upon the A or B nature of the ribosomal subunits under the same conditions of ionic strength, nature of added salts and temperature.

Since our ribosome preparations were obtained from fresh exponentially growing cultures, conversion of A- into B-type couples is thought to be related to the purification method, that involves depletion of polyamines and of certain cations. Anyway, modification of A-type ribosomes is not reversed by spermidine at 25 °C. From our data, the loss of some specific polyamine would hardly account for the structural differences between A- and B-type couples, unless (a) the specific polyamine in question is located within the ribosome core and is essential for the preservation of the native conformation related to the A type, and (b) the subsequent alteration of such a native conformation, e.g., via formation of new hydrogen bonds, is energetically favored: then, a large activation energy would be required to reincorporate the polyamine in the right place. So, it could be of value to investigate the possibility for a reversible A $\rightleftarrows$ B conversion in light of the results of Wireman and Sypherd (1975).

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Allosteric Interpretation of Mg^{2+} Binding to the Denaturable *Escherichia coli* $tRNA^{Glu}_2$†

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ABSTRACT: The Mg^{2+} binding properties of the denaturable $tRNA^{Glu}_2$ from *E. coli* in 0.1 M Na^+, pH 7, are characterized by equilibrium dialysis. At 34 °C, where the native and denatured conformers are in equilibrium, Mg^{2+} binding is cooperative. By trapping the tRNA completely in the native conformation at 4 °C it is shown that native $tRNA^{Glu}_2$ possesses one strong binding site, $K_1 = 7.5 \times 10^4$ M^{-1} and approximately 36 weak sites with $K_2 = 8.3 \times 10^2$ M^{-1}. A significantly lower affinity for the denatured conformer is indicated. We show that Mg^{2+} effects an allosteric transition from the low affinity denatured conformational state to the high affinity native state and develop the appropriate equations to fit the Mg^{2+} binding data with physically meaningful parameters. Our results also suggest the previously reported cooperative cation binding to tRNA arises from a cation induced conformational change to the native tRNA conformation and does not reflect the inherent Mg^{2+} binding properties of the native conformer.

It is now well known that there are macromolecules which undergo conformational changes in the course of their function and that allosteric effects may be important in the regulation of cellular activities (Monod et al., 1963). Evidence is accumulating which suggests that tRNA[1] may function in a similar fashion. In protein synthesis tRNA interacts with a number of different proteins. Although the generality of tRNA conformational changes accompaning interactions with various cellular components is not yet established, the results of Schwartz et al. (1974) and Erdman et al. (1973) indicate that the compact native structure of tRNA (Kim et al., 1973; Robertus et al., 1974; Ladner et al., 1975) is altered when tRNA is bound to the 30S ribosomal subunit. Possible roles for tRNAs in regulating cell differentiation are suggested by variations observed in chromatographic patterns of isoacceptor tRNAs in differentiating cells, different organs, and in response to hormones (Littauer and Inouye, 1973). The strong binding of tRNAs to some feedback-sensitive enzymes (Littauer and Inouye, 1973) and the high affinity of tRNA for the lysine-rich histones (Ilyin et al., 1971) provide further support for this idea. In addition, it has been suggested that tRNAs may be involved in regulating the biosynthetic pathways for their cognate amino acids (Goldberger and Kovach, 1972).

Mechanisms whereby tRNAs can function as regulatory molecules are therefore of interest. It is known that under certain conditions tRNAs bind divalent metal cations and polyamines cooperatively (Schreier and Schimmel, 1974, 1975). The reason for cooperative oxygen uptake in hemoglobin has been sought since the beginning of the century (Hill, 1910). Through extensive study over many years it has been shown that a conformational change between low and high affinity states is responsible for the cooperativity, in accordance with the ideas of Monod et al. (1965), and the triggering of the conformational transition can be explained in terms of chemical structure (Shulman et al., 1975). Most importantly, it is generally believed that the phenomenon exhibited by hemoglobin, a ligand-induced conformational change, may be a general feature of regulatory systems. Since tRNA may have regulatory functions, it could be of significance that ligands as diverse as divalent cations and polyamines interact with tRNA in a very similar fashion as evidenced by their binding properties and effect on tRNA conformation (Cohen, 1971; Schreier and Schimmel, 1975; Sakai et al., 1975).

In this paper we characterize a Mg^{2+} effected allosteric transition in a tRNA. *Escherichia coli* $tRNA^{Glu}_2$ was chosen because it is one of the "denaturable" tRNAs for which the native conformer exists in equilibrium with a nonnative, biologically inactive conformer at pH 7.0 over a range of Mg^{2+} concentrations in fairly high Na^+ (0.1 M), thus minimizing purely electrostatic effects. We show that Mg^{2+} binding to $tRNA^{Glu}_2$ is cooperative, and as for hemoglobin the cooperativity arises from a ligand affinity difference between two states. By kinetically trapping the tRNA in its native conformation for all degrees of ligation, it is shown that Mg^{2+} binds noncooperatively to this state with a high affinity, whereas a low affinity is indicated for the biologically inactive state. The relation between the degree of ligation and the extent of the conformational transition is expressed quantitatively using equations similar to those of Monod et al. (1965).

Experimental Section

Transfer RNA. Partially purified $tRNA^{Glu}_2$ from Oak Ridge National Laboratory was kindly provided by Drs. Z. Egan, A. Kelmers, and P. E. Cole. Further purification was

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[1] Abbreviations used: EDTA, ethylenediaminetetraacetic acid; tRNA, transfer ribonucleic acid; NMR, nuclear magnetic resonance.