DIRECT MEASUREMENT AND KINETIC ANALYSIS OF THE ASSOCIATION OF *E. COLI* RIBOSOMAL SUBUNITS

A.D. WOLFE* , P. DESSEN, and D. PANTALONI
Laboratoire d'Enzymologie, Centre National de la Recherche Scientifique, 91190 Gif-sur-Yvette, France

Received 26 July 1973

1. Introduction

Reversible association of bacterial ribosomes is thought necessary for synthesis of protein, and therefore must be considered a reaction of unusual biological importance. Such reactions were first discovered to be dependent upon the concentration of Mg^{2+} [1], but more recently initiation factors, in conjunction with mRNA and other molecular species, have been shown to dissociate ribosomes, or to associate ribosomal subunits [2]. However, in neither instance have the kinetics of ribosomal association been studied, for the methods of analysis were exceedingly slow in comparison with the rates of reaction between the ribosomal forms themselves. Toward study of the nature of reversible association of ribosomes in general, and the function of the initiation factors, in particular, we have first investigated, by light scattering stopped-flow techniques, the kinetics of the Mg^{2+}-dependent association of ribosomal subunits from *E. coli,* and have found this reaction to occur at two distinct rates. Concentrations of Mg^{2+} at or above 3.5 mM did not appear to influence either rate, but an increase in the concentration of Mg^{2+} produced a commensurate increase in the number of ribosomes participating in the more rapid reaction. Thus not only have the kinetics of subunit association been analyzed, but the analysis has confirmed the existence of a Mg^{2+}-dependent equilibrium recently observed by use of light scattering [3].

* On leave from the Department of Molecular Biology, The Walter Reed Army Institute of Research, The Walter Reed Army Medical Center, Washington, D.C., U.S.A.

2. Materials and methods

E. coli MRE 600-ribosomes were prepared by conventional techniques, including washing with 1.0 M NH_4Cl, and activation for 15 min in a buffer containing 10 mM Mg^{2+} , 50 mM NH_4Cl, 10 mM Tris–HCl at pH 7.5, and 6.6 mM mercaptoethanol. Under appropriate conditions, the ribosomes used in these experiments could bind 0.8 mole F-met tRNA/1.0 mole of ribosome, and exhibited complete and reversible association by static-light scattering analysis. Such analysis of ribosome weight–average molecular weights was carried out on a FICA 50 light scattering apparatus at a wavelength of 436 nm. The kinetics of association of ribosomal subunits were performed on a Durrum stopped-flow D 110 model, using the apparent absorbance in the 2 cm cell at 310 nm, in a manner similar to measurement of turbidity. The wavelength of 310 nm was chosen to provide substantially maximal turbidity relative to absorbance. All experiments were performed at 25°C.

3. Results

Fig. 1 presents a photograph of an oscilloscope recording of changes in the turbidity of scattered light upon stopped-flow mixture of 30 S and 50 S ribosomal subunits in 1 mM Mg^{2+} with a buffer containing 15 mM Mg^{2+}. The final concentration of ribosomes in the mixture was 10 A_{260} units, and the final concentration of Mg^{2+} was 8 mM. Turbidity in terms of units of absorbance is depicted on the ordinate, while time scales of 200 ms/cm and 5 sec/cm

North-Holland Publishing Company – Amsterdam

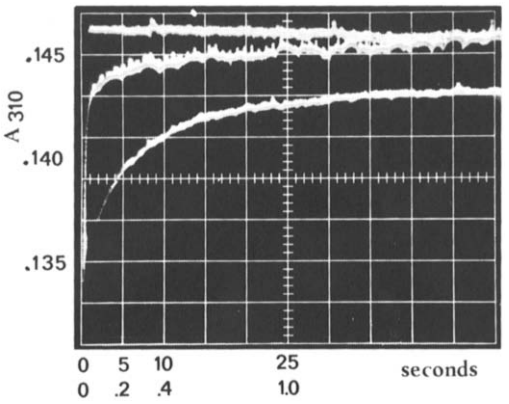
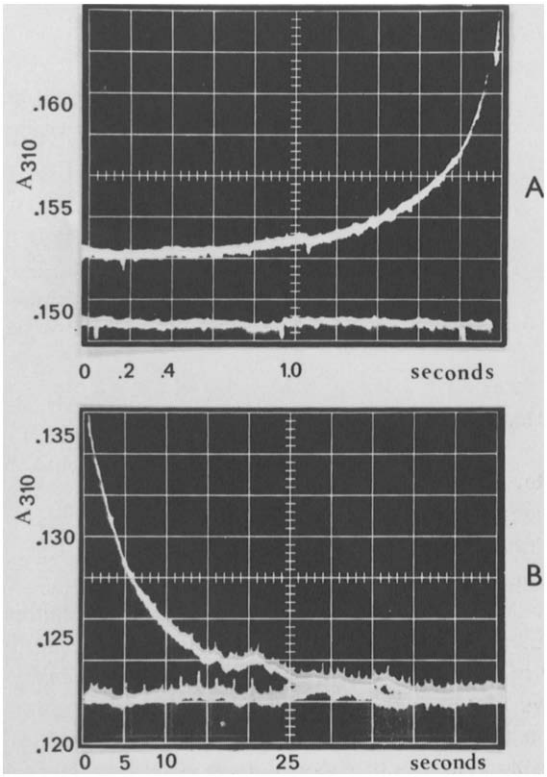


Fig. 1. Kinetics of ribosomal subunit association. Stopped-flow oscilloscope recording where ribosome solution in 1 mM Mg^{2+} (syringe I) was mixed with a 15 mM Mg^{2+} buffered solution (syringe II). The final Mg^{2+} concentration was 8 mM. Time scale on abscissa: 0.2 and 5 sec/division. Absorbance at 310 nm on ordinate: 0.002/division in a 2 cm cell.

are depicted upon the abscissa. Two separate, consecutive experiments are shown to illustrate the kinetics of association. Thus a rapid initial reaction, identified by the lower curve, occurred upon mixing, followed by a slower reaction, the final level of which is represented by the upper line recorded more than a minute later. The second experiment, shown by the intermediate curve, is related to the 5 sec/cm time scale, and continuously depicts the entire association process. The reactions shown in this recording suggest that the ribosomes associated at two considerably different rates, with the greater portion of the ribosomes participating in the more rapid reaction.

Fig. 2A illustrates the association of ribosomal subunits when mixed with a solution containing 30 mM Mg^{2+}, while fig. 2B illustrates the dissociating effect of a buffered solution containing 0.1 M EDTA upon 70 S ribosomes in a solution containing 30 mM Mg^{2+}. The magnitude of the changes is similar although association occurred considerably more rapidly than did dissociation. The latter process occurred at a single rate in the presence of EDTA, although simple dilution of ribosomes into solutions of lower Mg^{2+} concentration indicated that dissociation could also occur at two rates.

The relation between subunit association observed by turbidimetric analysis and conventional light scat-



tering is depicted in fig. 3. Demonstration of such relation was necessary because i) the stopped flow technique has not been used previously with ribosomes; ii) the maximal value attained for turbidimetrically-measured stopped-flow association of ribosomes was less than the theoretical maximum; iii) the change in turbidity related to subunit association is denoted on the ordinate, while the weight–average molecular weight is denoted on the abscissa. Values obtained by both types of measurements for the equilibrium concentration of 70 S ribosomes at selected concentrations of Mg^{2+} were plotted one against the other, on the respective axes, and their intercepts yielded the

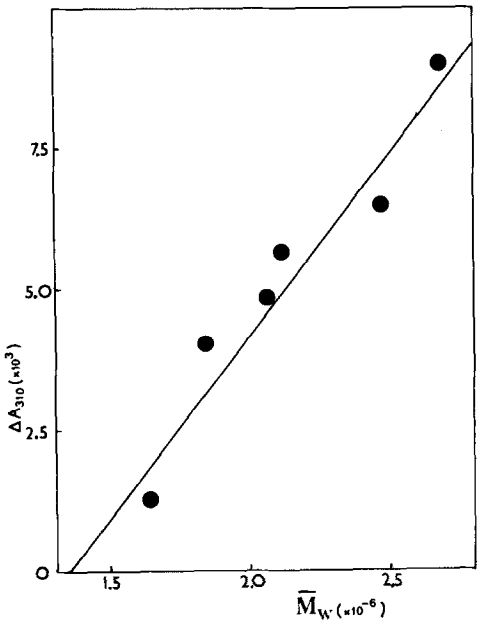


Fig. 3. Demonstration of a linear relation between stopped-flow increase in turbidity and static weight-average molecular weights of ribosomes as a function of the concentration of Mg^{2+}. The following Mg^{2+} concentrations were employed to obtain both kinetic and static measurements: 1.5, 3.5, 4.5, 5.5, 8.0, 15.5 mM. Ribosome concentrations were 5.2 and 7.0 A_{260} units.

linear relation shown in fig. 3. Such a linear relation demonstrates the validity of the results obtained by stopped-flow measurements, and it can reasonably be inferred that the relatively smaller increase observed upon subunit association when measured by stopped-flow in comparison with static light scattering was due to the true absorbance of ribosome chromophores at 310 nm.

Fig. 4 presents a graphical, semi-logarithmic representation and analysis of the association reaction shown in fig. 1. This typical reaction curve could be separated into two first order reactions with half-times of approximately 0.25 and 15 sec, respectively. Fig. 5 depicts the influence of Mg^{2+} upon the extent of association of different concentrations of subunits. Increase in turbidity is shown on the ordinate, while the abscissa depicts the concentrations of Mg^{2+}. Solutions of subunits of 5, 10, and 20 A_{260} units were mixed by stopped-flow with buffered solutions of Mg^{2+} to yield solutions of the indicated Mg^{2+} concentrations. The increases in turbidity were roughly proportional to the relative concentration of ribosomes, while the extent of increase was dependent upon the concentration of Mg^{2+}. More than 70% of the entire association occurred between 1.0 mM and 10.0 mM Mg^{2+}.

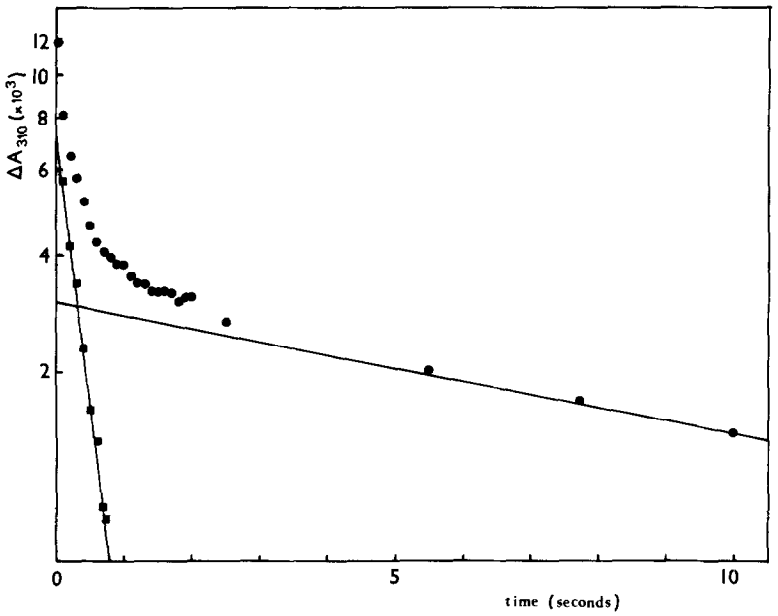


Fig. 4. Semi-logarithmic plot and analysis of the changes in turbidity associated with the rate of formation of 70 S ribosomes. Experimental conditions of fig. 1.

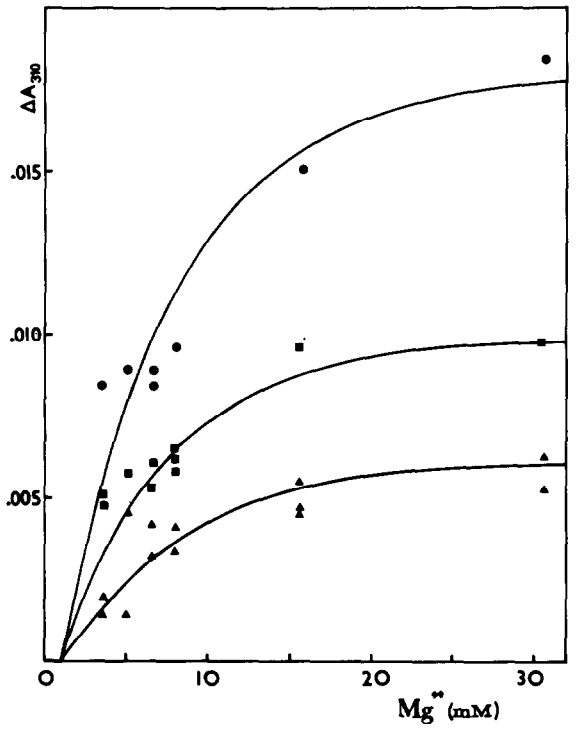


Fig. 5. The formation of 70 S ribosomes as a function of the concentrations of Mg^{2+} and of ribosomal subunits. (Final concentration: 2.5, 5 and 10 A_{260} units).

Table 1 presents an analysis and comparison between the two rates of subunit association as a function of the concentration of Mg^{2+}. A major increase was observed in the quantity of subunits associating by the faster rate, and this increase was responsible for the overall Mg^{2+}-dependent association of ribosomal subunits. Little variation, or perhaps a diminution, was observed in the quantity of subunits participating in the slower process, although at 3.5 mM Mg^{2+} more than one half of the subunits appeared to associate by this process. The rate constants for both processes were not influenced by variation in the concentration of Mg^{2+} at 3.5 mM or higher.

Table 1

Amplitudes and time constants of the two phases of the ribosomal subunit association induced by graded increases in the concentration of Mg^{2+}.

Final concentration of Mg^{2+} (mM)	1st Phase		2nd Phase		Total amplitude
	λ/τ_1 (sec^{-1})	ΔA_1 ($\times 10^3$)	λ/τ_2 (sec^{-1})	ΔA_2 ($\times 10^3$)	ΔA^* ($\times 10^3$)
3.5	3.2	3.1	0.07	4.6	7.7
5	2.3	5.1	–	–	–
6.5	2.8	5.8	–	–	–
8	3.1	7.0	0.04	2.2	9.2
15.5	2.7	9.1	–	–	–
30.5	2.5	11.3	0.12	2.4	13.7

The final ribosome concentration was 10 A_{260} units.
*ΔA is the absorbance at 310 nm in a 2 cm cell.

4. Discussion

Although Mg^{2+}-dependent formation of 70 S ribosomes was discovered in 1959 [1], the present communication is the first to describe the kinetics of the reaction. Association of the 30 S and 50 S subunits to form a 70 S ribosome occurred by a process separable into two first order reactions, the rates of which were apparently independent of the tested concentrations of Mg^{2+}. The extent of association by the more rapid process was Mg^{2+} dependent, however, and an increase in the concentration of Mg^{2+} from 1 mM to 10 mM resulted in rapid formation of more than 70% of all 70 S ribosomes.

The occurrence of two reaction rates suggests two different processes or reactions, or two ribosomal forms. It also brings to mind the existence of unitary and fractional ribosomal proteins [4]. Additionally, however, there exists the possibility of partial destruction of subunits through the hydrodynamic pressure inherent in the stopped-flow technique, although weight–average molecular weight determinations carried out by static light scattering procedures also suggested association to occur at two rates.

The significance of these experiments lies not only in the kinetic observations, but also in the property of the stopped-flow technique to measure subunit association directly. Thus the function of ribosomal proteins may be further characterized, and the influence of structural and conformational changes induced by mutations, and by a variety of ribosomotropic molecules, may be directly determined with respect to both the rate and the extent of association.

Acknowledgements

The authors are deeply indebted to M. Grunberg-Manago for her interest and advice, and to her and to J. Dondon and T. Godefroy-Colburn for generous gifts of ribosomes and their evaluation.

References

[1] Tissières, A., Watson, J.D., Schlessinger, D. and Hollingsworth, B.R. (1959) J. Mol. Biol. 1, 221.
[2] Grunberg-Manago, M., review, 1973 Mossbach Meeting of the Association of German Biochemists, in press.
[3] Zitomer, R.S. and Flaks, J.G. (1972) J. Mol. Biol 71, 263.
[4] Voynow, P. and Kurland, C.G. (1971) Biochemistry 10, 517.