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# PRESSURE-JUMP RELAXATION STUDIES OF THE ASSOCIATION–DISSOCIATION REACTION OF E. COLI RIBOSOMES*

Elfriede SCHULZ and Rainer JAENICKE
*Institut für Biophysik und physikalische Biochemie, FBB, Universität Regensburg, D-84 Regensburg, Germany*

and

Wilhelm KNOCHE
*Max-Planck-Institut für biophysikalische Chemie, D-34 Göttingen, Germany*

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The association–dissociation kinetics of ribosomal particles from E. coli have been studied using a pressure-jump apparatus with optical detection. Experiments on isolated subunits yield two relaxation times of about 10 and 700 ms, respectively. With mixtures of 30 S and 50 S particles an additional relaxation time of about 100 ms is observed, which is assigned to the equilibrium 30 S + 50 S ⇌ 70 S. The two other times are attributed to reversible equilibria between subunit monomers and subunit homo-associates.

## 1. Introduction

It is well known that high hydrostatic pressure inhibits bacterial growth and favours the deactivation of oligomeric enzymes [1,2]. A possible explanation of these findings is a pressure induced shift of association–dissociation equilibria towards dissociation due to a positive excess volume, e.g., in the process of forming electrostatic or hydrophobic interactions [3–5].

As shown by indirect evidence from sedimentation and fixation techniques [6–8] the association–dissociation equilibrium of ribosomal particles strongly depends on hydrostatic pressure. Direct proof for this effect has been put forward recently from turbidity measurements in solutions of E. coli ribosomes at elevated pressure [9,10].

Since ribosomal association is of crucial importance in protein biosynthesis, equilibrium and kinetic measurements on a simplified in vitro system may provide informations with respect to the mechanisms involved. Kinetic studies on this system have been reported [11–13] using the stopped-flow technique with light-scattering detection. The present relaxation experiments represent an independent approach to the problem which seems to be preferable for three reasons: (a) higher time resolution, (b) absence of appreciable changes in the solvent and (c) determination of characteristic parameters without arbitrary assumptions with respect to a distinct mechanism.

The specific technique used is pressure-jump relaxation with optical detection [14]. Since the association–dissociation equilibrium of E. coli ribosomes depends strongly on pressure, this technique appears to be suitable for the investigation of the association–dissociation kinetics.

## 2. Materials and methods

E. coli K12 strain A19 was kindly provided by

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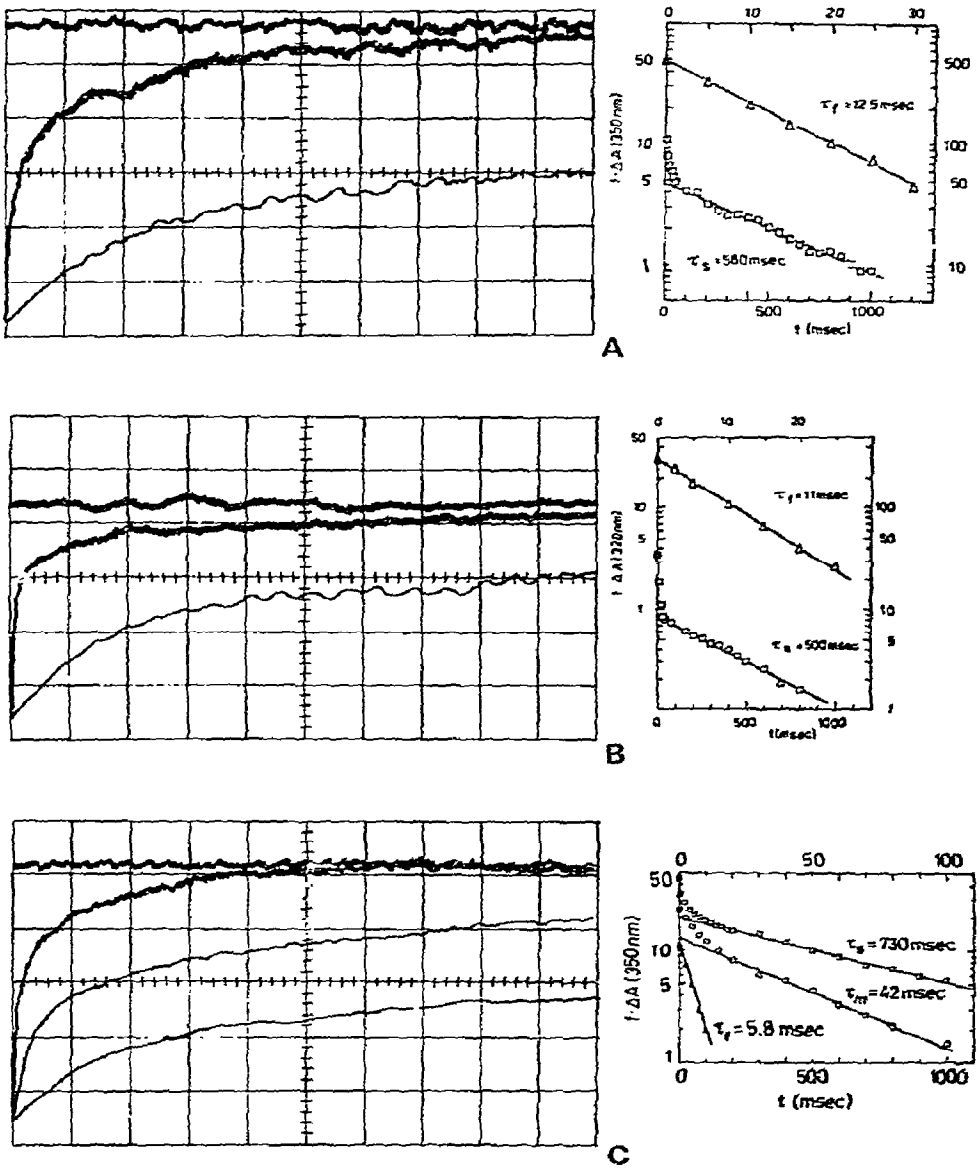


Fig. 1. Oscilloscope traces and analysis of relaxation curves of the pressure-jump relaxation of E. coli ribosomes.

(A) 30 S-subunits, $A(260\,nm) = 22.4$, 7 mM $Mg^{2+}$; detection wavelength, 350 nm, $\Delta p = 110$ bar. Oscilloscope settings: vertical, 20 mV/unit, horizontal, 5 ms/unit (lower trace), 200 ms/unit (upper trace); total intensity refers to 2 V.

(B) 50 S-subunits, $A(260\,nm) = 24.2$, 7 mM $Mg^{2+}$; detection wavelength, 320 nm, $\Delta p = 55$ bar. Oscilloscope settings: vertical, 20 mV/unit, horizontal, 5 ms/unit (lower trace), 200 ms/unit (upper trace); total intensity refers to 2 V.

(C) Mixtures of 30 S- and 50 S-subunits, $A(260\,nm)$ 30 S = 18.1, $A(260\,nm)$ 50 S = 36.4, 12.1 mM $Mg^{2+}$; detection wavelength, 350 nm, $\Delta p = 110$ bar. Oscilloscope settings: vertical, 50 mV/unit, horizontal, 5 ms/unit (lower trace), 50 ms/unit (medium trace), 500 ms/unit (upper trace); total intensity refers to 2 V.

Dr. A. Böck, Universität Regensburg. The cells were grown in a New Brunswick 40 ℓ fermentor at 37°C in rich medium and harvested in the early logarithmic phase. Ribosomal subunits were prepared as described in [15]. Stock solutions of the separated subunits were stored in liquid nitrogen. The relaxation technique used has been described elsewhere [14]; the dead-time of the instrument is 0.5 ms. The progress of the reaction was followed by observing the change in turbidity at 320, 330 and 350 nm. For each set of experiments solutions of 30 S and 50 S subunits were prepared by adding suitable amounts of the stock solutions to a standard buffer containing 10 mM Tris-HCl, 50 mM KCl, 1 mM EDTA, 0.1 mM dithioerythritol at pH 7.3 and varying concentrations of $Mg^{2+}$. Samples were prepared by suitable dilution or mixing, dialyzed exhaustively and filtered through nitrocellulose filters to remove dust particles. Concentrations were determined spectrophotometrically: $A^{0.1\%}_{260\,nm} = 15.5$. All measurements were performed at 22°C.

## 3. Results

*p*-Jump relaxation experiments have been performed with solutions containing only 30 S or only 50 S subunits and with mixtures of 30 S and 50 S subunits in equilibrium with 70 S particles. *p*-Jumps up to 150 bar do not cause irreversible turbidity changes as shown by the coincidence of the zero lines before and after the experiments monitored by turbidity changes at 320, 330 and 350 nm.

Fig. 1 shows oscilloscope traces of the relaxation processes caused by *p*-jumps of 55 or 110 atm, respectively. The relaxation process could be resolved completely, i.e., there is no relaxation effect faster than the dead time of the instrument. The change in absorption of solutions of 30 S or 50 S subunits can be fitted by superposition of two exponentials yielding one fast relaxation time, $\tau_f = 5-10$ ms, and one slow time, $\tau_s \approx 500-1000$ ms.

The validity of this analysis is demonstrated in figs. 1a and 1b by plotting the logarithm of the absorbance change versus time. Both relaxation times are very similar for both subunits. The pressure release is followed by an increase in turbidity indicating an increase of the weight-average molecular weight. In order to eliminate errors which may arise from the contributions of effects other than Rayleigh-scattering at the relatively short wavelengths used, measurements have been performed at different wavelengths. Investigations at 320, 330 and 350 nm yield identical relaxation times.

Table 1
Dependence of the relaxation times on ribosomal concentration.

| $A$(260 nm) 50 S | $A$(260 nm) 30 S | $Mg^{2+}$ (mM) | $\tau_s$ (ms) | $\tau_m$ (ms) | $\tau_f$ (ms) |
|---|---|---|---|---|---|
| 20.6 | 11.0 | 8.5 | 510 | 75 | 9.1 |
| 34.5 | 17.1 | 8.5 | 550 | 58 | 9.0 |
| 23.2 | 47.3 | 8.5 | 590 | 35.5 | 8.7 |
| 21.4 | 10.1 | 12.1 | 660 | 58 | 5.0 |
| 28.2 | 14.1 | 12.1 | 650 | 50 | 5.2 |
| 36.4 | 18.1 | 12.1 | 730 | 42 | 5.8 |

Fig. 1c shows oscilloscope traces of a *p*-jump experiment with a mixture of 30 S and 50 S subunits. The amplitudes of all processes indicate increasing weight-average molecular weight with decreasing pressure. The resulting relaxation spectrum can be analyzed by three relaxation times: a fast and a slow one, $\tau_f$ and $\tau_s$, being similar to those of separated subunits under the same experimental conditions, and one relaxation time $\tau_m$ in the medium time range of 40–100 ms.

Table 1 shows the dependence of the relaxation times on the initial concentrations of ribosomal particles. While the deviations for $\tau_f$ and $\tau_s$ are within the experimental error, the medium relaxation time $\tau_m$ decreases appreciably with increasing initial concentrations of the subunits.

Fig. 2 shows the dependence of the amplitudes of the three relaxation effects on the concentration of $Mg^{2+}$ ions for a mixture of 30 S and 50 S particles. The amplitudes of the slow and the fast process increase continuously within the concentration range investigated (2–20 mM $Mg^{2+}$), whereas the amplitude corresponding to the additional medium relaxation time $\tau_m$ forms a distinct peak with a maximum at about 12 mM $Mg^{2+}$. The [$Mg^{2+}$]-dependent equilibrium between 30 S, 50 S and 70 S particles has been determined under the same conditions following the absorbance at 350 nm. The results and their evaluation on the basis of the equilibrium 30 S + 50 S $\rightleftharpoons$ 70 S are given in fig. 3.

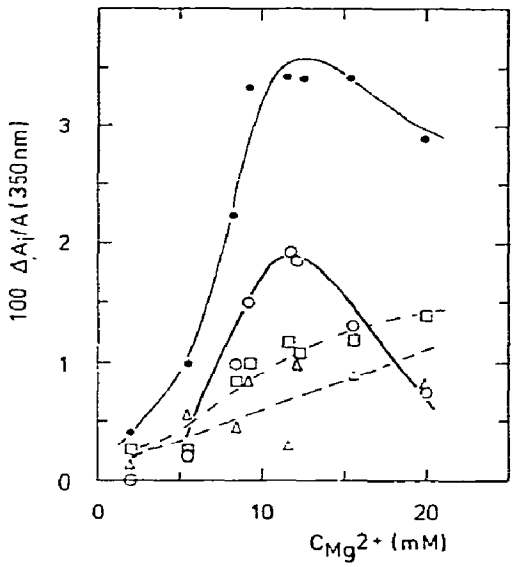


Fig. 2. $[Mg^{2+}]$-dependence of the relaxation amplitudes of the fast, (△); medium, (○); and slow, (□), relaxation processes of mixtures of 30 S and 50 S ribosomal particles from E. coli in standard buffer. $A$(260 nm) 30 S = 11.5, $A$(260 nm) 50 S= 21.0. (●), total relaxation amplitude.

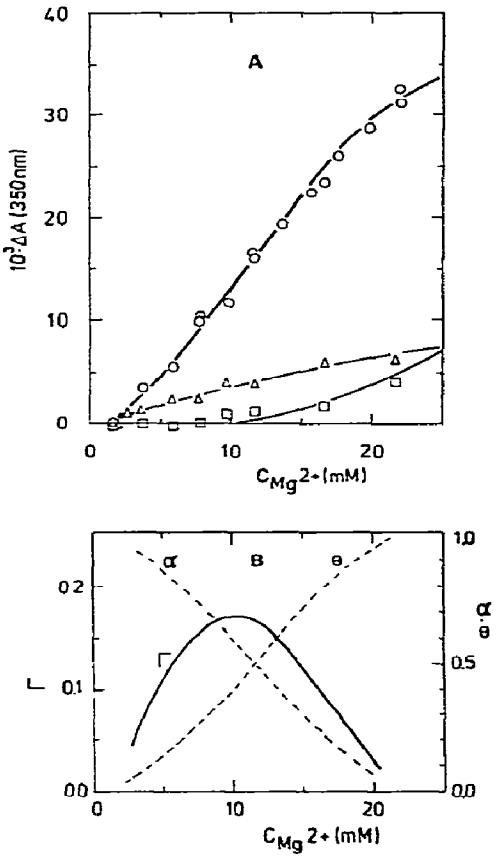


Fig. 3. $[Mg^{2+}]$-dependence of the association–dissociation equilibrium of ribosomal particles from E. coli.

(A) $[Mg^{2+}]$-titration curve followed by the absorbance change at 350 nm. (○), 30 S and 50 S-mixtures, $A$(260 nm) 30 S = 11.5, $A$(260 nm) 50 S = 21.0; (△), 50 S-subunits, $A$(260 nm) = 21.0; (□), 30 S subunits, $A$(260 nm) = 11.5.

(B) Evaluation of the titration curve with the assumption of the equilibrium 30 S + 50 S ⇌ 70 S. $\theta$: degree of association; $\alpha$: degree of dissociation; $\Gamma = (1/30\,S + 1/50\,S + 1/70\,S)^{-1}$.

## 4. Discussion

Three relaxation times ($\tau_f$ = 5–10 ms, $\tau_m$ = 40–100 ms, $\tau_s$ = 500–1000 ms) are observed when a pressure-jump is applied to mixtures of 30 S and 50 S ribosomal particles. Whereas the slow and the fast relaxation processes are also observed in solutions containing only 30 S or 50 S subunits, the medium effect is only present in solutions containing 30 S *and* 50 S subunits in equilibrium with 70 S particles. This indicates that the latter relaxation process may be assigned to the reaction:

$$30\,S + 50\,S \rightleftharpoons 70\,S. \qquad (1)$$

The amplitude $\Delta A_m$ of a single step relaxation process due to this reaction should depend on the concentrations of the particles involved, according to

$$\Delta A_m \propto \Gamma, \qquad (2a)$$

with

$$\Gamma = \left(\frac{1}{[30\,S]} + \frac{1}{[50\,S]} + \frac{1}{[70\,S]}\right)^{-1}. \qquad (2b)$$

As shown in fig. 3b, $\Gamma$ is characterized by a $[Mg^{2+}]$-dependence similar to that observed for $\Delta A_m$ (fig. 3), which indicates that $\tau_m$ has to be correlated to reaction (1). The presence of two further relaxation times ($\tau_f$, $\tau_s$) which are also detectable in solutions of separated subunits indicates that there must be additional processes participating in the overall association–dissociation reaction of ribosomal particles in the concentration range under investigation (0.5–5 mg/ml). This suggests aggregates to be involved in equilibria of the type

$$n\ 30\,S \rightleftharpoons (30\,S)_n. \quad (3)$$

$$n\ 50\,S \rightleftharpoons (50\,S)_n. \quad (4)$$

The occurrence of these homo-associates has been proved by sedimentation studies [16,17]. However, they have not been considered to participate in the association–dissociation reaction. Recent stopped-flow studies [12] show a single process without indicating an influence of homo-associates. The apparent difference in these results may be due to the fact that at the low concentrations of ribosomes and $Mg^{2+}$ used in the stopped-flow experiments the concentration of homo-associates may be negligible. Moreover, a change in the $Mg^{2+}$-concentration, especially in the lower concentration range, may not shift these equilibria appreciably, whereas the pressure dependence turns out to be significant.

It should be possible to use the concentration dependence of the relaxation times to evaluate rate constants and to prove the correct assignment. However, without further analysis of the different equilibria participating in the overall association–dissociation process, this evaluation cannot unambiguously be performed due to possible coupling between the different reactions. The occurrence of homo-associates has not been considered in the evaluation of the $[Mg^{2+}]$-titration given in fig. 3. The similarity of the $[Mg^{2+}]$-dependence of $\Delta A_m$ and $\Gamma$ however is of qualitative significance only, since eq. (2b) is certainly correct only in the case of a single-step reaction. Even in the case of a multi-step mechanism, the amplitude of the relaxation process corresponding to eq. (1) vanishes with decreasing concentration of reactants and reaches its maximum if all reactants involved have almost similar concentrations. Because this dependence of the relaxation amplitude upon equilibrium concentrations agrees qualitatively with eq. (2b), the use of this relation as an approximation seems to be justified.

The present measurements indicate different types of associates in solutions of ribosomal particles. They also show that *p*-jump relaxation with optical detection is a useful tool in the investigation of biomolecular association–dissociation reactions.

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