HIGH PRESSURE EQUILIBRIUM STUDIES ON THE DISSOCIATION–ASSOCIATION OF *E. COLI* RIBOSOMES

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Received 18 February 1976

1. Introduction

The dissociation and reassociation of ribosomal particles is an important feature of the involvement of ribosomes in protein biosynthesis [1–2]. In this process the reaction will only start after the ribosome takes on its 'functional state', i.e. after association or dissociation of a number of components to or from the ribosome. As has been shown in [3] the association–dissociation reaction of isolated ribosomal particles represents a chemical equilibrium with the equilibrium constant depending on the Mg^{2+} concentration.

Anomalies observed in sucrose gradient sedimentation patterns of ribosomes from different species were reported to result from the dissociation of ribosomal particles at high hydrostatic pressure [4–6]. On the other hand, experiments have been performed on sea urchin ribosomes in a high pressure cell where fixation was applied to stabilize the distribution of the ribosomal particles [4]. However, until recently, direct evidence was lacking for the pressure dependence of the association-dissociation equilibrium of ribosomal particles.

Pressure-jump relaxation experiments [7] have proved that homoassociates and 70S particles are involved in the association–dissociation equilibrium of *E. coli* ribosomes. The present study reports on static light scattering (turbidity) measurements in an optical high pressure cell. Elevated hydrostatic pressure is shown to shift the association-dissociation equilibrium in a reversible fashion under various ionic conditions and ribosomal concentrations. Besides the equilibrium $30S + 50S \rightleftharpoons 70S$ a significant contribution of homoassociates $(30S)_n$ and $(50S)_n$ has to be considered in the overall process under the given experimental conditions.

2. Materials and methods

E. coli K 12 strain A 19 was grown in a New Brunswick 40 litre fermentor at 37°C in rich medium and harvested in the early logarithmic phase. The preparation of the ribosomal subunits was performed as described earlier [8]. Ribosomal subunits were stored in liquid nitrogen. Sample solutions were prepared from stock solutions of 30S and 50S subunits by suitable dilution with standard buffer containing 10 mM Tris-HCl pH 7.3, 50 mM KCl, 1 mM EDTA, 0.1 mM dithioerythritol and varying concentrations of Mg^{2+} [2–25 mM]. Samples were dialyzed exhaustively and filtered through nitrocellulose filters to remove dust particles.

Ribosomal concentration was determined spectrophotometrically: $A_{260}^{0.1\%} = 15.5$. All measurements were performed at 22°C.

High pressure experiments made use of a thermostated transmission cell (optical path length 12 mm) mounted in a Gilford 2400 S spectrophotometer. For details see [9].

The dead time of the device, i.e. the time needed to build up the hydrostatic pressure isothermally to a desired final value, is of the order of 2 min. The association–dissociation equilibrium was followed by observing the turbidity at 320 and 350 nm. As shown by blank experiments at atmospheric pressure A_{320nm} depends linearly on the ribosomal concentration (fig.1, insert). This proves that the optical cell is

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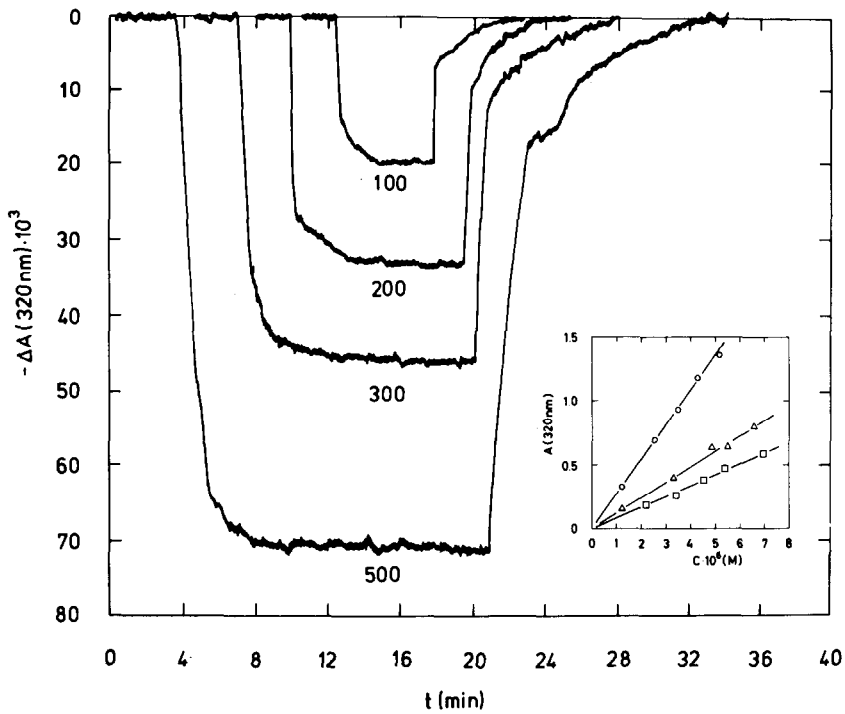

Fig.1. Turbidity profiles (A_{320nm} versus time) of mixtures of 30S + 50S ribosomal particles under high hydrostatic pressure; numbers on the experimental curves give the final pressure (in bar). Ribosomal concentration: A_{260nm} (30S)=30.2 A_{260nm} (50S) =61.0 (1cm) in standard buffer + 20 mM Mg^{2+}. Insert: Turbidity (αA_{320nm}) in the high pressure cell as a function of ribosomal concentration. Standard buffer + 25 mM Mg^{2+}.

applicable to light scattering measurements in the given wavelength range.

3. Results

High pressure experiments were performed with solutions containing only 30S or 50S subunits and with mixtures of both. Fig.1 shows traces of a pressure experiment with a solution containing 30S and 50S subunits under conditions favouring the formation of 70S particles at atmospheric pressure. Application of pressure produces a decrease in turbidity which indicates that the weight-average molecular weight is lower under high hydrostatic pressure. The kinetics of the pressure induced shift of the equilibrium could not be resolved in the present study. Pressure-jump relaxation experiments [7] prove that the relaxation times of the association reactions are below 1 sec, and hence the kinetics remain undetectable due to the dead time of the high pressure apparatus (~ 2 min). The turbidity changes induced by pressure are fully reversible and reproducible in the whole range of pressures investigated ($\leqq$ 1.5 kbar); this holds for both different solutions obtained from a given preparation of ribosomal particles and different ribosomal preparations. The results of experiments with 30S and 50S subunits, as well as with mixtures of both in 25 mM Mg^{2+} are shown in fig.2. The minor changes observed in the turbidity in 2 mM Mg^{2+} solutions (cf.fig.2 and 3) represent an upper limit for the pressure effects on the refractive index increment and prove that this effect is negligible under the experimental conditions. While the turbidity of the separated 30S and 50S particles is characterized by a slight decrease at $p \leqq$ 1 kbar, mixtures of both show a decrease which appreciably exceeds that of the separate subunits. The profile at high hydrostatic pressure ($>$ 0.8 kbar)

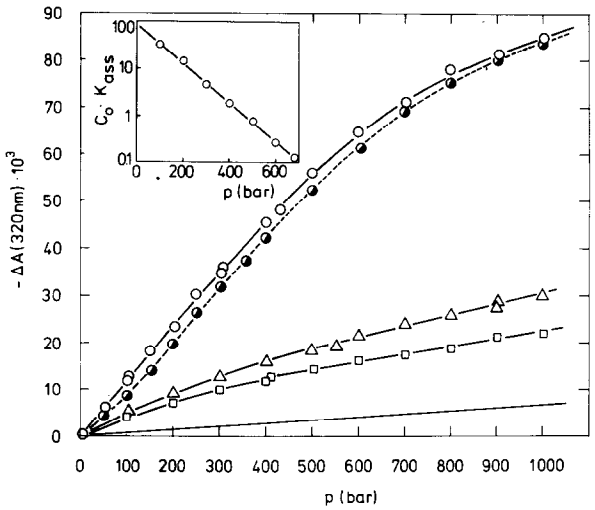

Fig.2. Change of turbidity ($\triangle A_{320nm}$) of ribosomal particles as a function of hydrostatic pressure. Ribosomal concentrations: 30S : A_{260nm} = 21.5 (□); 50S : A_{260nm} = 42.1 (△); 30 S + 50 S : A_{260nm} = 63.6 (○) and 30 S + 50 S : A_{260nm} = 91.2 (●), resulting from A_{260nm} (30S) = 30.2 plus A_{260nm} (50S) = 61.0; the latter data were reduced using the factor 63.6/91.2. Standard buffer containing 25 mM Mg^{2+}. 30 S + 50 S (–) in the presence of 2 mM Mg^{2+} (cf.fig.3). Insert: In C_0K_{ass} versus p plot for the association 30 S + 50 S ⇌ 70 S (data from curve (○)).

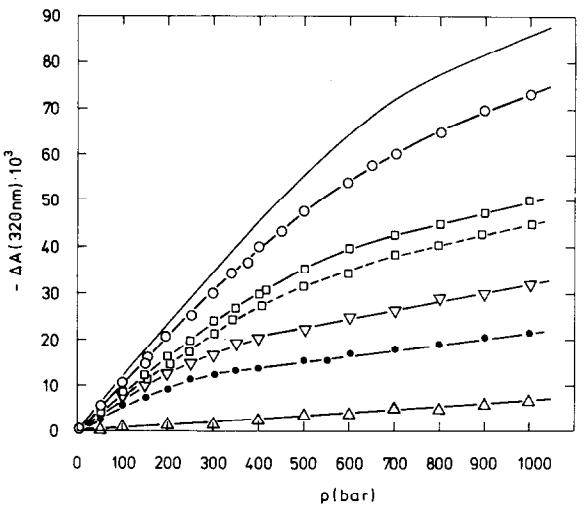

Fig.3. Change of turbidity (A_{320nm}) of mixtures of 30 S + 50 S ribosomal particles as a function of hydrostatic pressure and Mg^{2+} concentration [mM] : 2, (▽); 7, (●); 10, (△); 15, (□); 20, (○); 25, (–) taken from fig.2; standard buffer. (–□–), standard buffer with 15 mM Mg^{2+} and 100 mM KCl instead of 50 mM KCl. Ribosomal concentration: A_{260nm} (30S) = 21.5, A_{260nm} (50S) = 42.1.

reaches a similar limiting slope for all three sets of experiments with solutions containing 30S, 50S and 30S + 50S ribosomal subunits. As a consequence, the difference observed in the pressure dependence of the turbidity changes of subunit mixtures as compared to separated subunits may be attributed to the dissociation of 70 S particles into 30S and 50S subunits. The influence of the ionic conditions on the pressure induced dissociation is demonstrated in fig.3. Increase of the Mg^{2+} concentration at a fixed concentration of monovalent ions causes an increase in the change of the turbidity under constant high pressure. The maximum amplitude of the dissociation process is observed at $\geq$ 25 mM Mg^{2+} (fig.3). At a given Mg^{2+} concentration addition of monovalent cations decreases the degree of association. This is indicated by a smaller change in turbidity at comparable pressures. With constant composition of the buffer an increase of the ribosomal concentration leads to an enhancement of the association, in turn causing an upward shift of the corresponding tubidity–pressure profile (fig.2).

4. Discussion

The decrease in turbidity at 320 nm which has been observed in mixtures of 30S and 50S subunits of *E. coli* ribosomes at elevated hydrostatic pressure indicates a pressure dependent shift of the association-dissociation equilibrium:

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

towards dissociation. This finding is in qualitative agreement with previous results from sedimentation experiments in sucrose gradients [4–6]. However, the present measurements provide direct evidence in aqueous buffer solutions and allow a straight forward treatment of the data because of the absence of perturbations due to experimental procedures.

The evaluation of the results on the basis of the simple equilibrium (1) is complicated by the fact that solutions containing the separated 30S or 50S subunits

also show a pressure dependent change in turbidity. Pressure-jump relaxation experiments in the range of pressures up to 150 bar [7] confirm the assumption that these reversible changes in the weight-average molecular weight are attributable to association-dissociation equilibria between subunit monomers (30S, 50S) and their homo-associates, $(30S)_n$ and $(50S)_n$, respectively. Since there has been no quantitative determination of the self-association of the 30S and 50S particles the evaluation of the data according to eq. (1) can only be regarded as a first approximation. This approximation is based on the assumption that the turbidity changes observed in the solutions containing only 30S or 50S particles are not caused by the fraction of those subunits which are involved in equilibrium (1). As can be seen from the insert in fig.2 a plot of C_0K_{ass} versus p yields a straight line with a slope corresponding to a reaction volume of $\triangle V$ = +240 ml · mol^{-1}. This value represents the upper limit for the true value because of the simplifying assumption introduced into the calculation of K_{ass}. For sea urchin ribosomes a reaction volume in the range between 200 and 500 ml·mol^{-1} has been reported [4].

The sign of $\triangle V$ allows one to speculate about the nature of the intermolecular forces stabilizing the 70S ribosomal particle. Although there has been some disagreement regarding the dilatometric results with small aliphatic and aromatic model compounds, there is increasing evidence that, when hydrophobic interactions are involved, dissociation should result in an increase of volume ($\triangle V < 0$) [10]. On the other hand, splitting of inter-subunit salt linkages (salt bridges, ion pairs) would lead to a decrease of the overall volume upon dissociation. The high pressure results seem to indicate that the latter type of interactions is predominant in the volume changes observed for the 30S+50S ⇌ 70S equilibrium.

Acknowledgements

This investigation was supported by grants from the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie. The generous help of Drs V. A. Erdmann and H. G. Wittmann in the preparations of the ribosomes is gratefully acknowledged. E.S. wishes to thank Dr V. A. Erdmann for critical reading of the manuscript.

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