DEPENDENCE OF DISSOCIATION–ASSOCIATION OF UNCHARGED RIBOSOMES OF *ESCHERICHIA COLI* ON THE Mg^{2+} CONCENTRATION, IONIC STRENGTH, pH AND TEMPERATURE

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1. Introduction

In a previous communication [1] it was shown that in a suspension of uncharged ribosomes, that is particles devoid of mRNA, aminoacyl-tRNA and peptidyl-tRNA, there is an observable dynamic equilibrium between couples and subparticles:

$$50\ S\text{–}30\ S \rightleftharpoons 50\ S + 30\ S.$$

Sedimentation analysis of such equilibrium mixtures in the ultracentrifuge (both the moving boundary and moving zone methods) will to a greater less extent disturb the equilibrium. Consequently proportions of couples and subparticles apparent from sedimentation patterns may not reflect their real proportions in the equilibrium mixture. In principle, the ratio of the components in such a mixture could be investigated in the ultracentrifuge after both the forward and reverse reaction were in some manner quickly stopped.

In order to stop both the reaction of association of ribosomal subparticles and the reaction of dissociation of couples for a subsequent sedimentation analysis, a formaldehyde fixation technique was suggested [2]. It has been shown in this laboratory that the addition of formaldehyde to an equilibrium mixture of ribosomal particles 'freezes' the equilibrium existing at the moment the formaldehyde is added thus allowing subsequent changes of medium conditions or separation of the ribosomal components of the mixture without changing the proportions between the components. The present paper reports the results of utilizing the formaldehyde fixation technique followed by a sedimentation analysis for investigating the dependence of the dissociation–association state of ribosomal particles on the Mg^{2+} concentration, ionic strength (KCl or NaCl), pH and temperature in the medium.

2. Materials and methods

Preparations of both NH_4Cl-washed ribosomes [3] and 50 S–30 S couples obtained by associating washed isolated 50 S and 30 S subparticles [4] of *Escherichia coli* MRE-600 were used in the experiments. The results of experiments with both types of preparations were practically identical.

Prior to experiments the ribosomes were thoroughly dialyzed against a buffer containing 20 mM $MgCl_2$, 50 mM KCl and 1 mM K_2HPO_4, final pH 7.5. Reassociation of equimolar amounts of 50 S and 30 S subparticles was performed in the same buffer. The concentration of ribosomes after dialysis and reassociation was adjusted to 1 or 2 mg per ml.

For studying the effect of different cation concentrations (Mg^{2+}, K^+ or Na^+), equal (0.1 or 0.2 ml) portions of the ribosomal suspension were introduced into a series of buffer solutions ($MgCl_2$, KCl or NaCl, 1 mM K_2HPO_4, pH 7.5) of 1.9 or 1.8 ml; the solutions contained a salt concentration such that after

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mixing the required cation concentration was obtained. In all cases the final concentration of ribosomes after a dilution was 100 μg per ml. The diluted samples were incubated for 30 min at 20° to establish equilibrium (the time for establishing equilibrium was checked in special experiments) and then a 1/10 volume of 40% formaldehyde was added for fixation.

Experiments on the effect of pH were carried out in a similar way, except that portions of ribosomes were introduced into a series of solutions with 10 mM triethanolamine-HCl or K^+-acetate buffer of different pH; the K^+ concentration was adjusted to 50 mM; the final pH after addition of ribosomes was checked by a pH-meter.

In studies of temperature effects, the same portions of initial ribosomal suspension were introduced into a series of buffer solutions ($MgCl_2$, 50 mM KCl, 1 mM K_2HPO_4, pH 7.5) at different temperatures and after 30 min incubation the formaldehyde was added immediately at the corresponding temperatures. The samples fixed in this manner could be stored for any length of time at room temperature without changes in the proportions of couples and subparticles.

All the samples of the formaldehyde-fixed ribosomes were analyzed directly at 20° in the Spinco Model E (Beckman) ultracentrifuge using ultraviolet absorption optics. The amounts of 50 S–30 S couples and free subparticles in the analyzed suspensions were calculated from the sedimentation patterns. All details have been described previously [2, 5].

3. Results

As a rule, the maximal proportion of the 50 S–30 S couples in the mixture was about 80% (±10%). The remaining 20% of the preparation seem to consist of ribosomal subparticles incapable of associating under any conditions ('defective subparticles'). In connection with this, the fractions of couples observed at 20 mM Mg^{2+}–50 mM K^+ (pH 7.5, 20°) are taken as 1 in the figures presented below.

Fig. 1 shows the Mg^{2+}-dependence of dissociation of NH_4^+-washed ribosomes (curve 1, filled circles) and ribosomes obtained by reassociating washed 50 S and 30 S subparticles (curve 1, open circles). The Mg^{2+}-dependence of dissociation of unwashed (crude) ribosomes (curve 2), taken from a previous communication [2], is shown for comparison.

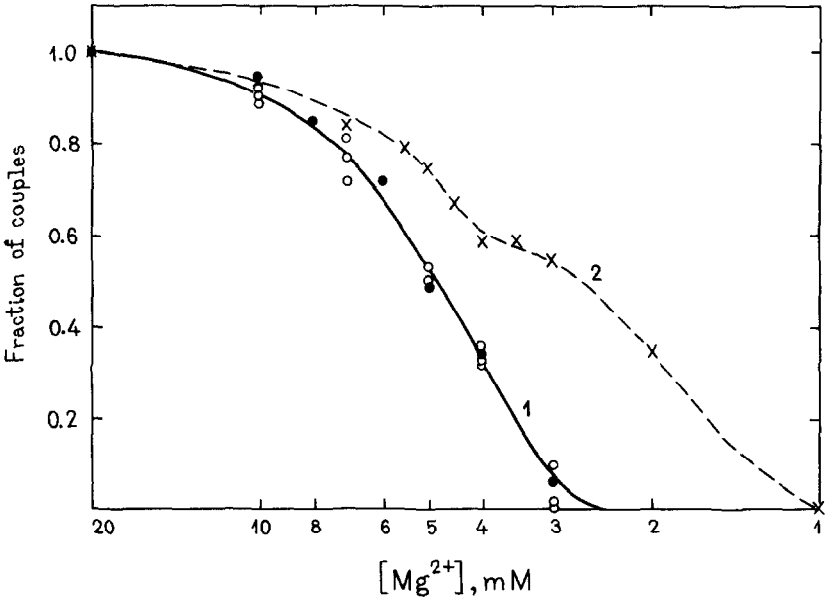

Fig. 1. Dependence of the amount of the 50 S–30 S couple fraction on the $MgCl_2$ concentration (at 1 mM K_2HPO_4, 50 mM KCl, pH 7.5, 20°). *Curve 1* (——): preparations of NH_4Cl-washed ribosomes (•) and ribosomes obtained by re-associating washed isolated subparticles (○); *curve 2* (- - -): preparation of unwashed 70 S monoribosomes [2].

It is seen that different preparations of uncharged ribosomes (fig. 1, curve 1) exhibit a well defined reproducible dependence of dissociation of the 50 S–30 S couples on the Mg^{2+} concentration. Half-dissociation of couples is observed at 5 mM Mg^{2+}; the main Mg^{2+} range of dissociation is from 8 mM to 3 mM Mg^{2+}.

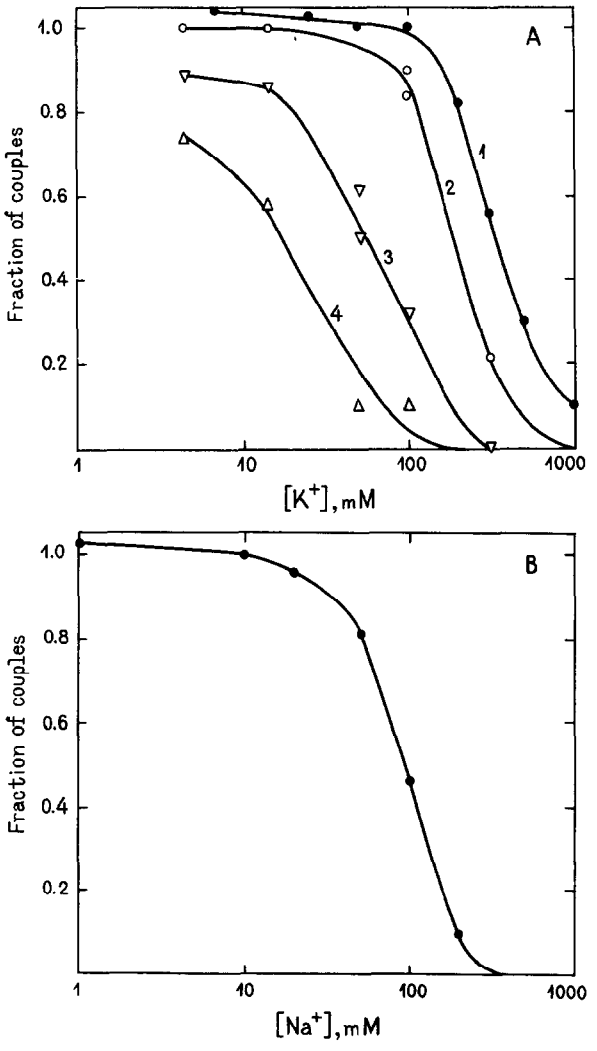

Fig. 2. Dependence of the amount of the uncharged 50 S–30 S couple fraction on the monovalent cation concentration.
(A) Dependence of the K^+ concentration at four different concentrations of $MgCl_2$: 20 mM (curve 1), 10 mM (curve 2), 5 mM (curve 3) and 3 mM (curve 4) (1 mM K_2HPO_4 is present everywhere; pH 7.5, 20°).
(B) Dependence on the NaCl concentration (at 20 mM $MgCl_2$, 1 mM K_2HPO_4, 5 mM KCl, pH 7.5, 20°).

It should be noted that the observed Mg^{2+}-dependence of dissociation of uncharged couples is valid only for the given ribosomal concentration in a mixture (100 μg per ml), at the particular ionic strength (50 mM KCl), pH (7.5) and the temperature (20°).

The preparations of unwashed ribosomes display a distinct heterogeneity in respect to their Mg^{2+}-dependence of dissociation (fig. 1, curve 2); a conclusion was made earlier that the fraction of the more stable monoribosomes (dissociating between 3 mM and 1 mM $MgCl_2$) represents 70 S particles stabilized by the presence in them of peptidyl-tRNA or aminoacyl-tRNA [2].

Fig. 2 shows that in all cases the increase of the monovalent cation concentration in the medium promotes the dissociation of uncharged ribosomal 50 S–30 S couples. It is seen from fig. 2A, that the higher the Mg^{2+} concentration in the medium, the greater the K^+ concentration required to induce the dissociation of ribosomes. For half-dissociation of ribosomes at 20, 10, 5 and 3 mM Mg^{2+}, a concentration of 300, 200, 50 and 20–30 mM K^+ is required, respectively.

It is seen from fig. 2B, that Na^+ possesses a much greater dissociating effect than K^+; at 20 mM Mg^{2+}

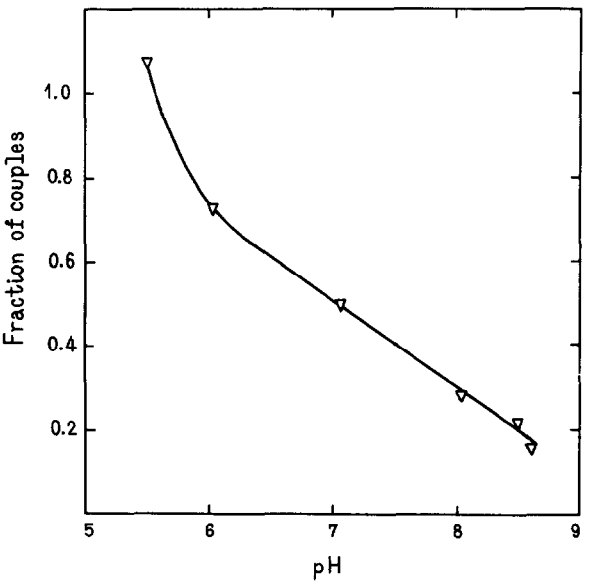

Fig. 3. Dependence of the amount of the uncharged 50 S–30 S couple fraction on pH at 5 mM $MgCl_2$.

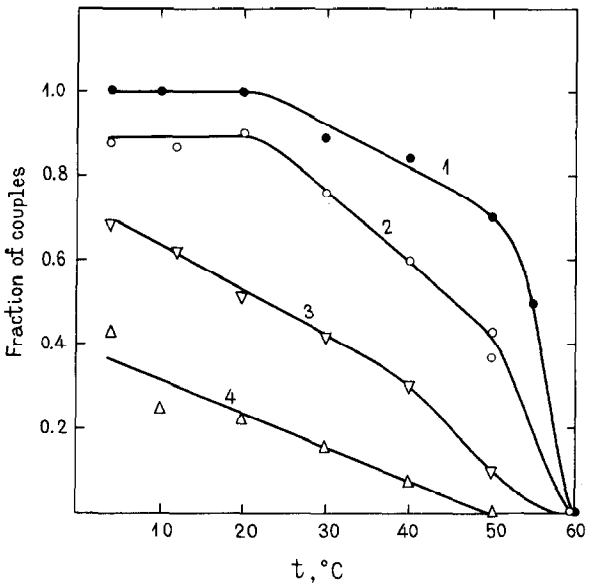

Fig. 4. Dependence of the amount of the uncharged 50 S–30 S couple fraction on temperature at four different concentrations of $MgCl_2$: 20 mM (curve 1), 10 mM (curve 2), 5 mM (curve 3) and 3 mM (curve 4) (1 mM K_2HPO_4 and 50 mM KCl were present in all experiments; pH 7.5).

half-dissociation of the uncharged couples is induced even by 100 mM NaCl.

From fig. 3 it follows that the increase in pH above 7–7.5 promotes the dissociation of ribosomes into subparticles. On the other hand, a decrease in pH leads to association of the ribosomal subparticles and stabilization of the couples. At pH below 7 the tendency of 70 S ribosomes to dimerize into 100 S particles strongly of 70 S ribosomes to dimerize into 100 S particles strongly increases; as the pH approaches 5, aggregation of the particles begins.

Finally, fig. 4 gives a pattern of dissociation of uncharged ribosomal couples under the effect of temperature at four different Mg^{2+} concentrations. The lower the Mg^{2+} concentration, the stronger is the dissociating effect of temperature. In all cases complete dissociation is observed at 60°. It was shown in special experiments that the presence of dithiothreitol had no effect on the observed thermal dissociation of couples, i.e., in this case dissociation is not stimulated by oxidation of ribosomal SH-groups (contrary to experiments by Miyazawa et al. [6]). The temperature dissociation of ribosomes up to 50° was reversible; however, at 60° dissociation was found to be irreversible (in the presence of dithiothreitol as well) and is most probably connected with the irreversible denaturation of particles.

4. Conclusion

The investigated 50 S–30 S couples seem to represent uncharged 70 S ribosomes (i.e., particles deprived of mRNA, aminoacyl-tRNA and peptidyl-tRNA [2, 4, 5]). It was shown that for such ribosomes a dynamic equilibrium with free subparticles exists: 50 S–30 S $\rightleftharpoons$ 50 S + 30 S [1]. In such a case the data reported here mean that Mg^{2+} ions, a decrease in pH and a decrease in temperature shift the equilibrium towards association, while K^+ ions, and to an even greater extent Na^+ ions, an increase in pH and high temperature shift the equilibrium towards dissociation.

The overall conclusion drawn from these data is that Mg^{2+} stabilizes, while an increase in the concentration of monovalent cations, an increase in pH and an increase in temperature destabilizes the association of ribosomal subparticles in a complete 70 S ribosome.

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