EFFECT OF NON-IONIC AGENTS ON THE STABILITY OF ASSOCIATION OF RIBOSOMAL SUBPARTICLES

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1. Introduction

It is well known that a certain Mg^{2+} concentration in the medium is necessary to maintain the associated state of 30 S and 50 S ribosomal subparticles in a whole 70 S ribosome [1, 2]. Mg^{2+} can be replaced by equivalent concentrations of Ca^{2+} or Mn^{2+} [3–5]. Spermine, spermidine and aminoglycoside antibiotics (neomycin, kanamycin, streptomycin) strongly stabilize the association of ribosomal subparticles [5–10]; in their presence much lower Mg^{2+} concentrations in the medium are required to dissociate ribosomes. An increase in the concentration of univalent cations, pH and temperature, on the contrary, destabilizes the association of ribosomal subparticles [11]. It has been shown that Na^+ and Li^+ exert an especially strong destabilizing action on the ribosomal 50 S–30 S couple, in comparison with the much weaker effect of K^+, NH_4^+, Rb^+ and Cs^+ [5].

In the present communication data are reported on the effect of a number of non-ionic agents on the stability of association of ribosomal subparticles. The stability of association was judged from the Mg^{2+} concentration at which the dissociation of 70 S particles into the subparticles takes place in the presence of appropriate agents (assuming that the more stable the association, the lower Mg^{2+} concentration required for dissociation). It is shown that dioxane (1 M), ethanol (1 M), methanol (1 and 2 M) and dimethyl sulphoxide (1 M) strongly stabilize association, glycerol (1 M) stabilized it only slightly, while urea (0.1 and 0.4 M) exhibits a destabilizing effect.

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2. Materials and methods

Ribosomes were prepared from *Escherichia coli* MRE-600, then dissociated into 50 S and 30 S subparticles in 0.5 M NH_4Cl with 0.01 M tris-buffer and 1 mM $MgCl_2$ (pH 7.3), and the subparticles were separated in a sucrose gradient, prepared against the same buffer, in the B-XIV zonal rotors of the MSE SS-65 ultracentrifuge; the ribosomal subparticles were precipitated with $(NH_4)_2SO_4$; the procedures were described in more detail previously [12].

Immediately before the experiment, the suspensions of 50 S and 30 S subparticles were thoroughly dialyzed against buffer containing 20 mM $MgCl_2$, 0.1 M NH_4Cl and 0.01 M tris-HCl, pH 7.2; the concentration of 50 S subparticles was adjusted to 1.32 mg/ml, and that of the 30 S to 0.66 mg/ml. For reassociation of subparticles, equal volumes of 50 S and 30 S subparticle suspensions were mixed and incubated for 30 min at 20° (a longer incubation time did not increase the amount of 50 S–30 S couples in the mixture). In order to study dissociation of the 50 S–30 S couples formed on decreasing Mg^{2+} concentrations in the presence of a non-ionic agent, 0.2 ml aliquots of the 50 S–30 S couple suspension were introduced into a series of test-tubes each containing 1.8 ml solutions with 0.1 M NH_4Cl, 0.01 M tris-HCl, pH 7.2, the corresponding agent and $MgCl_2$ to final concentrations of 20, 10, 9, 8, 7, 6, 5, 4 and 3 mM, respectively; introduction of an 0.2 ml aliquot into 1.8 ml solutions without $MgCl_2$ gave a final Mg^{2+} concentration of 2 mM. The final concentration of ribosomes was 0.1 mg/ml in all cases. After 30 min incubation at 20° (further incubation did not lead to visible changes),

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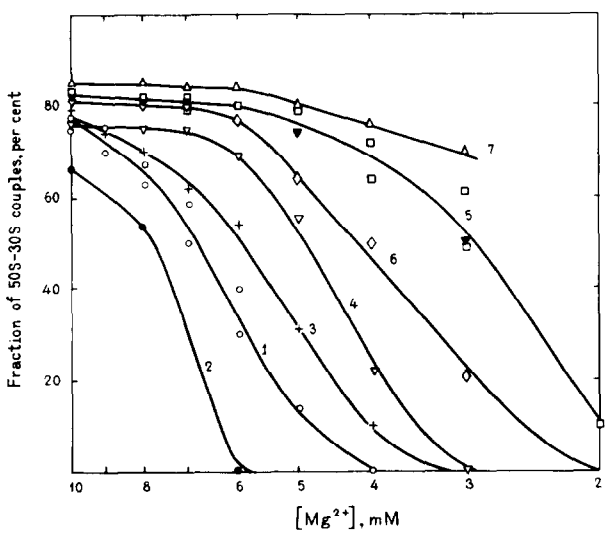

Fig. 1. Relative content of non-dissociated 50 S–30 S couples (68 S component) depending on the Mg^{2+} concentration, according to analytical ultracentrifugation data. 0.1 M NH_4Cl and 0.01 M tris-HCl, final pH 7.2, were present in all the cases.

1) (○——○): control (without addition of non-ionic agent);
2) (●——●): 0.1 M urea;
2) (+——+): 1 M glycerol;
4) (▽——▽): 1 M methanol;
5) (▼——▼ and □——□): 2 M methanol and 1 M ethanol, respectively;
6) (◇——◇): 1 M dimethyl sulphoxide;
7) (△——△): 1 M dioxane.

all samples were subjected to sedimentation analysis at 42,040 rpm using the ultraviolet absorption method in 12 mm analytical cells of the Spinco E ultracentrifuge. The relative amounts of non-dissociated 50 S–30 S couples (68 S component) and separate 50 S and 30 S subparticles were calculated from sedimentation densitograms. The percentage of non-dissociated 50 S–30 S couples was plotted against corresponding Mg^{2+} concentrations.

The non-ionic agents studied were dioxane, dimethyl sulphoxide, ethanol, methanol, glycerol and urea. All these except urea were tested at a 1 M concentration (methanol was also tested at 2 M); urea was tested at 0.1 M.

3. Results

In a control experiment (fig. 1, curve 1), dissociation of 50 S–30 S couples was studied in buffer containing 0.1 M NH_4Cl, 0.01 M tris-HCl and decreasing $MgCl_2$ concentrations, in the absence of an non-ionic agent. The initial degree of association, i.e. the amount of 50 S–30 S couples at 0.02 M $MgCl_2$, was usually 80–85%; a fraction of 50 S and 30 S subparticles remained incapable of associating. The 50 S–30 S couples had a sedimentation coefficient $S_{20,w}$) of 68 S ($\sigma = \pm 1$ S). A decrease of Mg^{2+} concentration from 20 to 10 mM led to a negligible dissociation of 50 S–30 S couples. A decrease of the Mg^{2+} concentration to 4 mM caused complete dissociation into 50 S and 30 S subparticles. Semi-dissociation was usually observed at 6 mM or between 7 and 6 mM Mg^{2+}.

In experiments with non-ionic agents (fig. 1, curves 3–7), the amount of 50 S–30 S couples at 0.02 M Mg^{2+} was approximately the same as in the control series, i.e. about 80–85%. This amount decreased only slightly or not at all at 10 mM Mg^{2+} in the presence of all the non-ionic agents studied, except 0.1 M urea.

As seen from fig. 1, curve 2, 0.1 M urea strongly destabilized 50 S–30 S couples; they dissociated noticeably (approximately 20%) even at 10 mM Mg^{2+} and at 6 mM Mg^{2+} complete dissociation into 50 S and 30 S subparticles was observed. 0.4 M urea caused complete dissociation of ribosomes into subparticles even at 10 mM Mg^{2+}.

1 M glycerol (fig. 1, curve 3) had no strong influence on the Mg^{2+}-dependence of dissociation of 50 S–30 S couples, though some stabilizing effect in comparison with the control was observed: complete dissociation was obtained at 3 mM Mg^{2+}, and semi-dissociation between 6 mM and 5 mM Mg^{2+}.

As seen from fig. 1, curves 4–7, methanol, ethanol, dimethyl sulphoxide and dioxane, i.e. hydrophobic agents, strongly stabilized 50 S–30 S couples; 50 S–30 S couples were still quite stable and did not dissociate at 6 mM Mg^{2+}. Dioxane (fig. 1, curve 7) exhibited the strongest effect; not more than 20% of initial 50 S–30 S couples dissociated even at 3 mM Mg^{2+}. 1 M ethanol and 2 M methanol were approximately equally effective (fig. 1, curve 5); the semi-dissociation region was at 3 mM Mg^{2+} or a little lower. 1 M dimethyl sulphoxide (fig. 1, curve 6) stabilized 50 S–30 S couples noticeably more weakly than 1 M ethanol. 1 M methanol was even less effective; the semi-dissociated state was observed between 5 mM and

4 mM Mg^{2+} (fig. 1, curve 4), whereas, in the controls, dissociation was already nearing completion at these Mg^{2+} concentrations.

4. Conclusion

Thus, different non-ionic agents present in the solution, strongly influence the association of ribosomal subparticles. Urea, even at a very low concentration (0.1 M), strongly destabilizes association. Hydrophobic agents, on the contrary, promote the association between subparticles. Glycerol, a non-hydrophobic alcohol, only weakly influences binding between the subparticles. Although from this no unambiguous conclusion can be drawn on the character of bonds between the subparticles, the data cast doubt on the significant participation of hydrophobic interactions and, on the other hand, favour some kind of participation of hydrogen bonds in the association of ribosomal subparticles.

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