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Pressure-induced dissociation of tight couple ribosomes

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Ribosomes from *Escherichia coli* have been shown to undergo subunit dissociation at elevated hydrostatic pressure. This holds for both crude and highly purified ribosomes. No inhibitory effect could be detected by addition of either the S100 supernatant, or tRNA, polyuridylic acid, and spermine. Light scattering experiments at pressures up to 1000 bar reveal different susceptibility of tight couple and loose couple ribosomes toward pressure dissociation. Tight couples are subjected to EF-Tu-catalyzed binding of aminoacyl-tRNA, thus yielding a model system of the elongating ribosome before the peptidyl transfer step. High pressure dissociation of this compound suggests that enzymatic binding converts tight couples into loose couples. A hypothesis referring to conformational changes during the elongation cycle is presented.

Elongation cycle; High pressure; Light scattering; Ribosome; Subunit dissociation; Tight couple

1. INTRODUCTION

The dissociation-association equilibrium of ribosomal subunits has been shown to be highly sensitive toward hydrostatic pressure. Previous light-scattering equilibrium studies on *E. coli* ribosomal subunit dissociation [1] led to the conclusion that ribosome dissociation might be responsible for bacterial growth inhibition at high pressures [2]. However, these results obtained with highly purified ribosomal subunits may not be relevant for the growth limitation in vivo, since the equilibrium of subunit association of uncomplexed ribosomes is involved neither in initiation nor in the elongation cycle.

Therefore, in the present study, 'programmed ribosomes' [6] representing the ribosomal state after EF-Tu-catalyzed binding of aminoacyl-tRNA, were prepared and subjected to high pressure light-scattering measurements.

According to Burma et al. [3], the process of enzymatic binding of aminoacyl-tRNA to tight couple ribosomes converts them to loose couples. The difference between both species is supposed to be a change in the conformation of 23 S rRNA resulting in a higher stability against dissociation induced by moderate pressures or low concentrations of Mg^{2+}.

Whereas Burma et al. only referred to sedimentation analysis and kethoxal modification as criteria for tight or loose couple behavior, the present results clearly indicate that altered susceptibility toward high pressure dissociation may be applied to distinguish tight couples or loose couples in elongation cycle intermediates.

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2. MATERIALS AND METHODS

Crude ribosomes were prepared from *E. coli* K12 according to Cronenberger and Erdmann [4]. The standard buffer for preparation and storage was TMA I (10 mM Tris-HCl, pH 7.4; 10 mM $MgCl_2$; 30 mM NH_4Cl; 0.5 mM DTE). For high pressure experiments TMA I was supplemented with 50 mM KCl and 1 mM EDTA. The standard temperature was 20°C.

Tight couples, prepared according to Rheinberger et al. [5], were generously provided by Dr K. Nierhaus (Berlin).

Enzymatic binding of aminoacyl-tRNA to ribosomes was performed as described by Wagner and Sprinzl [6], using deacylated tRNA for P-site binding and S100 supernatant instead of purified tRNA synthetase.

High pressure experiments were performed in a thermostated optical transmission cell-mounted on a Gilford 2400 S spectrophotometer as described by Schmid et al. [7]. Light scattering was monitored as absorption at 320 nm. For standardization, A_{320} was divided by the concentration measured in A_{260} units.

3. RESULTS

High pressure dissociation measurements with crude ribosomes resulted in dissociation curves similar to those described by Schulz et al. [1] for ribosomes reassociated from purified subunits. No significant stabilization could be detected by adding S100 supernatant, tRNA, polyuridylic acid or spermine (Fig. 1). As illustrated by the lower profiles, tight couple ribosomes show a significantly different pressure-dissociation behavior, exhibiting sigmoidal profiles. The sigmoidal

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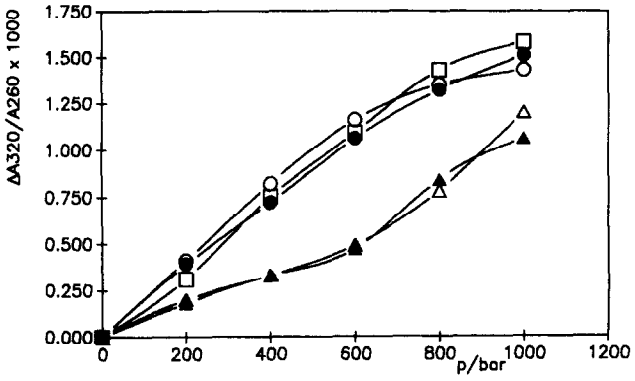

Fig. 1. Pressure-induced dissociation of crude ribosomes and tight couples. (○) 51 A_{260} units/ml crude ribosomes; (●) 39 A_{260} units/ml crude ribosomes; (□) 39 A_{260} units/ml crude ribosomes in the presence of S100 supernatant. (△) 45 A_{260} units/ml tight couples; (▲) the same sample of tight couples after freezing and thawing.

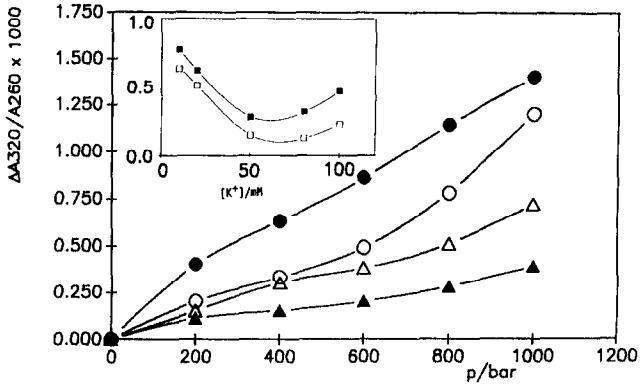

Fig. 2. Pressure-induced dissociation of tight couples: labilization by EF-Tu-dependent binding (upper profile) and stabilization by higher Mg^{2+} concentrations (lower profiles). (○) 45 A_{260} units/ml tight couples; (●) 18 A_{260} units/ml charged ribosomes. (○,●) 10 mM; (△) 15 mM; (▲) 20 mM Mg^{2+}. Insert: pressure sensitivity of tight couples as a function of K^+ concentration (identical ordinate as in the large frame). (□) Turbidity changes at 400 b ar; (■) turbidity changes at 600 bar. Ribosome concentration: 47 A_{260} units/ml.

characteristics seem to be typical for tight couples. It is reproducible even after freezing and thawing the sample. However, after dissociation at low Mg^{2+} concentration, and subsequent reassociation, sigmoidicity is no more detectable.

Tight couples were subjected to enzymatic charging with [^{14}C]Phe-tRNA^{Phe}. Depending on the nucleotides used in the charging procedure, different results were obtained: using three different ribosome preparations, no enzymatic charging could be accomplished with EF-Tu GTP. On the other hand, EF-Tu GMPPCP, in the presence of tight couples yielded 70% incorporation of radioactivity. After dialysis no change in the charging capacity could be detected. On the other hand, dialysis and storage at −70°C led to a drastic decrease in the amount of bound radioactivity. Only 10% of the radioactivity was still attached to ribosomes; the rest was found in the solution, thus indicating that dissociation of the aminoacyl-tRNA-ribosome complex had taken place during freezing or thawing.

High pressure experiments on this sample led to a dissociation profile essentially identical with the result obtained for crude ribosomes containing loose couples (Fig. 2). From this result we conclude that the binding procedure converts tight couples into loose couples.

Crude ribosomes subjected to the above charging procedure yielded nonenzymatic binding only, exhibiting no significant alteration of their pressure-dependent dissociation pattern.

The effect of Mg^{2+} ions on the pressure-induced dissociation of tight couples was examined at concentrations from 10 to 25 mM Mg^{2+}, the limit where high pressure effects become irreversible. In this range, a slight stabilization of tight couples by Mg^{2+} could be observed (Fig. 2). Varying the K^+ concentration in the range between 10 and 100 mM revealed optimum pressure stability of tight couples at 50–80 mM KCl (insert Fig. 2).

4. DISCUSSION

Burma et al. [3] showed in a two-step in vitro system that during protein biosynthesis the EF-Tu-dependent binding, followed by the peptidyl transfer reaction, converts tight couples into loose couples, whereas the EF-G-dependent translocation step converts loose couples into tight couples (Fig. 3). These results suggested the loose and tight couple conformations to be the 'natural states' of the elongation cycle intermediates I3 and I5. Experiments in which EF-G release was inhibited by fusidic acid clearly indicated that the I4 intermediate is in the loose couple conformation [3].

The present results provide some evidence that the I2 intermediate is in the loose couple conformation. A clearer distinction between I1 and I2 would be possible if tight couples could be enzymatically charged with EF-Tu GTP and the EF-Tu release could be inhibited by kirromycin, thus leading to a model system for the I1 state.

In principle, model systems for each of the five intermediates in the elongation cycle could be tested for tight couple or loose couple behaviour by direct measurements of the characteristics of their high pressure dissociation. The following working hypothesis seems to be compatible with available data: since both the change in affinity of the E-site and A-site in the three-site-model of the elongation cycle [8], and the conformational change from tight to loose couples are supposed to be located in the 23 S rRNA, a close relationship (or identity) of these two conformational switches is assumed. In this context, the tight couple conformation is proposed to be correlated with the high-affinity E-site, and the loose couple conformation to the high-affinity A-site.

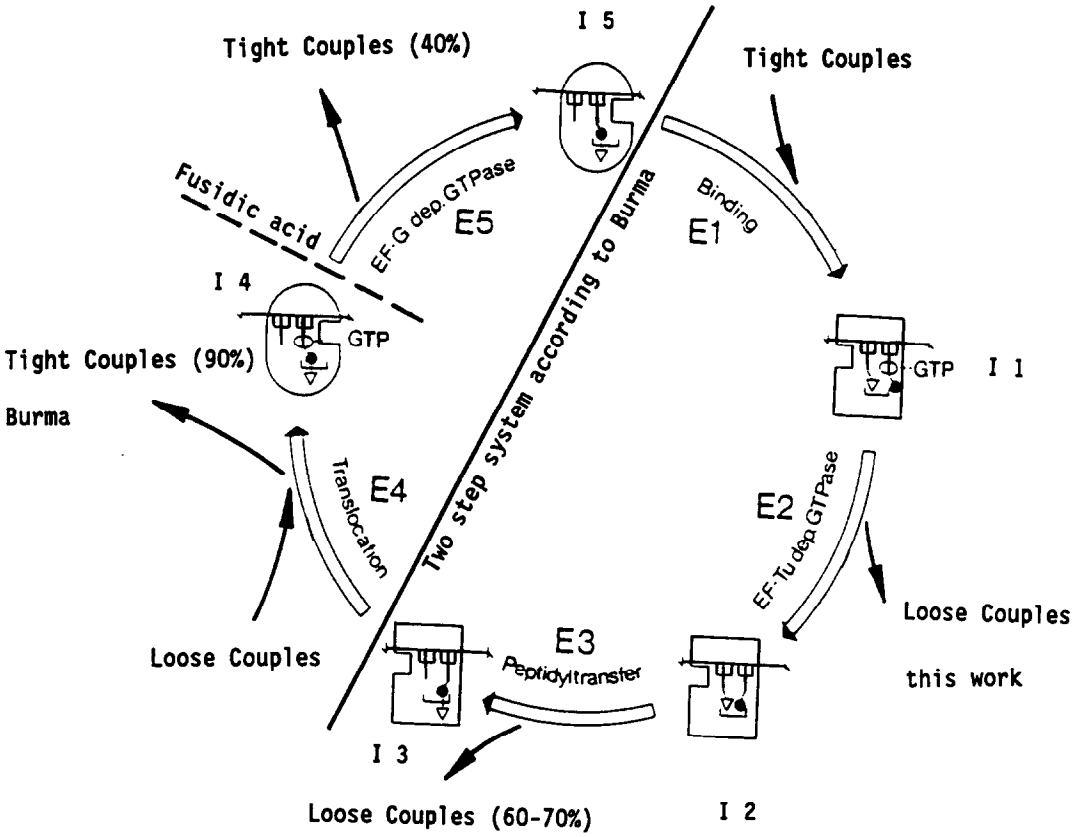

Fig. 3. Synopsis of the elongation cycle according to Nierhaus [8], together with the results of Burma [3] and the present results. Rectangular symbols represent ribosomes with high-affinity A-site, rounded symbols indicate ribosomes with high-affinity E-site.

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