Instability of Three-Dimensional Structures in Ribosomal Cores Evidenced by Microcalorimetric Studies

Adalberto Bonincontro[a], Stefania Cinelli[b], Giuseppe Onori[b], and Gianfranco Risuleo[c,*]

[a] INFM-Dipartimento di Fisica, Università di Roma "La Sapienza"
[b] INFM-Dipartimento di Fisica, Università di Perugia
[c] Dipartimento di Genetica e Biologia Molecolare, Università di Roma "La Sapienza", Piazzale Aldo Moro 5 - 00185 ROMA Italy.
Fax: 396 4440812. E-mail: risuleo@axcasp.caspur.it

* Author for correspondence and reprint requests

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In this paper we show a microcalorimetric investigation carried out on the so-called cores, *i.e.* ribosomes deprived of select proteins by LiCl treatment. Thermal degradation of native ribosomes gives rise to two thermal transitions occurring at different temperatures. In the cores the high temperature peak persists even after treatment at very high ion strength (2 M LiCl). This strongly suggests the existence of a very stable structure that was previously observed also in particles treated with agents that hydrolyze the RNA moiety. The low temperature peak gradually but dramatically decreases even though it never disappears completely. This indicates that the treatment to obtain ribosomal cores does not cause complete unfolding of the particle but only the destabilization of a structural three-dimensional domain present in native ribosomes. These data are discussed in the light of previous results obtained by dielectric spectroscopy and microcalorimetric studies on ribosomal particles.

Introduction

Extensive studies were performed to investigate the structure/function relationships in the ribosome of *E. coli* (Chambliss *et al.,* 1979; Hill *et al.,* 1990; Nierhaus *et al.,* 1993). We adopted different experimental strategies such as dielectric spectroscopy (DS), fluorescence and microcalorimetry (DSC) [recently reviewed by Bonincontro *et al.* (2000)]. The typical dielectric behavior of the ribosome consists of two subsequent relaxation processes occurring at about 200 kHz and 2 MHz. The first relaxation is due to counterion movement along segments of rRNA exposed to the solvent and its characteristic parameters, dielectric increment and relaxation time, allow the estimation of the persistence length of the nucleic acid (Mandel, 1977; Bonincontro *et al.*, 1991). The high frequency relaxation was attributed to the protein-RNA complex and possibly is due to oscillation of proteins linked to rRNA (Bonincontro *et al.*, 1997). These two dielectric dispersions were related in a phenomenological manner to two typical denaturation peaks observed in microcalorimetric experiments (Bonincontro *et al.*, 1998). In particular, the first dielectric dispersion may be associated to the low temperature peak and the second dispersion indicates a more stable structure present in the ribosomal particle. In experiments performed by both techniques, DS and DSC, native 70S ribosomes were subjected to increasing concentration of RNase, an enzyme that hydrolyzes the phosphodiester bonds in the nucleic acid backbone. The dielectric measurements showed an effect only on the first dispersion (Blasi *et al.*, 2000). This occurred at higher frequency and was characterized by a reduced dielectric increment as compared to the native ribosome. The effect is clearly due to the reduction of RNA persistence length, caused by the RNase treatment which fragments the nucleic acid polymer. The calorimetric investigation evidenced the deletion of the first peak, while the second remained unvaried. These results strongly suggest that at least one stable structure, resistant to the enzyme, exists inside the ribosomal particle. To this structure is associated a persistent dielectric response in MHz region. The nucleolytic action of RNase demolishes a vulnerable structure that is paralleled by the dielectric modifications reported above. In a recent paper we demon-

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strated that selective extraction of proteins from the ribosomal particle again has effect only on the first dispersion (Bonincontro *et al.*, 1999). This is shifted towards a lower frequency with an augmentation of dielectric increment. The logical interpretation of this data is that elimination of a limited number of proteins causes a higher exposure of rRNA. Furthermore the persistence of the second dispersion indicates that the stable structure, associated with the dielectric response, still exists. Therefore we felt urged to explore the calorimetric behavior of core particles where a few select proteins are eliminated.

Materials and Methods

Ribosome preparation

Ribosomes from *E. coli* (strain MRE600) were prepared as previously reported (Gualerzi *et al.*, 1981). Prior to LiCl treatment ribosomes were dialyzed against buffer A [10 mM $MgCl_2$, 10 mM tris-(hydroxymethyl)-aminomethane HCl, pH 7.5, 40 mM NH_4Cl, 6 mM β-mercaptoethanol]. Cores were obtained adding the appropriate amount of 10 M LiCl in buffer A to the final concentrations of 0.5, 1.0, 1.5 and 2.0 M (for details see Bonincontro *et al.* 1999). Prior to each measurement, ribosome and core samples were dialyzed against measuring buffer (0.8 mM $MgCl_2$, 3 mM KCl, 1 mM tris-HCl pH 7.5) and diluted to a final concentration of 5 mg/ml. At this relatively low ion strength ribosomal aggregation is prevented and thermal transitions are accurately defined (Bonincontro *et al.*, 1998).

Differential scanning calorimetry

For calorimetry experiments a differential scanning microcalorimeter 11 Setaram (Lyon, France) was used at a scan rate of 0.5 °C/min (temperature range 25–100 °C). The mass of the measured sample was 850 mg. Reference and sample cell weights matched. An excess power vs. temperature scan for the ribosome transition was obtained subtracting scan of the buffer vs. buffer from the power input scan of the ribosome solution, to minimize systematic differences between the measuring cells. This quantity referred to 1 mg of particles in the measuring sample gives the excess heat capacity, C_P.

Results and Discussion

Figure 1 demonstrates the thermal degradation profiles of native ribosomes and core particles obtained from treatment with increasing concentrations of LiCl. The typical profile of native ribosomes is observed. These particles melt in two thermal irreversible transitions as also previously shown (Bonincontro *et al.*, 1998). The profiles of thermal degradation of cores evidence that there is little or no difference, with respect to native particles, in the case of 0.5 and 1 M LiCl. At higher concentration of salt the picture changes quite dramatically. In particular the low temperature peak seems to be significantly more vulnerable to the LiCl wash as it disappears almost completely already at 1.5 M LiCl. This result resembles the data observed on RNase treated ribosomes (Blasi

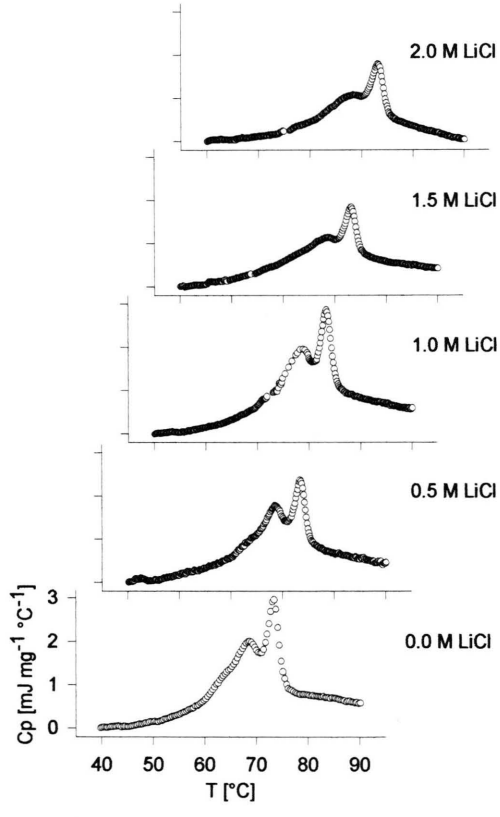

Fig. 1. Thermal profile of excess heat capacity (C_P) relative to native and LiCl treated 70S ribosomal particles. From bottom to top the effect from 0 to maximum concentration of LiCl is reported.

et al., 2000) where the low temperature peak was also affected by nucleolytic hydrolysis. Therefore the three-dimensional denaturation proposed for RNase-treated ribosomes occurs also after partial elimination of the proteinaceous component from the native ribosome (Kaltschmidt *et al.*, 1971). Recent dielectric data (Bonincontro *et al.*, 1999) showed that the low frequency dispersion was affected in cores obtained with the same treatments. Therefore the increase of the persistence length estimated from the dielectric measurements is paralleled by the same structural denaturation caused by hydrolysis of the phosphate bond in the rRNA backbone.

Finally the resistance of the high temperature peak to both RNase and LiCl treatment, as well as the stability of the high frequency dispersion in the same samples, highlights the existence of an extremely stable "core". This is essentially involved in conservation of the ribosome configuration but its functional role remains to be assessed.

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