Dielectric Properties of Ribosomal Core Particles Lacking a Select Population of Proteins

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In this communication we present a comparative investigation of the dielectric properties of native *E. coli* 70S and ribosomal cores obtained by LiCl treatment. Previous data obtained in our laboratory showed that ribosomes exhibit two different dielectric dispersions. We show that elimination of some select proteins modifies only the first one and therefore the overall dielectric properties of the ribosome result altered. Ribosomal RNA and proteins remaining in the core particle are mainly responsible for the second dielectric dispersion. Our experimental approach allows an estimation of the size of RNA traits exposed to solvent both in native ribosomes and in core particles where a higher portion of rRNA interacts with the external environment. Furthermore our results are consistent with the idea that proteins remaining after high salt treatment are necessary and sufficient for the maintenance of the basic structural properties of the ribosome.

Introduction

Dielectric spectroscopy is a powerful tool that has been successfully used to evaluate the physical parameters characterizing many relevant biological systems and macromolecules (Mandel, 1977; Bonincontro and Mariutti, 1988; Asami *et al.*, 1989; Pedone and Bonincontro, 1991; Bone, 1994; Bonincontro *et al.*, 1996). We exploited this approach to investigate the fine structure and the biophysical properties of the ribosome of the bacterium *E. coli*. This supramolecular organelle was intensely studied during the last thirty years by means of other experimental strategies (Nomura *et al.*, 1974; Chambliss *et al.*, 1979; Nierhaus *et al.*, 1993). Measuring the dielectric properties of *E. coli* 70S ribosomes we demonstrated that they exhibit two distinctive relaxations, the first in the kHz and the second in the MHz range, respectively. Furthermore, the low frequency dispersion is strongly dependent on the ion charge of the solvent while the relaxation time of the higher frequency process is rather constant in all experimental conditions (Bonincontro *et al.*, 1991). We have further extended our studies to the investigation of 30S and 50S subunits by fluorescence measurements (Bonincontro *et al.*, 1993). The high frequency dielectric dispersion previously reported (Bonincontro *et al.*, 1991) is also present in suspensions of separated subunits and the relaxation frequency is limited within a few MHz. From these data we hypothesized that the ribosomal proteins play a significant role in the dielectric behavior of the whole particle (Bonincontro *et al.*, 1996). This hypothesis was corroborated by a recent work where isolated ribosomal proteins were studied (Bonincontro *et al.*, 1997). To define the role of the proteins in the maintenance of the particle structure, we investigated the dielectric behavior of ribosomal particles deprived of a select subpopulation of proteins. In this communication we discuss the effects of the elimination of a limited number of proteins on the overall dielectric properties of the ribosome.

Materials and Methods

Ribosome preparation

Ribosomes 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$_4$Cl, 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. Treatment was continued for about 12 hrs at 4 °C under stirring. Core particles were recovered by centrifugation, resuspended in buffer A and dialyzed against the same buffer, divided in aliquots, frozen and kept at −80 °C. 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 7 mg/ml. This buffer was previously adopted in both dielectric (Bonincontro *et al.*, 1997) and thermographic measurements (Bonincontro *et al.*, 1998). This relatively low ion strength prevents ribosomal aggregation and allows accurate permittivity measurements.

Dielectric measurements

Permittivity (ε') and dielectric loss (ε'') were measured by means of a computer controlled Hewlett Packard impedance analyzer Mod. 4194A in the 0.1–100 MHz range. The measuring cell, previously described (Bonincontro *et al.*, 1996), is a section of a cylindrical waveguide which can be partially filled with the sample solution. The system behaves as waveguide excited far beyond its cut-off frequency mode and therefore only the stray-field of the coaxial line-waveguide transition is used in the measurement. Cell constants were determined by an interpolation method based on measurements with electrolyte solutions of known conductivities similar to those of the samples under test (Athey *et al.*, 1982). The errors on ε' and ε'' are within 1%. The measuring cell was thermally controlled within 0.1 °C.

Results and Discussion

In Fig. 1A and B, we present two typical dispersion curves obtained at a measuring temperature of 25 °C on native 70S particles and on 2 M LiCl cores. The best fit was in both cases obtained considering two subsequent relaxation processes that are the sum of a Debye followed by a Cole-Cole relaxation characterized by the frequencies f_1^*, f_2^* and dielectric increments $\Delta\varepsilon_1$, $\Delta\varepsilon_2$ (Hasted, 1973) A critical analysis of the experimental data allowed the choice of these best fit procedures. Results thus obtained, validate previous observations attributing the low frequency dispersion to the part of the RNA moiety exposed to the solvent
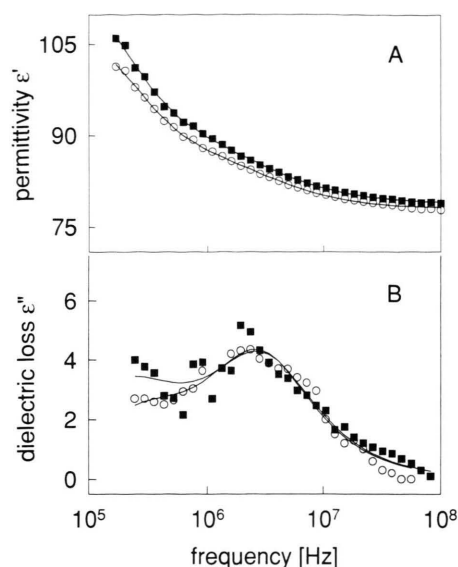

Fig. 1.A. Permittivity ε' (real part of complex dielectric constant) vs. frequency of native 70S (○) and 2M (■) washed cores in aqueous solution.
Fig. 1.B. Dielectric loss ε' (imaginary part of complex dielectric constant) vs. frequency of native 70S (○) and 2M (■) washed cores in aqueous solution. The continuous lines are the result of a best fit based on a sum of Debye and Cole-Cole relaxations (Hasted, 1973).

(Bonincontro *et al.*, 1991). The second derives very likely from the intrinsic association of proteins with RNA (Bonincontro *et al.*, 1997). In Table I we summarize the parameters of the dielectric dispersions referred to all samples utilized in this study. Comparing native ribosomes to cores, it is evident that the removal of proteins has an effect mainly on the first relaxation at low frequency, while the second one is essentially unaffected. It is difficult to establish a correlation between salt concentration used in each treatment and dielectric behavior of the particles. However, in the cores a shift of the relaxation to lower frequencies

Table I. Relaxation frequencies (f_1^*, f_2^*) and dielectric increments ($\Delta\varepsilon_1$, $\Delta\varepsilon_2$) of the two dispersions exhibited by native and LiCl treated ribosome samples.

SAMPLE	f_1^* [kHz]	$\Delta\varepsilon_1$	f_2^* [MHz]	$\Delta\varepsilon_2$
Native 70S	260 ± 20	19 ± 1	2.8 ± 0.6	10 ± 1
Core 0.5 M LiCl	190 ± 20	24 ± 1	2.7 ± 0.4	10 ± 1
Core 1.0 M LiCl	180 ± 20	29 ± 2	2.6 ± 0.7	11 ± 2
Core 1.5 M LiCl	200 ± 20	23 ± 2	2.5 ± 0.7	11 ± 2
Core 2.0 M LiCl	210 ± 20	23 ± 1	2.6 ± 0.4	13 ± 2

and an increase of dielectric increment are evident. This phenomenon is selfconsistent since the dispersion may be interpreted with the oscillating counterion model proposed by Mandel (1977). A decrease of f_1^* and an increase of $\Delta\varepsilon_1$ imply an overall augmentation of the portions of ribonucleic acid exposed to the solvent. This is obviously in agreement with the removal of proteins from the particle. Applying the Mandel model to calculate the average length of the portions of the RNA exposed in the native 70S, a value of about 86 nm is obtained. This is surprisingly similar to the maximum circumpherence of the ribosome grossly assumed as quasi spherical. This assumption is plausible since the differences in value of the ribosomal axes, usually cited in the literature, appear to be not dramatic. In the cores the same calculation produces a value of the exposed RNA about 10% higher than the former one. Possibly, the assumed quasi spherical shape is no longer valid since a polar collapse might have occurred. In any case, the internal structure of the RNA/ protein complex still remains as inferred from the stability of the high frequency dispersion that we have assigned essentially to this complex (Bonincontro *et al.*, 1997). Results also give evidence that the proteins remaining in the core are sufficient for the maintenance of the structural integrity of the particle. As a matter of fact core particles run on sucrose density gradients at low magnesium (0.3 mM in buffer A) show that after LiCl treatment they migrate as a homogeneous population but at lower sucrose density with respect to the homologous particles (not shown). In any case, we would like to stress that our evaluation is only of

structural nature. It is known in fact, that treatment with LiCl promptly inhibits ribosome function (see for instance Nierhaus and Montejo, 1973 and Moore *et al.*, 1975). Our data support the idea that the structural "framework" of the ribosome remains even after removal of some proteins. With respect to this, we checked the proteins expelled from the ribosome after LiCl washing by polyacrylammide gel electrophoresis (not shown). Our results are in agreement with previous observations (Kaltschmidt *et al.*, 1971) that only few proteins are expelled. In particular, the following proteins are most efficiently removed: S1, S3, S9, S10, S20, L1, L8, L9 and L10. Also S2, S5, L6 and L7 are removed although to a lesser extent. The role and the chemico-physical nature of ribosomal proteins were addressed in a number of studies on the structure/function relationships in the ribosome (Kaltschmidt *et al.*, 1971; Chambliss *et al.*, 1979). In the light of the results presented here, the abovementioned proteins are evidently not required for the maintenance of the structural features of what we would define "basic" ribosome. In conclusion we envisage that ribosomal proteins might be divided into two subgroups; the first is necessary for the maintenance of the "basic" structural core of the ribosome while the second one is essentially involved in the performance of its biological functions.

Asami K., Takhashi Y. and Takashima S. (1989), Dielectric properties of mouse lymphocytes and erythrocytes. Biochim. Biophys. Acta, **1010**, 49–55.

Athey T. W., Stuckly M. A. and Stuckly S. S. (1982), Measurements of radio frequency permittivity of biological tissues with an open-ended coaxial line: Part I. IEEE Trans. MTT. **30**, 82–86.

Bone S. (1994), Dielectric studies of native, unfolded and intermediate forms of β-lactamase. Phys. Med. Biol. **39**, 1801–1809.

Bonincontro A. and Mariutti G. F. (1988), Influence of hyperthermia, pH and culturing conditions on the electrical parameters of chinese hamster V79 cells. Phys. Med. Biol. **33**, 557–568.

Bonincontro A., Giansanti A., Pedone F. and G. Risuleo (1991), Radiofrequency dielectric spectroscopy of ribosome suspensions. Biochim. Biophys. Acta **1115**, 49–53.

Bonincontro A., Briganti G., Giansanti A., Pedone F. and Risuleo G. (1993), Effects of magnesium ions on ribosomes: a fluorescence study. Biochim. Biophys. Acta **1174**, 27–30.

Bonincontro A., Iacoangeli A. and G. Risuleo (1996), Electrical conductivity dispersion as a probe of membrane modificationsin mouse poliomavirus infected cells in culture. Biosci. Rep. **16**, 41–48.

Bonincontro A., Briganti G., Giansanti A., Pedone F. and Risuleo G. (1996) Electrical conductivity and dielectric dispersion of *E. coli* 70S ribosomes and of 30S and 50S subunits: effects of magnesium. Colloids Surfaces **6**, 219–226.

Bonincontro A., Mari C., Mengoni M. and G. Risuleo (1997), A study of dielectric properties of *E. coli* ribosomal RNA and proteins in solution. Biophys. Chem. **67**, 43–50.

Bonincontro A., Cinelli S., Mengoni M., Onori G., Risuleo G. and Santucci A. (1998), Differential stability of *E. coli* ribosomal particles and free RNA towards thermal degradation studied by microcalorimetry. Biophys. Chem. **75**, 97–103.

Chambliss G., Craven G. R., Davies J. and Nomura M. Eds. (1979), Ribosomes: Structure, Function and Genetics. University Park Press, Baltimore, USA.

Gualerzi C., Osawa H., Risuleo G. and Pon C. L. (1981), In: Structural Aspects of Recognition and Assembly of Biological Macromolecules (Balaban, Sussman, Traub and Yonath eds.). ISS Philadelphia pp. 793–803.

J. B. Hasted (1973), Aqueous Dielectrics, Chapman and Dole eds. London.

Kaltschmidt E., Rudloff V., Janda H.-J., Cech T., Nierhaus K. H. and Wittmann H. G. (1971), Isolation of proteins from 70S ribosomes of *Escherichia coli* Hoppe-Seyler's Z. Physiol. Chem. **352**, 1545–1552.

Mandel M. (1977), Dielectric properties of charged linear macromolecules with particular reference to DNA. Ann. NY Acad. Sci. **303**, 74–87.

Moore V. G., Atchison R. E., Thomas G., Moran M. and Noller H. F. (1975), Identification of a ribosomal protein essential for peptidyl transferase activity Proc. Natl. Acad. Sci. USA **72**, 844–848.

Nierhaus K. H., Franceschi F., Subramanian K., Erdmann V. and Wittmann-Liebold B. Eds. (1993), The Translational Apparatus. Plenum Press, New York.

Nierhaus K. H. and Montejo V. (1973), A protein involved in the peptidyltransferase activity of *Escherichia coli* ribosomes. Proc. Natl. Acad. Sci. USA **70**, 1931–1935.

Nomura M., Tissières A. and Lengyel P. Eds. (1974), Ribosomes. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA.

Pedone F. and Bonincontro A. (1991), Temperature dependence of DNA dielectric dispersion at radiofrequency. Biochim. Biophys. Acta **1073**, 580–584.