Intrinsic structural differences between 'tight couples' and Kaltschmidt–Wittmann ribosomes evidenced by dielectric spectroscopy and scanning microcalorimetry

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Received 27 November 2000; accepted 5 December 2000

First published online 28 December 2000

Edited by Lev Kisselev

Abstract Measurements of dielectric spectroscopy (DS) and microcalorimetry (differential scanning calorimetry (DSC)) of *Escherichia coli* 70S, 50S and 30S were performed on particles prepared according either to the 'classical' twice NH_4Cl-washed ribosomes, also known as loose couples (LC), or to the 'tight couples' preparative protocol (TC). Results show that 70S particles prepared according to the two different protocols exhibit different structural properties. Two subsequent relaxation processes occur in both samples as measured by DS. However, in LC ribosomes the first one is shifted towards a lower frequency with a higher dielectric increment. This is suggestive of a more extensive exposure of RNA to the solvent and of an overall more relaxed structure. The smaller LC subunit exhibits only one relaxation while the TC 30S shows two dielectric dispersions as well as 70S. No substantial differences were evidenced in either 50S species. Two typical melting peaks were observed by DSC both in LC and TC 70S as well as in 50S. Thermograms obtained from the TC 30S show a single well structured peak while LC particles produce a large unstructured curve. On the basis of these results we conclude that TC 70S particles are more compact than LC ribosomes and that in the former ones the rRNA is less exposed to the solvent phase. Furthermore 30S particles obtained from TC show a more stable structure with respect to LC 30S. We conclude that the 30S subunit gives a major contribution to the compact character of the whole TC 70S. These differences might be related to the intrinsic and well documented functional difference between the two ribosome species.

Key words: Tight-couple (ribosome); Washed ribosome; Ribosome, gross structure; Dielectric spectroscopy; Microcalorimetry

1. Introduction

The structure and function of *Escherichia coli* ribosomes is a field of intensive research [1,2]. We applied biophysical techniques to investigate structural properties of *E. coli* ribosomes: dielectric spectroscopy (DS) and differential scanning calorimetry (DSC) were adopted to probe the three-dimensional organization of native particles [3,4] or the structural alteration after different treatments (e.g. mild RNase T_1 or selective protein stripping with LiCl; see for instance [5,6]). In our investigation we used the 'classical' Kaltschmidt–Wittmann ribosomes, also known as 'loose couples' (LC), since this preparative protocol is of high yield and produces a homogeneous particle population with good functional activity. Our results indicated that the small and the large subunits exhibit different behavior and structural features. In particular the 30S particle is floppy with respect to the 50S which shows a more rigid character. In addition, both the large subunit and the 70S ribosome show substantially similar dielectric and thermal behavior.

It is known however that the so called tight couples (TC, [7]) permit the production of ribosomes far more active than the former ones, even though gross structural differences were never evidenced [8,9]. This prompted us to carry out a comparative structural study in an attempt to correlate the better functional performance of TC in vitro with possible structural intrinsic features. We would like to point out, however, that by 70S monomer we mean a particle formed by a complex of 30 plus 50S subunits that structurally and spatially closely resembles the active ribosome.

2. Materials and methods

2.1. Ribosome preparation

Twice NH_4Cl-washed ribosomes (LC) were prepared as previously reported [10] and TC according to [7].

Prior to each measurement both ribosome species were dialyzed against measuring buffer (0.8 mM $MgCl_2$, 3 mM KCl, 1 mM Tris–HCl pH 7.5). Particle measuring concentration was: 7 mg/ml (70S and TC 30S) and 5 mg/ml (LC 30S). At this relatively low ion strength ribosomal aggregation is prevented thus permitting both thermal and dielectric accurate measurements [5]. As previously discussed, analysis by sucrose density gradients of ribosomal particles suspended in the measuring buffer shows that at this Mg^{2+} concentration, the bulk of ribosomes sediment as intact 70S monomers [5].

2.2. DS

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 [4], is a section of a cylindrical waveguide which can be

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PII: S0014-5793(00)02415-7

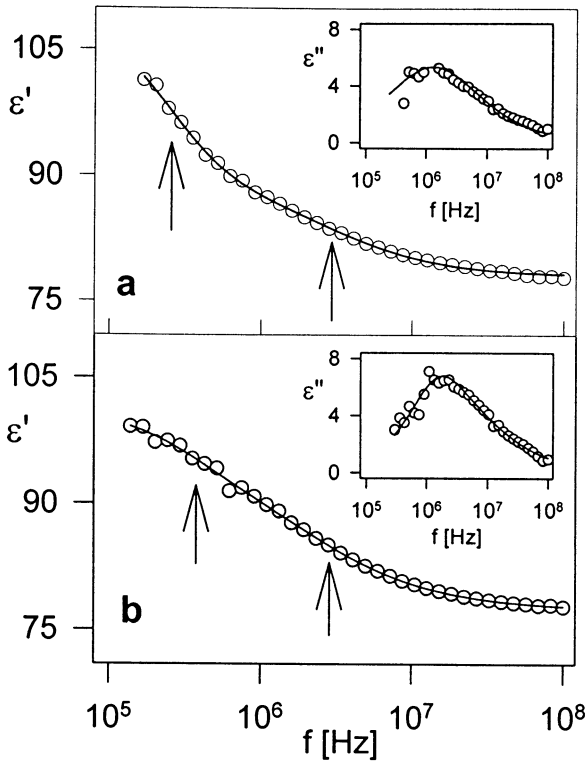

Fig. 1. Permittivity ε' versus frequency of native 70S LC ribosomes (a) and TC ribosomes (b). In the inset dielectric loss ε'' versus frequency is reported. Arrows visualize the frequencies of relaxations referred to the different samples. It is evident that the first dispersion of LC samples occurs at lower frequency (see text for comments). Ribosome concentration was 7 mg/ml and temperature 25°C. The continuous lines are the result of a best fit based on a sum of Debye and Cole–Cole relaxations.

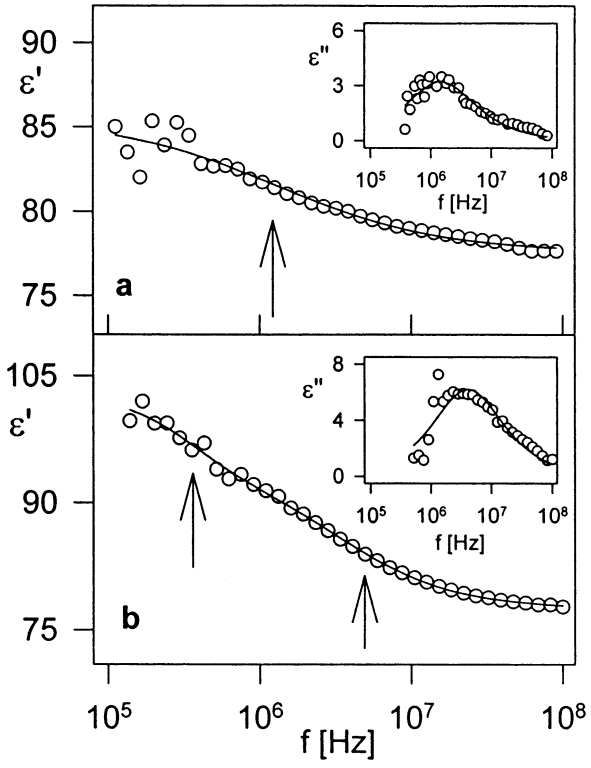

Fig. 2. Permittivity ε' versus Frequency of LC 30S subunits (a) and TC subunits (b). In the inset dielectric loss ε'' versus frequency is reported. In LC samples the low frequency dispersion is out of the measured field and the second is at lower frequency than the one measured in TC (see text for comments). TC ribosome concentration was 10 mg/ml and the temperature 25°C. The continuous line is the result of a best fit based on Cole–Cole relaxation (a) while a sum of Debye and Cole–Cole relaxations is presented in (b).

partially filled with the sample solution. The system behaves as a 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 [11]. The errors on ε' and ε'' are within 1%. The measuring cell was thermally controlled within 0.1°C.

2.3. DSC

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 versus temperature scan for the ribosome transitions was obtained subtracting scan of the buffer versus buffer from the power input scan of the ribosome solution to minimize systematic differences between the measuring cells. This quantity is referred to 1 mg of particles in the measuring sample and gives the excess heat capacity, C_p.

3. Results and discussion

We performed comparative DS measurements on LC and TC ribosomes (Fig. 1a,b, respectively). The dielectric behavior of these particles consists in two relaxations at two different frequencies. The first occurs within the range 0.1–0.3 MHz and second at 1.0–3.0 MHz. As far as the TC 70S is concerned a significant difference is observed for the low frequency dispersion (Fig. 1b). In this case the frequency of relaxation is higher while the dielectric increment is lower with respect to LC particles. Since this phenomenon is essentially due to counter-ion travelling along the RNA not bound to ribosomal protein, the variation of these parameters suggests that longer traits of exposed RNA in LC 70S exist [3,12]. Table 1 summarizes the different dielectric features of the two ribosome species. These data, as previously discussed [4], are

Table 1
Relaxation frequencies (f_1*, f_2*) and dielectric increments ($\Delta\varepsilon_1$, $\Delta\varepsilon_2$) of the two dispersions exhibited by all ribosome samples are reported

Sample	f_1* (kHz)	$\Delta\varepsilon_1$	f_2* (MHz)	$\Delta\varepsilon_2$
LC 70S	260 ± 20	19 ± 1	2.8 ± 0.6	10 ± 1
TC 70S	370 ± 40	8 ± 2	2.6 ± 0.5	15 ± 2
LC 30S	ND	29 ± 2	1.2 ± 0.2	8 ± 2
TC 30S	350 ± 70	10 ± 1	3.7 ± 0.7	15 ± 2

The parameter f_1* for LC 30S was out of the measuring range and not defined (ND). Error values derive from the fitting procedure and are therefore statistical.

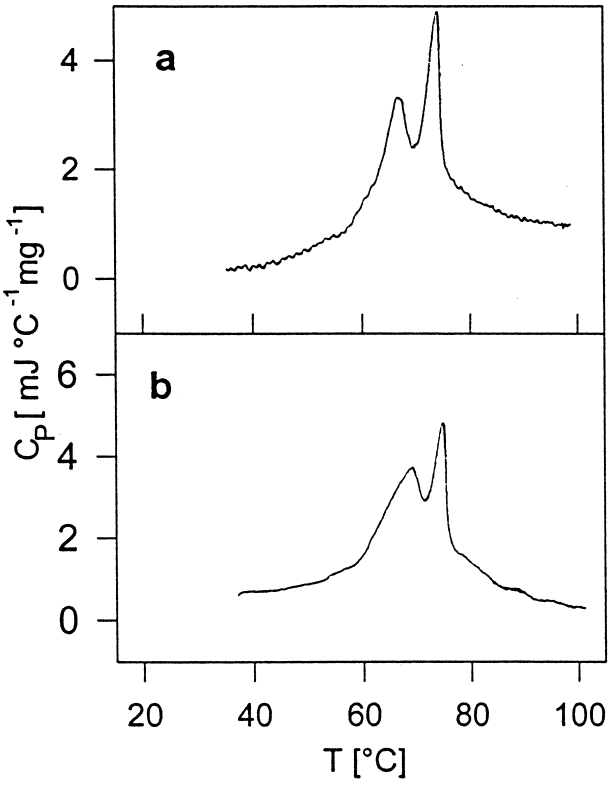

Fig. 3. C_p excess thermal profile of 70S ribosomal particles (LC in a, TC in b, respectively). The curve was calculated by the subtraction of a baseline from the recorder tracing as described in Section 2. Different scale plotting accounts for the apparent differences in thermal profile, in any denaturation peaks correspond.

indicative of a decreased particle compactness. An analogous phenomenon was observed in core ribosomes stripped of a select population of r-proteins [13]. Therefore we can conclude that TC ribosomes are 'denser' than LC particles. It is known that in our measuring buffer, ribosomes tend to dissociate [14–16]. However the results we present here are consistent with previous ones [3–6] showing the existence of a particle that can still be defined as 70S. This complex, under an exquisitely structural point of view, is formed by closely interacting 30S and 50S subunits.

The same study was performed on the separate subunits. Large 50S subunits exhibit two relaxations occurring at the same frequencies for both types of particles (data not shown). The dielectric behavior of TC 30S resembles that of 70S, i.e. two dispersions at about the same frequencies as monitored in the previous analyses. On the contrary, LC 30S subunits show only the second relaxation but the phenomenon is shifted to a lower frequency (Fig. 2a,b). The disappearance of the first dispersion in LC particles is due to its movement towards frequencies below our measuring window [4]. This overall shift of the two dispersions is consistent with the 'floppier' structure of the LC small ribosomal subunit as compared to the TC 30S.

Table 2
Melting temperature monitored in all samples

Sample	70S T (°C$_{(first)}$)	70S T (°C$_{(second)}$)	30S T (°C)
LC 70S	68.6 ± 0.2	72.8 ± 0.2	–
TC 70S	69.9 ± 0.2	72.9 ± 0.2	–
LC 30S	–	–	ND
TC 30S	–	–	66.9 ± 0.2

The broad denaturation profile exhibited by LC 30S did not permit the definition of a denaturation temperature (ND).

Microcalorimetric measurements show two levels of structural organization as revealed by the characteristic denaturation peaks in 70S (Fig. 3a,b) and 50S (data not shown): in other words the ribosomes obtained by the two different protocols do not exhibit substantial differences by calorimetric analysis. The small 30S subunits differ, on the other hand, in their microcalorimetric response (Fig. 4a,b). Small subunits obtained according to the LC protocol namely give rise to a broad and unstructured thermal profile. Particles from TC preparations produce, on the contrary, a well defined peak that indicates a single denaturation event. A further difference is represented by an evident exothermic peak denoting a significant particle aggregation. It should also be noted that the first denaturation peak in LC 70S also occurs at a slight lower temperature than in TC particles (Table 2). We therefore suggest that the intrinsic floppiness monitored in LC 30S influences the overall structure of the 70S particle. These data also confirm that 70S particle remains in a physical configuration strongly resembling the native 70S monomer as also by the thermograms performed on LiCl 70S cores where the gradual disappearance of the sole first transition was monitored [17].

The functional activity of the ribosomes used in this study was measured both by poly(U) directed poly(Phe) incorporation and by formation of the ternary complex aa-tRNA, 30S and the cognate synthetic poly-ribonucleotide. In both cases the activity was satisfactory. However, it is known that TC

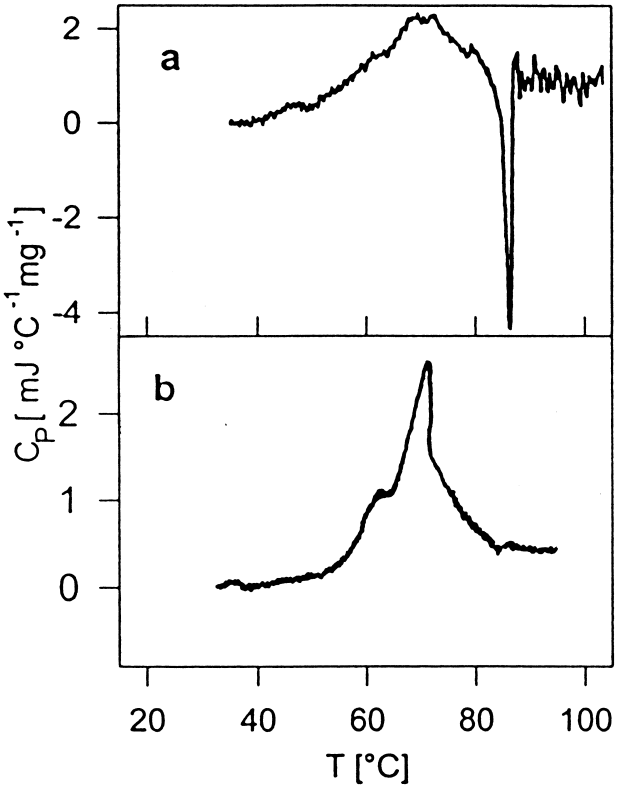

Fig. 4. C_p excess thermal profile of 30S subunits (LC in a, TC in b, respectively). A single well defined peak is present in TC particles, while LC show a broad thermal profile with evident aggregation (see text for comments).

particles exhibit a notably higher in vitro functional activity. Functional and structural transitions from TC to LC and vice versa [9] and some differences between the two forms were reported. It was shown that TC ribosomes resist dissociation caused by low ion strength while LC ribosomes quantitatively dissociate [14–16]. However, these 'classical' data were obtained by high speed sedimentation analysis on sucrose density gradients; this approach is known to induce spontaneous subunit separation because of hydrodynamic shearing. The spectrum and extent of hybridization of oligo-DNA probes complementary to the rRNA also show some differences between LC and TC ribosomes [18]. Furthermore, a spatial particle rearrangement during the translocation phase was claimed to occur and was thought to reflect an inter-conversion from TC to LC ([9], see also [18]). Data presented here are consistent with the hypothesis that in TC particles the rRNA is less exposed than in LC particles. TC ribosomes have a more compact structure as observed by our experimental approach. Therefore, the data strongly suggest that the loss of 'compactness' observed in ribosomes twice washed with ammonium chloride is related to its decrease in protein synthesis ability.

Acknowledgements: Cells of *E. coli* used for the preparation of twice NH_4Cl-washed ribosomes were obtained from the Servizio Biologico of Istituto Superiore di Sanità, Rome. The authors thank Dr. Silvia Baccaro for the participation to some experimental measurements.

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