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A study of the dielectric properties of *E. coli* ribosomal RNA and proteins in solution

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Abstract

The permittivity of ribosomal proteins and ribosomal RNA (rRNA) in solution was measured in the range 100 kHz to 1 GHz at four different temperatures (5, 15, 25 and 35 °C). The experimental dielectric relaxation was analysed by the Cole–Cole equation and, from the best-fit parameters, the average values of the dipole moment and molecular radius of the proteins were obtained. The activation enthalpy was calculated from an Arrhenius plot of the relaxation time. The energy involved in the dielectric polarization of free proteins has a magnitude of about one hydrogen bond. The data on RNA were analysed according to the Mandel model. This analysis allowed the calculation of the "subunit b" as defined by Mandel. This parameter is dependent on the temperature and therefore the relaxation time does not follow the Arrhenius law. Our data thus show that, in solution, the rRNA structure is thermally rather unstable and highly flexible. © 1997 Elsevier Science B.V.

Keywords: Dielectric properties; *E. coli*; Ribosomal proteins; Ribosomal RNA

1. Introduction

The ribosomal particle plays a key role in the process of protein biosynthesis in both prokaryotic and eukaryotic cells. The ribosomes of the bacterium *E. coli*, in particular, have been studied in detail during the last 30 years [1–3]. An enormous amount of knowledge exists on the genetics, molecular biology and biophysics of these bacterial organelles.

Dielectric spectroscopy is a powerful tool which has been successfully used to evaluate the physical parameters characterizing many biological systems and macromolecules of biological relevance, e.g. plasma membranes, isolated proteins and nucleic acids [4–9]. However, to our knowledge, this approach has never been exploited to investigate the fine structure and biophysical properties of the ribosome.

To address this point, we initiated a study of the chemicophysical properties of the prokaryotic ribosomal particles of *E. coli* based on dielectric spec-

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troscopy measurements. This study was performed in conditions which permit or abolish the biological functions of the ribosome, i.e. mainly in the presence or absence of magnesium, since this ion is principally involved in the maintenance of the ribosomal activity both in vivo and in vitro. By measuring the dielectric properties of *E. coli* 70S ribosomes, we demonstrated that they exhibit two distinctive dielectric relaxations, the first in the kilohertz and the second in the megahertz range. Furthermore, the low frequency dispersion is strongly dependent on the presence of Mg^{2+}, whereas the relaxation time of the higher frequency process is fairly constant in all experimental conditions [10]. We have also shown, by subsequent fluorescence studies on ribosomal RNA (rRNA) in solution, that the presence of Mg^{2+} ions, at concentrations spanning the range 0–10 mM, triggers a conformational rearrangement of the ribosomal nucleic acid. This is suggestive of a larger exposure to aqueous solvent of the RNA moiety with respect to the protein moiety [11]. In a recent development of our study, we have performed dielectric investigations on separate ribosomal subunits. Conductivity measurements show the existence of an interfacial conductivity modulated by bound magnesium ions. Furthermore, we also inferred the hydrated volume of the single isolated ribosome; thus it is worth noting that significant structural information can be obtained by relatively rapid and low cost conductivity experiments [12]. Moreover, we observed that the high frequency dispersion previously reported [10] is also present in suspensions of separate subunits and the relaxation frequency is limited within a few megahertz. From these data, we hypothesized that the ribosomal proteins are responsible per se for the observed dispersion [12]. In the light of these results, we decided to investigate the dielectric behaviour of purified total rRNA and proteins in solution. In this paper, we present the results of permittivity measurements, validating the assumption that the relaxation in the narrow megahertz range, observed in both the 70S and separate subunits, is attributable to ribosomal proteins. Average values for the dipole moment and molecular radius of the ribosomal proteins in solution are also reported. Finally, from our data, an informative estimate of the energy involved in the binding between rRNA and proteins can be obtained.

2. Materials and methods

2.1. Ribosomal protein and RNA preparation

Ribosomes were prepared as reported previously [13]. Proteins and RNA were extracted according to the 67% acetic acid procedure and resuspended at a final concentration of 6.5 mg ml^{-1} in the following buffer: 0.1 M magnesium acetate, 0.006 M β-mercaptoethanol, 0.05 M tris-HCl at pH 7.5. Two volumes of glacial acetic acid were added dropwise with stirring. Stirring was continued for 45 min at 4 °C, the solution was centrifuged at 25 000 × g for 15 min and the pellet was extracted again in the same conditions. After centrifugation, the protein supernatant was dialysed exhaustively against 50% acetic acid followed by 25% and 0.5% acetic acid. The protein concentration, 5.1 mg ml^{-1}, was determined colorimetrically using a commercial kit (Biorad). An RNA pellet was resuspended in 1 mM EDTA at pH 7.0. Protein and RNA integrity was checked by polyacrylamide gel electrophoresis (for details, see Ref. [14]).

2.2. Permittivity measurements

The permittivity (ϵ') of ribosomal protein and RNA solutions was measured by two computer-controlled Hewlett Packard impedance analysers (models 4194A and 4191A). The first was used in the 0.1–100 MHz range and the second in the 1 MHz–1 GHz interval. The measuring cell, described previously [12], is a section of a cylindrical waveguide which can be 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 conductivity similar to those of the samples under test [15]. The errors in ϵ' are within 1%. The measuring cell was thermally controlled within 0.1 °C.

3. Results

3.1. Dielectric dispersion of ribosomal proteins

We measured the permittivity of the total ribosomal proteins in the 0.1–100 MHz range at four different temperatures: 5, 15, 25 and 35 °C (Fig. 1). Proteins were dissolved in 0.5% (v/v) acetic acid at a concentration of 5.1 mg ml^{-1}. This concentration is sufficiently dilute to rule out possible protein–protein interactions. The results show a very well-defined β dispersion, fitted according to the Cole–Cole equation [16] written for the real part of the dielectric constant

$$\epsilon' = \epsilon_\infty + \Delta\epsilon\left[1 + (f/f^*)^{(1-\alpha)}\sin(\pi\alpha/2)\right] / \left[1 + 2(f/f^*)^{(1-\alpha)}\sin(\pi\alpha/2) + (f/f^*)^{2(1-\alpha)}\right] \quad (1)$$

where $\Delta\epsilon$ is the dielectric increment, f^* is the relaxation frequency, ϵ_∞ is the permittivity extrapolated at high frequency and α is a parameter taking into account the spread of the relaxation times. Table 1 summarizes the dispersion parameters after best-fit treatment. Column 6 reports the average dipole moment of the proteins estimated by the Oncley equation [17]

$$\mu^2 = 2\epsilon_0 MKT\Delta\epsilon/Ncg \quad (2)$$

where μ is the dipole moment, M is the average ribosomal protein molecular mass [1] expressed in kilodaltons, T is the temperature expressed in kelvin, K is the Boltzmann constant, ϵ_0 is the vacuum dielectric constant, N is the Avogadro number, c is the protein concentration expressed in kilograms per cubic metre and g is the molecular correlation parameter assumed to be unity in dilute solutions [18]. The values of μ are expressed in debye, 1 D being equivalent to 3.33×10^{-30} C m. The last column of

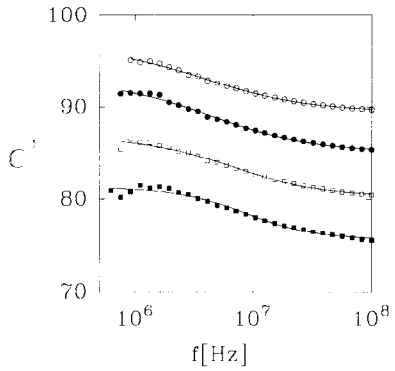

Fig. 1. Permittivity ϵ' vs. frequency of an acidic solution of ribosomal proteins at a concentration of 5.1 mg ml^{-1}. The figure shows the permittivity measured at four different temperatures: ○, 5 °C; ●, 15 °C; □, 25 °C; ■, 35 °C. The full line represents the Cole–Cole best fit.

Table 1 shows the molecular radius (r) obtained from the equation

$$f^* = KT/8\pi^2\eta r^3 \quad (3)$$

where η is the viscosity of the solution [18]. This model considers the protein as an ideal sphere; therefore the parameter thus obtained is a rough approximation of the ribosomal protein average molecular radius. Table 1 clearly shows that μ and r do not depend on the temperature, which demonstrates that the conformation of the ribosomal proteins is stable in the measurement buffer.

We further analysed the temperature-dependent shift of the dielectric relaxation time following the Arrhenius law. The Arrhenius plot reported in Fig. 2 does not deviate from linearity, and therefore it allows an evaluation of the activation enthalpy of the protein orientation in the electric field. This value is $\Delta H = 4.1$ kcal mol^{-1} which has the magnitude of about one hydrogen bond.

It is known that ribosomal proteins are unfolded in aqueous solution at low ionic strength; therefore we deliberately subjected a ribosomal protein sus-

Table 1
Parameters of the Cole–Cole fit of the dielectric data, average dipole moment and molecular radius of ribosomal proteins in acidic solution

t (°C)	$\Delta\epsilon$	f^* (MHz)	ϵ_∞	α	μ (D)	r (Å)
5	7.4 ± 0.4	4.0 ± 0.3	89 ± 2	0.30 ± 0.04	370 ± 20	20.0 ± 0.5
15	8.0 ± 0.5	4.3 ± 0.4	85 ± 2	0.29 ± 0.04	390 ± 20	21.8 ± 0.8
25	6.8 ± 0.5	6.7 ± 0.6	80 ± 2	0.24 ± 0.05	370 ± 20	20.6 ± 0.6
35	5.8 ± 0.4	8.7 ± 0.8	76 ± 2	0.14 ± 0.06	350 ± 20	20.5 ± 0.6

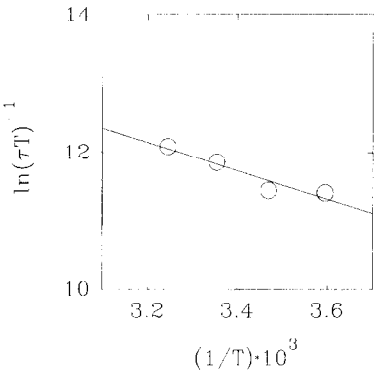

Fig. 2. Arrhenius plot of the dielectric relaxation time of the ribosomal proteins in acidic solution (Fig. 1). From the slope, an activation enthalpy of $\Delta H = 4.1$ kcal mol^{-1} can be calculated.

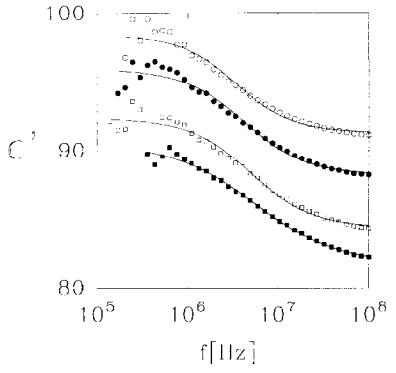

Fig. 3. Permittivity ϵ' vs. frequency of an aqueous solution of ribosomal proteins at a concentration of 3.6 mg ml^{-1}. These are the proteins remaining in solution after dialysis against water (see text). The figure shows the permittivity measured at four different temperatures: ○, 5 °C; ●, 15 °C; □, 25 °C; ■, 35 °C. The full line represents the Cole–Cole best fit.

pension to exhaustive dialysis against water in order to proceed with a new set of measurements to verify the stability conditions previously observed. As expected, on dialysis, a certain amount of proteins precipitated irreversibly. We observed by polyacrylamide gel electrophoresis [14] that the precipitated proteins were qualitatively the same as those still remaining in solution. This strongly suggests that sufficiently dilute proteins remain soluble in water. This protein solution at a concentration of 3.6 mg ml^{-1} was measured in the same experimental conditions as reported previously. The results are shown in Fig. 3, where clearly the β-dispersion is again well resolved. Even in this case the Cole–Cole fit allows an estimation of the dielectric parameters reported in Table 2. In this second set of measurements, the values of μ and r are larger than those previously observed. In particular, while r shows about a 10% increment, μ is almost doubled. Moreover, both parameters increase as a function of the temperature which indicates, on average, a lower stability of the protein population which is prone to rapid unfolding. If an Arrhenius plot based on these data is made (Fig. 4), a reduced activation enthalpy equal to $\Delta H = 2.4$ kcal mol^{-1} is calculated.

3.2. Dielectric dispersion of rRNA

To verify our initial hypothesis that the dielectric dispersion observed at megahertz frequencies on entire ribosomes and reported previously [12] is mainly due to the protein moiety, we decided to measure the dielectric properties of rRNA. We measured the permittivity of rRNA in aqueous solution at a concentration of 17.8 mg ml^{-1} at the same four temperatures as the proteins. rRNA shows a dielectric relaxation in the radiofrequency region. By trial and error we observed that the best resolution of the dielectric dispersion was obtained at a higher frequency range (1 MHz–1 GHz) compared with the protein solution. We report the measured permittivity in Fig. 5, where the Cole–Cole curve is also shown. In Table 3, we summarize the best-fit dielectric parameters. The relaxation frequency is in dramatic

Table 2
Parameters of the Cole–Cole fit of the dielectric data, average dipole moment and molecular radius of ribosomal proteins in aqueous solution

t (°C)	$\Delta\epsilon$	f^* (MHz)	ϵ_∞	α	μ (D)	r (Å)
5	7.2 ± 0.7	3.4 ± 0.5	91 ± 2	0.15 ± 0.09	660 ± 40	21 ± 1
15	7.8 ± 0.5	4.2 ± 0.5	88 ± 2	0.19 ± 0.06	700 ± 30	22.0 ± 0.9
25	8.1 ± 0.6	5.0 ± 0.7	84 ± 2	0.19 ± 0.07	730 ± 30	23 ± 1
35	9.0 ± 0.5	5.7 ± 0.5	82 ± 2	0.31 ± 0.04	780 ± 30	23.6 ± 0.7

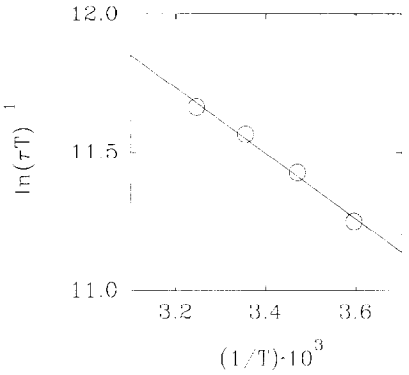

Fig. 4. Arrhenius plot of the dielectric relaxation time of the ribosomal proteins in aqueous solution (Fig. 3). From the slope, an activation enthalpy of $\Delta H = 2.4$ kcal mol^{-1} can be calculated.

disagreement with the trend predicted by the Arrhenius law. This implies that, depending on the temperature, the molecular state of the polyion changes; this statement is corroborated by the magnitude of b reported in the last column of Table 3; this value was estimated on the basis of the Mandel model [19]. In this model, the polyion is represented by a sequence of thin rod-like subunits of identical length b, also defined as "subunit b". As pointed out by Mandel, the subunit length is assumed to be independent of the molecular weight for a macromolecule with a sufficiently high degree of polymerization. Counterions can freely move along each subunit, but cannot cross from one subunit to another unless a potential barrier, at the junction between each subunit, is

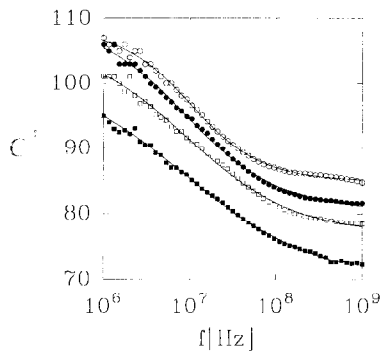

Fig. 5. Permittivity ϵ' vs. frequency of an aqueous solution of ribosomal RNA at a concentration of 17.8 mg ml^{-1}. The figure shows the permittivity measured at four different temperatures: ○, 5 °C; ●, 15 °C; □, 25 °C; ■, 35 °C. The full line represents the Cole–Cole best fit.

Table 3
Parameters of the Cole–Cole fit of the dielectric data and length of "subunit b" of ribosomal RNA in aqueous solution

t (°C)	$\Delta\epsilon$	f^* (MHz)	ϵ_∞	α	b (nm)
5	24.2±0.4	10.1±0.1	85±2	0.27±0.01	10.8±0.1
15	30±1	8.6±0.6	80±2	0.38±0.02	13.7±0.5
25	30±2	9±1	77±2	0.44±0.03	15.3±0.9
35	35±3	8±2	72±2	0.53±0.03	18±2

overcome. In the presence of external electric fields, the uniform distribution of counterions is perturbed and an induced dipole moment arises. From this induced polarization, normally a relaxation is triggered in the radiofrequency range. The size of the subunit is closely related to the frequency of relaxation according to the following equation

$$b = (\pi KTu/2f^*)^{1/2} \tag{4}$$

where u is defined as the mobility of the counterions moving along the rigid linear subunit of length b. This mobility is expressed as a ratio between velocity and force. Following Mandel, we assumed for u the values 2.0×10^{11}, 2.6×10^{11}, 3.3×10^{11} and 3.9×10^{11} m s^{-1} N^{-1} at the respective experimental temperatures. These values correspond to ionic mobilities at infinite dilution. The results for b obtained from Eq. (4) are reported in Table 3. In the Mandel model, the parameter b is also related to the dielectric increment $\Delta\epsilon$ of the dispersion as

$$b = \left(36KT\epsilon_0\Delta\epsilon/e^2FNC\gamma\right)^{1/2} \tag{5}$$

where N is the total number of counterions of charge e per polyelectrolyte molecule, C is the macromolecule concentration per cubic metre, F is the average fraction of bound counterions, γ, which may be assumed to be close to unity, is the ratio of the actual electric field acting on the polyion and the average Maxwell field in the solution. Values reported in the literature were used for an estimation of F [20]; thus we obtained b values compatible with those calculated from the relaxation time. Therefore the validity and consistency of our data, within the model employed, are ensured.

The values derived from our measurements are rather small compared with previously published data on nucleic acids [19]. This suggests that our polyion is more flexible and has a more complex spatial

configuration. Finally, the b value increases with temperature which shows an opening of the spatial arrangement of the RNA molecule; this accounts for the observed temperature-dependent relaxation curve.

4. Discussion

In our previous studies [10–12], we investigated certain physical properties of the *E. coli* ribosome. By fluorescence intensity measurements of the intercalating agent ethidium bromide bound to rRNA, we observed a different behaviour of free RNA with respect to the whole ribosomal particle. In fact, Mg ions change the conformation of RNA within the ribosome; this conformational alteration is strongly reduced in protein-free RNA. In this case, as deduced by a Scatchard plot, Mg only has an ion strength effect on the association constant, whereas the number of excluded sites is practically unaffected. We interpreted these results as an indication that the presence of proteins is required for the structural rearrangement of the RNA polymer mediated by Mg ions. In addition, by conductivity measurements, we were able to demonstrate the presence of an intrinsic conductivity modulated by bound Mg ions. We associated this phenomenon essentially with an interfacial specific conductivity of the ions in the buffer. In addition, the estimation of the gyration radius of the ribosomal particle deduced from the same measurements was in excellent agreement with previously published data obtained by neutron scattering [21]; therefore our relatively simple experimental approach provides an economical and powerful tool to investigate, among other biophysical parameters, the geometry of the cell organelle in suspension. For a characterization of the average dielectric properties of the ribosomal components, we associated permittivity measurements with those cited above.

We observed two distinct relaxations in suspensions of 70S ribosomes and separate subunits. The first appears at around 100 kHz and shows a strong dependence on Mg ions. In fact, this relaxation shifts to lower frequencies in the absence of the ion. The shift is consistent with a larger exposure of the RNA moiety to the solvent [10] and is in agreement with results of fluorescence experiments [11]. The second relaxation appears in the megahertz range and is not affected by Mg ions. The results presented in this paper support the idea that this relaxation is essentially due to the ribosomal proteins, which maintain a certain degree of flexibility even when assembled in the ribosomal particle. While RNA relaxes around 10 MHz, the protein mixture relaxes at lower frequency (about 6 MHz at 25 °C). The protein relaxation observed at this frequency is consistent with that previously observed on entire ribosomes. In any case, we rule out the possibility that the RNA is responsible for this relaxation on the basis of the following considerations. First, we deduced the size of "subunit b" according to the Mandel model [19] as illustrated in Section 3. The value of "subunit b" calculated by Mandel on calf thymus DNA in solution was 62 nm at 25 °C. On rRNA, we measured a 15 nm long "subunit b"; this strongly suggests a large flexibility of rRNA and confirms the literature model proposing a highly complex tertiary structure for both 16S and 23S rRNA [3]. The lower value estimated for "subunit b" in the case of rRNA is consistent with conformation A assumed by double helical RNA molecules. In fact, in this conformation, the pitch of the double helix is smaller than that measured for DNA in conformation B. The length of "subunit b" indicates an oscillatory extension of the counterions at the RNA polymer surface and, therefore, cannot be directly applied to the length of the double helix tracts along the rRNA molecule: indeed, although the amount of double helix proposed for the rRNA molecules is high (see, for instance, Brimacombe et al. in Ref. [2]), it infrequently exceeds one entire helical turn.

A second important observation is that the conformation of rRNA in solution is strongly affected by the temperature, since the length of "subunit b" increases by about a factor of two between 5 and 35 °C. If the dielectric dispersion observed at 2 MHz in whole ribosomes was attributed to the RNA moiety, this would imply a much larger value for "subunit b"; however, this does not seem compatible with the highly folded and complex tertiary structure present in the ribosomal particle.

In the light of these results, we decided to examine in more detail the dielectric properties of the ribosomal proteins in solution. Proteins were first measured in a 0.5% (v/v) acetic acid solution. This

acidic environment allows complete solubilization of the ribosomal proteins, since they are predominantly basic and therefore hardly soluble in low ionic strength aqueous solution. From dielectric measurements, we obtained information essentially on two different parameters: the molecular radius and electric dipole moment. As reported in Table 1, these two values are largely independent of the temperature, which demonstrates that, in our experimental temperature range, ribosomal proteins remain very stable in solution. We estimated an average molecular radius of 20.5 Å and this value is consistent with the gyration radius measured by neutron scattering [22]. It is surprising that the average molecular radius estimated on total ribosomal proteins is in such good agreement with the gyration radius obtained on select ribosomal proteins and by a much more sophisticated technique. It should be borne in mind, however, that our molecular radius is an average value essentially derived from proteins with a molecular weight of 13–18 kDa (the arithmetic average being 15 kDa calculated on all 55 ribosomal proteins). This interval comprises the bulk of the proteins present in the ribosome. Therefore the few proteins exceeding the average molecular weight, e.g. S1, S2, S20, S21 or L1, L2, L33, L34, should not contribute significantly to an increase in the average molecular radius estimated by us. This interpretation is supported by the fact that the gyration radius obtained by neutron scattering was measured on proteins within the same molecular weight range [22].

As far as the dipole moment is concerned, we estimated a value of 370 D, which is similar to that measured for proteins of biological relevance, such as oxyhaemoglobin and carboxyhaemoglobin (dipole moment, 400 D) and bovine serum albumin (384 D) [16]. We wish to point out that both the molecular radius and dipole moment measured on proteins dissolved in water are greater and depend strongly on the temperature. At 25 °C, the molecular radius is 23 Å and the dipole moment is 730 D. The increase in these physical parameters and their temperature dependence indicate that, in water, the ribosomal proteins are found in a partially unfolded state. However, the relatively small difference in radius suggests that, in water, the proteins are in a molecular conformation strongly resembling that in acetic acid. This small difference in the overall size between the folded and unfolded state has also been observed by Bone [7] on the enzyme β-lactamase. On the other hand, the almost two-fold increase in dipole moment is probably due to the rearrangement of charges exposed to the solvent. This may cause a dramatic variation of the dipole moment in the absence of a significant variation of the molecular geometry of the proteins.

As reported in Section 3, the activation enthalpy of orientational polarization of the proteins in water is lower than that measured in acetic acid. Therefore, in a partially unfolded condition, ribosomal proteins exhibit a lower friction with the solvent. One way to rationalize this is that, in water, more hydrophobic interactions between the amino acid side-chains are established. We speculate that this conformational rearrangement involves a redistribution of polar groups which enhances the dipole moment. On the other hand, this redistribution may cause a reduced interaction with the solvent due to the larger exposure of non-polar groups. This structural modification may account for the presence of a large amount of bound water at the surface of the macromolecule. This may justify the values of ϵ_∞ estimated by best fit (column 4, Table 2); these are, in fact, significantly higher than the permittivity of bulk water. The ϵ_∞ values obtained for acidic protein and RNA solution, on the other hand, coincide within the error margins with the static permittivity of water. We are aware that our data do not allow a clearer interpretation. Further developments will involve measurements performed on partially purified or purified proteins and individual RNA molecules.

The last parameter discussed concerns the activation enthalpy of dielectric relaxation of total ribosomal proteins in acidic solution. The estimated value of 4.1 kcal mol^{-1} is equivalent to about one hydrogen bond. This low activation energy probably reflects the interactions between proteins and solvent; however, different interactions, such as protein–protein and protein–RNA, play a role in the determination of the thermal stability of the entire ribosomal particle. As reported previously [10], the activation enthalpy associated with the megahertz relaxation observed in a 70S suspension is 8.7 kcal mol^{-1}. In this paper, we present evidence to indicate that this relaxation is in fact attributable to the protein moiety. With respect to this, we wish to point out that the

activation enthalpy measured on free proteins in solution is not dramatically different from that previously estimated on whole 70S. Therefore the apparent physical stability of the ribosomal particle is essentially due to the extraordinarily large number of secondary interactions established within the organelle. In fact, it is of interest to note that, in an entire functional ribosomal particle, the energy required for conformational rearrangement is only two-fold higher than that observed in an artificial measuring system such as ribosomal proteins in acetic acid. This may suggest that the establishment or breakage of one or two secondary interactions may have a cascade effect and determine the major conformational alterations within the ribosome.

5. Conclusions

The main results discussed in this paper show that the characteristic dielectric dispersion present in the megahertz region in ribosome suspensions can be attributed to the proteins. Furthermore, we have characterized the dielectric properties of these proteins in solution and obtained an estimate of the average molecular radius and electric dipole moment. The first is in agreement with neutron scattering observations and the second is comparable with the dipole moments measured for other proteins of biological significance. In addition, we evaluated the average length of "subunit b" of rRNA in solution by applying the Mandel model to the dielectric measurement data. The results validate the idea that rRNA is a highly flexible molecule. Finally, the activation enthalpy of free proteins in solution is not dramatically different from that measured on whole ribosomes.

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