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The secondary structure of E. coli ribosomes and ribosomal RNA's: a spectrophotometric approach

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ABSTRACT

The thermal denaturation spectra of E. coli 50S and 30S ribosomal subunits and of their isolated RNA's were studied over the wavelength range of 2300-3000 Å.

It was possible to fit the experimental denaturation spectra with superpositions of the reference spectra for denaturation of A - U and G - C base pairs derived from model polyribonucleotides. The coefficients of these linear combinations were used to calculate the fractions of A - U and G - C base pairs in the samples.

It was found that the helical content of the RNA's inside the subunits is smaller than that of the isolated RNA's, thus suggesting that proteins may affect the secondary structure of RNA in the ribosome.

INTRODUCTION

It is known that temperature largely affects the conformation of nucleic acids in solution. Heating can break the hydrogen bonds between complementary base pairs (thermal denaturation) and so lead to less ordered conformation. At the same time, a change in the U. V. absorption spectrum occurs, this due to the change in the interaction between neighboring bases. The analysis of the difference spectrum upon denaturation (denaturation spectrum) may give information on the helical content of the macromolecules and on the base composition of the double helical regions. This approach has been largely used with natural and synthetic polynucleotides [1 - 5], but not with ribonucleoproteins. In particular, we found no study in the literature on the denaturation spectra of ribosomes. Only the melting profiles at a fixed wavelength received attention [6-9]. This was probably due to the difficulties encountered in the spectrophotometric measurements. In fact, heating the solution may cause the appearance of some turbidity in the sam

ple owing to particle aggregation[9]. The spectrum observed in this case is affected by a significant contribution from the scattered light, that cannot be evaluated with sufficient precision. In this work we present the analysis of the denaturation spectra for E. coli ribosome subunits. We were able to overcome the ribosome aggregation upon thermal denaturation by choosing the proper ionic environment. Analogous measurements were taken for the respective RNA's. The comparison between the two sets of results provided that the conformations of intraribosomal and isolated RNA's are not the same.

MATERIALS AND METHODS

Preparation of ribosomes.

Ribosomes were extracted from E. coli RNase I^-_{10} (Gesteland[10]) with a method derived from that of Tissières et al.[11] as described elsewhere[12]. The 70S ribosomes so obtained were dissociated into subunits by overnight dialysis in a 10^{-2}M Tris-HCl, 10^{-4}M magnesium acetate buffer (pH 7.4), then layered onto a 5-30% linear sucrose gradient and centrifuged in a B-IV zonal rotor of a Beckman L-4 centrifuge at 40,000 rpm for 6h. The 50S and 30S fractions were precipitated with 40% cold ethanol and pelletted by centrifugation at 30,000 rpm for 6h at 4°C. The pellets were washed and resuspended in a relatively low ionic strength buffer (10^{-3} Tris-HCl, $8 \cdot 10^{-5}$M magnesium-acetate, pH 7.4), then dialyzed against the same buffer in order to remove sucrose traces.

Preparation of rRNA

Ribosomal RNA's were directly isolated from the separated subunits by the phenol method[13]. The subunits were first suspended in a buffer at high ionic strength (10^{-2}M Tris-HCl, 10^{-2}M magnesium-acetate, pH 7.4), then a freshly distilled phenol was added. The phenol was previously saturated with 10^{-2}M Tris-HCl buffer (pH 8), 10^{-2}M magnesium-acetate, 10^{-4}M EDTA, according to Kurland[14]. The extracted RNA was then dialyzed against a low ionic strength buffer (10^{-3}M tris-HCl, $8 \cdot 10^{-5}$M magnesium-acetate, pH 7.4).

In the following, the RNA's extracted from 30S and 50S subunits will be referred to as RNA_{30S} and RNA_{50S} respectively.

Analytical ultracentrifugation

For sedimentation coefficient measurements, ribosome and RNA samples were analyzed in a Spinco Beckman mod. E, ultracentrifuge by means of band sedimentation technique, as already described [15].

Spectrophotometric measurements

The U. V. absorption spectra were recorded by means of a Cary mod.15 spectrophotometer. 3ml cuvets, 1 cm long, with teflon stoppers were used. All the samples were at OD about one at 2600 Å. Before OD measurements, the samples were filtered through a Millipore membrane (8 μ pore size)in order to remove large aggregates. The sample heating was accomplished by external water circulation. The temperature was measured by means of a thermistor in contact with the sample through the teflon stopper. The samples were divided into two parts, one of which was mantained at 20°C and used as a reference while the other was used for filling the cuvet to be heated. In this way we were able to directly record the denaturation spectra with an OD sensitivity of 0.001.

The melting profiles have been drawn by measuring the OD at 2600 Å as a function of temperature. The rate of heating was approximately 1°C/min

All the OD values were corrected for thermal expansion of water.

RESULTS

The UV absorption spectrum at 20°C of a sample of 30S subunit dissolved in a buffer with a relatively high magnesium concentration (10^{-3}M Tris-HCl, 10^{-3}M magnesium-acetate, pH 7.4) is shown in Fig. 1(curve a). Heating the ribosomes up to 90°C leads to an OD increase in the entire wavelenth range, including the region 3150-3500 Å in which neither RNA nor ribosomal proteins absorb (curve c). The OD in this region was proved to be only due to the light scattered by aggregating particles, as a linear relationship was found between the OD and the logarithm of the wavelength. Extrapolation into the true absorption region showed that the scattering became comparable with the hyperchromicity, thus not allowing a sufficiently accurate evaluation of the latter. The aggregation was ruled out by lowering the magnesium concentration in the buffer [9]. Curve b shows the spectrum registered

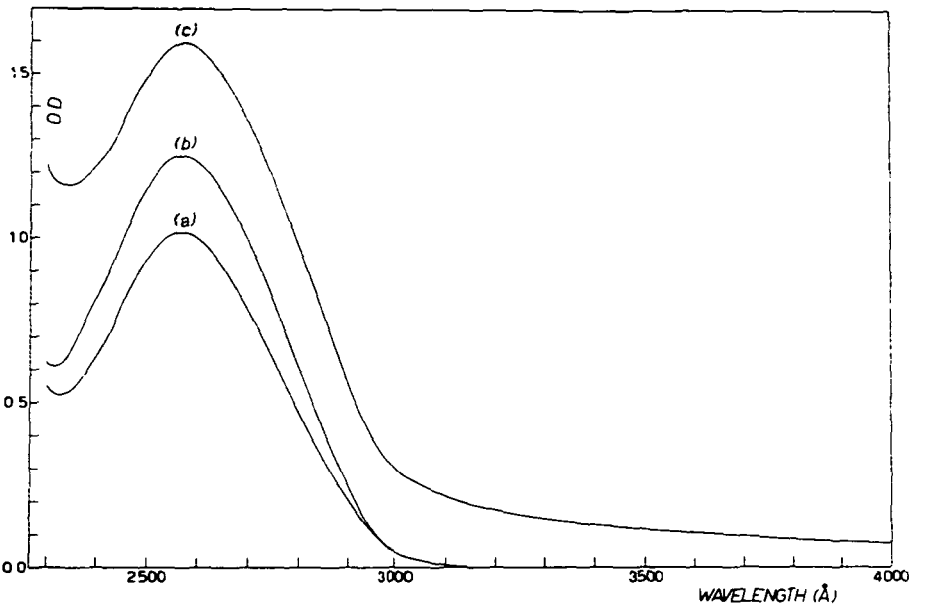

Fig 1 - Absorption spectra of 30S subunits a) at 20°C in 10^{-3}M tris-HCl, $8 \cdot 10^{-5}$M (or 10^{-3}M) magnesium acetate (pH 7.4) b) at 90°C in 10^{-3}M tris-HCl, $8 \cdot 10^{-5}$M magnesium acetate (pH 7.4), c) at 90°C in 10^{-3}M tris-HCl, 10^{-3}M magnesium acetate (pH 7.4)

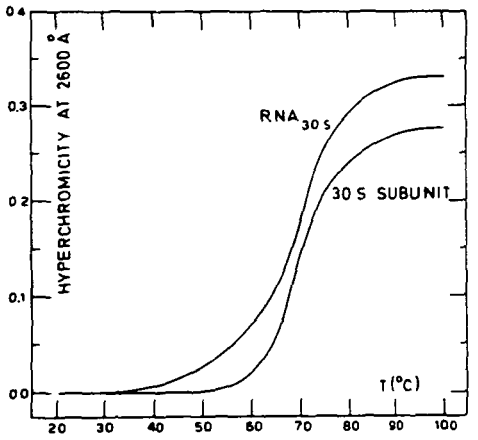

Fig. 2 - Melting profiles of 30S subunit and its RNA

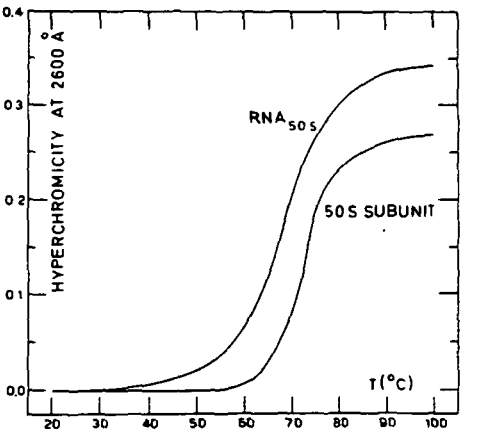

Fig. 3 - Melting profiles of 50S subunit and its RNA.

at 90°C for the same sample when the concentration of magnesium - acetate in the buffer was lowered to $8 \cdot 10^{-5}$ M. This magnesium concentration prevents aggregation, as proved by the absence of OD in the region of no absorption (that is, for wavelengths greater than 3150 Å). On the other hand, it is well known that ribosomes may undergo conformational changes when

magnesium concentration is lowered[8,16,17]. These changes may result, for instance, in the lowering of the sedimentation coefficient. Actually, the ultracentrifuge analysis proved that the sedimentation coefficients of subunits remained unchanged on lowering the magnesium concentration from 10^{-3} M to $8 \cdot 10^{-5}$ M, thus excluding a significant change in the ribosome conformation in this range. A magnesium concentration of $8 \cdot 10^{-5}$ M was therefore used for our spectrophotometric studies in order to prevent particle aggregation.

In Figs. 2 and 3, the hyperchromicity at 2600 Å as a function of the temperature is reported for the isolated subunits and their RNA's. One can see that the melting profiles for ribosomes are quite different from those for RNA's. The transition is narrower and less pronounced for ribosomes than for RNA's. The maximum hyperchromicity were about 26% for ribosomes and 33% for RNA's. In order to elucidate whether these differences reflect a difference in the helical content of ribosome and of RNA's, a number of denaturation spectra for ribosomes and RNA's were collected in the range 2300-3000 Å as described in "Materials and Methods". Each denaturation spectrum was recorded by heating the sample from 20°C up to 90°C. It must be pointed out that at 20°C the transition had not yet begun and that at 90°C it was practically completed (see Figs 2 and 3).

Typical denaturation spectra for subunits and RNA's are reported in Figs. 4 and 5. They are normalized by bringing the OD of the samples at 20°C to unity at 2600 Å. The spectra for subunits are lower than that for their RNA's.

They can all be obtained as a superposition of the reference spectra for denaturation of A-U and G-C base pairs in double helical polyribonucleotides, according to the equation:

$$\Delta OD(\lambda) = a \cdot \Delta \varepsilon_{AU}(\lambda) + b \cdot \Delta \varepsilon_{GC}(\lambda)$$

where $\Delta OD(\lambda)$ is the observed denaturation spectrum, a and b are the coefficients of the linear combination, $\Delta \varepsilon_{AU}(\lambda)$ and $\Delta \varepsilon_{GC}(\lambda)$ are the reference spectra on a molar nucleotide basis for the denaturation of A-U and G-C base pairs, respectively[5].

Best fits of the experimental data were obtained by the aid of an IBM

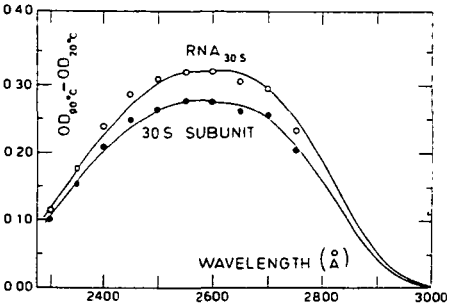

Fig 4 - Denaturation spectra of 30S subunit and its RNA The values refer to OD=1 at 2600 Å and 26°C. Solid lines experimental spectra. Circles best fits.

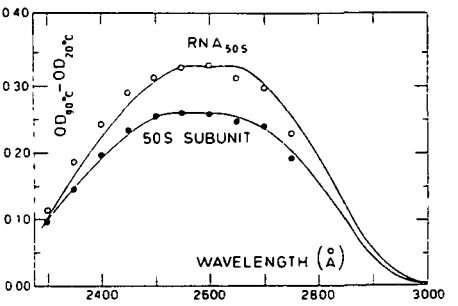

Fig 5 - Denaturation spectra of 50S subunit and its RNA The values refer to OD=1 at 2600 Å and 20°C Solid lines: experimental spectra. Circles best fits

7040 computer, using a MINUIT program[18]. The circles shown in Figs. 4 and 5 are the values drawn from the best fits. The good agreement between experimental and interpolated spectra indicates that the analysis reported by Cox for RNA[5] may be extended to E. coli ribosomes. Consequently, the calculated coefficients a and b can be related to the fractions of the total number of nucleotides that are in A-U and G-C base pairs. By indicating these fractions by f_{AU} and f_{GC} respectively, we have:

$$a = c \cdot f_{AU} \quad \text{and} \quad b = c \cdot f_{GC}$$

where c is the molar nucleotide concentration in solution.

In order to obtain f_{AU} and f_{GC} separately, it is necessary to evaluate the concentration c. In our case this was obtained by the assumption that at 90°C (i. e., in the denatured form) the molar extinction coefficient at 2600 Å is the same both for ribosomes and for RNA's. This assumption implies that at this temperature there is no residual bi - helical structure (this is supported by the observation that a further increase of temperature to 100°C does not lead to appreciable changes in the OD) and that absorption by ribosomal proteins may be neglected at this wavelength. In fact, it is unlikely that the proteins contribute more than 2-3%[19]. The results summarized in the table below are obtained by the best fit analysis by taking a value of 9, 800 for the molar extintion coefficients.

	f_{AU}	f_{GC}	% dh
30 S subunit	0.34 ± 0.1	0.39 ± 0.1	73 ± 2
50 S subunit	0.33 ± 0.1	0.37 ± 0.1	70 ± 2
RNA_{30S}	0.41 ± 0.1	0.45 ± 0.1	86 ± 2
RNA_{50S}	0.41 ± 0.1	0.42 ± 0.1	83 ± 2

Each value represents the average of four determinations. The above errors are the standard deviations. The total helical content is obtained by adding f_{AU} to f_{GC} ; it is shown as the percent of nucleotides in "double helical" regions (% dh). This can be seen to be significantly larger for RNA's than for ribosomes.

DISCUSSION

Determination of the f_{AU} and f_{GC} values is based on the reference spectra and on the extinction coefficients. Therefore, in addition to the experimental errors reported in Tab. 1, they may be affected by possible systematic errors due to uncertainties in the extinction coefficients and the reference spectra. With regard to this aspect, it must be pointed out that the reference spectra were drawn from measurements made in phosphate buffers.

The number of nucleotides in base pairs found in the RNA's is close to the maximum permissible number as can be calculated by their base composition[20], that is 87% for 16S RNA and 84% for 23S RNA. These values are higher than those reported by Cox[3, 5], concerning RNA from E. coli 70S ribosomes at 25°C in phosphate buffer. The differences are mainly in the f_{AU} values. One possible explanation of this discrepancy is given by the observation that at 25°C the E. coli rRNA is not fully structured in the phosphate buffer. In fact, we found that in this buffer the melting profiles of RNA's had shifted towards lower temperatures, and that at 25°C the transition had already started. This suggests that in these conditions some of the regions richer in A-U are melted. On the other hand, it must be pointed out that the hyperchromic effect may depend, to a certain extent, on the ionic environment, as it is shown by the rather large variability of the results (from 24 to 35%) reported by different authors[6, 8, 20, 21].

As far as the ribosomes are concerned, we have shown that their denaturation spectra may be described in terms of superposition of the reference spectra, as in the case of RNA. This makes it possible to get information on the secondary structure of the RNA inside the ribosome.

Other authors have reported results based on hyperchromism[6,8,21], ORD[7] and X-ray diffraction[19] indicating that the RNA inside the ribosome has a structure similar to that of the isolated RNA, with a high double helical content. Our approach qualitatively confirms this conclusion but also gives evidence of some differences between the secondary structure of intraribosomal RNA and that of isolated RNA, at least in the ionic environment we used. This result suggests that proteins may play a role in determining the secondary structure of RNA inside the ribosome.

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