OPTICAL ABSORBANCE PROPERTIES OF MITOCHONDRIAL RIBOSOMAL RNA

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SUMMARY

The differential melting profiles of mitochondrial and cytoplasmic ribosomal RNA from the fungus, Aspergillus nidulans were examined. Both rRNAs presented a biphasic melting profile. However, mitochondrial rRNA differed from its cytoplasmic counterpart in the relative proportions and temperature ranges of the various melting phases. Differences were also found between the melting patterns of the heavy and light mitochondrial rRNA components. Thermal denaturation of formaldehyde-treated rRNA showed that mitochondrial rRNA has a partially ordered structure in solution which is stabilized by base pairing in helical regions as well as by base stacking. These results indicate that the forces which contribute to the ordered structure of mitochondrial rRNA resemble those operative in other ribosomal RNAs. In the absolute conformation, however, the unique nature of mitochondrial rRNA becomes apparent.

The conformation of mitochondrial ribosomal RNA has been found to differ from that of cytoplasmic ribosomal RNA in several fungal species (1, 2). In Aspergillus nidulans, thermal denaturation studies showed the heavy and light mitochondrial rRNA components to have different transition midpoints and hypochromicities than those of the corresponding cytoplasmic rRNA, suggesting differences in the length and nucleotide compositions of the ordered regions of the molecules (1). Polyacrylamide gel electrophoresis of rRNA from Trichoderma viride and Neurospora crassa in buffers of low ionic strength also emphasized the unusual structural characteristics of mitochondrial rRNA (2).

In the present communication, the differential melting profiles of mitochondrial and cytoplasmic rRNA from Aspergillus nidulans were determined in order to further analyze the nature of the thermal denaturation curves. In addition, the contribution of base stacking to the conformation of mitochondrial and cytoplasmic rRNA was examined. The experiments revealed that the same forces act to hold mitochondrial and cytoplasmic rRNA in ordered conformation. However, the absolute conformation of the two ribosomal RNAs are quite distinct.

MATERIALS AND METHODS

Ribosomal RNA was isolated from purified mitochondria and cytoplasmic ribosomes of Aspergillus nidulans, as well as from Escherichia coli cells by methods previously described (1). This same procedure was used to obtain mitochondria and mitochondrial rRNA from hyphae of Trichoderma viride.

Thermal Denaturation Analyses. In general, a procedure described previously (1) was followed. Peak rRNA fractions isolated from sucrose density gradients were diluted tenfold with 50 mM NaCl, 50 mM sodium acetate buffer, pH 5.7. Absorbancy at 260 nm or 280 nm was recorded at 3 to 5° intervals. After heating to 95°, the solutions were cooled and their absorbancies measured once again. Readings in each case returned to within 2-3% of the initial values showing that degradation of the RNA samples was negligible. The rate of change in absorbance was calculated from the hypochromicity data. For each interval of 2.5° the difference in the relative absorbance was plotted against temperature.

To study the effect of formylation on the thermal denaturation patterns of rRNA, formaldehyde (37% reagent grade, Fisher and Co.) was added to a final concentration of 1% (3, 4). The samples were then heated to 95° and cooled to 10° and absorbancies at 260 nm recorded as described above. The heating and cooling cycle was repeated to ensure completion of the formaldehyde reaction (3, 5).

RESULTS AND DISCUSSION

Differential Denaturation Profiles. A careful analysis of the temperature-absorbance profiles of Aspergillus rRNA at both 260 nm and 280 nm reveals indications of more than one 'melting' phase. This is clearly seen when the stepwise difference in absorbance is recorded versus temperature. Figure 1 describes the differential denaturation profiles for the separated components of mitochondrial and cytoplasmic rRNA as well as for E. coli rRNA. Results at 280 nm have been chosen for presentation since denaturation of ordered G·C base pairs can be monitored at this wavelength without interference from thermally-induced transitions in A·U base pairs (6, 7). It is apparent that both mitochondrial and cytoplasmic rRNA have bimodal melting profiles. Under the particular conditions of salt and hydrogen ion used in these experiments, the patterns obtained for E. coli rRNA show just the barest indication of a biphasic nature, an observation which is in agreement with the data of Cox (8).

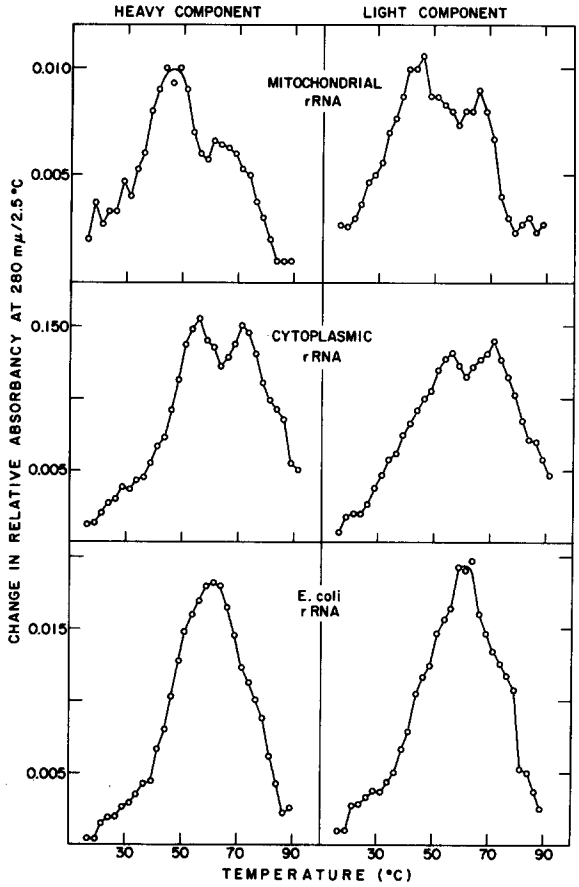

Figure 1. Differential denaturation profiles of the isolated components of mitochondrial and cytoplasmic rRNA from A. nidulans and E. coli rRNA. Ten to twenty μg of rRNA in 50 mM-NaCl, 50 mM-sodium acetate buffer (pH 5.7) was subjected to thermal denaturation in an appropriately modified spectrophotometer. The change in relative absorbancy at 280 nm per interval of 2.5°, was determined and plotted versus temperature. Values are corrected for thermal expansion of water.

However, in acidic solutions below pH 4.0, E. coli rRNA does show a clear biphasic melting profile (8). Bi-componental melting patterns have also been indicated for rRNAs from mesophillic and thermophillic Bacilli (9), yeast and rabbit reticulocytes (8). Thus, this appears to be a general feature of all ribosomal RNA types.

Figure 1 shows that both the first and second melting phases of the heavy and light mitochondrial rRNA components have maxima at lower temperatures (45-47° and 62-66°) than do those of the homologous cytoplasmic rRNA (56-57° and 71-72°).

Thus, the conclusions drawn from the integrated thermal denaturation profiles (1) concerning the lower G+C content of the ordered regions and shorter helices in mitochondrial rRNA hold true at this finer level of resolution as well. The differential melting profiles also show that absorbancy changes in mitochondrial rRNA occur mainly in the low-temperature phase as opposed to cytoplasmic rRNA where the two temperature phases contribute about equally to the denaturation pattern. Another feature revealed by these measurements is that the differences between the melting profiles of the heavy and light components of the mitochondrial rRNAs are more pronounced than for cytoplasmic or E. coli rRNAs. This is consistent with the oligonucleotide pattern encountered when the ionopherograms of T_1 ribonuclease digests of the heavy and light components of the three rRNA types were measured. The most striking differences between components was shown by mitochondrial rRNA (10). Thus, in conformation as well as nucleotide sequence the heavy and light components of mitochondrial rRNA differ considerably.

Thermal Denaturation of Mitochondrial rRNA in the Presence of Formaldehyde. Figure 2 shows the absorbance-temperature profiles of mitochondrial and cytoplasmic rRNA from Aspergillus nidulans treated with formaldehyde. Curves I depict the patterns obtained upon raising the temperature of the rRNA solution from 10° to 95° while curves II show the behavior of the rRNA samples after the solutions were recooled and subjected to a second heating cycle. The inserts show the thermal denaturation profiles of the untreated rRNA samples. The thermal denaturation midpoints of both mitochondrial and cytoplasmic rRNA from Aspergillus are depressed several degrees in the presence of formaldehyde as compared to the values obtained in the absence of the denaturant (compare curves I with inserts, and with Fig. 6 of ref. 1). The same phenomenon has been noted for mammalian cytoplasmic rRNAs (11). It can be seen from curves II that after the reaction with formaldehyde is completed, both mitochondrial and cytoplasmic rRNA retain more than a third of their hypochromicity at low temperatures. Since formylation of the base residues in the RNA chain blocks their participation in hydrogen bonding (12-14), the remaining hypochromicity can be interpreted to be due mainly to single-stranded base stacking (5).

The patterns shown in Fig. 2 are of the heavy rRNA components from Aspergillus nidulans. Thermal denaturation of the light rRNA components under comparable conditions yielded similar curves (not shown). In addition, mitochondrial rRNA extracted from another fungus, Trichoderma viride, and subjected to analysis in the presence of formaldehyde as above, also showed absorbance-temperature profiles similar to those

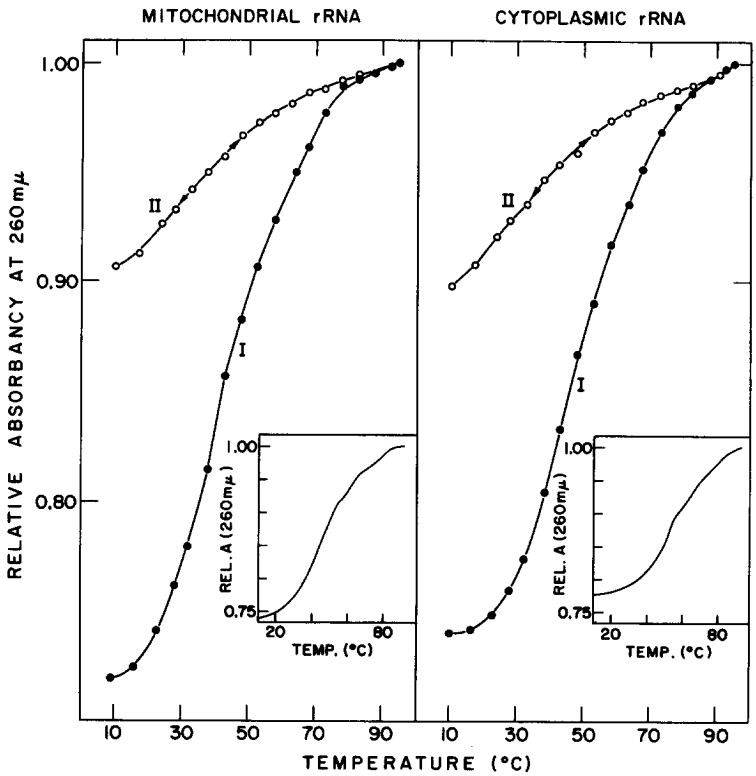

Figure 2. Thermal denaturation patterns of the heavy components of Aspergillus mitochondrial and cytoplasmic rRNA in the presence of formaldehyde. Ribosomal RNA samples were treated as in Figure 1 except that formaldehyde was added to a final concentration of 1%. To calculate the relative absorbancy (rel. A), readings at 95° were set equal to one. Inserts, without formaldehyde. Curves I, in the presence of formaldehyde, first heating (10° to 95°). Curves II in the presence of formaldehyde, second heating cycle (10° to 95° and 95° to 10°).

found for the isolated components of Aspergillus mitochondrial rRNA. Indeed, a residual of 35-40% in hypochromicity after reaction with 1% formaldehyde characterizes cytoplasmic rRNA from rabbit reticulocytes (3, 11), Saccharomyces cerevisiae (4) and Escherichia coli (our unpublished observation). However, the reationship between the remaining hypochromicity and a quantitative estimation of the fraction of the RNA molecule involved in base stacking is complicated by several factors. For example, it is not clear to what extent the phenomenon of base pairing and base stacking are independent (15). It is possible that the geometry of the stacked bases is altered after formaldehyde treatment with a concomitant change in the hypochromicity properties of the reacted bases (14). Moreover, hypochromism in rRNA is a sensitive function of nucleotide composition and sequence (6, 7, 9). Consequently, hypochromicity values obtained for

formaldehyde-treated rRNA cannot presently be used to obtain a precise measure of the extent of base stacking in the mitochondrial and cytoplasmic polynucleotides. From the results presented, however, it is clear that these two ribosomal RNA species in Aspergillus require both base pairing and base stacking to stabilize their ordered structure. A similar conclusion has been reached from a study of the circular dichroism properties of formaldehyde-treated mitochondrial rRNA from Trichoderma (15).

DISCUSSION

The present investigation supports the hypothesis that mitochondrial rRNA, like cytoplasmic rRNA, possesses a substantial ordered structure in solution which is stabilized both by hydrogen bonding of paired bases in helical segments and by nearest-neighbor base stacking. Both rRNA species show two melting phases in solution, however, extensive differences between the mitochondrial and cytoplasmic rRNAs were revealed in the contribution of the G·C interactions to the melting profiles. Thus, while mitochondrial rRNA has certain structural features in common with other ribosomal RNAs, other characteristics identify it as a unique molecular species within the cell.

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