CIRCULAR DICHROISM OF MITOCHONDRIAL RIBOSOMAL RNA FROM *TRICHODERMA VIRIDE*

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1. Introduction

Mitochondrial ribosomal RNA (rRNA) from the fungus, *Aspergillus nidulans*, has been shown to be a unique molecular species, differing from its homologous cytoplasmic rRNA in nucleotide sequence, shape and size [1–3]. For example, *Aspergillus* mitochondrial rRNA showed a greater percentage change in relative absorbance at 260 nm than at 280 nm, while cytoplasmic and *E. coli* rRNA samples exhibited quite the opposite pattern. Thermal denaturation midpoints also occurred at considerably lower temperatures for mitochondrial rRNA. Additionally, studies with formaldehyde treated RNA indicated that the secondary structure of mitochondrial and cytoplasmic rRNA results from stacking of the bases along the polynucleotide chain as well as from base pairing [4]. Thus, in terms of temperature dependent changes, *Aspergillus* mitochondrial rRNA has certain structural features in common with cytoplasmic and bacterial rRNA but other features which set it clearly apart from these two molecular species. Similar results have recently been obtained for the corresponding rRNA's from the fungus, *Trichoderma viride* [5]. As with *Aspergillus nidulans*, base composition studies of *Trichoderma* mitochondrial rRNA revealed a much lower G + C content (33%) than the cytoplasmic rRNA component (51%).

In view of the potential of circular dichroism (CD) as a probe to gain insight into the forces stabilizing RNA structure, it seemed worthwhile to apply this technique to mitochondrial rRNA. A similar approach has recently been employed for yeast mitochondrial DNA [6]. In the present study, the contribution of base paired regions and base stacking to the RNA structure was investigated under two sets of conditions. In the first, single-stranded-stacked conformations predominated over base-paired regions. This was achieved by reducing the number of hydrogen-bonded nucleotides in mitochondrial rRNA by any of three treatments: formylation, protonation or reduction of the ionic strength of the medium. In the second, ethylene glycol was used to preferentially break base stacking.

The results presented are consistent with a role for both hydrogen bonding and base stacking in stabilizing mitochondrial and cytoplasmic rRNA structure, and indeed suggest that the two forces are not independent of one another. They also show that mitochondrial rRNA has less ordered structure than the corresponding cytoplasmic rRNA.

2. Materials and methods

The growth of *Trichoderma viride*, harvesting of mycelia and isolation of mitochondrial rRNA from purified mitochondrial fractions was carried out as described previously [1, 5]. Cytoplasmic rRNA was isolated from purified ribosomes by phenol–sodium dodecyl sulfate method [1].

Ultraviolet CD measurements were carried out on a Cary model 6001 CD attachment to a Cary 60 recording spectropolarimeter. Constant nitrogen flushing was maintained over the wavelength range examined (200 - 00 nm). Measurements were made in 2 nm cells at RNA concentrations of about 0.001%. The results are reported in terms of mean residue molecular ellipticity (θ), given by the relation $(\theta) = \theta M/100\,lc$, where M is the mean residue weight (342 atomic mass units) [7], θ is the observed ellipticity in degree, l is the pathlength in decimeters and c is

the concentration of RNA in g/ml. On the basis of signal-to-noise ratios, the error in ellipticity over the wavelength range explored is estimated to be no greater than ±500°.

3. Results and discussion

The CD peak of about 265 nm was chosen for scrutiny, since nearly all experimental structural interpretations have relied upon this band. It has been proposed [8, 9] that a red shift of this band, coupled to a small decrease in magnitude, indicates the breaking of hydrogen bonds between base pairs. On the other hand, a decrease in amplitude, with no wavelength change, points to the unstacking of adjacent bases. Representative CD scans of mitochondrial rRNA under various reaction conditions are presented in fig. 1, and major ellipticity values are summarized in table 1. Additionally, positions of ellipticity bands and their magnitudes are summarized for the homologous cytoplasmic rRNA of *Trichoderma viride.*

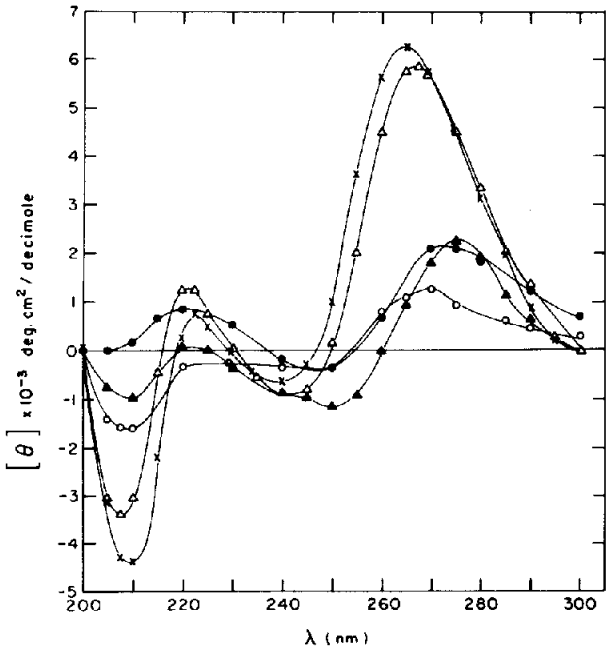

Fig. 1. Molar ellipticities per residue of mitochondrial rRNA of *Trichoderma viride.* Experimental results in the presence of: 0.1 M phosphate, 5×10^{-3} M Mg^{2+} (x——x); 0.001 M phosphate (△——△); 80 volumes percent ethylene glycol in 0.10 M PO^{2-} buffer (●——●) and 1 mM H_3PO_4, pH 3.3 (○——○). Also included are the molar ellipticity values for RNA in 0.1 M phosphate, heated in the presence of 1% formaldehyde to 63° for 10 min and then fast coolled to room temperature (▲——▲).

3.1. *The effect of ionic strength*

In 0.1 M phosphate containing 5×10^{-3} M Mg^{2+} (pH 7), mitochondrial rRNA exhibits maxima at 265 and 223 nm, as well as troughs at 238 and 209 nm. The positions and magnitudes of the peaks and troughs parallel previously published data on *E. coli* rRNA [10]. Upon reduction of the ionic strength to 0.001 M phosphate, a small reduction (∼6%), is observed in the 265 nm maxima as well as a red shift of 3 nm for both the peak and crossover, consistent with a predominantly single-stranded structure. If we visualized the mitochondrial rRNA molecule as being composed of short DNA-like, helical-chain segments connected by single-stranded regions, then at low ionic strength the coulombic repulsion between phosphates on opposite strands favors a more single-stranded structure over the helical base-paired one [11–14].

3.2. *The effect of protonation*

The effects of the presence of excess protons on RNA structure have been discussed elsewhere [15, 16] and are thought to arise from the protonation of cytosine residues and the resulting disruption of hydrogen bonding between GC base pairs. In addition to cytosine, adenine is also susceptible to protonation in this pH range. However, Sarker and Yang [16] have shown that protonation of the adenine ribonucleotides did not alter their cotton effects significantly. Table 1 shows that homologous cytoplasmic rRNA, which has a higher GC content than mitochondrial rRNA, undergoes a more extensive reduction in the amplitude of the 265 nm peak at both pH 4.6 and 3.3, reflecting the presence of a larger number of GC residues per ordered region in these rRNA chains than is present in the mitochondrial system. A similar conclusion has already been drawn from comparative absorbance–temperature measurements of the mitochondrial and cytoplasmic rRNA from *Aspergillus nidulans* [1].

Table 1
Circular dichroism parameters of *Aspergillus nidulans* and *E. coli* rRNA.

rRNA species	Solvent system	Maximum I		Crossover	Minimum I		Maximum II		Crossover	Minimum II	
		λ(nm)	[θ]	λ(nm)	λ(nm)	[θ]	λ(nm)	[θ]	λ(nm)	λ(nm)	[θ]
Mitochondrial	0.1 M PO_4^{2-} $5X10^{-3}$ M Mg^{2+}	265	+6255	247	238	−750	223	+751	219	209	−4754
Mitochondrial	0.001 M PO_4^{2-}	267.5	+5879	249.5	240	−600	221	+1257	216	208	−3377
Mitochondrial	0.02 M sodium acetate 0.1 M magnesium acetate pH 4.6	265	+5000	240	240	0	227	+450	220	210	−3640
Mitochondrial	10^{-3} M H_3PO_4, pH 3.3	270	+1252	252.5						210	−2800
Mitochondrial	80 volumes % ethylene glycol in 0.1 M PO_4^{2-}	272.5	+2085	255	245	−700	223	+869			
Mitochondrial	Heated in presence of 1% HCHO to 63° for 10 min and then fast cooled at room temp.	275	+2250	260	250	−1125	223	+150	220	210	−940
Cytoplasmic	0.1 M PO_4^{2-} $5X10^{-3}$ M Mg^{2+}	265	+5833	246	235	−700	223	+550	218	209	−4100
Cytoplasmic	0.02 M sodium acetate 0.01 M magnesium acetate pH 4.6	265	+2268	246	235	−500				209	−2352
Cytoplasmic	10^{-3} M H_3PO_4, pH 3.3	275–278	+588	255						210	−840
Cytoplasmic	Formylated as mitochondrial RNA	275	+1350	258	250	−500	223	+150	215	210	−380
*E. coli**		265	+6820		236	−880	223	+500		209	−5050

* Data taken from Sarkar and Yang [10].

3.3. *The effect of ethylene glycol*

The position of the 265 nm peak for mitochondrial rRNA in 80 volumes percent ethylene glycol in 0.10 M PO_4^{2-} buffer undergoes a 7 nm red-shift and additionally, is reduced in magnitude to $\sim$ 35% of the value in 0.1 M phosphate, 5×10^{-3} M Mg^{2+} (pH 7.0). Since ethylene glycol is assumed to promote unstacking of bases and stabilization of hydrogen bonding [17, 18], any secondary structure which still persists in the RNA after exposure to this reagent should reflect the presence of hydrogen-bonded base pairing. However, the additional red shift in the 265 nm band which accompanies this process implies that hydrogen bonds are being disrupted as well under the conditions described. These observations are therefore consistent with a role for both hydrogen-bonding and base stacking in the stabilization of mitochondrial rRNA structure, and suggest that the two forces are not independent of one another.

3.4 *The effect of formylation*

The reaction of formaldehyde with RNA primarily formylates the amino and imino groups of the bases in the RNA chain [19, 20] and blocks their participation in hydrogen bonding. Fig. 1 reveals that the 265 nm peak for mitochondrial rRNA in 0.1 M phosphate, 5×10^{-3} M Mg^{2+} (pH 7.0) undergoes a red shift by 10 nm due to formylation, and the amplitude decreases by about 65%. The persistence of a small ellipticity at 275 nm, implies that an asymmetric structure is being maintained in the essential absence of hydrogen bonding. Other forces such as base stacking must be contributing to the intensity of the observed bands in formylated mitochondrial rRNA.

Table 1 also shows that a smaller ellipticity (an 80% decrease relative to the native state) persists at 275 nm upon formylation of homologous cytoplasmic rRNA, again reflecting the larger number of exocyclic amino groups available for formylation with this system as compared with mitochondrial rRNA.

4. Conclusions

The present investigation supports the hypothesis that mitochondrial rRNA, like RNA of bacterial and plant systems [10, 12], possesses a substantial ordered structure in solution which is stabilized by hydrogen bonding of paired bases in helical segments, and by nearest-neighbor base-stacking. The data are also consistent with a role for both hydrogen bonding and base-stacking in stabilizing mitochondrial rRNA structure and indeed suggest that the two forces are not independent of one another. Similar conclusions may be drawn for the homologous cytoplasmic rRNA from *Trichoderma viride*, in terms of a parallel CD behaviour under the reaction conditions explored; however, this system does undergo more extensive protonation and formylation, reflecting its higher GC content. Additionally, the data suggest that mitochondrial rRNA has less ordered structure than the homologous cytoplasmic rRNA.

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