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Optical Rotatory Dispersion and Circular Dichroism Properties of Yeast Mitochondrial DNA's

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Optical rotatory dispersion and circular dichroism spectra of mitochondrial DNA's from two "grande" and three cytoplasmic "petite" strains of *Saccharomyces cerevisiae* were determined.

The optical rotatory dispersion spectra showed a blue shift compared to those of control DNA's; furthermore, they exhibited a shoulder at 270 mμ. The Samejima–Yang relationship between [α] and base composition was not followed. In the circular dichroism spectra, the broad maximum at 276 to 278 mμ exhibited by control DNA's was replaced by a peak at 273 mμ and two shoulders at 263 and 281 mμ, respectively. The shoulder at 263 mμ had a higher intensity in the DNA's derived from the petite mutants compared to those prepared from the grande strains. In both optical rotatory dispersion and circular dichroism spectra, the negative bands, at 255 and 247 mμ, respectively, were greatly increased in intensity.

A comparison of the spectra of yeast mitochondrial DNA's with those of poly(dAT:dAT) and poly(dA:dT) showed that the peculiar features of the yeast mitochondrial DNA spectra may be due to contributions from both alternating poly(dAT:dAT) and non-alternating poly(dA:dT) structures.

1. Introduction

The advent of a chromatographic method for the preparation of yeast mitochondrial DNA (Bernardi, Carnevali, Nicolaieff, Piperno & Tecce, 1968) has recently enabled Bernardi, Faurès, Piperno & Slonimski (1970) to prepare the mitochondrial DNA's from two "grande" yeast strains and three cytoplasmic "petite" mutants in relatively large amounts; several physical and chemical properties of these DNA's have also been characterized. The guanine plus cytosine contents of the DNA's from the grande strains were found to be 17·4 and 16·8%; those from the petite mutants were 15·5, 15·6 and 12·6%. For all mitochondrial DNA's, the base compositions calculated from their buoyant densities and melting temperatures were quite different from those obtained from analysis. The melting curves of mitochondrial DNA's indicated a striking heterogeneity, and were characterized by distributions of melting components which were different for DNA's derived from different strains. Distributions were

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broad and asymmetric in the case of the DNA's from the grande strains, whereas they were multimodal, with a small number of components, in the case of the DNA's from the petite mutants. In contrast to the DNA's from the grande cells, the DNA's from the petite mutants showed very little or no increase in their buoyant densities and ultraviolet absorptions after heating and rapid cooling. These findings suggest that the cytoplasmic mutation is accompanied by a variable decrease in G+C contents of mitochondrial DNA and leads to a greatly increased rate of its renaturation.

The results of Bernardi *et al.* (1970) prompted the present investigations on the optical rotatory dispersion and circular dichroism of yeast mitochondrial DNA's. A preliminary report on part of this work was presented at the 13th Annual Meeting of the Biophysical Society (Los Angeles, Calif., February 1969).

2. Materials and Methods

(a) *Materials*

The yeast mitochondrial DNA's investigated in the present work are those described in the preceding paper (Bernardi *et al.*, 1970). Two DNA's were obtained from grande *Saccharomyces cerevisiae* strains designated A and B, respectively; three DNA's were obtained from cytoplasmic petite mutants designated a_1, a_2 and b; the first two strains were related to strain A; the third to strain B. For more details on the genetic properties of the strains used, as well as for the physical and chemical properties of the mitochondrial DNA's, see the preceding paper.

Yeast nuclear DNA was obtained from strain B by chromatography on hydroxyapatite columns (Bernardi *et al.*, 1970). Other DNA samples were obtained from *Escherichia coli*, *Haemophilus influenzae*, T2 phage and calf thymus by accepted standard procedures. All non-mitochondrial DNA's will be referred to as control DNA's in the present paper.

Poly(dAT:dAT) and poly(dA:dT) were biosynthetic products.

(b) *Methods*

All ORD† and CD spectra were obtained on a Cary 60 recording spectropolarimeter equipped with a model 6001 CD accessory. ORD spectra were measured between 360 and 230 mμ in fused quartz cells of 2·0- and 5·0-cm path lengths, depending on the DNA concentration. CD spectra were measured between 320 and 230 mμ using 1·0- and 2·0-cm path lengths. The results of the ORD measurements were calculated as $[\alpha]$, to have a direct comparison with the data of Samejima & Yang (1965). All CD results were calculated as $[\theta']$, i.e. the ellipticity in deg. cm^2/decimole of residues, corrected for the index of refraction of the solvent (Adler & Fasman, 1968). All ORD and CD experiments were carried out in SSC buffer (0·15 M-NaCl, 0·015 M-sodium citrate, pH 7·2) at room temperature.

3. Results

The ORD results are summarized in Table 1 and typical curves are presented in Figure 1. Control DNA's have ORD spectra similar to those reported by Samejima & Yang (1965). Yeast mitochondrial DNA's exhibit ORD spectra which are grossly similar to those of the control samples. In detail, however, they differ in three features.

(1) The peak and trough are shifted to lower wavelengths—from 290 mμ and 257 mμ to 288 mμ and 255 mμ, respectively.

(2) A shoulder is present at about 270 mμ. This feature is not apparent in the ORD spectra of the control samples.

(3) The rotations at the peak and trough are no longer nearly identical, the negative rotation at the trough being much larger, in absolute value, than that at the peak, so that the ratio $|[\alpha]_{255}|/[\alpha]_{288}$ varies between 1·5 and 2·0, instead of being close to unity.

† Abbreviations used: ORD, optical rotatory dispersion; CD, circular dichroism.

TABLE 1

Optical rotatory dispersion parameters of various DNA's

DNA	Peak		Trough		$\lvert[\alpha]_{tr}\rvert/[\alpha]_p$
	Wavelength (mμ)	[α]	Wavelength (mμ)	[α]	
Calf thymus	290	2045	258	−2165	1·06
E. coli	290	2150	256	−2390	1·07
H. influenzae	290	2010	257	−2710	1·35
T2 phage	290	1530	257	−2800	1·83
Yeast nuclear (strain B)	290	1760	255		1·32†
Yeast mitochondrial:					
strain A	288	1660	255	−2885	1·74
strain B	288	1390	256	−2700	1·94
strain a_1	288	1810	255	−2740	1·51
strain a_2	288	1865	255	−3190	1·71
strain b	287	1590	255	−3035	1·90
Poly(dAT:dAT)	283	2300	255	−5010	2·18
Poly(dA:dT)	289	700	278	− 670	
	264	1230	253	−4980	

† Ratio obtained from a separate run in which the DNA concentration had not been determined.

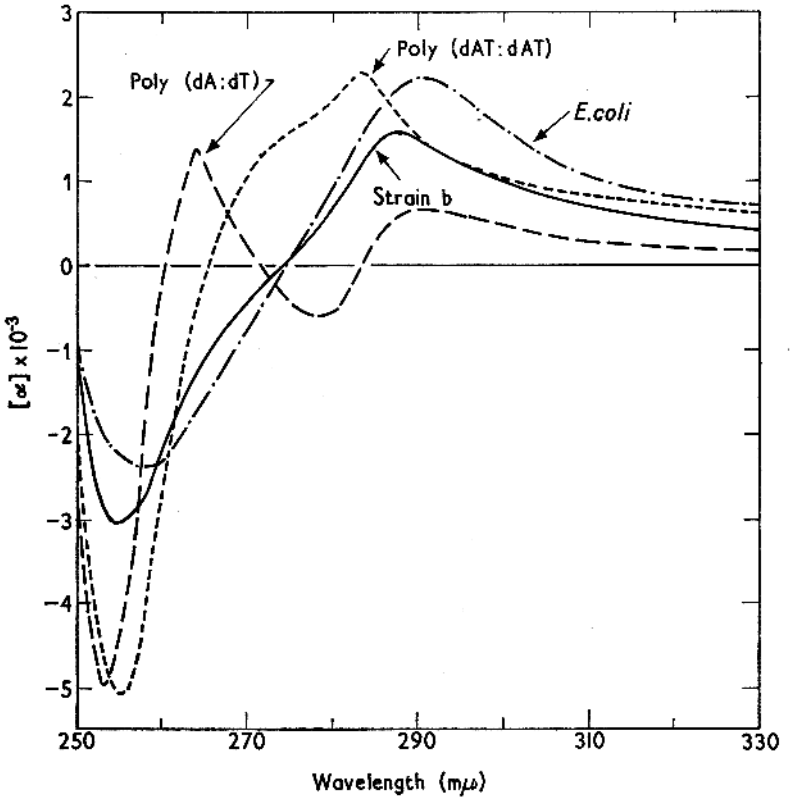

FIG. 1. Optical rotatory dispersion of control *E. coli* DNA, mitochondrial DNA from the petite mutant b and two polynucleotides.

A comparision of these ORD spectra with those of poly(dAT:dAT) and poly(dA:dT), also given in Figure 1, shows that the departures from the usual spectra observed in the yeast mitochondrial DNA's are in a direction toward the spectra of both poly(dAT:dAT) and poly(dA:dT). The ORD spectrum of poly(dAT:dAT) was found to be characterized by a peak at 283 mμ, a marked shoulder at about 270 mμ and a trough at 255 mμ, in agreement with previous results by Samejima & Yang (1965). The ORD spectrum of poly(dA:dT) was found to show two peaks, at 289 mμ and 264 mμ, and two troughs, at 278 and 253 mμ, respectively, in accordance with data of Ts'o, Rapaport & Bollum (1966). Thus, the peak at 288 mμ in the mitochondrial DNA spectra is very close to the 289-mμ peak of poly(dA:dT); its very slight blue shift may reflect a displacement toward the poly(dAT:dAT) peak at 283 mμ. The shoulder at 270 mμ may be related to that observed at the same wavelength in poly(dAT:dAT); at the same time, it may also reflect a contribution from the trough and peak of poly(dA:dT) at 278 and 264 mμ, respectively. Finally, the increased negative rotation at 255 mμ also recalls the spectra of both biosynthetic polymers.

In order to identify the transitions involved in the ORD spectral shift, CD spectra were measured on the same DNA samples between 230 and 320 mμ. The results are summarized in Table 2 and typical curves are shown in Figures 2 and 3. The control DNA's show a broad positive band which comes to a maximum at 276 to 278 mμ and

TABLE 2

Circular dichroism of various DNA's

DNA	Positive band Wavelength (mμ)	$[\theta']$	Negative band Wavelength (mμ)	$[\theta']$	$\lvert[\theta']_{neg}\rvert/[\theta']_{pos}$
Calf thymus	276	6175	247	− 6585	1·07
E. coli	275	5850	246	− 7270	1·25
H. influenzae	278	5850	247	− 8140	1·38
T2 phage	279	4940	247	−12600	2·55
Yeast nuclear (strain B)	277;282sh†	5565	247	− 6720	1·21
Yeast mitochondrial:					
strain A	273;281sh;263sh	4280	247	−11230	2·63
strain B	273;280;263sh	4650	247	−10200	2·19
strain a_1	273;282sh;262sh	5230	247	−10870	2·08
strain a_2	273;282sh;263sh	4720	247	−11220	2·38
strain b	272;281sh;263sh	5680	247	−10200	1·80
Poly(dAT:dAT)	263;278sh	7555	247	−10880	1·44
Poly(dA:dT)	283	3480	269	− 900	
	259	6015	248	−18325	

† sh, shoulder.

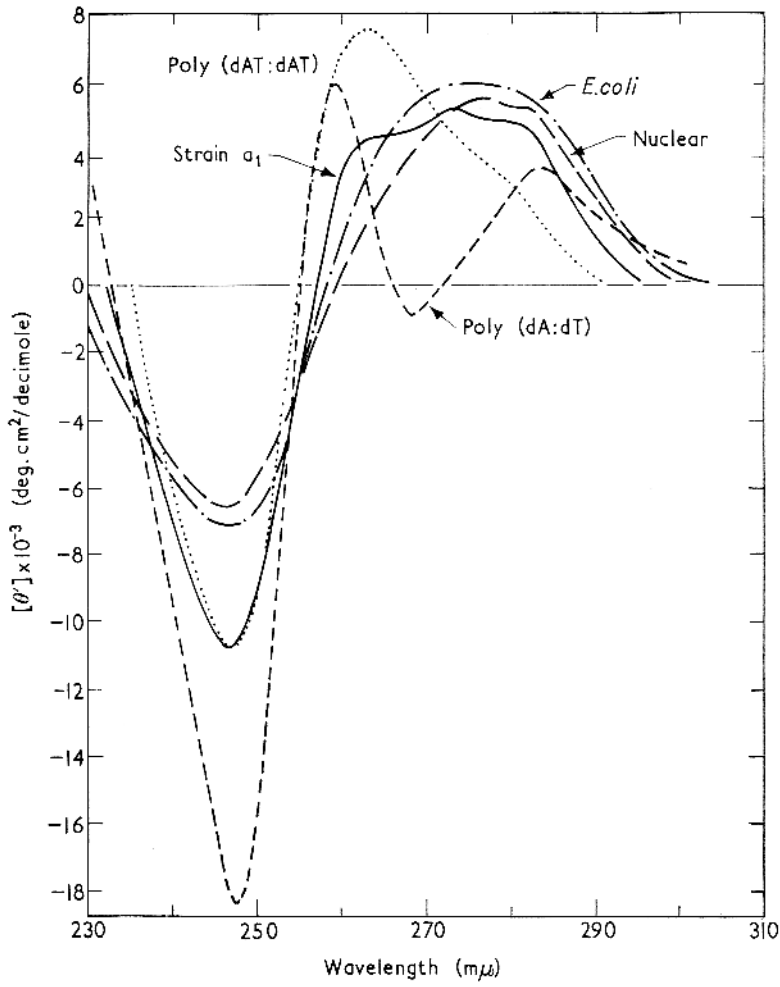

FIG. 2. Circular dichroism spectra of control *E. coli* DNA, mitochondrial DNA from the petite mutant a_1, yeast nuclear DNA and two polynucleotides.

a negative band with a maximum at 246 to 247 mμ. The amplitudes of the positive and negative absorptions are about the same, with the ratio $|[\theta']_{247}|/[\theta']_{276}$ varying between 1·0 and 1·4 (except for T2 DNA), essentially in agreement with previously reported spectra (Brahms & Mommaerts, 1964; Brahms, 1965; Sarkar, Wells & Yang, 1967). The yeast mitochondrial samples (Fig. 3) give spectra with a positive maximum at 273 mμ, with shoulders at 281 mμ and 263 mμ and a deep negative band at 247 mμ. The ratio $|[\theta']_{247}|/[\theta']_{273}$ varies between 1·8 and 2·6. Thus, when the CD spectra of yeast mitochondrial DNA's are compared to those of the control samples, three different features are apparent:

(1) The positive maximum is shifted from between 276 and 278 mμ to 273 mμ;

(2) Two positive shoulders appear at 263 and 281 mμ, respectively;

(3) The intensity of the negative band at 247 mμ is greatly increased in mitochondrial DNA's.

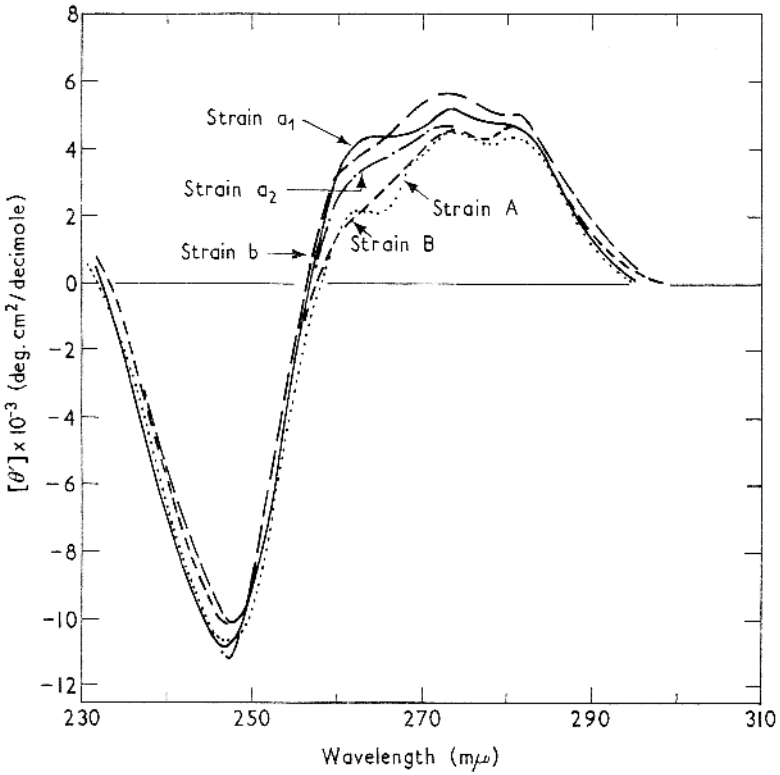

FIG. 3. Circular dichroism spectra of the mitochondrial DNA's from various yeast strains.

Comparison with the CD spectra of poly(dAT:dAT) and poly(dA:dT) shows again that the observed changes in the DNA spectra are related to those of the polynucleotides. The CD spectrum of poly(dAT:dAT), shown in Figure 2, has a strong positive band at 263 mμ with a shoulder at about 278 mμ, and a deep negative band at 257 mμ. The intensities of these two bands are considerably greater than those of the corresponding bands of control DNA. The CD spectrum of poly(dA:dT) shows two maxima, at 283 and 259 mμ, respectively, and two minima at 269 and 248 mμ, respectively. It is quite striking that, in mitochondrial DNA spectra, two shoulders appear, at 263 and 281 mμ, which are wavelengths close to the peaks of poly(dAT:dAT) and poly(dA:dT). The higher negative bands at 247 mμ also recall those present in the spectra of both biosynthetic polymers. Surprisingly, the yeast nuclear DNA shows a shoulder at 281 mμ.

In order to verify whether the mitochondrial DNA's obey the Samejima & Yang relationship (1965), the apparent base compositions were calculated from the height of the ORD peaks at 288 mμ. The results are presented in Table 3, where they are compared with the base compositions as determined by analysis (Bernardi *et al.*, 1970). It is evident that the calculated G+C contents are systematically much higher than those determined by analysis. It can be concluded, therefore, that, in the case of ORD as well as for buoyant density and T_m (Bernardi *et al.*, 1970), the usual linear relationships between base composition and physical parameters are not obeyed by yeast

TABLE 3

Base composition of various DNA's from ORD and analysis

DNA	Calculated† (%)	Analytical (%)
Calf thymus	45·2	42·0
E. coli	49·2	50·0
H. influenzae	37·2	38·0
Yeast nuclear (strain B)	34·3	39·7
Yeast mitochondrial:		
strain A	30·7	17·4
strain B	20·4	16·8
strain a_1	36·2	15·5
strain a_2	38·3	15·6
strain b	27·8	12·6

† According to Samejima & Yang, 1965.

mitochondrial DNA's. In contrast, the control bacterial DNA's precisely obey the Samejima & Yang relationship (1965). A less satisfactory agreement between calculated and analytical base composition was found for the two nuclear DNA's from calf thymus and yeast. The discrepancy may perhaps be due to the presence in these DNA's of satellites which are known to have different base compositions and repeating sequences. Finally, the DNA from T2 phage shows spectra which are different from those of both mitochondrial and other control DNA's. This difference is, in all likeliness, due to the glucosylation of this DNA. Interestingly enough, the Samejima–Yang relationship is not obeyed by T2 DNA. In contrast to mitochondrial DNA's, however, the calculated G+C content (25·6%) is lower than the analytical value (34%).

4. Discussion

Our results indicate that subtle differences exist between the secondary structures of control and mitochondrial DNA's. Both the ORD and CD spectra of these nucleic acids display some characteristics of the corresponding spectra of poly(dAT:dAT) and poly(dA:dT).

Displacements in ORD spectra are reflections of the observed shifts in CD band position and intensity. Since ORD bands are infinite, whereas CD bands have discrete widths, a change in the CD characteristics of any band will be manifested over the entire ORD spectrum (Moscowitz, 1960). Thus, displacements of both the peak and trough position and the increase in trough to peak rotation ratio in ORD are the results of the appearance of the 263 mμ shoulder in the positive CD band and the increase in intensity of the negative band. The positive peak in ORD in the 290 mμ region is the sum of the high wavelength end of the Cotton effects in the 260 to 282 mμ region. The appearance in mitochondrial DNA of an additional positive Cotton effect at 263 mμ should result in a blue shift of the ORD peak; this indeed is observed. The ORD trough in the 257 mμ region consists essentially of the sum of the low wavelength ends of the 260 to 282-mμ positive Cotton effects and the high wavelength end of the 247 mμ negative Cotton effect. Thus, in the trough, changes in the two bands are additive.

It would be expected, therefore, that the appearance of an additional positive CD band at a lower wavelength than in control DNA would result in a blue shift in the trough, while an increase in intensity of either the positive or negative CD bands would lead to an increase in the negative rotation of the 255 to 257 mμ trough. In fact, both effects are observed. A new positive Cotton effect appears in mitochondrial DNA at 263 mμ, causing a blue shift of the trough, and the increase in CD intensity at 247 mμ leads to an increase in negative rotation at 255 mμ and an increase in the $|[\alpha]_{255}|/[\alpha]_{288}$ ratio. It is evident, therefore, that the deviations of the ORD behaviour of mitochondrial DNA's from the Samejima–Yang relationship are a result of the appearance of a new transition at 263 mμ and the increased intensity of the negative band at 247 mμ.

As pointed out above, the observed deviations of the ORD and CD spectra of mitochondrial DNA from those of control DNA's reflect the appearance of poly(dAT:dAT) and poly(dA:dT) spectral characteristics in the spectra of mitochondrial DNA's. That *both* alternating (dAT:dAT) and non-alternating (dA:dT) structures contribute to the spectra of mitochondrial DNA's is suggested by the following considerations: (1) the contributions to the 281 mμ and 263 mμ CD peaks seem to derive predominantly from (dA:dT) and from (dAT:dAT) structures, respectively; (2) the intensity of the CD trough at 247 mμ is identical to that of poly(dAT:dAT); since mitochondrial DNA's are not formed by pure (dAT:dAT) structures, it appears that non-alternating (dA:dT) structures, which have a higher intensity of the negative band than the alternating ones, must contribute to the trough of mitochondrial DNA's; (3) the very moderate blue shift of the 288-mμ ORD peak is easily explained if (dA:dT) structures (peak at 289 mμ) contribute to its intensity.

The results obtained with T2 DNA suggest that the deviation of this DNA from the Samejima–Yang relationship is quite different from that shown by mitochondrial DNA's and, indirectly, rule out the possibility that the latter are glucosylated. It may be relevant to point out here that yeast mitochondrial DNA's are the first natural DNA's in which non-alternating (dA:dT) structures have been demonstrated. The buoyant density results previously obtained (Bernardi *et al.*, 1968,1970) lend independent support to this conclusion.

If the CD spectra of the various mitochondrial DNA's, shown in Figure 3, are examined in detail and compared with each other, it is quite evident that the CD spectra above 255 mμ can be divided into two classes, which are distinguished by the intensity of the 263 mμ CD band. The first class, having a high intensity at 263 mμ consists of the petite mutants, a_1, a_2 and b; the second class, characterized by a much weaker dichroic band at that wavelength, consists of the two grande strains A and B. The small difference within the classes must be regarded at present as within experimental error.

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