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Abstract

Ring DNA from rat liver mitochondria has been examined by circular dichroism (CD) in the region of the 225 to 320 m/∼ and the followings have been clarified. The ring DNA gives a CD spectral curve somewhat different from linear DNA from nuclei, showing a big positive peak at 266 m/∼ and a small negative band at 243 m!∼. That is, the positive CD band of ring DNA shifted by about 7 m/∼ to the shorter wavelength side from the band of the ordinary nuclear DNA, 273 m!∼. Negative band appeared at the same region as that of linear DNA but reduced in depth. Heat denaturation of the ring DNA induced a red shift of the positive band, by about 4 mp., but no change in negative band. From these experimental results it has been concluded that the ring DNA has highly twisted conformation and high in G.C contents, both of which are responsible for the blue shift of the CD spectrum.

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CONFORMATIONAL STUDIES OF MITOCHONDRIAL DNA

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Recent observations of mitochondria and chloroplasts revealed that they have DNA, the ring DNA, similar to that of some microorganisms. Each ring DNA molecule should has the complete set of gene needed for the construction of the organella or the microorganism (1～3). It appears as a ring on the spread and negatively stained specimen, and its duplicating pattern has also been observed under electron microscope. But the morphologic pictures of the DNA appearing as ring on the section of the organellae or the microorganism suggest some complicated three dimentional structure of the DNA in the living cell. It may undergo some conformational changes according to the changed functional state, resting, duplicating or transcription stage. At present we have no information concerning how it assumes the three-dimentional structure of ring DNA and its possible conformational changes. However, the recent development of the analytical method, the spectrography by circular dichroism or optical rotatory dispersion, makes it possible to reveal the three-dimentional conformation of large melecules, like polypeptide and protein. And it has become quite promising to obtain a reliabe information on the three dimentional structures of DNA by the same method. In the present paper the authors present the spectral pattern of the circular dichroim (CD) of the ring DNA of rat liver mitochondria, which suggests a highly twisted conformation of the DNA in its native state or in the living cell.

MATERIALS AND METHODS

Isolation of mitochondria: The mitochondria were isolated from the liver of 25- to 35-day old wister rats by the modified method of Hogeboom (4). Immediately after sacrifice of the animals livers were removed and homogenized by glass homogenizer adding about 5 volumes of 0.25 *M* sucrose containing 50 μM EDTA and 1 m*M* Tris-HCl buffer (pH 7.4). The homogenate was centrifuged at 50×g for 10 min. The upper half of the supernatant was taken carefully and superimposed on the equal volume of 0.34 *M* sucrose containing 50 μM EDTA and 1 m*M* Tris-HCl (pH 7.4), centrifuged at 700×g for 10 min. and the precipitant was removed. Repeating the same process the contaminated nuclei were eliminated as

precipitate. The supernatant obtained through three centrifugations was further centrifuged at 5000×g for 10 min, and the mitochondrial fraction was obtained as the precipitate. The mitochondria were washed twice with the homogenizing medium and twice again with 0.15 *M* NaCl (pH 8.0) containing 0.1 *M* EDTA by the repeated centrifugation and stored at −20°C being suspended in an appropriate volume of the washing medium.

Isolation of DNA from the mitochondria : The stored mitochondrial suspension was frozen-thawed and the mitochondrial pellet was obtained by centrifugation at 7,000×g for 10 min. By using the pellet DNA was isolated by the modified method of MARMUR (5). That is, the pellet was deproteinized by shaking with isoamylalcohol-chloroform vigorously, the denatured protein was removed by centrifuging at 800×g for 10 min, and DNA was precipitated by treating the aqueous layer with 95% cold ethanol. The DNA was dissolved with citrate-saline solution and the process of the deproteinizaiotn was repeated further three times. The final citrate-saline solution was treated with RNase in a final concentration of 50 μg/ml for 30 min at 37°C. The digest was subjected to the deproteinization process as just described, repeating two to three times. Finally the sample was suspended in citrate-saline, and added with an equal volume of 80% phenol by the method of MIURA, and washed with ether. The DNA suspension obtained was used for the physical analyses.

Isolation of DNA from hamster thymus nuclei: Hamster thymus nuclei were isolated by the method of CHAUVEAU *et al.* (6) and the DNA was purified by the method of MARMUR as described above.

Measurement of the CD specrta of DNA: With the DNA suspension just mentioned the spectrogram of circular dichroism of the DNA was obtained by using the JASCO spectropolarimeter model ORD/UV-5 having CD attachment. Measurement was carried out with the samples in a quartz cell on the light paths varying from 0.1 to 1.0 cm. The circular dichroism was expressed in terms of the difference of the molar extinction coefficient for left- and right-handed circularly polarized light(ε_L-ε_R) at the frequency ν. The molar ellipticity can be found from those as $[\theta]=3300(\varepsilon_L-\varepsilon_R)$.

Absorption spectra of the DNA were obtained by using the Hitachi autorecording spectrophotometer.

RESULTS

The DNA solution from rat liver mitochondria, which was carefully prepared by the method just described, shows the typical absorption spectra of DNA (Fig. 1). The absorption ratios were $A_{260} : A_{230}=1.77$ and $A_{260} : A_{280}=2.61$, and indicate that the sample contains neither phenol nor protein. This suggests that the DNA is of double strand and splits into two single strands by heating. The mitochondrial DNA shows a typical circular dichroic spectrum in the 0.15 *M* NaCl containing 0.015 *M* sodium citrate

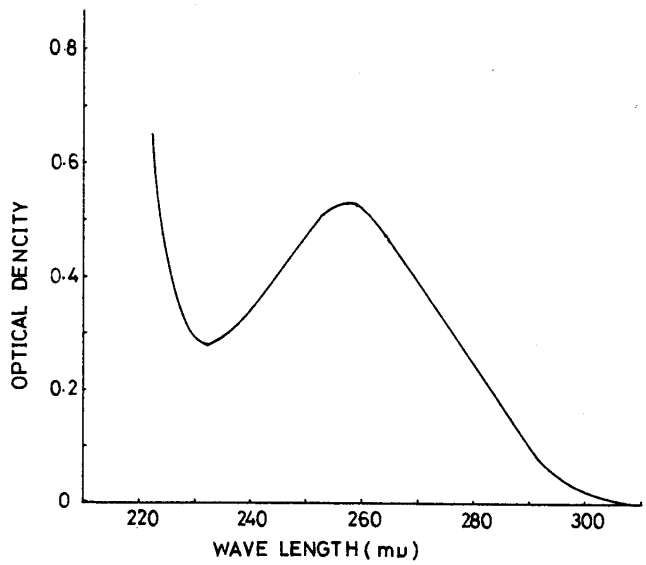

Fig. 1 UV-absorption spectrum of the isolated liver mitochondrial DNA

at pH 7.0 (SSC solution). It displays one peak and one trough; a peak having a broad base with the summit at 266 mμ, and a small trough at around 243 mμ (Fig. 2, curve a). By heating at 90°C for 10 min and rapid cooling, the DNA was denatured and the CD spectrum showed

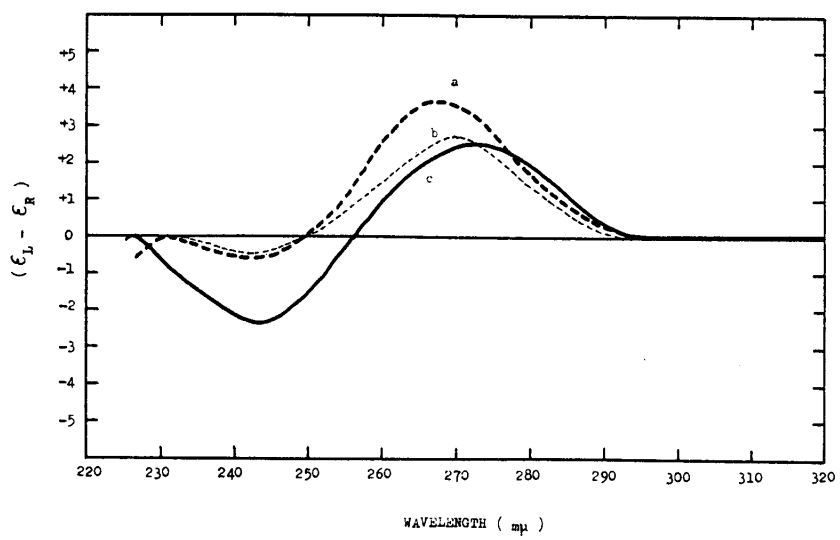

Fig. 2 Circular dichroism of isolated liver mitochondrial DNA
a: mitochondrial DNA, b: mitochondrial DNA, heated at 90°C for 10 min and cooled rapidly, c: nucelar DNA from hamster thymus. For details refer to the text.

some change in its positive peak, *i. e.* a slight red shift of the summit from 266 to around 270 mμ with decrease in height (Fig. 2, curve b). The decrease in the height of the peak means the loss of secondary structure. The nuclear DNA from hamster thymus dissolved in SSC solution at pH 7.0 gave also one peak having the summit at 273 mμ and one trough at around 243 mμ (Fig. 2, curve c). The curve represents the typical pattern of CD spectrum of nuclear DNA of linear type as reported on the DNA from the nucleus of calf thymus, fowl erythrocytes, rat liver as well as *E. coli* in aqueous saline solution. The data indicate clearly that the CD spectra of ring DNA of rat liver mitochondria is largely different from that of nuclear linear DNA, *i. e.* the native ring DNA has a positive CD band at 266 mμ while the nuclear DNA at 273 mμ, though the troughs appeared at around 243 mμ in both DNAs. By heat denaturation the CD band of the ring DNA shifted by about 3 mμ toward the blue side.

The optical rotatory dispersion (ORD) of the nuclear DNA has been extensively studied by YANG and his associates (7, 8) and it has been revealed that Cotton effect appearing around 260 mμ reflects the conformation of DNA. Recently CD method has been also applied to the study of the conformation of nuclear DNA and the ribonucleoside by BRAHMS and his associates (9—11) and HASHIZUME and IMAHORI (12). Generally, theoretical evaluation of spectroscopic evidence has led to an assignment of electric transition in purines and pyrimidines (13—16). The strong absorption at around 260 mμ is ascribed to a $\pi \rightarrow \pi^*$ transition and another transition is the region of 280 mμ which is attributable to an $\pi \rightarrow \pi^*$ transition. Besides these, DNA shows negative CD peak at 243 to 245 mμ but RNA does not give the similar peak. For the characteristic negative CD band by DNA HASHIZUME and IMAHORI (12) explain as follows: (I) The dA+dT shows the characteristic big negative CD band at 243 mμ, and dG+dC gives the positive CD band around 255 mμ which is rather week. So the former negative band is retained without neutralization. (II) The absence of 2'OH in the ribose ring of the DNA reflects week negative band of CD spectra at around 245 mμ. Therefore, it is reasonably deduced that the broad positive band of ring DNA from 250 to 290 mμ as revealed in the present experiment is the integrated feature of 2 to 3 bands appearing in the region.

The most important point of this experiment is the blue shift of positive band of mitochondrial DNA in CD spectra as compared to that of nuclear DNA. This blue shift may be explained by the two possible ways. The one possibility is the high G-C contents of mitochondrial DNA, because the maximum positive band of the CD spebtra of double strand

poly (G+C) helix appears at 261 mμ. As is well known, high G-C contents mean the increase of Tm. Actually, it has been reported that the G-C content of mitochondrial DNA is slightly higher than that of nuclear DNA (1) and also it was found that the Tm of mitochondrial DNA is slightly higher than that of nuclear DNA (17). The other possibility is that the blue shift of the positive CD peak of ring DNA is due to its highly twisted conformation. The electron microscope observation of monolayer preparation of mitochondrial DNA from rat liver revealed three kinds of molecular conformation. The first one is the highly twisted ring, the second is an open ring and the third is the linear form probably of artifact (18—30). HASHIZUME and IMAHORI (12) reported a close relation between molecular conformation and the CD spectra in several natural and synthetic polynucleotides. Namely, the peak of CD band showed a red shift when base pairing between two strands of polynucleotides was ruptured. Essentially the similar results were obtained on hypochromic Cotton effect as observed by the ORD (8, 31). From these facts it is deduced that the blue shift of the positive band of ring DNA from mitochondria reflects the increase in the G-C contents in certain region of the DNA molecule and the highly twisted molecular conformation. Probably, in the highly twisted condition of the molecule the stacking of the bases will become closer. Explanation of the decrease in the depth of the negative CD band in ring DNA seems to need further experiments and informations.

SUMMARY

Ring DNA from rat liver mitochondria has been examined by circular dichroism (CD) in the region of the 225 to 320 mμ and the followings have been clarified. The ring DNA gives a CD spectral curve somewhat different from linear DNA from nuclei, showing a big positive peak at 266 mμ and a small negative band at 243 mμ. That is, the positive CD band of ring DNA shifted by about 7 mμ to the shorter wavelength side from the band of the ordinary nuclear DNA, 273 mμ. Negative band appeared at the same region as that of linear DNA but reduced in depth. Heat denaturation of the ring DNA induced a red shift of the positive band, by about 4 mμ, but no change in negative band. From these experimental results it has been concluded that the ring DNA has highly twisted conformation and high in G-C contents, both of which are responsible for the blue shift of the CD spectrum.

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