Use of the 295- to 300-Nanometer Circular Dichroism Trough of Ribonucleic Acid to Study Helix Winding: Effect of Acridine Orange

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Acridine orange decreases the amplitude of the 295-nm circular dichroism (CD) trough of ribosomal ribonucleic acid (rRNA) where the trough has been related to coil character. Since acridine orange is known from earlier work to intercalate between base pairs of nucleic acids, causing an unwinding of the coil, and our studies show a decrease in the 295-nm CD trough, it appears that CD measurements may be used to observe relative unwinding of rRNA. Under similar solution conditions, melting temperatures with acridine orange indicate no significant change in the stabilization of rRNA structure by acridine orange. Hypochromicity studies show no increase in the percent base pairing in rRNA when 0.1 M tris(hydroxymethyl)aminomethane (pH 7.6) with 1.35 M KCl is used. These results indicate that CD changes in the amplitude of the 295-nm trough of rRNA are related to helix winding in rRNA.

One technique frequently used for studying macromolecular conformation is circular dichroism (CD) (14). The CD spectrum of ribosomal ribonucleic acid (rRNA) (Fig. 1) contains two Cotton effects in the spectral region above 250 nm. One of these, at approximately 265 nm, contains the spectroscopic contribution of those bases which are in linear, noncoiled regions of the molecule. When the bases are fixed in space relative to one another at a discrete angle deviating substantially from 180°, the bases interact spectroscopically, resulting in so-called exciton coupling (9) and the generation of a characteristically S-shaped band whose centroid is approximately at the position of the maximal ultraviolet absorption. The second, or positively signed lobe, adds intensity to the peak at approximately 265 nm, as previously described. The first lobe has a negative sign and occurs at longer wavelengths. This band is only partially compensated for by the positive peak of the contribution of the linearly arranged bases and may be seen in the spectrum of rRNA at about 295 nm. When this relatively weak trough in the 295- to 300-nm region of the CD spectra of various nucleic acids was first observed, it was suggested that this negative peak was due to an n-π* transition in the helical regions of the molecules (13). More recent investigations have suggested that this trough results from an asymmetry induced by coiling of the tertiary structure of ribosomal RNA; the greater the amplitude of the trough, the tighter the coil (2). Acridine orange, an antibacterial and mutagenic agent, is known to intercalate between the bases of nucleic acids (3, 8, 15). This process of intercalation causes the helix to unwind by about 12° (10, 11). In light of this property, it was thought that amplitude decreases in the 295- to 300-nm CD trough of rRNA should be observed in the presence of acridine orange and could be directly related to helix unwinding. This would provide a convenient spectroscopic tool for measuring the interactions of intercalating drugs with nucleic acids.

Extrinsic Cotton effects occur when acridine orange binds to nucleic acids (12). The asymmetry induced in the spectrum of acridine orange by binding to nucleic acids is observable at long wavelengths (420 to 550 nm). These earlier studies in essence followed the interaction of the dye with nucleic acids through induced rotatory changes in the dye. These observations suggested that the dye might modify the conformation of the polymer, but this was not observed directly (12). In this paper, on the other hand, evidence is presented that conformational
changes in the polymer may be observed directly when ellipticity changes at 295 nm are used.

MATERIALS AND METHODS

Yeast ribosomal RNA was purchased from Worthington Biochemical Corp., Freehold, N.J. Hypochromicity studies (4, 5; Table 1) showed that approximately 70% of this RNA was doubly stranded. Ultraviolet difference spectra (7) indicated that between 70 and 90% of these base pairs were guanosine-cytidine. The RNA concentrations are expressed as phosphate units with a calculated molecular weight of 670. The acridine orange HCl, a gift of E. J. Eyring of The Ohio State University, had been recrystallized from aqueous ethanol as prescribed (1). In a solution of 0.1 M LiCl in 50% (vol/vol) methanol, the acridine orange HCl gave an $e_{440nm} = 6.81 \times 10^4$ in agreement with the literature value (1). All solutions were prepared in 0.1 M tris(hydroxymethyl)aminomethane buffer at pH 7.6 with 1.35 M KCl by using demineralized, double-distilled water again distilled over ethylenediaminetetraacetic acid. The high ionic strength was necessary to preclude any electrostatic interaction of the cationic acridine orange and the anionic rRNA, thus allowing only the intercalation of acridine orange into the rRNA to occur (6).

Circular dichroism measurements were made by using a Durrum-Jasco ORD/UV-5 spectropolarimeter with the Sproul Scientific SS-20 modification. All determinations were made at 22.0 ± 0.1 C by using a water-jacketed cell. The optical train was kept under constant nitrogen flush.

A Beckman model DU spectrophotometer with a thermostatic cell compartment was used in the determination of melting temperatures. Melting curves were constructed from the observed increase in absorbance at 260 nm with increasing temperature of RNA solutions. Hypochromicity studies were made in a Cary 15 recording spectrophotometer with a thermostatic cell compartment. The entire ultraviolet-visible spectrum of each solution was determined at both low (11 C) and high (90 C) temperatures and by using absorbance at 255 nm. The hypochromicity and, subsequently, percent base pairing was calculated according to the method of Boedtker (4, 5).

RESULTS AND DISCUSSION

Figure 2 shows the typical result obtained when the CD spectra of acridine orange, rRNA, and their admixture at the same concentrations are determined in the 295- to 300-nm region. Acridine orange caused a marked decrease in the amplitude of the 295-nm trough of rRNA. When the difference between the amplitude of rRNA alone and rRNA with acridine orange (at 295 nm) is compared with that of rRNA alone, a measure of the percent decrease in amplitude due to acridine orange can be determined.
Various ratios of acridine orange to rRNA were used, and their CD spectra were measured and analyzed in a similar fashion. The percent decrease found for the different mixtures is represented in Fig. 3. As the log of ratio of acridine orange to rRNA (whose concentration is based on phosphate units) increased, it is seen that there was a sigmoidal decrease in the amplitude of the 295-nm trough. At a ratio of about 0.25, the amplitude of the trough had essentially been reduced to zero. Based on these data, it appears that the unwinding effects of acridine orange on rRNA structure can be studied through the CD amplitude changes at 295 nm. The assumption that the 295-nm trough of rRNA reflects coil character seems valid since absorbance spectra of acridine orange under similar solution conditions do not show an absorbance peak at 295 nm, although a shoulder does appear at about 285 nm. Usually an extrinsic Cotton effect due to an induced asymmetry in an otherwise symmetrical molecule occurs at the wavelength maximum of the chromophore of the molecule. Acridine orange additionally shows an absorption maximum at about 260 nm where rRNA also has an absorption peak. The absorption spectrum obtained with a mixture of acridine orange and rRNA was less than would be predicted from a simple summation of the spectra obtained for the individual components. However, the CD spectrum at 265 nm of rRNA (4 \times 10^{-8} M) with acridine orange (10^{-8} M) was identical to that of rRNA alone at the same concentration. Acridine orange alone did not have an ellipticity at about 265 nm. Taking into consideration all of these spectroscopic properties, it would thus appear that the 295-nm trough is indeed reflecting coil character in rRNA. To show that coil effects are not due to percent base pair changes in RNA, hypochromicity studies were made with rRNA alone and acridine orange-to-rRNA mixtures at ratios of 0.1:1 and 0.25:1. The results show essentially no change in percent base pairing of rRNA (Table 1) under the same solution conditions as the CD measurements. The ratios used are the same as those where maximal decrease in the 295-nm CD trough was observed. It does not, therefore, appear that base pairing changes affect the conclusion drawn from the CD results. When the melting curves for rRNA and rRNA with acridine orange (at a ratio of 0.5:1) were determined, the melting temperature for the mixture was found to increase slightly (Table 1). This difference is insignificant, suggesting that no stabilization of gross rRNA structure occurs under these solution conditions. In 0.01 M tris(hydroxymethyl)- aminomethane buffer containing no KCl, acridine orange increased the melting temperature of rRNA by about 20°C. Thus, stabilization of structure does occur under such conditions as would be expected for nucleic acids (11) but not
under the conditions of our CD measurements. It is concluded that structural stability is not complicating the CD measurements.

The application of this technique to the study of analogous drug systems where an interaction is believed to lead to perturbations in the conformation of the deoxyribonucleic acid or RNA molecule should be profitable.

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LITERATURE CITED