OPTICAL ROTATORY DISPERSION OF E. coli sRNA IN THE FAR ULTRAVIOLET REGION

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Received June 21, 1965

The anomalous rotatory dispersion (ORD) of E. coli sRNA has been recently reported by Lamborg et al. (1965), and of yeast sRNA by Samejima and Yang (1965). It was observed by the former authors that with respect to the Cotton effect centered at 264 mμ, a bathochromic shift and loss of amplitude resulted from such various conditions as treatment with urea, heating, acid or base titration, bromination in aqueous media, and alkaline hydrolysis. It was concluded that a large component of that Cotton effect was due to the conformational asymmetry of the macromolecule.

This communication reports the presence at 198 mμ of an additional ORD peak which is at least three times larger than the 278 mμ peak. The relative contribution of secondary structural asymmetry to the various E. coli sRNA Cotton effects is discussed.

MATERIALS AND METHODS

sRNA from E. coli was purchased from General Biochemical Corp. (Chagrin Falls, Ohio) and was stripped of esterified amino acids by the method of Sarin and Zamecnik (1964). The hydrolysis of sRNA and the calibration of the Cary 60 spectropolarimeter were carried out as previously described (Lamborg et al., 1965). ORD measurements below 220 mμ for sRNA and for the alkali hydrolyzed product of sRNA were carried out at room temperature, with cells having a 0.1 mm light

path (Opticell, Brentwood, New Jersey). At elevated temperatures, thermojacketed cells of the same dimensions were connected to an external circulating water bath. The concentration of sRNA and of hydrolysate varied from 1.2 to 1.5 mg/ml in a solution of potassium phosphate:potassium fluoride (0.015 M:0.15 M). ORD measurements at pH 5.7 and 7.0 showed no significant changes either in amplitude or wavelength. Boiled, glass-distilled water was used in the preparation of solutions used for these measurements, and the spectropolarimeter was exhaustively purged with nitrogen before any measurements were made. Measurements were made at minimum scan speed, and at various pen periods consistent with maximum sensitivity and minimum pen deflection. Low light levels below 220 mμ led to high noise levels which produced variations in amplitude of 10-15 per cent in separate measurements. Each curve shown in Fig. 1 is therefore an average of three to five separate measurements.

From 320 mμ to 220 mμ, the concentration of sRNA and of its hydrolysate varied from 0.6 to 1.0 mg/ml, with the use of 1.0 mm light path cells. Saline:citrate buffer (0.15 M:0.015 M, pH 7.1), as well as those solutions previously mentioned, was used in this range. No significant variation in ORD measurement was observed in changing either solvents or pH. The error of separate measurements in this wavelength range was 0.5 to 1.0 per cent.

RESULTS

E. coli sRNA has multiple Cotton effects in the ultraviolet region of the spectrum (Lamborg *et al.*, 1965). A minor Cotton effect is centered between 210 and 235 mμ. The peak of a third Cotton effect has now been found at 198 mμ, in agreement with the observations of Samejima and Yang (1965), reported since the completion of these experiments. Its amplitude (+ 11650°) is more than three times greater than that of the 278 mμ peak (Fig. 1).

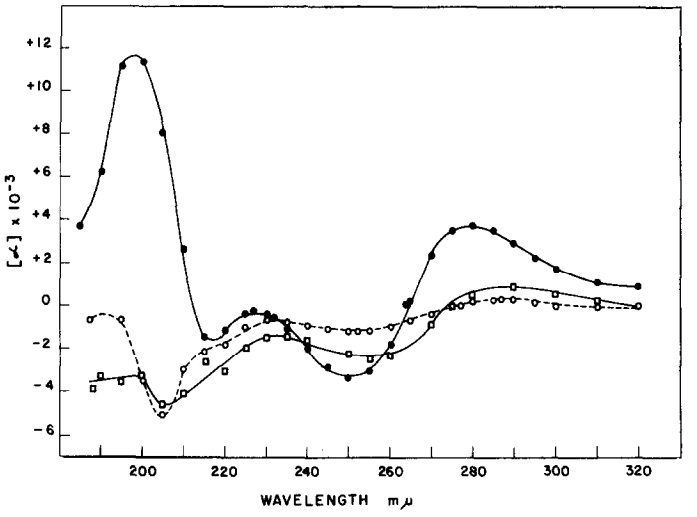

Fig. 1. The effect of heating and hydrolysis on the optical rotatory dispersion of E. coli sRNA. E. coli sRNA (●) was dissolved in potassium phosphate:potassium fluoride (0.015 M, 0.15 M). Above 220 mμ the concentration was 1.0 mg/ml, pH 5.7 or 7.0; below 220 mμ the concentration was 1.5 mg/ml. Similar conditions were used for heated sRNA (□) except that the solution was heated to 90° for 10 min before measurements were made, and was maintained at this temperature throughout the measurement period. Alkali-hydrolyzed, perchloric acid-extracted sRNA hydrolysate (○) was measured in the same solution, at a concentration of 0.75 mg/ml above 220 mμ, and at 1.2 mg/ml below 220 mμ. See Methods for further details. Lamborg et al. (1965) reported that the hydrolysate of E. coli sRNA has a λ_o of 269 mμ and an amplitude of 1900° in succinate-Mg^{++} buffer (0.1 M, 5 x 10^{-3} M, pH 5.1). These values differ from measurements made in potassium phosphate, potassium fluoride (0.016 M, 0.15 M, pH 7.0 or 5.7), which are as follows: λ_o = 277 mμ, amplitude 1477°.

The ORD of E. coli sRNA is thus quite similar to that reported for rat liver and for yeast RNA by Samejima and Yang (1964, 1965). Either maintenance of sRNA solutions at 90° or alkaline hydrolysis causes a bathochromic shift of each Cotton effect and a marked diminution in amplitude (Fig. 1). Both treatments produce an inversion of rotation from the (positive) peak at 198 mμ to a (negative) trough at 205 mμ. A consequence of the appearance of the 205 mμ trough is the almost complete abolition of the trough previously centered at 215 mμ.

The Cotton effect centered at 264 mμ is symmetrical about the

line of zero rotation - both upper and lower limbs having equivalent amplitude. The amplitude of this Cotton effect becomes asymmetric as a result of heating or hydrolysis, as shown in Fig. 1. When the ORD is measured at 90°, the 278 mμ peak loses 75 per cent of its total amplitude, while the 250 mμ trough loses only 26 per cent of the original amplitude. Changes of the 227 mμ peak are difficult to evaluate because the original amplitude is small. Similar effects are noted as a result of hydrolysis. The effect of elevation of temperature and of alkaline hydrolysis on the ORD pattern at this lower range of the Cotton effect whose peak is centered at 198 mμ was not reported by Samejima and Yang (1965). Such a comparison is necessary in assessing the relative contributions of primary and secondary structural asymmetry to the ORD observed.

DISCUSSION

In comparing the ORD of sRNA with that of its hydrolysate, a possible source of error may be due to alteration in magnitude or sign of the optical rotation of the constituent monoribonucleotides when polymerized into a polynucleotide chain - a possibility discussed by Tinoco et al. (1963) and by Samejima and Yang (1964). This possibility seems likely to be small, judging from ORD measurements of brominated sRNA. Bromination of sRNA in aqueous solution results in bromonium ion addition to the cytosine, uracil and (to a slight extent) guanine bases of sRNA. Absorptivity of these chromophores in the region of 260 mμ is lost, but no hydrolysis occurs (Yu and Zamecnik, 1963). The ORD of sRNA in which all of the cytosine, uracil and a small fraction of the guanine residues have been brominated strongly resembles that of AMP and GMP (Lamborg et al., 1965), suggesting that the configurational Cotton effects of the monoribonucleotides do not undergo great change as a result of polymerization.

The data show that E. coli sRNA exhibits at least three Cotton

effects in the ultraviolet region of the spectrum. One Cotton effect centered at 264 mμ is due to primary and to secondary structural asymmetry. Based on amplitude changes of the 278 mμ peak, approximately 90 per cent of this Cotton effect is due to the secondary structure of sRNA. The peak at 198 mμ seems to be entirely due to secondary structural asymmetry. Samejima and Yang (1965) suggested that their 195 mμ peak might be sensitive to secondary structure, based on the hyperchromicity of absorption bands below 200 mμ. Brahms (1965) reached a similar conclusion, inasmuch as absorption bands in the region of 320-230 mμ could not account for observed positive rotations in the visible region. The data presented here provide direct evidence in agreement with these suggestions. The fact that the amplitude of the 250 mμ trough is relatively resistant to change (compared to the 278 mμ peak), coupled with the relatively flat area in the vicinity of 230 mμ (Fig. 2) suggests that the Cotton effect originally centered between 200-250 mμ is, on the other hand, due almost entirely to primary or configurational asymmetry. Samejima and Yang (1965) have recently reported a linear relationship between the cytosine + guanine content of DNA and the peak amplitude at 290 mμ. Measurement of several synthetic heterpolyribonucleotides (Lamborg, M. R. and Zamecnik, P. C., unpublished data) suggests that a similar relationship may also exist for the 278 mμ peak of sRNA. On the basis of the results presented here we would predict no linear relationship between the base composition and the 198 mμ peak.

The interesting feature of the large positive Cotton effect observed for sRNA at the 198 mμ peak is that it provides a sensitive measure of the secondary structural conformation of sRNA. Inasmuch as it is likely that changes in conformation of sRNA may accompany and may indeed play a determining role in its multiple biological functions in amino acid esterification and transfer, this ORD prop-

erty of sRNA seems propitious for future exploration of conformational changes in sRNA.

ACKNOWLEDGMENT

This work was supported by the Atomic Energy Commission Contract AT(30-1)-2643 and by grants-in-aid No. E-277 and E-278B from the American Cancer Society, Inc. and R1O 02421-11 CY from the U. S. Public Health Service. This is publication No. 1214 of the Cancer Commission of Harvard University.

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