CIRCULAR DICHROISM OF MIXTURES OF POLYRIBO-ADENYLIC AND -URIDYLIC ACIDS IN THE NEAR ULTRAVIOLET

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SUMMARY

The circular dichroism of various combinations of polyribo-uridylic and -adenylic acids in the near ultraviolet were resolved into Gaussian elements. The resolved peaks exhibited wave length shifts as a function of sample composition which were distinctly different from those of the unresolved spectra. This discrepancy is shown to result from the combination of the resolved peaks into the original spectra. Higher order structure and electronic transitions involved with these spectra are more easily rationalized in terms of the resolved spectra than the unresolved.

Synthetic homopolynucleic acids have long been used as model systems for investigating the properties of nucleic acids. Circular dichroism (CD) of these polymers have been studied with the aim of interpreting the conformation of RNA and DNA, natural and synthetic, under a variety of conditions. The present communication demonstrates that the spectra resolved into Gaussian components are more easily interpretable in terms of molecular and electronic events.

MATERIALS AND METHODS

Nucleic acid solutions were prepared which contained 8.33×10^{-5} M (base concentration) polyriboadenylic acid (poly A) or polyribouridylic acid (poly U), 5×10^{-4} M EDTA and 0.1 M sodium phosphate at pH 7.8 (1). These solutions were mixed in varying proportions to give eleven samples whose polymer content varied by 10% mole-fraction increments from 0% poly U, 100% poly A to 100% poly U, 0% poly A and whose total base content was identical. The polymers, under these conditions, were shown to form a (1:1) two-stranded complex [poly (A + U)] by the method of continuous variations (2) as applied to nucleic acids (3, 4). After formation of the poly (A + U) double-helix, any poly A or poly U in excess formed single-stranded helix (5, 6, 7) or random coil (8), respectively.

The CD of each sample was determined from 235 to 205 nm at 23°C by the use of an extensively modified (11) prototype Beckman Far UV-CD spectrophotometer, cell length 10 mm, slit 0.6 mm, in conjunction with a computer of average transients using 5 scans (12). Each resulting spectrum was resolved into non-skewed Gaussian components by the use of a Dupont Model 310 curve resolver.

RESULTS

The wave lengths of several characteristic points in the spectra varied with sample composition. The eleven spectra obtained with the different mixtures clearly illustrated the spectral shifts which occurred as a function of composition (see, for example, Fig. 1). Absolute rotational strengths of the peaks were not determined since the extinction coefficients of various polymer combinations were not known. However, comparisons are valid within the group since each sample contained an equal number of base residues.

The wave lengths of the CD maximum, minimum and crossover point for each sample are plotted as a function of sample composition in Fig. 2. With increasing mole-fraction of poly U, the CD maxima shifted toward longer wave lengths while the minima shifted toward shorter wave lengths. The cross-

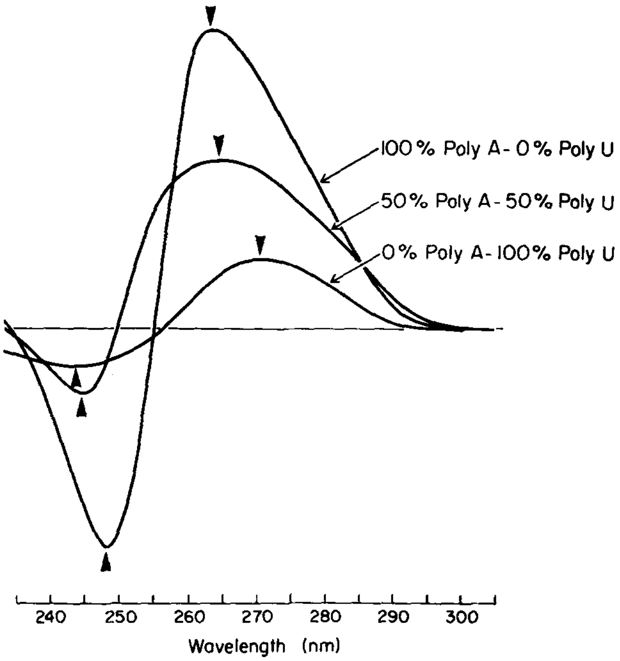

Figure 1

Circular dichroism of several polymer combinations. Arrows indicate wave lengths at maxima and minima.

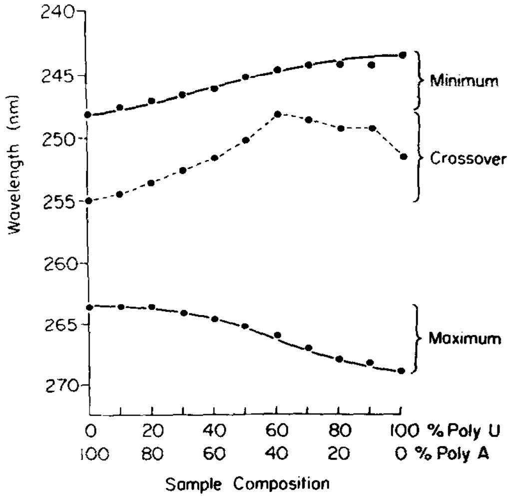

Figure 2

Wave lengths of characteristic points of unresolved spectra as a function of sample composition.

over point failed to show any regular behavior. Hashizume and Imahori (9) observed that in the CD spectrum of poly (A + U) (i.e., 50% poly A-50% poly U), "the positions of the positive and negative peaks have shifted to somewhat shorter wave lengths than those of poly A." The present findings agree with the downward shift of the minimum. The validity of the shift in the opposite direction found for the CD maximum in the present studies is supported by the smooth curve obtained for these values with a continuous range of different polynucleotide mixtures (Fig. 2).

A typical unresolved spectrum, the resolved Gaussian peaks, and the summation of the resolved peaks are shown together in Fig. 3. Since curve resolution does not necessarily provide a unique mathematical solution for this

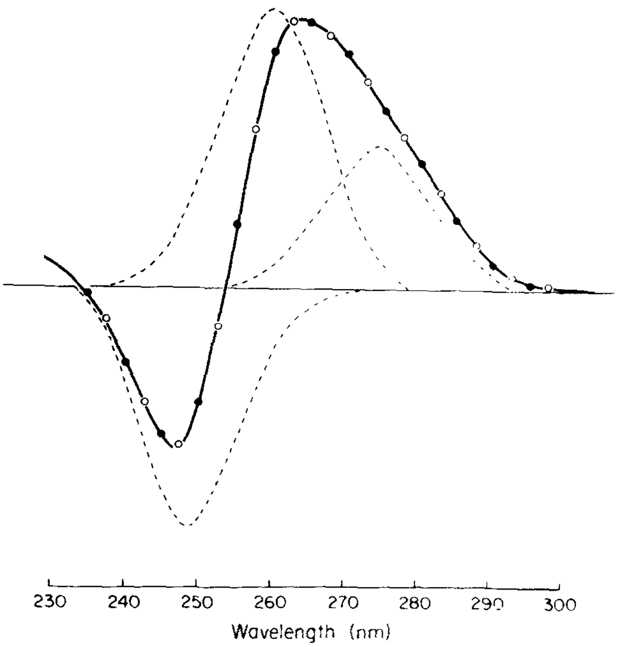

Figure 3

Circular dichroism of 80% poly A-20% poly U (closed circles) and resolved Gaussian elementals (dotted lines) showing the summation of Gaussian elementals (open circles).

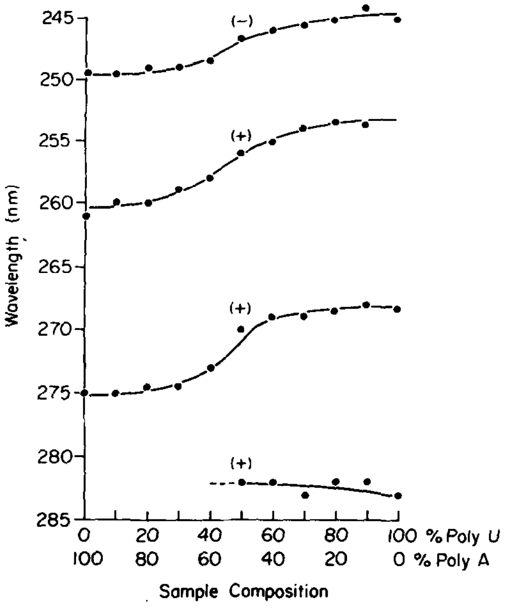

Figure 4

Wave lengths of positive (+) and negative (-) Gaussian elementals as a function of sample composition resolved from circular dichroic spectra.

situation, the spectra must be resolved into the minimum possible number of peaks to assure consistency. Using this procedure, two positive peaks and one negative peak were resolved from the spectra; an additional positive peak in the 282 nm region is discerned in the spectral analysis of solutions containing more than 50% mole-fraction of poly U.

The spectral shifts of the resolved peaks are summarized in Fig. 4.

Peak at 282 nm. This peak showed no significant wave length shift in those spectra where it appeared. Since its magnitude was very small compared to that of the other three resolved peaks, it was difficult to determine its exact wave length at maximum amplitude. This peak may occur in the other spectra but may not be detected by use of the curve resolver. Other means of investigating this peak are now being used (14).

Peak at 269-275 nm. This resolved peak showed a definite shift toward the shorter wave lengths with increasing mole-fraction of poly U. This behavior indicates that the chromophore associated with this transition was strongly affected by sample composition and/or conformation.

Peaks at 245-250 and 254-262 nm. The third (positive) and fourth (negative) resolved peaks will be considered together (10, 14). In common with the second resolved peak, both the wave lengths at maximum and minimum shifted toward shorter values with increasing mole-fraction of poly U. Thus, the single chromophore (10) underlying this transition was strongly affected by sample composition.

DISCUSSION

The summation of the Gaussian elementals into the CD spectrum indicates that the CD maximum in Fig. 1 was primarily the summation of the second (269-275 nm) and third (245-262 nm) resolved peaks in Fig. 3. The first resolved peak (282 nm) may contribute to the CD maximum in some of the spectra, but because of its small rotational strength this contribution was probably negligible. The negative resolved peak (254-262 nm) (Fig. 3) was the primary contributor to the CD minimum (Fig. 1), while moderate positive contributions to the CD minimum arose from the second (269-275 nm) and third (245-262 nm) resolved peaks. Thus, the observed CD spectrum must be reasonably considered as a complex combination of at least three resolvable Gaussian peaks.

The most striking feature of the comparison is that the maximum in Fig. 2 shifted toward longer wave lengths while none of the curves in Fig. 4 shifted in this direction. The shift toward longer wave lengths is thus a trivial consequence of the summation of at least two Gaussian peaks which, as they shifted, actually toward shorter wave lengths, varied in amplitude. This amplitude. This amplitude effect more than compensated for the shifts toward shorter wave lengths of the individual components, while the summation of the peaks resulted in an apparent shift toward longer wave lengths. Thus, the interpretation of CD spectra, especially with regard to the electronic events underlying spectral shifts in wave lengths, must be approached with extreme caution.

Studies being completed show that the spectral shifts (Fig. 4), the amplitudes of the resolved peaks (14), and the parallel behavior of the third and fourth resolved peaks, reflect the predicted excitron splitting of the π-π^* transition (10). Similarly, the response of the second resolved peak confirms the location of an n-π^* transition in poly A (13) and identifies it in poly U and poly A-poly U complexes (14). By further analysis of the amplitudes of these transitions, the total amount of double-helix, single-helix, and random coil can be estimated (14).

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