The Structure of Polyguanylic Acid and its Complex with Polycytidylic Acid[1]

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THE preparation of polyguanylic acid (poly G) has recently become possible,[2] and some physical studies on this polymer have been described.[3] It was shown that it forms a 1 : 1 complex with polycytidylic acid (poly C), in disagreement with a previous report.[4] An understanding of the helical structure and of the stability of these substances is of importance because of the crucial contributions of the G–C base pairs to the structure of ribonucleic acid. We now report the result of optical rotatory dispersion (o.r.d.) studies on these polymers over a wide pH range.

Poly G at neutral pH gives an o.r.d. curve with two peaks and a trough (the extrema are at 266, 243, and ~222 mμ; see Fig. 1) characteristic of helical polynucleotides with stacked bases.[5–7] This curve changes little as the pH is reduced; at pH 1 the extrema are at 269, 244, and ~226 mμ respectively, and the amplitude of the first Cotton effect is slightly reduced (from +394 at pH 7 to +363). Since poly G has a basic pK_a of 3·0 [3] it is evident that the helical structure survives complete protonation. On the other hand, ionization of the –NH–CO-group in poly G (pK_a = 11·3 in 0·15 M-salt[3]) leads to a complete loss of secondary structure. The o.r.d. curve at pH 12·2 (Fig. 1) shows a *small negative* Cotton effect, characteristic of purine β-mononucleotides.[8,9] This differential behaviour to protonation and ionization is probably at least partly due to the fact that, except in strong acid, the phosphate groups carry a negative charge, and additional negative charges in the polymer lead to repulsion effects and disruption of the secondary structure, whereas positive charges can be accommodated. Poly(7-methylguanylic acid), for example, has a stable secondary structure at pH 7, although all the bases carry a positive charge at this pH.[10]

The o.r.d. of poly C has been studied before, though only over very limited ranges of wavelength and pH.[6,11] We have found that the single-stranded helical form of poly C previously observed at neutral pH is quite stable in alkali (see curve at pH 12·2, Fig. 1). In acid solution, poly C is known to form a two-stranded helix stable from pH 5·7 to pH 3·0,[12] and this appears to persist down to pH 2 (see Fig. 1). The extrema at this pH [301, 271, 250—240 (shoulder), and 232 mμ] are very similar to those at pH 4·1 (302, 273, 252, 240, and 233 mμ), though the amplitude of the first Cotton effect (peak 301, trough 271) is reduced from +980 to +826. At pH 1 the secondary structure has disappeared, some poly C comes out of solution and the o.r.d. now shows only a plain positive curve (down to 220 mμ).

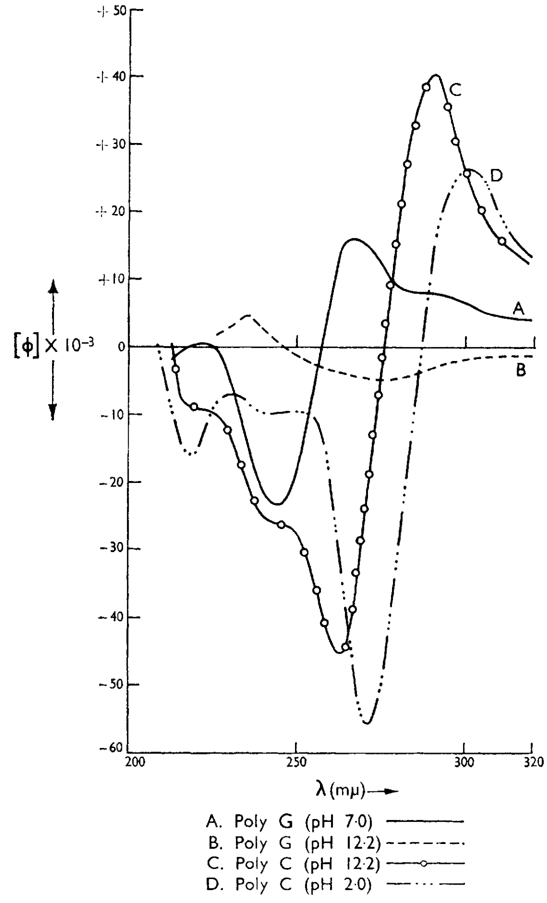

FIGURE 1

The o.r.d. curves of the 1 : 1 complex poly G + C (Fig. 2) indicate that a complex exists over the pH range 1—12·2. The curve at pH 12·2 is similar to that shown for pH 7·0, but the amplitude of the first Cotton effect (peak 287, trough 260) is reduced from +285 to +175, indicating that at pH 12·2 the complex is undergoing structural change. The pK_a of guanine in poly G + C is 12·27[3] and the curve at pH 13·0 clearly shows that the complex has dissociated and that the species now present are poly C (single-stranded; *cf.* the poly C pH 12·2 curve, Fig. 1) and poly G (coil). Since there is half as much poly C in this solution as in the poly C solution at pH 12·2, the amplitude of the first Cotton effect should be about half (actual values: +403 and +859. The negative poly G curve will reduce the amplitude of the mixture slightly). At acid pH, different extrema are found. The curve at pH 3·4 (see Fig. 2) is clearly different from those of the separate polynucleotides, as is that at pH 1, indicating interaction between the two polymers. This interaction is reduced at pH 1, as shown by the low amplitudes of the Cotton effects observed.

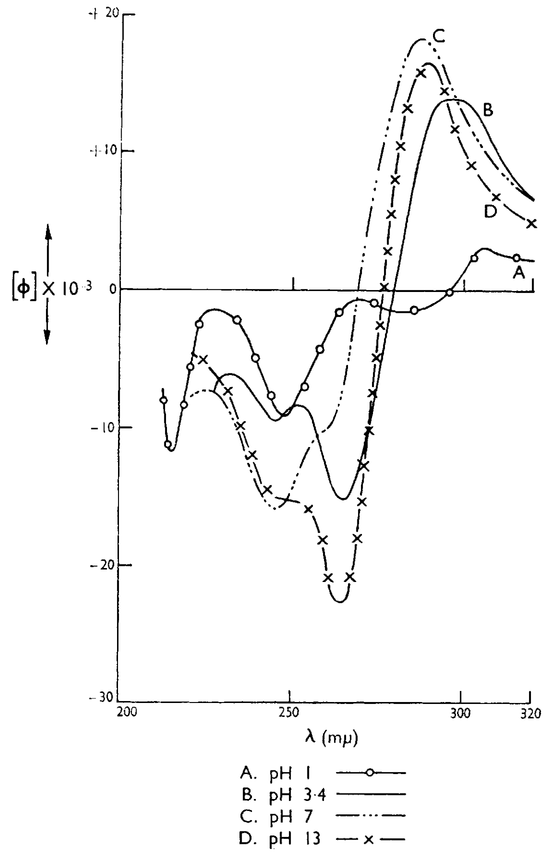

FIGURE 2

The o.r.d. curves of poly G + C differ from those of the constituent polynucleotides over a wide range of pH, and the shape of the curves indicate that a helical complex is present which is remarkably stable to these changes in pH, and which is probably similar to the complex of poly C with polyinosinic acid.[13]

All the o.r.d. measurements were made at a total ionic strength of 0·15 M, with the Bellingham and Stanley/Bendix-Ericsson Spectropolarimeter "Polarmatic '62", at room temperature.

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