INTERACTION OF POLYURIDYLIC ACID AND SPERMINE*

G.T. Rogers, T.L.V. Ulbricht, and W. Szer

Twyford Laboratories, Elveden Road,
London, N.W.10., England

and

Institute of Biochemistry and Biophysics,
Polish Academy of Sciences, Warsaw, Poland.

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It has been known for some time that spermine stabilises DNA and certain double-stranded polynucleotide complexes to thermal denaturation (Tabor, 1962; Mandel, 1962; Szer, 1966a; Matsuo and Tsuboi, 1966; Higuchi and Tsuboi, 1966). Spermine also interacts with turnip yellow mosaic virus RNA to make it more compact (Mitra and Kaesberg, 1963) and recently has been shown to stabilise s-RNA to thermal denaturation (Goldstein, 1966). The interaction of spermine, as well as other di- and polyvalent cations, with polymer phosphate groups has been termed "strong" (Felsenfeld, 1962) since it proceeds stoichiometrically, in contrast to the "weak" backbone charge neutralization effected by monovalent cations. In addition, spermine exerts a specific stabilizing effect as compared to divalent metal ions. It is not clear, however, why the increase in T_m of DNA brought about by spermine is related to the AT content of DNA (Mandel, 1962).

One of us has recently reported that spermine was the most effective of a number of di- and poly-amines (and considerably more effective than Mg^{++}) in stabilising poly U and polyribothymidylic acid (Szer, 1966a;

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* Optical Rotatory Dispersion of Nucleic Acid Derivatives, Part IX. Part VIII: Emerson et al, 1967.

1966b). In the presence of one equivalent of spermine, for example, polyuridylic exhibits considerable hypochromism, its T_m is raised, and the thermal transition profile is much sharper than in poly U itself. This effect was observed in dilute solution, under conditions where aggregates were not formed (no absorption at 340mμ). We now report results on the pH-dependence of the poly U-spermine interaction, and on the optical rotatory dispersion (ORD) of the complex.

Experimental The poly U was a commercial sample (Sigma) which was further purified by a method based on Kirby's procedure (Kirby, 1962, 1965) to remove any traces of protein, and low molecular weight oligomers. The following buffers were used: 0.01M phosphate, pH 5.5, 7.0 and 8.5; no additional salt was added. Crystalline spermine tetrahydrochloride

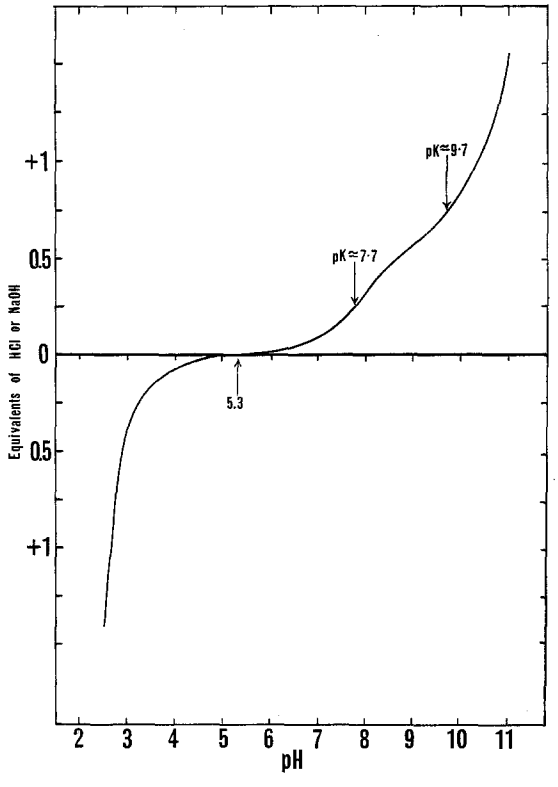

Figure 1. Titration of spermine

(Koch-Light Laboratories) was dissolved in water and titrated to the appropriate pH. The complete titration (fig. 1) was carried out using a Radiometer (Copenhagen). ORD Measurements were made with the Bellingham and Stanley/Bendix-Ericsson recording spectropolarimeter "Polarmatic '62" as described elsewhere (Emerson et al, 1967).

Results Titration of spermine (fig. 1) shows that, as is to be expected, the pK_a's are close together ($\sim$7.7, 9.7). From the curve it appears that spermine carried four protons at pH $\sim$5.3 (the neutralisation point), two at pH $\sim$8.5 (at which point half an equivalent of acid or alkali have been added) and an average of three at pH $\sim$7.0.

On examining the interaction of spermine with poly U at different pH values, a hypochromic effect (31%) was observed at 20° at pH 5.5 and at pH 7.0, but not at pH 8.5. The ORD results confirm this (Table 1). The ORD of poly U itself is unchanged in the pH range 5.5 - 8.5, and

Table 1

Sample (20°C unless otherwise stated)	1st Peak, λ	1st Trough, λ	Mean residue amplitude a
Poly U pH 5.5	286	260	332
" " pH 7.0	286	260	335
" " pH 8.5	286	260	330
Poly U + Spermine, pH 5.5	286	256	1,320
" " " pH 7.0	286	256	1,220
" " " pH 8.5	286	260	328
Poly U, pH 5.5, 45°	286	260	240
Poly U + Spermine, pH 5.5, 45°	290	263	216
" " " pH 5.5, heated to 45° and cooled to 20°	286	256	1,180

spermine has no effect on the ORD of poly U at pH 8.5. (See figure 2.) However, at pH 5.5 and 7.0 the mean residue amplitude of the first (long wavelength) Cotton Effect of poly U is increased fourfold in the presence of one equivalent of spermine at 20° (Table 1 and figure 2). There is no further change on adding a second equivalent of spermine.

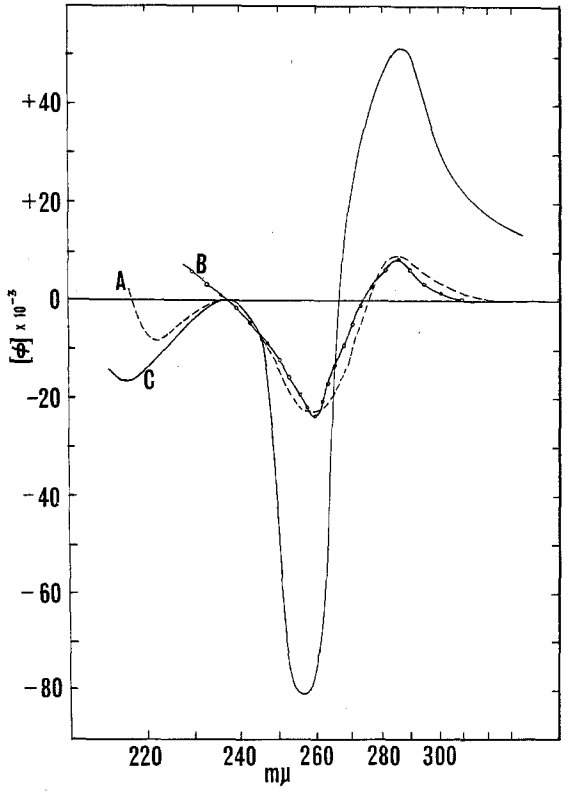

Figure 2. A (- - - -) ORD of poly U, pH 5.5. B (-O-O-O-) ORD of poly U + spermine, pH 8.5. C (————) ORD of poly U + spermine, pH 5.5.

The ORD of poly U at 45° shows a reduction in amplitude (Table 1 and figure 3). At this temperature, which is significantly above the T_m (28°) of the poly U-spermine complex (Szer, 1966), spermine has no effect on the ORD of poly U, but when the solution is cooled to 20° the same increase in amplitude is again observed (figure 3).

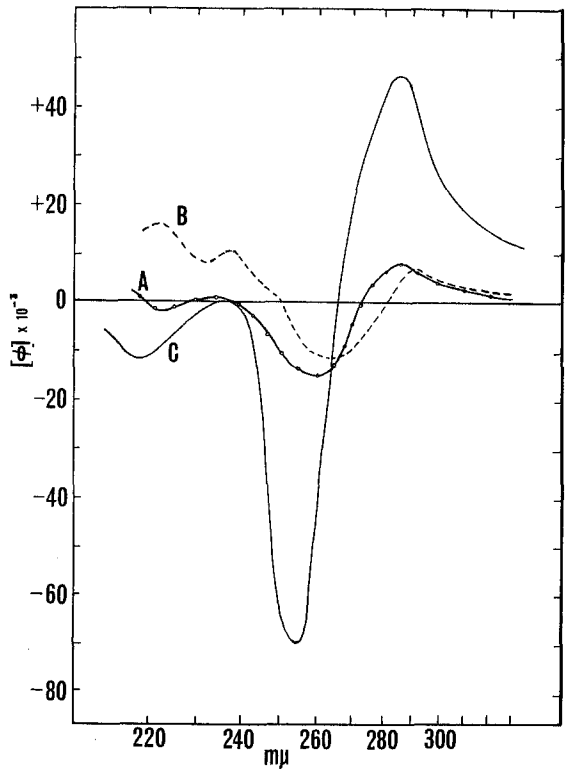

Figure 3. A (-O-O-O-) ORD of poly U, pH 5.5, 45°. B (- - - -) ORD of poly U + spermine, pH 5.5, 45°. C (————) ORD of poly U + spermine, pH 5.5, heated to 45° and cooled to 20°.

Discussion It is interesting to note that the amplitude of the first Cotton Effect in poly U-spermine at pH 5.5 - 7.0 is considerably higher than that of single stranded polynucleotides which have appreciable helical character, such as poly C (Ulbricht et al, 1966), poly A (Michelson et al, 1966), and poly U at low temperature and high salt (Michelson and Monny, 1966), and of similar magnitude to that of double-stranded poly A in acid (Michelson et al, 1966). This fact, together with the sharpness of the thermal transition observed by Szer (1966a, 1966b), which indicates a co-operative melting, suggests that the poly U-spermine complex may involve a double-stranded poly U structure. At the least, one can say

that the structure must exhibit a higher degree of base-stacking than any known single-stranded polynucleotide, since the double Cotton Effect is a measure of base stacking (Warshaw and Tinoco, 1966). We are investigating the structure of the complex by X-ray diffraction.

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