Interaction between acridine orange and polyriboadenylic acid

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The absorption spectra and circular dichroism of aqueous solutions of acridine orange mixed with poly(riboadenylic acid) [poly(rA)] have been measured for different mixing ratios at acid and neutral pH. The binding ratio of dye to poly(rA) has been determined by equilibrium dialysis. At acid pH where poly(rA) is in a double-stranded helix, monomeric dye molecules are intercalated between base pairs, first sparsely and then at neighbouring sites with mutual coupling, as the nucleotide-to-dye mixing ratio decreases. In the presence of excess dye, dimeric dye molecules of antiparallel type are bound to phosphate groups electrostatically and stack together to form linear sequences along a poly(rA) chain. At neutral pH where poly(rA) is single-stranded, isolated intercalation of monomeric dye molecules can occur in the helical parts. At intermediate mixing ratios, half-intercalated dimeric dye molecules are bound to adjacent sites and electronically coupled, inducing characteristic circular dichroism. In the presence of higher amounts of dye, external stacking of dimeric dye molecules of antiparallel type occurs along a poly(rA) chain. The binding of dye cations is suppressed to some degree at acid pH compared to that at neutral pH, owing to the repulsion exerted by protonated adenine bases.

Introduction

Poly(riboadenylic acid) [poly(rA)] in aqueous solution undergoes conformational changes induced by changes of pH and temperature[1-11]. It has a parallel double-stranded helical conformation at acid pH, in which base pairs are formed and their molecular planes slightly tilt from the direction perpendicular to the helix axis[12]. The double-stranded helix in solution melts into random coils above a certain definite temperature[5-7]. At neutral pH poly(rA) is single-stranded and its helical parts are stabilized by pairwise stacking interactions between adjacent bases[5,8]. With rising temperature, its helical parts gradually convert into random coils[5-8].

When acridine dye is added to an aqueous solution of poly(rA), it interacts with the poly(rA) in different ways, depending on the pH of the solution or the state of ionization of the adenine bases. Acridine orange has an absorption band at 502 nm at high [P]/[D] (nucleotide residue/added dye) molar mixing ratios, while it has a band around 465 nm at low [P]/[D] ratios[13,14]. The shift of absorption band occurs at a different value of [P]/[D] ratio, depending on whether the pH of solution is acid or neutral, as was first found by Bradley and Wolf[13]. Extrinsic Cotton effects of proflavin induced in the presence of poly(rA) were investigated by Blake and Peacocke[15]. Whereas the visible absorption band of proflavin exhibits Cotton effects at acid pH, no anomalous rotatory dispersion has been observed at neutral pH. Furthermore, some studies on the binding process, including its kinetic behaviour, of acridine dye with poly(rA) were performed more recently[16-19].

It is known that acridine dye binds with polynucleotides through two types of binding interaction[20]. One of them is strong binding, in which a dye molecule is inserted between two neighbouring bases of a polynucleotide chain, and the other is weak binding caused by electrostatic attraction between a dye cation and phosphate ion. The two kinds of binding modes should influence the absorption spectra and circular dichroism of bound dye, and the induced optical activity of acridine orange interacting with DNA[21-27] and RNA[28,29] has been investigated in great detail. We have also carried out some calculation on the induced circular dichroism of acridine orange bound to DNA, assuming plausible structure of complexes composed of sequences of intercalated dye molecules or of externally bound dimeric dye molecules[30].

In this work we will study the binding interaction of acridine orange with poly(rA) by measurements of absorption spectra, circular dichroism and equilibrium dialysis. and propose possible binding modes of the dye with poly(rA) at acid and neutral pH.

Experimental

Poly(rA) as K salt was purchased from P-L Biochemicals Inc., lot number 441102, and used without further purification. Acridine orange was purified as previously described[31], and it has a molar extinction coefficient, $\varepsilon_{496} = 6.76 \times 10^4$ l mol^{-1} cm^{-1}, in chloroform. Solutions were prepared by the method similar to that given in a previous paper[32]. A stock solution of poly(rA) in 0.001 M NaCl was made up, and its concentration was determined spectroscopically by using the residue extinction coefficient, $\varepsilon_{257} = 1.01 \times 10^4$ l mol^{-1} cm^{-1}, at pH 7 in 0.001 M NaCl[3,5]. The pH was adjusted by HCl or NaOH solutions,

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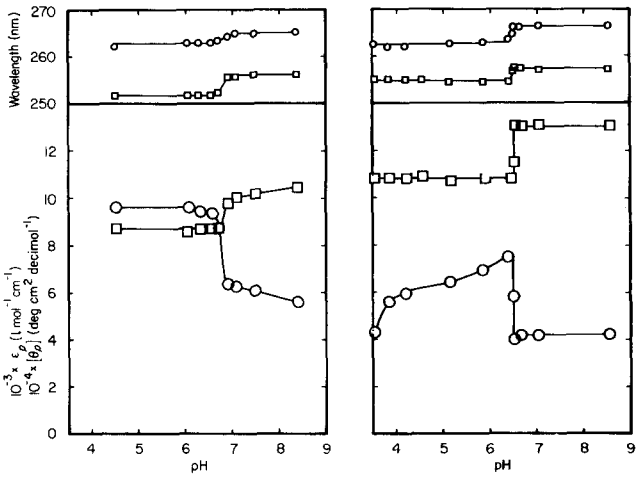

Figure 1 The pH dependence of the ultraviolet absorption and circular dichroic bands for solutions of poly(rA) with and without acridine orange. Left: poly(rA) of 2.05×10^{-4} N in 0.001 M NaCl at 25°C; right: acridine orange–poly(rA) mixture of [P]/[D] = 9, $[D] = 2.00 \times 10^{-5}$ M, in 0.001 M NaCl at 25°C. □, absorption; ○, circular dichroism

followed by the addition of a stock solution of dye. The NaCl concentration in the final mixed solution was kept to 0.001 M in most cases and the concentration of acridine orange was 2.00×10^{-5} M in all the experiments. The pH measurements were carried out by a Hitachi–Horiba $F\text{-}7_{SS}$ pH-meter after spectroscopic measurements.

The absorption spectra and circular dichroism were measured on a Shimadzu UV-200S spectrophotometer and a Jasco J-20 circular dichrometer, respectively. Quartz cells having path length, 20, 5 and 1 mm, were used. Temperature of sample cell chambers was adjusted to 25°C in most cases, by circulating water of constant temperature from a Haake or Tamson Thermobath. Residue extinction coefficient, ε_P, and residue ellipticity, $[\theta_P]$, were expressed on the basis of residue mole of poly(rA), and molar extinction coefficient, ε_D, and molar ellipticity, $[\theta_D]$, were based on the total number of mols of dye added.

Equilibrium dialysis was carried out by modifying the method described by Fredericq and Houssier[27]. The dialysis cell consisted of two Teflon vessels, each having a cylindrical compartment of radius 1 cm and a depth of 1 cm, and the vessels were separated by a sheet of Visking membrane. The Visking membrane was used after being boiled in a 1% $NaHCO_3$ solution for 1 h and rinsed with distilled water[33]. A mixed solution of dye and poly(rA) was put in the inner compartment, and an NaCl solution of the same pH and NaCl concentration was put in the outer compartment. The cell was kept for 60 h in a water bath at 25°C with intermittent rocking and the time for equilibration was checked. After having reached the dialysis equilibrium ethanol was added to the solution in each compartment to make the ethanol concentration 60% (v/v), and the total dye concentration in each compartment was determined spectroscopically from a calibrated curve.

For the construction of a binding isotherm, the initial dye concentration was always kept at 2.00×10^{-5} M and the poly(rA) concentration was varied in such a way as to give the desired [P]/[D] value. However, it was found that considerable amounts of dye were adsorbed on Visking membrane, especially when the amount of unbound dye was high, i.e. when [P]/[D] was 0.6. Thus it is not necessarily certain whether or not a [P]/[D] value in the dialysis experiments corresponds accurately to that in the optical measurements.

The molar concentration of unbound dye in the inner compartment, i.e. in equilibrium with poly(rA), was assumed to be equal to that of free dye in the outer compartment, since the compartments have an identical volume. The total molar concentration of dye in the inner compartment was obtained by direct determination. The mole of bound dye was derived from their difference, and the molar ratio of bound dye to total dye, f'_b, was used to represent the results. Correction for the Donnan effect was not applied, since the binding was almost complete for [P]/[D] close to 10 and the correction was negligible at [P]/[D] around 1.

Results

Figure 1 shows the pH dependence of ultraviolet absorption and circular dichroic bands for the solutions of poly(rA) with and without acridine orange. The absorption spectra of poly(rA) solution of concentration 2.05×10^{-4} N have an absorption band at 252 nm when the pH is lower than 6.8, while the band shifts to 256 nm when the pH is neutral. The residue extinction coefficient of the band also undergoes a sudden change at pH 6.8. The circular dichroism also changes with pH in a corresponding way. An aqueous solution of poly(rA) at acid pH has a pair of dichroic bands in the ultraviolet region, i.e. a strong positive band at 262 nm and a weak negative band at 242 nm. When the pH is raised beyond 6.8 the positive dichroic band shifts to 265 nm and becomes weaker while the negative band also shifts to the red but becomes stronger; in addition, a shoulder around 275 nm disappears and a positive band appears at 220 nm[6]. Similar spectral changes, which occur at pH 6.5 in this case, are observed in the ultraviolet region for solutions of poly(rA) mixed with acridine orange at [P]/[D] = 9. They can be attributed to the transition of poly(rA) from a double-stranded helix to single strands.

The visible absorption spectra of the dye–poly(rA) solutions at acid pH are shown in *Figure 2* for several [P]/[D] values. The absorption band is located at 506 nm for [P]/[D] = 300. With decreasing [P]/[D] value, the band shifts to the blue and becomes weaker, but an isosbestic point is present at 491 nm down to [P]/[D] = 50. For [P]/[D] = 10, the band is at 495 nm and a stronger band appears at 470 nm. The band at 506 nm can be assigned to an intercalated monomeric dye molecule, as in the case of binding to DNA[27,34], and the band at 470 nm is associated with a dimeric dye molecule. For [P]/[D] = 2 the observed hypochromism is the strongest. However, the spectra for [P]/[D] = 0.6 resemble those of free acridine orange, indicating that the amount of bound dye is low.

For the dye–poly(rA) solution is induced the circular dichroism in the visible region, as shown in *Figure 3*. A negative dichroic band is located at 501 nm for [P]/[D] = 300. It is noticed that the band shapes of the absorption and circular dichroism are identical, which supports the assignment to the single intercalation. Solutions of [P]/[D] = 100 and 50 exhibit, besides a negative band at 496 nm, a pair of bands i.e. a weak positive band at 522 nm and a strong negative band at 478 nm. The pair of bands

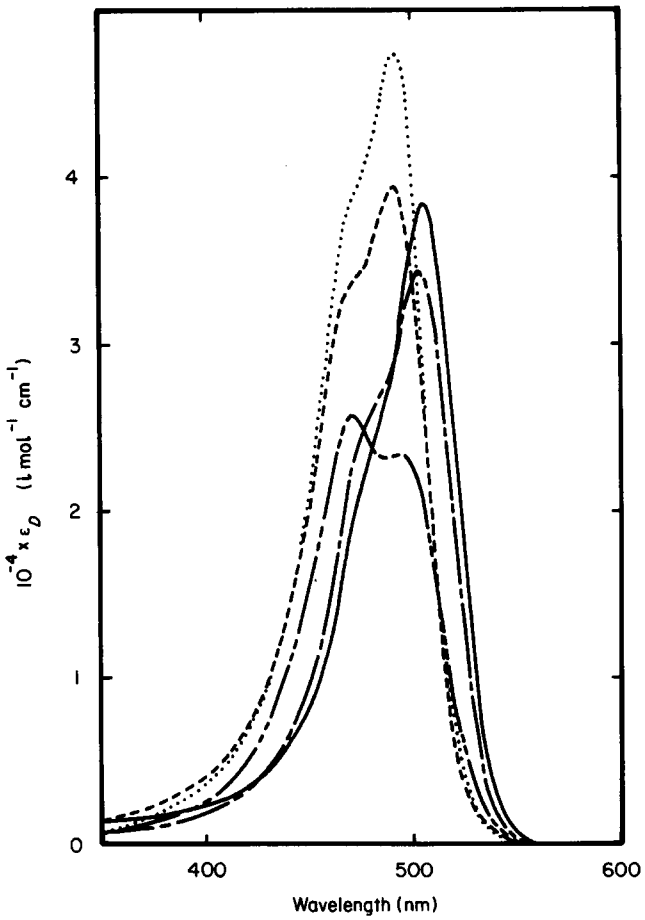

Figure 2 Visible absorption spectra of acridine orange–poly(rA) mixtures in 0.001 M NaCl at acid pH. —, [P]/[D] = 300 (pH 4.39); – –, [P]/[D] = 50 (pH 4.31); – – –, [P]/[D] = 10 (pH 4.32); – – –, [P]/[D] = 0.6 (pH 4.07); ···, free acridine orange

can be attributed to the electronic coupling of monomeric dye molecules intercalated at the adjacent sites. For [P]/[D] = 10 and 2 the strong negative band at 478 nm shifts to the blue and the negative shoulder at 496 nm becomes weaker. A solution of [P]/[D] = 0.6 has a negative dichroic band at 470 nm and a positive band at 435 nm, both being weak.

At neutral pH different absorption behaviour is observed for the dye–poly(rA) solutions, as shown in *Figure 4*. For [P]/[D] = 609 the band at 502 nm indicates intercalation of a monomeric dye molecule. For [P]/[D] = 41 and 9 a strong band is observed at 462 nm which can be assigned to dimeric dye molecules. An isosbestic point is manifest at 470 nm over [P]/[D] ratios from 609 to 2, and this means that an equilibrium exists between intercalated monomers and dimeric molecules of dye. Negligible amounts of free dye are left, as demonstrated by the equilibrium dialysis below. However, the spectrum for [P]/[D] = 0.6 does not pass any more through the isosbestic point but strong hypochromism of the band for dimeric dye occurs. This suggests strong interaction between bound dimers or the formation of their stacked sequences.

The visible circular dichroism of the dye–poly(rA) solutions at neutral pH is shown in *Figure 5*. For [P]/[D] = 609 a positive band is induced at 513 nm. Again the band shapes of the absorption and circular dichroism are identical, suggesting the single intercalation. The circular dichroism of the solutions of [P]/[D] from 180 to 2 is characterized by a conservative pair of bands, i.e. a positive band at 477 nm and a negative band at 455 nm, accompanying a weaker band at 502 nm. The conservative pair centred at 467 nm may be associated with an electronically coupled pair of dimeric dye molecules. A solution of [P]/[D] = 0.6, however, exhibits a weak negative band at 475 nm and a weak positive band at 442 nm; the spectrum is similar to that observed for the same solution at acid pH.

All the results given above were obtained with solutions in 0.001 M NaCl. In the presence of 0.1 M NaCl, the dye–poly(rA) solutions at acid pH precipitates, but the solutions were stable at neutral pH and their spectra were examined. While the increase in NaCl concentration scarcely influences the absorption and circular dichroism for [P]/[D] = 9 at neutral pH, it markedly alters both spectra for [P]/[D] = 0.6. For the low [P]/[D], the absorption of free dye becomes stronger and the sign of dichroic bands is reversed by the increase in ionic strength.

Thus the binding of dimeric molecules for [P]/[D] = 0.6 at neutral pH is primarily electrostatic, owing to the attraction of dimeric dye cations with phosphate ions, and their sequences are formed by stacking interactions between bound dimers. The dimer molecule of dye would have an antiparallel stacked structure, as originally suggested by Zanker[35]. The same mode of binding would hold for [P]/[D] = 0.6 at acid pH, as will be seen below.

On the other hand, for [P]/[D] from 41 to 2 at neutral pH the binding of dimeric dye molecules is less electrostatic and, as was shown above, the circular dichroism is composed of a characteristic pair of dichroic bands. Thus the dimeric dye molecule referred to here should be

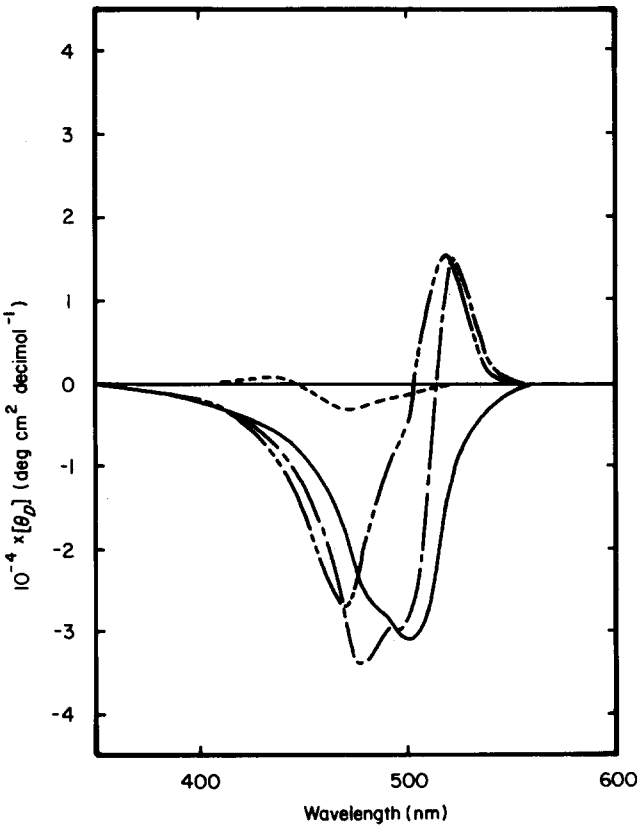

Figure 3 Visible circular dichroism of acridine orange–poly(rA) mixtures in 0.001 M NaCl at acid pH. Legends are the same as in *Figure 2*

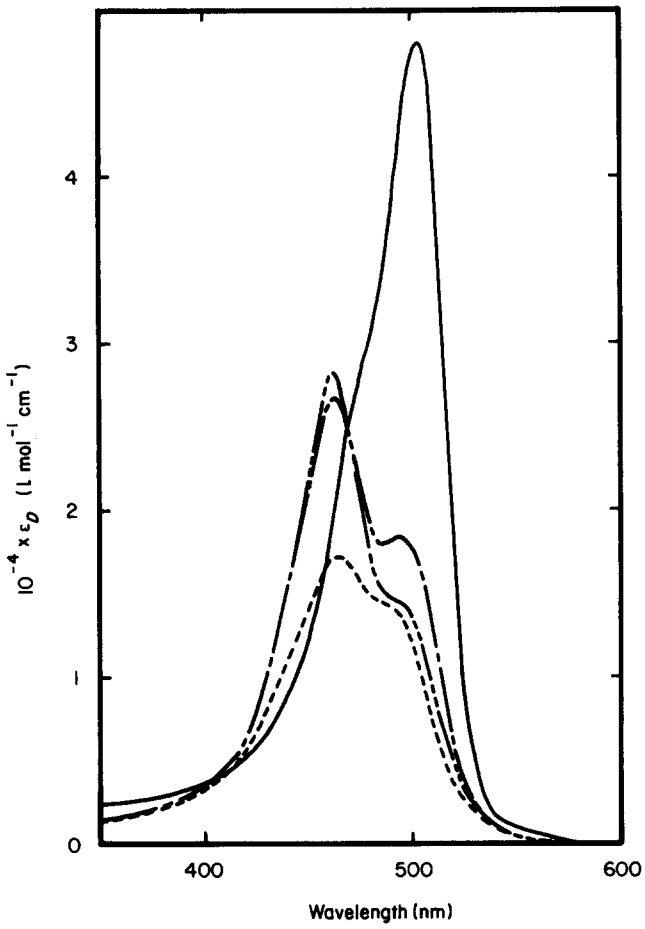

Figure 4 Visible absorption spectra of acridine orange–poly(rA) mixtures in 0.001 M NaCl at neutral pH. —, [P]/[D] = 609 (pH 6.77); – –, [P]/[D] = 41 (pH 7.20); – – –, [P]/[D] = 9 (pH 7.15); – – –, [P]/[D] = 0.6 (pH 7.30)

distinguished from that forming external stacked sequences at low [P]/[D] ratios. Such a bound dye molecule would have a partially stacked structure consisting of a partly intercalated monomer molecule and another monomer molecule incompletely overlapping with it. It might be similar to the dimer species first proposed by Armstrong *et al.*[34] and, subsequently, by Fredericq and Houssier[27] for the binding to DNA. We will call this dimeric molecule a half-intercalated or a partially intercalated dimeric molecule.

In *Figure 6* the molar ratio of bound dye to total dye, f'_b, is plotted against the [P]/[D] ratio. At neutral pH almost all dye molecules are bound to poly(rA), except in solutions of [P]/[D] < 1, but at acid pH a significant amount of unbound dye molecules is generally present in solution. Consequently, the solution of [P]/[D] = 0.6 should show hypochromism at the visible band even at acid pH as it does at neutral pH, if the absorption spectrum is corrected for the amount of bound dye.

Furthermore, it was found that the binding of dye is somewhat lower at acid pH than at neutral pH. The suppression of binding at acid pH would be mainly caused by the electrostatic repulsion exerted by protonated adenine bases of poly(rA), as the potentiometric titration has shown a strong buffering action at pH 6.8 in 0.001 M KCl[9].

Absorption spectra and circular dichroism of the dye–poly(rA) solution of [P]/[D] = 10 at neutral pH were measured at three different temperatures, 5, 25 and 70°C. The absorption band at 258 nm becomes gradually stronger at higher temperatures, but the two bands at 492 and 462 nm undergo abrupt changes at 70°C, indicating desorption of bound dye.

All the circular dichroic bands become weaker at higher temperatures, as illustrated in *Table 1*. The molar ellip-

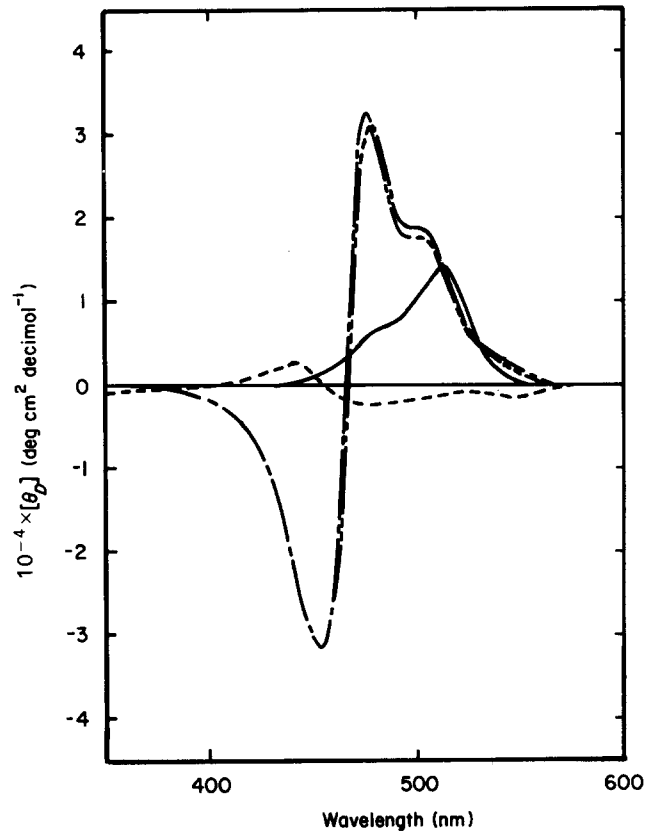

Figure 5 Visible circular dichroism of acridine orange–poly(rA) mixtures in 0.001 M NaCl at neutral pH. Legends are the same as in *Figure 4*

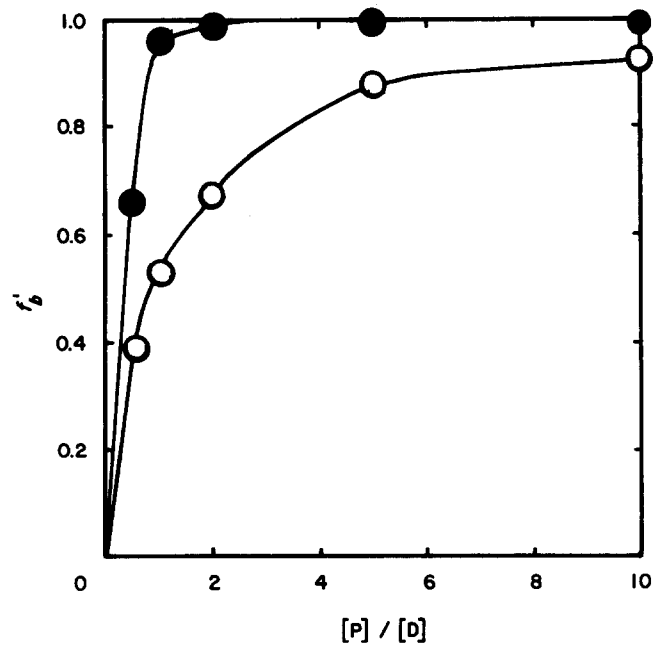

Figure 6 Binding isotherms of acridine orange on poly(rA) for low [P]/[D] ratios in 0.001 M NaCl at 25°C. [D] = 2.00×10^{-5} M ○, pH 4.0–4.2; ●, pH 6.8–7.0

Table 1 Effect of temperature on the circular dichroic bands for [P]/[D] 10 at pH 6.77

Temperature (°C)	λ (nm)	$[\theta_P]$	λ(nm)	$[\theta_D]^a$	λ (nm)	$[\theta_D]^a$	λ (nm)	$[\theta_D]^a$	$F_h{}^b$ (%)
5	265	56900	503	24000	478	40000	456	−39400	85
25	265	45100	503	22300	478	37400	456	−38500	68
70	268	18700	503	6000	476	14400	453	−19800	27

[a] The molar ellipticity $[\theta_D]$ was calibrated on the basis of mole of bound dye
[b] The helical content F_h was estimated from the data in *Figure 7* of Brahms *et al.*[7]

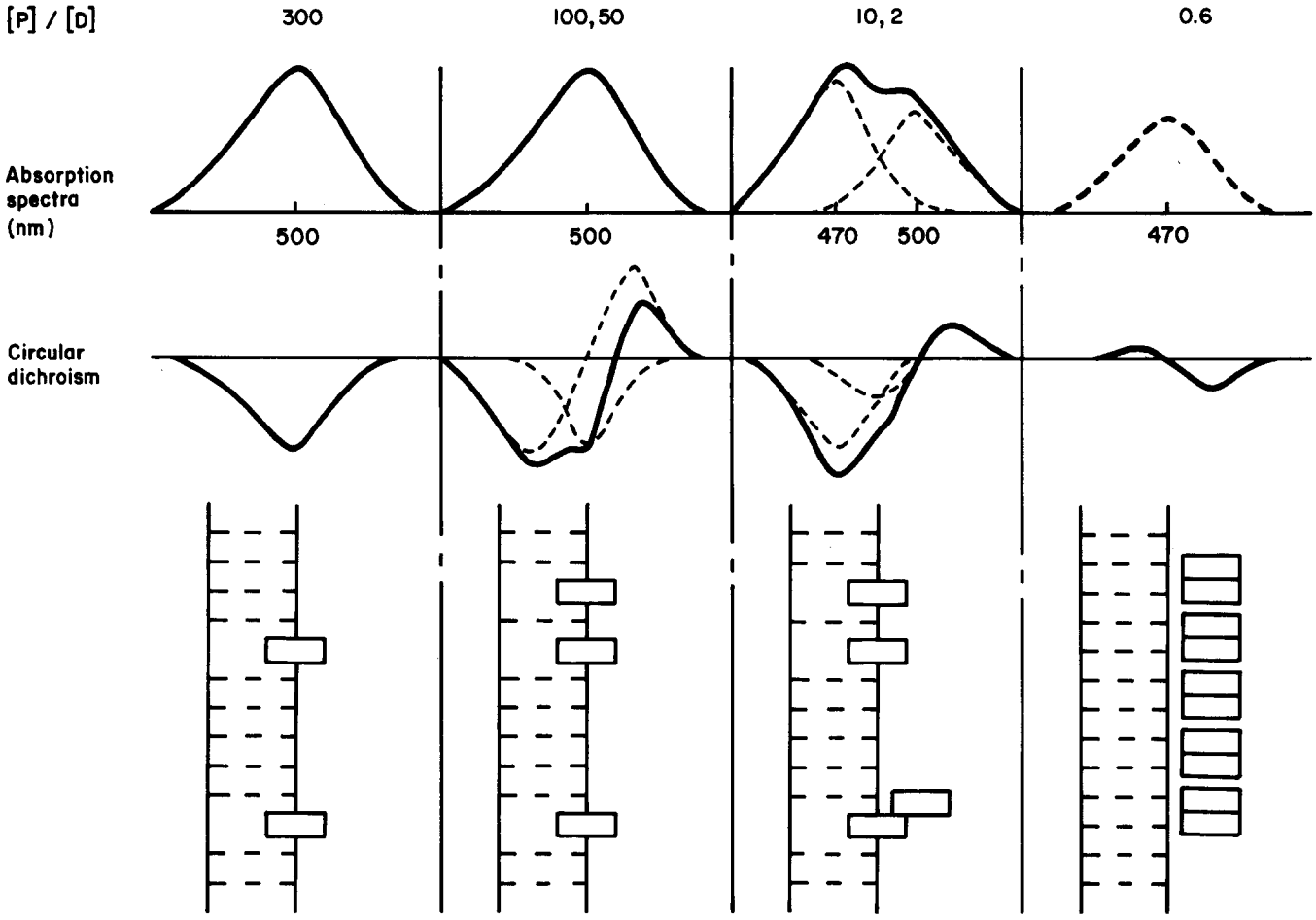

Figure 7 Proposed structural scheme for the mode of binding of acridine orange on poly(rA) at acid pH

ticity of visible dichroic bands associated with bound dye was calibrated for the number of mols of bound dye obtained in the following way. If the fraction of bound dye is f_b, the molar extinction coefficient at 492 nm may be given by $\varepsilon = f_b\varepsilon_b + (1-f_b)\varepsilon_u$, where ε_b and ε_u are the molar extinction coefficients of bound and unbound dyes. The value of ε_b was taken as 14300 from the absorption spectrum at 5°C, and the value of ε_u was 47200 as observed for free acridine orange. Then f_b can be evaluated from the observed spectrum, and, if multiplied by [D], the mole of bound dye per litre of solution is obtained.

The helical content, F_h, of single-stranded poly(rA) listed in *Table 1* was estimated from the equilibrium constant K for the helix–coil transition, where $K = (1-F_h)/F_h$. Values of K at given temperatures were read from Figure 7 of Brahms *et al.*[7]. The residual ellipticity of a dichroic band around 265 nm for the dye–poly(rA) solution was found to be proportional to the helical content. However, the molar ellipticity of visible dichroic bands is not necessarily proportional to the helical content but diminishes less sharply than it does, as the temperature rises.

Discussion

The conformational transition of poly(rA) between single-stranded helices and a double-stranded helix has been observed to occur at pH 6.8 in 0.001 M NaCl and it was found that the transition was not influenced very much by the addition of acridine orange even to [P]/[D] as low as 9, for which the transition pH was 6.5.

The absorption spectra of the dye–poly(rA) solutions of different [P]/[D] values have shown that at least three kinds of binding species are present for bound acridine orange irrespective of whether the poly(rA) helix is double-stranded or single-stranded. One is monomeric molecules participating in the strong binding at high [P]/[D] ratios and intercalated between adenine bases.

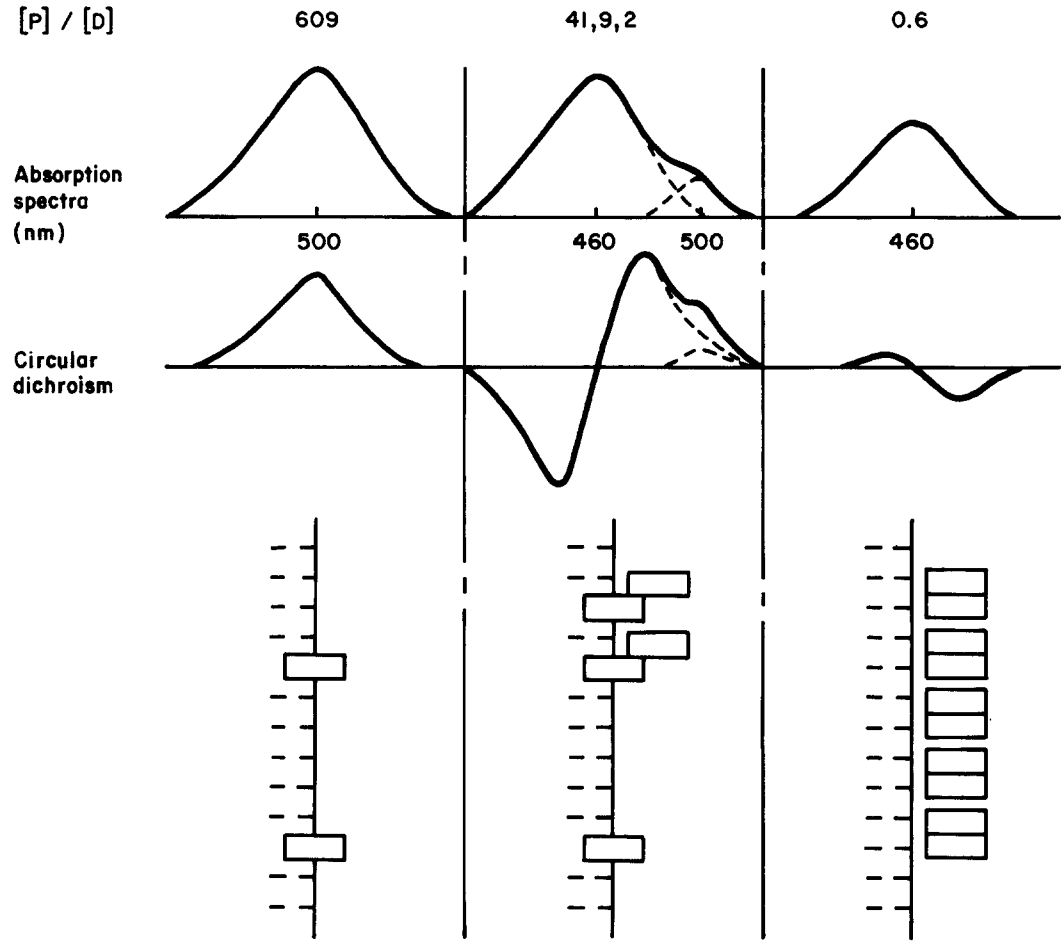

Figure 8 Proposed structural scheme for the mode of binding of acridine orange on poly(rA) at neutral pH

The other is half-intercalated dimeric molecules which appear at intermediate [P]/[D] ratios. These two dye species are in mutual equilibrium, unless [P]/[D] is very low. For very low [P]/[D] ratios dimeric dye molecules of antiparallel type[35] bind electrostatically with phosphate groups and form stacked sequences externally along a poly(rA) chain. It should be noted that the binding species for external association are not monomeric molecules but are dimeric molecules of antiparallel type.

For the induction of optical activity of a symmetrical dye bound to polynucleotide, two types of mechanism have been considered[20]. In the first mechanism a bound dye molecule undergoes a dissymmetric perturbation from the bases in helical conformation through the dipole–dipole interaction. The other mechanism is caused by the electronic coupling of monomeric or dimeric dye molecules bound to neighbouring sites, in which the dye molecules are arranged in a helical way[30]. The first mechanism induces a single circular dichroic band at the absorption band of dye, while the second one gives rise to a pair of dichroic bands centred at the absorption band of the dye.

Examination of the circular dichroism based on the two mechanisms has revealed the modes of binding involved in more detail. Acridine orange is bound to poly(rA) in one of the following five stages: a sparsely intercalated monomer, an electronically coupled pair of intercalated monomers, a half-intercalated dimer, an electronically coupled pair of half-intercalated dimers, and a stacked sequence of externally bound dimers. The binding schemes at different [P]/[D] ratios are illustrated in *Figure 7* for the case at acid pH and in *Figure 8* for the case at neutral pH.

At acid pH where poly(rA) is double-stranded and helical, the binding process of dye would be similar to that for DNA[27,30].

(a) When [P]/[D] is 300, the absorption band and the negative circular dichroic band is located around 506 nm, and a monomeric dye is intercalated between adjacent bases on the same poly(rA) chain. Strong stacking interaction between dye and bases must be operative for the binding, and the intercalation should occur in such a way as to reduce possible electrostatic repulsion between dye cation and charged adenine bases.

(b) For [P]/[D] = 100 and 50, the circular dichroic bands may be divided into a negative band and a conservative pair of bands, centred around 500 nm. The former can be ascribed to a singly intercalated monomer as above, and the latter pair of bands is associated with a neighbouring pair of intercalated monomers that are electronically coupled.

(c) For [P]/[D] = 10 and 2, an absorption band appears at 470 nm, in addition to the band due to intercalated monomers. The band at 470 nm may be attributed to the half-intercalated dimer. The circular dichroic bands can be divided into a conservative pair around 500 nm and a negative band at 470 nm, in agreement with the assignment of absorption bands.

(d) For [P]/[D] = 0.6 the band at 470 nm of bound dye should be hypochromic, and externally bound dimers of

antiparallel type form stacked sequences along a poly(rA) chain. The dimers are bound to poly(rA) electrostatically, as supposed from the observation of analogous dichroic bands and their dependence on ionic strength at neutral pH.

At neutral pH a single-stranded poly(rA) is stable and it is partly helical and partly coiled. Since the induced circular dichroism is observed to be parallel to the helical content, the dye bound to helical parts should be made optically active, and the intercalation and external association will occur in a manner similar to those at acid pH. (1) When [P]/[D] is 609, the absorption and circular dichroic bands are around 502 nm, and intercalated monomers are sparsely distributed along a poly(rA) chain. (2) For [P]/[D] = 41, 9 and 2, another absorption band appears at 462 nm, which can be ascribed to the half-intercalated dimer molecules. An equilibrium is established between intercalated monomers and half-intercalated dimers. The circular dichroic bands consist of a positive band at 502 nm and a characteristic conservative pair of bands centred at 467 nm. The former is associated with the sparsely intercalated monomers and the latter pair is with the electronically coupled pair of half-intercalated dimers. (3) For [P]/[D] = 0.6, stacked sequences of externally bound dimers are formed along a poly(rA) chain. The nature of binding mode is largely electrostatic, since it is sensitive to the ionic strength.

The induced circular dichroism of dye bound to single-stranded poly(rA) suggests that a dye molecule is inserted between neighbouring adenine bases through stacking of dye with bases on the same chain, in such a way as proposed by Pritchard *et al.*[36] for DNA. The original intercalation model due to Lerman[37] assumes that a dye molecule is inserted between hydrogen-bonded base pairs of DNA. Since the optical activity is induced on acridine orange bound by poly(rA) at neutral pH as well as to denatured DNA, it is concluded that a single-stranded helix can intercalate acridine orange and confer dissymmetry on it.

It was also deduced from an observation on circular dichroism of acridine orange mixed with tRNA[29] that intercalation of dye occurs only on the double-stranded helix but not on the single-stranded part, because a dichroic band assigned to the dye intercalated on the double-stranded helix disappears when tRNA is treated with formaldehyde. This deduction is not in accord with the present results.

Next we will consider the stability of three dye species bound to poly(rA) at acid or neutral pH and to DNA or, more exactly, the formation of three complexes consisting of mainly one of these bound dye species. The stability of intercalated monomer or intercalation complex can be measured by the reciprocal of the lowest [P]/[D] value for preponderance of the absorption band around 506–502 nm over that around 470–462 nm. Thus we have the following order for the stability of intercalation: DNA > double-stranded poly(rA) > single-stranded poly(rA), with the corresponding [P]/[D] value, 10, 50 and 180, respectively. Similarly, the stability of externally stacked sequence of antiparallel dimers or external association complex can be assessed by the [P]/[D] value below which the spectrum ceases to pass the isosbestic point at 491 or 470 nm or hypochromism occurs. Then we have the following order for the stability of external association: double-stranded poly(rA) > DNA > single-stranded poly(rA), with the [P]/[D] value, 10, 4 and 0.9, respectively. Here we have taken the data on DNA from Figure 5 of Frederieq and Houssier[27]. Between these two regions of [P]/[D] value, the half-intercalated dimer is predominantly formed. It is evident that the intercalation and external association are less stable for single-stranded poly(rA), but the half-intercalated dimer is formed over a wide [P]/[D] region at neutral pH. This is reasonable for a relatively open structure of single-stranded poly(rA).

When we compare the binding process of dye on various polynucleotides we find that some of the stages of binding process proposed above are absent from some particular cases. For poly(rA) at acid pH and also for DNA[27], four stages are manifest except for the electronic coupling of half-intercalated dimers. A similar situation can be seen for tRNA[29]. For single-stranded poly(rA) three stages are evident, among which the coupled pair of half-intercalated dimers is predominant over a very wide range of [P]/[D] ratios. However, only the isolated intercalation and external association occur with double-stranded cytoplasmic polyhedrosis virus RNA[29].

Blake and Peacocke[23] failed to observe the induced Cotton effect of proflavin mixed with poly(rA) at neutral pH. The apparent difference between acridine orange and proflavine in the interaction with poly(rA) may be explained in terms of the difference in stacking tendency of the two acridine dyes[38]; the former stacks more strongly.

Dourlent and Hogrel[19] suggested reaction schemes for the binding of proflavin on poly(rA) and DNA. If their complexes, $C_{II,1}$ and $C_{II,2}$, for DNA are assigned to those of sparsely intercalated monomeric dye molecules and of coupled pairs of intercalated monomers, respectively, their reaction scheme will be in agreement with the present one for poly(rA) of high [P]/[D] ratios at acid pH. Similarly, if their complex, C_{II}, for poly(rA) is identified with that of sparsely intercalated monomers, the same is true for poly(rA) at neutral pH. However, our half-intercalated dimer is not represented in their schemes.

It is relevant to note here that the magnitude of induced circular dichroism of acridine orange is generally much lower when bound to poly(rA) than to DNA, especially in the case of an external association mechanism. This could be related to the flexibility of poly(rA), which is greater than that of DNA, as revealed in the smaller exponent of the intrinsic viscosity–molecular weight relation for poly(rA) than for DNA, i.e. 0.65 for poly(rA) at neutral pH and 0.92 for poly(rA) at acidic pH[39], compared with 1.32 for DNA[40].

It is known that a poly(rA) segment consisting of as many as 220 nucleotides constitutes an accessory polynucleotide sequence linked to 3′ end of eukaryotic mRNA[41]. The present work could provide a method applicable to the elucidation of its physiological significance, which is not yet understood.

References

1 Beers, Jr., R. F. and Steiner, R. F. *Nature* 1957, **179**, 1076
2 Fresco, J. R. and Doty, P. *J. Am. Chem. Soc.* 1957, **79**, 3928
3 Rawitscher, M. A., Ross, P. D. and Sturtevant, J. M. *J. Am. Chem. Soc.* 1963, **85**, 1915
4 Luzzati, V., Mathis, A., Masson, F. and Witz, J. *J. Mol. Biol.* 1964, **10**, 28
5 Holcomb, D. N. and Tinoco, Jr., I. *Biopolymers* 1965, **3**, 121
6 Van Holde, K. E., Brahms, J. and Michelson, A. M. *J. Mol. Biol.* 1965, **12**, 726

7 Brahms, J., Michelson, A. M. and Van Holde, K. E. *J. Mol. Biol.* 1966, **15**, 467
8 Leng, M. and Felsenfeld, G. *J. Mol. Biol.* 1966, **15**, 455
9 Holcomb, D. N. and Timasheff, S. N. *Biopolymers* 1968, **6**, 513
10 Small, E. W. and Peticolas, W. L. *Biopolymers* 1971, **10**, 69
11 Stannard, B. A. and Felsenfeld, G. *Biopolymers* 1975, **14**, 299
12 Rich, A., Davies, D. R., Crick, F. H. C. and Watson, J. D. *J. Mol. Biol.* 1961, **3**, 71
13 Bradley, D. F. and Wolf, M. K. *Proc. Natl. Acad. Sci. US* 1959, **45**, 944
14 Steiner, R. F. and Beers, Jr., R. F. *Arch. Biochem. Biophys.* 1959, **81**, 75
15 Blake, A. and Peacocke, A. R. *Biopolymers* 1967, **5**, 383
16 Hammes, G. G. and Hubbard, C. D. *J. Phys. Chem.* 1966, **70**, 2889
17 Dourlent, M. and Hélène, C. *Eur. J. Biochem.* 1971, **23**, 86
18 Dourlent, M., Hogrel, J. F. and Hélène, C. *J. Am. Chem. Soc.* 1974, **96**, 3398
19 Dourlent, M. and Hogrel, J. F. *Biopolymers* 1976, **15**, 29
20 Blake, A. and Peacocke, A. R. *Biopolymers* 1968, **6**, 1225
21 Neville, Jr., D. M. and Bradley, D. F. *Biochim. Biophys. Acta* 1961, **50**, 397
22 Mason, S. F. and McCaffery, A. J. *Nature* 1964, **204**, 468
23 Blake, A. and Peacocke, A. R. *Biopolymers* 1966, **4**, 1091
24 Yamaoka, K. and Resnik, R. A. *J. Phys. Chem.* 1966, **70**, 4051
25 Gardner, B. J. and Mason, S. F. *Biopolymers* 1967, **5**, 79
26 Zama, M. and Ichimura, S. *Biopolymers* 1970, **9**, 53
27 Fredericq, E. and Houssier, C. *Biopolymers* 1972, **11**, 2281
28 Ito, T., Zama, M. and Amagasa, J. *Biopolymers* 1972, **11**, 1583
29 Zama, M. and Ichimura, S. *Biopolymers* 1976, **15**, 1693
30 Imae, T. and Ikeda, S. *Polym. J.* 1976, **8**, 531
31 Ikeda, S. and Imae, T. *Biopolymers* 1971, **10**, 1743
32 Imae, T. and Ikeda, S. *Biopolymers* 1975, **14**, 1213
33 Kubota, Y., Eguchi, Y., Hashimoto, K., Wakita, M., Honda, Y. and Fujisaki, Y. *Bull. Chem. Soc. Jpn* 1976, **49**, 2424
34 Armstrong, R. W., Kuruesev, T. and Strauss, U. P. *J. Am. Chem. Soc.* 1970, **92**, 3174
35 Zanker, V. *Z. Physik. Chem.* 1952, **199**, 225
36 Pritchard, N. J., Blake, A. and Peacocke, A. R. *Nature* 1966, **212**, 1360
37 Lerman, L. S. *J. Cell. Comp. Physiol.* 1964, **64**, Supplement 1
38 Ikeda, S. and Yamaguchi, M. *Kolloid Z. Z. Polym.* 1972, **250**, 311
39 Fresco, J. R. and Doty, P. *J. Am. Chem. Soc.* 1957, **79**, 3928
40 Eigner, J. and Doty, P. *J. Mol. Biol.* 1965, **12**, 549
41 Brawerman, G. in 'Progress in Nucleic Acid Research and Molecular Biology' (Ed. W. E. Cohn) Academic Press, 1976, Vol. 17, p. 177