Biophysical Chemistry, 48 (1994) 353–358
Elsevier Science B.V., Amsterdam

BIOCHE 01793

Cooperative binding of quinacrine to inorganic polyphosphate

V.N. Zozulya * and I.M. Voloshin

Institute for Low Temperature Physics and Engineering, Ukrainian Academy of Sciences, 310164 Kharkov (Ukraine)

(Received 1 June 1993; accepted 1 July 1993)

Abstract

The cooperative binding of quinacrine to inorganic polyphosphate in aqueous solutions of different ionic strengths at pH 7 has been investigated by means of absorption and polarized fluorescence spectroscopy. In a wide range of molar phosphate-to-dye ratios, P/D, at a low ionic strength, the quinacrine molecules form aggregates by the chromophore stacking at the linear polyanion. The aggregation is characterized by a bathochromic shift of the visible absorption maxima accompanied by a hypsochromic effect and a nearly total quenching of the quinacrine fluorescence. On monomeric binding, which dominates at $P/D \geq 10^4$, the spectroscopic properties of the dye do not change. The parameters of cooperative binding were estimated by Schwarz's method: the number of binding sites per monomeric unity of the polyphosphate $g = 0.45$, the cooperativity parameter $q \simeq 130$, the cooperative binding constant is dependent on the ionic strength with $\mathrm{d} \log K / \mathrm{d} \log [\mathrm{Na}^+] \simeq -1.65$.

Keywords: Quinacrine; Cooperative binding; Polarized fluorescence; Spectroscopy of dye aggregation; Chromosomal fluorescence staining

1. Introduction

The phenomenon of cooperative stacking of organic cationic dyes electrostatically attached to linear polyanions is well-known [1–7]. When the stacking of dye molecules occurs at the polyanionic matrix of a nucleic acid, the external electrostatic binding is greatly enhanced as a result of the cooperativity process, so that it can compete effectively with the strong intercalation mechanism. Li and Crothers observed such an effect for the proflavine binding to DNA [8]. The cooperative stacking is of still more significance for the dye binding to single-stranded nucleic acids [9,10]. Stacked aggregates and intercalated dye molecules differ essentially in their absorbance and fluorescence [11], that allows to distinguish quantitatively two binding types using the standard spectroscopic methods.

Up to now the aggregation on linear polyanions of monocationic dyes was studied. It is interesting to investigate this phenomenon for dicationic acridine dye quinacrine (Fig. 1) used widely for staining of chromosomes. The possible effects of the dye aggregation must be taken into account when analyzing the fluorescence banding pattern. In the present work the occurrence of

* Corresponding author.

SSDI 0301-4622(93)E0047-9

the stacked quinacrine aggregates on the model polyanion–sodium polyphosphate in aqueous solutions was demonstrated and their spectroscopic properties were studied. The effect of the solution ionic strength on the aggregation was investigated and the cooperative binding parameters were estimated.

2. Materials and methods

Quinacrine dihydrochloride was purchased from Serva, BRD, and sodium polyphosphate with degree of polymerisation ≥ 75 was from Sigma Chemical Co. Deionized distilled water and 1 mM sodium cacodilate buffer, pH 7, 0.5 mM Na_2 EDTA were used as solvents. The ionic strengths of the solutions were adjusted to the preset values by addition of NaCl. The concentrations of quinacrine were determined spectrophotometrically using the extinction coefficient, $\varepsilon_{424} = 9750$ M^{-1} cm^{-1} from the paper [12]. The polyphosphate concentration was estimated from the dissolved substance weight (1% solution corresponds to 98 mM phosphates [13]).

The quinacrine–polyphosphate interaction was studied by visible absorption and laser polarized fluorescence spectroscopy. The instruments and methods are described earlier [14]. The absorption and fluorescence were measured alternately in the same 1-cm path length quartz cuvette at the environment temperature from 22 to 24°C. The quinacrine solution was titrated at constant concentration $C_T = 5 \times 10^{-5}$ M (in all the experimental runs) with increasing amounts of the polyphosphate solution containing the same concentration of dye.

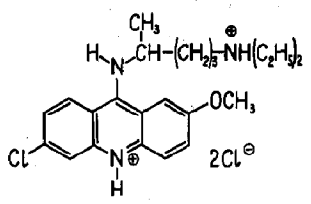

Fig. 1. Molecular structure of quinacrine.

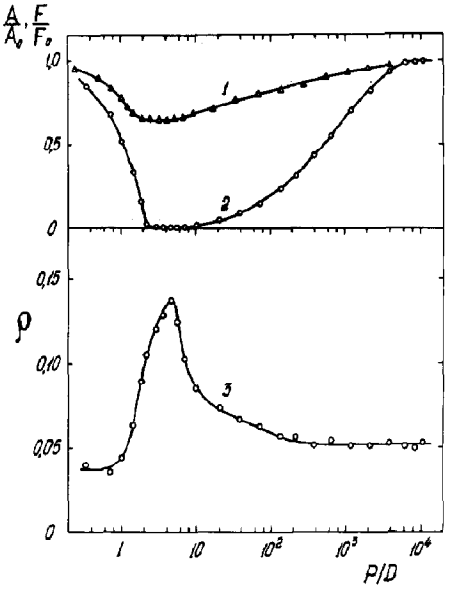

Fig. 2. Changes in the relative absorption (1), relative fluorescence intensity (2) and degree of fluorescence polarization (3) of quinacrine in the maxima of respective spectra on titration by the polyphosphate in water (A_0 and F_0 are absorption and fluorescence intensities of the free dye). Weighed-in total concentration of quinacrine is constant, i.e. $C_T = 5 \times 10^{-5}$ M.

3. Results and discussion

3.1. Spectroscopic properties of quinacrine–polyphosphate complexes

The most strong quinacrine binding to polyphosphate is observed in deionized distilled water because of the absence of competitive interaction with other cations. The curves of absorption and fluorescence titrations of quinacrine with polyphosphate in water are shown in Fig. 2 as P/D-dependences of the changes in the relative absorption A/A_0, relative fluorescence intensity F/F_0 and degree of fluorescence polarization ρ in the maxima of the absorption and fluorescence spectra. The first two curves have the shapes typical of cooperative binding of cationic dyes to linear polyanions [5–7,11]. The strong fluorescence quenching suggests a stacking interaction of the quinacrine chromophores. The lowest residual fluorescence observed at $P/D = 4.5$ is $\sim 0.6\%$ of the fluorescence intensity of the dye solution without the polyphosphate. The

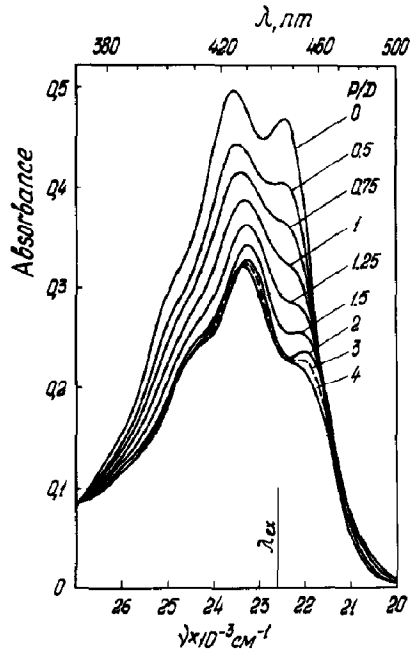

Fig. 3. Change in the visible absorption spectrum of quinacrine upon binding to the polyphosphate in water. Dye concentration is 5×10^{-5} M, P/D values are given in the figure, and λ_{ex} = 441.6 nm is the wavelength of fluorescence excitation.

maximum of the curve of the P/D dependence of ρ (Fig. 2, curve 3) corresponds to this F/F_0 minimum. This means undoubtedly that, besides the nonbound quinacrine molecules, dye aggregates contribute to the residual glow, the fluorescence polarization of which is essentially higher than that of the free molecules. The increase of ρ is caused by the limitation of the chromophore mobility in the stacks or by the decrease of the fluorescence lifetime. We could not determine the exact values of F/F_0 and ρ for quinacrine stacks but the fact is that on dye aggregation its fluorescence intensity decreases by about 200 times.

The most interesting changes in the quinacrine absorption spectra occur in the range $P/D = 0$–4 (Fig. 3). The visible absorption spectrum of the free quinacrine has two pronounced bands with maxima at 424 and 445 nm. On binding of quinacrine to polyphosphate both the bands reveal hypsochromic and bathochromic shifts but in different ways. The short-wave band intensity decreases monotonically as P/D increases and reaches the minimum at $P/D = 4$–5 in accordance with the maximum fluorescence quenching. The hypsochromic process in the long-wave band is not uniform: up to $P/D = 1$ the band decreases faster than the short-wave one and transforms into a shoulder, then appears again at $P/D = 2$, whereupon it becomes a shoulder in the spectrum again. The highest value of the bathochromic shift of the short-wave band maximum is 7 nm at $P/D = 2$, while at $P/D = 4$ when the aggregated dye fraction is largest it is 4 nm. For the long-wave band the maximum red shift is 10 nm at $P/D = 2$. These specific changes in the absorption spectrum and the absence of the isosbestic point are indicative of the aggregate reconstruction resulting in a change of mutual positions of the dye chromophores. The bathochromic shift of the absorption bands suggests the ethidium bromide type of the quinacrine aggregation on the polyphosphate [6], at which planes of chromophores are displaced towards "a head to tail" configuration [11].

At $P/D > 5$ the run of the titration curves reflect decomposition of the quinacrine aggregates into monomeric complexes, the process being completed at $P/D \simeq 10^4$ (Fig. 2). The absorption and fluorescence spectra become similar to those of the free dye but the fluorescence polarization degree remains greater (Fig. 2, curve 3). The latter fact evidences the monomeric binding.

The presence of Na^+ ions in the solution reduces strongly cooperative binding of quinacrine to the polyphosphate. Figure 4 presents the curves of fluorescence titration at low P/D values with varying concentration of Na^+ ions. It is seen that under the dye concentration used, $C_T = 5\times10^{-5}$ M, the aggregation occurs only at the low ionic strengths. At $[Na^+] \gtrsim 0.05$ M no changes was observed in the spectroscopic dye properties.

3.2. *Parameters of the cooperative binding*

Parameters of the cooperative binding of quinacrine to polyphosphate were evaluated using the fluorescence titration data. Under assumption that all dye is bound in the aggregated form and since the aggregate fluorescence is neg-

ligible, the curves of P/D-dependences F/F_0 can be considered as P/D-dependences of an unbound fraction of dye, γ_0, in the range P/D = 0–5 (Fig. 4), i.e. $\gamma_0 \cong F/F_0$. The straight-line parts of these curves correspond to stoichiometric binding conditions and, being extrapolated to the P/D-axis yield a binding site value of $n = 2.2$ monomeric units of the polyphosphate per quinacrine molecule. Thus, the number of binding sites per monomeric lattice unit is $g = 0.45$.

The cooperative binding parameters were estimated by Schwarz's method [15]. For the determination of the cooperative binding constant K, the relation

$$K = 1/C_T\gamma_0^*. \quad (1)$$

was used, where C_T is the total weighed-in dye concentration and γ_0^* is the fraction of the free ligand when the lattice is half occupied. The γ_0^* values were determined from the intercept of the F/F_0 versus P/D curves by means of the straight line drawn at half the negative slope of the stoichiometric part of these curves (i.e., the dashed line in Fig. 4). The results were obtained for five $[Na^+]$ values and are collected in Table 1. The $[Na^+]$ values were evaluated taking into account the contribution of Na^+ ions introduced by the polyphosphate, the cacodilate buffer and EDTA.

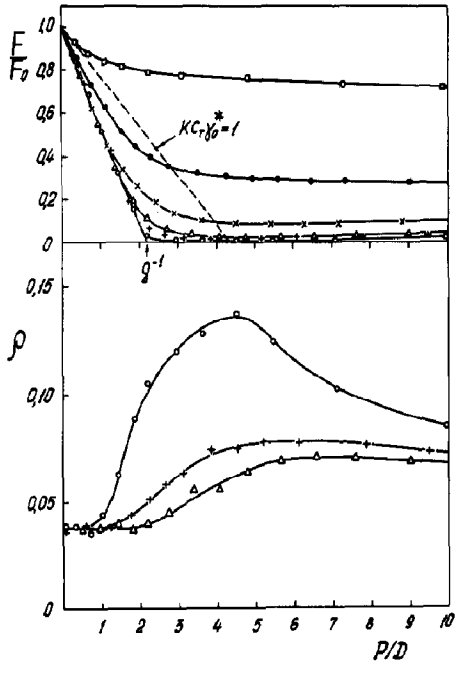

Fig. 4. Curves of fluorescence titration of quinacrine by polyphosphate in deionized water (○) and in the buffer with various Na^+ ion concentrations: 1 mM (+), 2 mM (△), 5 mM (×), 10 mM (●), 20 mM (□). Total dye concentration $C_T = 5\times10^{-5}$ M is constant. Extrapolation procedures to determine parameters g and K are discussed in the text.

Table 1

Estimation of cooperative binding constants for the interaction of quinacrine with polyphosphate according to Schwarz, $K = 1/C_T\gamma_0^*$, using the fluorescence titration curves shown in Fig. 4. Concentration of the dye is $C_T = 5\times10^{-5}$ M

$[Na^+]$ (mM)	γ_0^* [a]	K (M^{-1})
0.22	0.007	2.9×10^6 [b]
	0.005	4.0×10^6 [b]
1.2	0.012	1.7×10^6
2.2	0.024	8.3×10^5
5.2	0.10	2.0×10^5
10.1	0.35	5.7×10^4

[a] γ_0^* is the fraction of free dye, when the lattice is half occupied.

[b] Two independent series of measurements in deionized distilled water.

The binding cooperativity parameter q was estimated by using the following equation [15]

$$q = g\frac{P}{D}\frac{1-\sqrt{\gamma_m}}{\gamma_m}. \quad (2)$$

The fraction of the dye bound as monomers γ_m was obtained from the data on fluorescence titration in deionized water at high P/D values (curve 2 in Fig. 2) as $\gamma_m \cong F/F_0$, since for these conditions the fraction of free dye can be neglected. The mean value of q calculated from the data in the range of $P/D = 20–2\times10^3$ was $q = 130 \mp 20$.

Since a polymer with the moderately long lattice, the number of monomeric units M being ≥ 75, was used in the given work, the question arises as to how far the determined binding affinity $K = K_M q$ (K_m is the monomeric binding constant) will differ from that of an infinite lattice. To answer this question, we used the results of Epstein's work [16]. According to Epstein, the relation $KC_T\gamma_0^* = 1$ (which is the same as $K_M qL = 1$, where L is the free ligand concentration),

from which K was determined, corresponds to the situation most unfavourable for the strongly cooperative binding when the approach to the infinite lattice limit with increasing lattice length becomes quite slow. For the combination of the parameters $n = 2$, $q = 100$, $K_M L = 0.01$—which approximately corresponds to our case—it can be see from Table 1 [16] that the polyphosphate lattice with length $M = 75$ is by about 20% less filled is than an infinite lattice. Hence, the obtained values of γ_0^* are larger, and consequently the values of constant K smaller, by about 20% than that those for an infinite lattice.

Perhaps, the fact that the determined binding site value $n = 2.2$ is somewhat higher than the quinacrine cation charge, which equals 2, is explained by the effects of the finite lattice length.

It is interesting to verify the prediction of the polyelectrolyte theory [17,18] on the ionic strength effect, applied to the system studied. According to this theory the sodium ion effect on the binding constant for the interaction of an oligocation with a rod-like polyanion obeys the equation

$$-\frac{\mathrm{d}\log K}{\mathrm{d}\log [\mathrm{Na}^+]} = 0.88Z\,. \qquad (3)$$

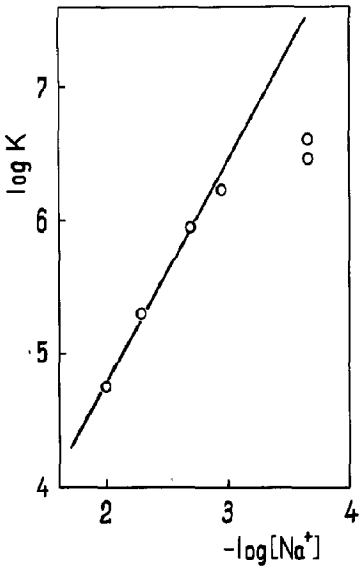

Fig. 5. Effect of Na^+ ions on the cooperative binding constant K.

where Z is a cation charge. For quinacrine $Z = 2$, so that $-\mathrm{d}\log K/\mathrm{d}\log [\mathrm{Na}^+] = 1.76$. Figure 5 shows the data from Table 1 as a plot of log K versus $-\log [\mathrm{Na}^+]$. The experimental points fall onto a straight line, excluding two points at $[\mathrm{Na}^+] \leq 1.2$ mM, which is explained by the fact that under these ionic conditions the fluorescence fraction of the bound quinacrine in F/F_0 becomes significant (this is evidenced by the increased fluorescence polarization as shown in Figs. 2 and 4), which results in the overestimation of γ_0^* and, hence, in the underestimation of K. The slope of this linear plot is 1.65 close to the theoretical value of 1.76. This result demonstrates the applicability of the polyelectrolyte theory to the highly cooperative binding proces.

4. Conclusions

Though the self-association of free quinacrine in aqueous solutions is slight [12], its stacking aggregation on the polyphosphate is characterized with the relatively high parameter of the binding cooperatively, $q \simeq 130$. The cooperative constant is very affected by the ionic strength, decreasing with the increase of $[\mathrm{Na}^+]$ according the equation

$$-\frac{\mathrm{d}\log K}{\mathrm{d}\log [\mathrm{Na}^+]} = 1.65\,. \qquad (4)$$

The present work observed the significant fraction of quinacrine bound as aggregates only at the low ionic strengths of solutions, $[\mathrm{Na}^+] \leq 20$ mM. The binding effectiveness is determined by the so-called binding strength, KC_T [15], therefore, to preserve it at the increase of $[\mathrm{Na}^+]$, it is necessary to increase the ligand concentration C_T according to decreasing K as defined by eq. (4). For example, in order to have ~ 90% fraction of quinacrine bound to the polyphosphate in the aggregated form (for medium values of P/D) at $[\mathrm{Na}^+] = 0.1$ M, that takes place at $[\mathrm{Na}^+] = 5$ mM for $C_T = 5 \times 10^{-5}$ M (see Table 1), it is necessary to use the dye concentration $C_T = 7 \times 10^{-3}$ M.

The chromosomal staining techniques uses a high quinacrine concentration of $C_T \simeq 10^{-2}$ M

[19], therefore the stacking dye aggregation is possible, especially in the case when non-buffered solutions are used, i.e. the ones prepared from distilled water. However, as the aggregate fluorescence is negligible, it does not contribute to the fluorescence banding pattern.

Finally, it should be noted that when studying quinacrine interactions with DNA *in vitro* by absorption spectroscopy, it is difficult to reveal the surface-bound dye aggregates because the absorption spectra on quinacrine molecule stacking and on their intercalation into the double helix of DNA change similarity (i.e., they display hypsochroism and a red-shift).

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