A MECHANISM FOR THE ENTRAPMENT OF DNA AT AN AIR-WATER INTERFACE

THOMAS H. EICKBUSH AND EVANGELOS N. MOUDRIANAKIS,
Department of Biology, The Johns Hopkins University, Baltimore, Maryland 21218

ABSTRACT Addition of the intercalating dye quinacrine to a low ionic strength solution of DNA in quantities sufficient to saturate the high affinity sites in the DNA will result in the accumulation of the DNA at the solution interface. This entrapment of DNA at the air-water interface has been assayed by the adsorption of DNA to untreated carbon-coated electron microscope grids touched to the solution surface. Other intercalating dyes can also bring about this entrapment, if they possess a side arm large enough to occupy one of the DNA grooves when the dye is intercalated into the DNA. The extension and unwinding of the DNA helix brought about by the intercalating chromophore of the dye molecules are not requirements for the entrapment process. Spermidine, a simple polyamine that will bind to the DNA minor groove but that has no intercalating chromophore, was found to bring about this entrapment. Even simple mono- and divalent cations in the absence of the above ligands were found to promote a low level of surface entrapment. A model for the entrapment of DNA at the air-water interface is proposed in which one (or both) of the hydrophobic grooves of the DNA becomes a surface-active agent as a consequence of the association of various ligands and charge neutralization.

INTRODUCTION

Routine observation of DNA by means of the electron microscope was made possible with the development of the protein monolayer technique of Kleinschmidt and Zahn (1). In this technique, DNA is trapped in a layer of denatured protein, usually cytochrome *c*, formed at an air-water interface. While the use of this protein technique in the study of DNA or RNA is well established (see ref. 2 for review), its usefulness in the observation of DNA in specific association with protein has been much less certain. First, the wrapping of a cytochrome *c* layer about the DNA will shield specific DNA-protein complexes from view. Second, since this denatured cytochrome *c* behaves as a polycation in its binding to DNA, possible structural modifications can result in DNA-protein complexes, which are stabilized by such ionic forces. More recently, the detergent benzyldimethylalkylammonium chloride has been used as a surface-active agent in the entrapment of DNA at the air-water interface (3). Since the coat of this detergent absorbed onto DNA brings less mass than that of cytochrome *c*, the resulting apparent DNA diameter is closer to its real diameter, and therefore was expected to make the analysis of DNA-protein complexes easier. However, that the agent facilitating the spreading is a cationic detergent again introduces the possibility of artifacts when used in the study of structures such as chromatin, which are naturally stabilized by ionic and hydrophobic interactions.

In this report we demonstrate that upon addition of certain intercalating dyes to DNA solutions, the DNA is trapped at the air-water interface of those solutions. We also describe the characteristics of the intercalating dye molecule necessary to bring about this entrapment. DNA trapped in this manner is easily adsorbed at low ionic strength conditions to untreated carbon films for subsequent examination in the electron microscope. The significance of this method of DNA "spreading" lies in its application to reconstituted DNA-histone complexes (4, 5) or chromatin, which when "spread" in this way would allow detection of "naked" DNA, i.e., areas of the DNA molecule not covered by protein. Furthermore, since the same intercalating dyes used in our spectrofluorimetric studies on the structure of DNA or deoxynucleoproteins in solution (6, 7) were used here to induce the entrapment of DNA at the air-water interface, it is expected that a closer correlation can be achieved between the results from the spectrofluorimetric studies of these polyelectrolytes while in solution and the results from electron microscopic studies of the dehydrated polyelectrolyte.

MATERIAL AND METHODS

Material

DNA from the phage PM 2 was a gift from Steve Rogers of this department. This DNA was composed of approximately equal amounts of linear and open circular DNA separated from supercoiled (closed circular) DNA on a hydroxyapatite column (8).

The DNA ligands (obtained from Calbiochem, San Diego, Calif.; Allied Chemical Corp., Morristown, N.J.; Mann Research Laboratories, Inc., New York; National Biochemical Co., Chicago, Ill., or Sigma Chemical Co., Inc., St. Louis, Mo.) were dissolved in either distilled water or 5 mM Tris, pH 8, and stored frozen until immediately before use. The acridine orange derivative 3,6-*bis*(dimethylamino)-9-{[2-(1-pyrrolidinyl)ethyl]thio} acridine was a gift from Dr. Edward F. Elslager of Parke, Davis & Co., (Detroit, Mich.). The concentrations of the following compounds were determined spectrophotometrically with the indicated extinction coefficients; ethidium bromide at 460 nm, $4.8 \times 10^3 mol^{-1} cm^{-1}$ (9); quinacrine dihydrochloride at 420 nm, $7.6 \times 10^3 mol^{-1} cm^{-1}$ (10); and chloroquine dihydrochloride at 344 nm, $1.9 \times 10^4 mol^{-1} cm^{-1}$ (11).

Adsorption of DNA

Carbon support films were made by the slow evaporation of carbon onto freshly cleaved mica in a Kinney evaporator type SC-3 (Kinney Vacuum Co., Boston, Mass.) at a pressure of 0.5–0.20 μm of mercury. The carbon films were floated off the mica and onto 300-mesh copper electron microscope grids precoated with a thin layer of polybutane (12). Grids were dried, stored in a desiccator, and used for periods of up to several weeks.

Aliquots of 0.1 ml of DNA in 5 mM Tris, pH 8, and each of the various salts or dyes used in this study, as indicated, were placed as small droplets on a piece of Parafilm, Teflon, or similar hydrophobic surface and allowed to equilibrate at room temperature for 2 min. Grids were lightly touched to the surface of each drop and removed carefully at a 45° angle so that no solution remained adhering to the grids. Any small amount of solution that occasionally adhered was immediately removed with filter paper. Grids were allowed to dry face up on filter paper in the absence of any washes. Circular shadowing was performed at a 10:1 angle in a Kinney high-vacuum evaporator with sublimation of platinum monitored by a Kronos QM-1 digital thickness monitor (Kronos, Inc., Carson, Calif.). Grid observations were made with a

JEM-100B electron microscope (JEOL U.S.A., Medford, Mass.) at 80 KeV (40-μm objective aperture).

To quantitate the relative number of DNA molecules adsorbed to carbon films, 12 widely spaced, randomly sampled areas of each grid were photographed at a magnification of 10,000. Consequently each negative represented approximately 67 μm^2 of carbon-film surface area. This entire area was treated as one micrograph. The average number of DNA molecules countable per negative is presented (along with its standard deviation) for each time point or ligand concentration in the figures of this paper.

RESULTS

Entrapment at the Interface

When slightly hydrophilic carbon films are touched to DNA solutions of low ionic strength, very little DNA is adsorbed to the carbon. Those molecules that occasionally become adsorbed are of little use since extensive intra- and intermolecular lateral aggregation of the DNA occurs. One way in which we attempted to eliminate this aggregation was with intercalating dyes. Since intercalating dyes are able to rigidify the DNA molecule, as measured by an increase in viscosity (13) or in persistence length (14), the so-stiffened helixes should be less likely to fold back upon themselves or align with other molecules. When this prediction was tested, aggregation was indeed found to be reduced. More importantly, however, certain of these dyes drastically increased the amount of DNA adsorbed to the carbon films and also changed the manner in which the DNA was adsorbed. At a final concentration of 3×10^{-5}M quinacrine the smooth, sweeping appearance of the DNA adsorbed to the film (Fig. 1) is remarkably similar to the appearance of DNA spread by the protein monolayer technique (see figures in ref. 2). Since in the protein monolayer technique, DNA is entrapped at the air-water interface, it seemed reasonable to assume that quinacrine might also be capable of trapping DNA at the interface of a solution.

To test this hypothesis, 0.1-ml aliquots were drawn from a test tube containing a solution of PM 2 DNA in 5 mM Tris, pH 8, with 3×10^{-5}M quinacrine, and allowed to incubate for various periods of time in droplet form on a piece of Parafilm. As seen from Fig. 2, the number of DNA molecules adsorbed to carbon films touched to these droplets increased as a function of the length of time that the mixture was left to stand as a droplet. This increase was independent of the period of preincubation since the amounts of DNA adsorbed were identical whether the solutions of quinacrine and DNA were preincubated in the test tube for 1 h or used immediately. Only the length of time the solutions were in droplet form on the Parafilm before the grids were touched to their surfaces determined the amount of DNA adsorbed onto the grids. A similar adsorption of DNA as a function of time has been reported with the protein monolayer technique by Lang and Mitani (15). While both their results and ours suggested the buildup of DNA at the interface of the solution, Lang and Mitani reported that no DNA molecules could be adsorbed to grids within the first 2 min of incubation, since time was required to form a film of denatured protein at the surface of the droplet. With quinacrine, on the other hand, well-spread DNA

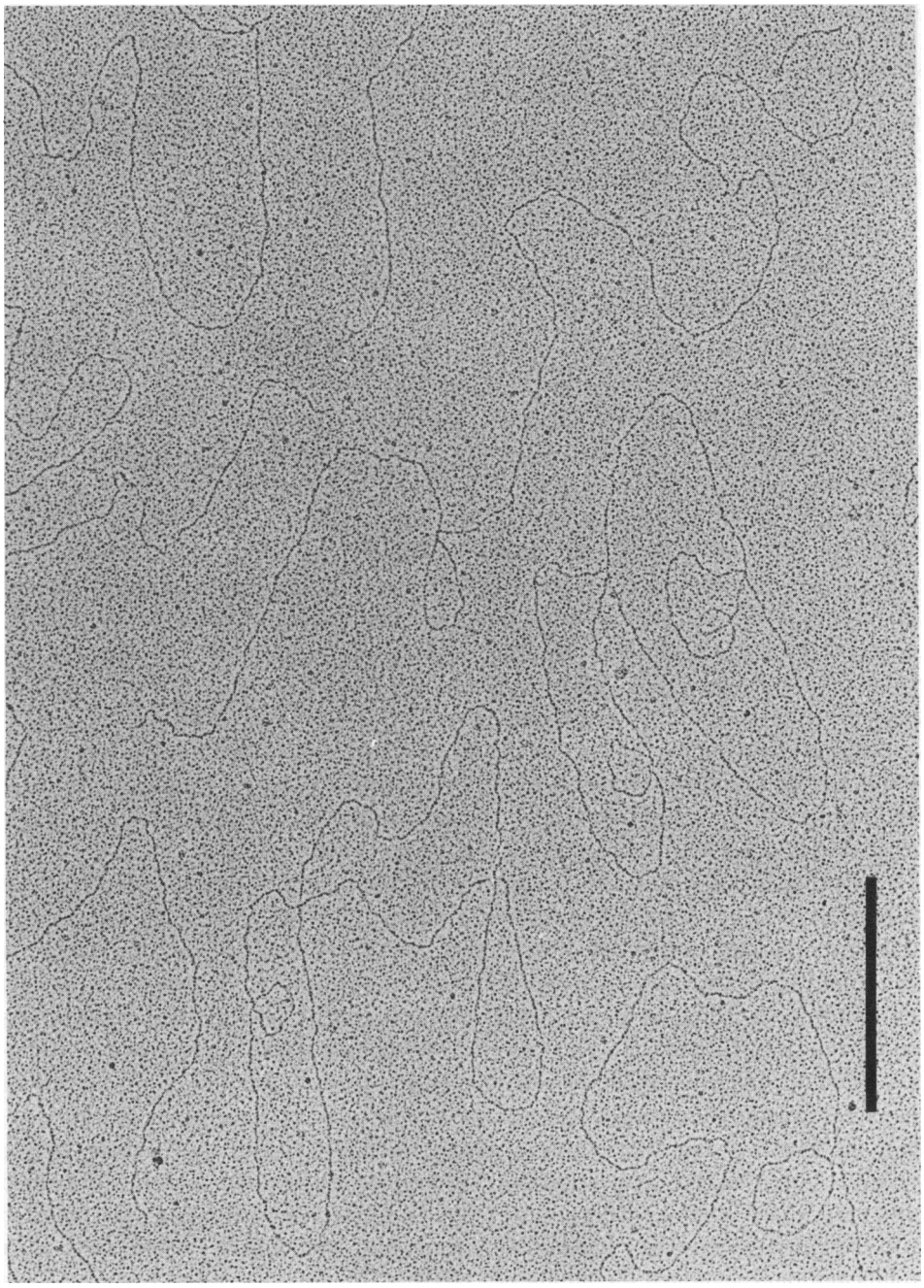

FIGURE 1 Quinacrine-spread DNA. Adsorption conditions were 0.2 μg/ml PM 2 DNA, 5 mM Tris (pH 8), and 3×10^{-5} M quinacrine dihydrochloride. Scale bar represents 0.5 μm.

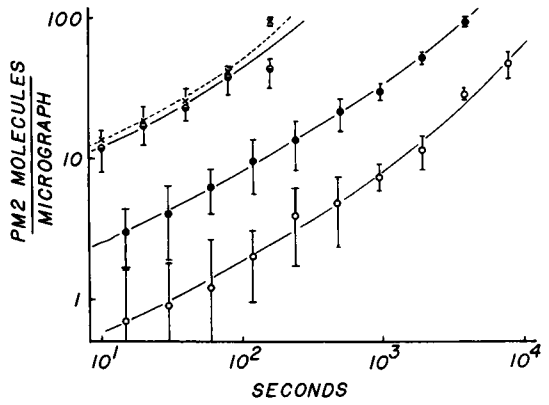

FIGURE 2 Adsorption kinetics of quinacrine-spread DNA. DNA was adsorbed from the surfaces of 0.1-ml aliquots withdrawn from a preincubated solution (1 h) of 3×10^{-5}M quinacrine and the indicated PM 2 DNA concentrations in 5 mM Tris (pH 8). The time in seconds represents the interval from the moment the droplet is formed on the hydrophobic surface until a carbon-coated grid is touched lightly to its surface, as described in Material and Methods. (○) 0.025 μg/ml PM 2 DNA; (●) 0.1 μg/ml PM 2 DNA; (⊖) 0.4 μg/ml PM 2 DNA; (×) 0.4 μg/ml PM 2 DNA without preincubation (aliquot incubations were started immediately after the addition of quinacrine to the DNA).

can be found at the interface at the shortest time point tested (10 s). This was the first indication that DNA entrapment by quinacrine does not depend upon the formation of a dye monolayer.

Intercalating Dyes that Support Entrapment

The binding of quinacrine to DNA is believed to be typical of many intercalating compounds (16, 17). To investigate the nature of the entrapment process further, a number of commonly available intercalating dyes were tested for their ability to entrap DNA at the air-water interface. For convenience of comparison, the structural formulas of these dyes are presented in Fig. 3. As summarized in Table I, although a variety of molecules were found to be capable of entrapping DNA at the interface in a manner similar to quinacrine, other molecules could not promote entrapment. A comparison of the structures of these dyes suggests that the ability to entrap DNA at an interface does not correlate with a specific structure of the intercalating chromophore of the molecule. Derivatives of phenanthridinium (ethidium), thioxanthene (hycanthone), quinoline (chloroquine), and acridine (quinacrine) are all capable of trapping DNA at the droplet interface, yet their planar chromophore rings show significant structural differences. Three other acridine derivatives (proflavine, acriflavine, and acridine orange), whose chromophores are quite similar to that of quinacrine, show no ability to trap DNA at the interface. The only apparent characteristic common to all compounds that bring about successful entrapment of DNA is the presence of a side arm projecting from the chromophore of the molecule. To examine the importance of this side arm, a derivative of acridine orange was tested which differed from the parent compound only in the presence of a pyrrolidinyl-ethyl-thio side arm

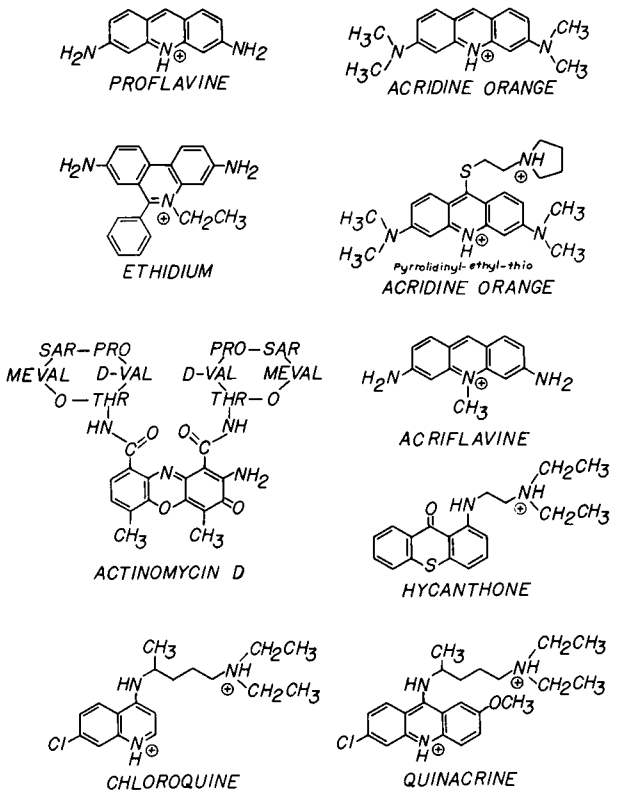

FIGURE 3 Structural formulas of the intercalating compounds used in this study. The charged cationic forms expected at pH 8 are drawn.

at carbon 9 (Fig. 3). This compound was indeed capable of entrapping DNA at the interface (Table I) and thus provided direct support for the correlation between the presence of a side arm and DNA entrapment. To date, the only exception to this correlation has been actinomycin D. A possible explanation for this will be offered later in this paper.

Entrapment Does Not Depend upon the Formation of a Dye Monolayer

The presence of an alkyl side chain on the dye molecules that promote entrapment suggests that the dye might simply act as a surface-active compound and entrap the DNA at the interface in a monolayer of dye. If this mechanism of entrapment were correct, it would basically be the same as that of the protein monolayer technique (1) or the benzyldimethylalkylammonium chloride method (3). As mentioned above, DNA was found at the interface of the solution at the shortest time points testable. This suggested that the formation of a layer of dye was probably not responsible for DNA entrapment. More direct evidence for this assumption can be derived from a comparison of the published surfactant properties of three acridine derivatives. Albert et al. (18) have shown that proflavine, quinacrine, and acridine orange cannot be

TABLE I

INTERCALATING COMPOUNDS TESTED FOR THEIR ABILITY TO ENTRAP DNA AT AN AIR-WATER INTERFACE*

Compound tested	Ability to entrap DNA at the interface
Acridine orange hydrochloride	–
Acriflavine hydrochloride	–
Chloroquine diphosphate	+
Ethidium bromide	+
Hycanthone methansulfonate	+
Proflavine hydrochloride	–
Quinacrine dihydrochloride	+
3,6-*bis*(dimethylamino)-9-{[2-(1-pyrrolidinyl)ethyl] thio}acridine hydrochloride	+
Actinomycin D	–

*Each compound was tested over the range 10^{-7}–10^{-3}M for its ability to promote the adsorption of unoriented DNA to carbon films as described in Material and Methods. (+) indicates that fully extended DNA molecules similar to that seen in Fig. 1 could be adsorbed. (–) indicates that fully extended molecules were never seen adsorbed to carbon films. However, compounds scored as (–) sometimes gave rise to the adsorption of low numbers of highly aggregated DNA molecules at the highest concentration ranges tested (see Discussion).

classified as surface-active compounds. Indeed, if any statement is to be made about their surfactant properties, it is that acridine orange is slightly more effective in reducing the surface tension of water than either quinacrine or proflavine, yet quinacrine can trap DNA at the interface while acridine orange cannot. This lack of a correlation between surface entrapment and surface activity suggests that the formation of a monolayer to bring about the entrapment of DNA at the air-water interface is not the mechanism for the spreading of DNA by intercalating dyes.

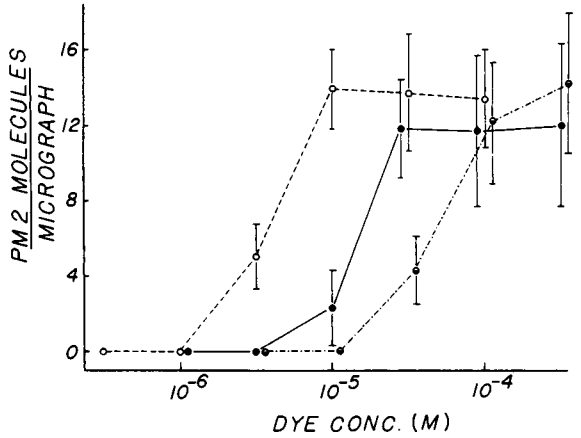

FIGURE 4 DNA adsorption as a function of dye concentration. Adsorption conditions were 0.15 μg/ml PM 2 DNA, 5 mM Tris (pH 8), and the indicated dye concentrations. Droplets were incubated for 2 min. (-----o-----), quinacrine; (——●——), chloroquine; (·-·-●-·-·), ethidium.

Another argument that the DNA adsorbed to carbon films is not being trapped in a monolayer of dye stems from an analysis of the concentration of dye required to bring about entrapment. Since the values for the association constants of quinacrine, chloroquine, and ethidium to DNA have been reported, these three dyes have been selected for study. Fig. 4 indicates the number of PM 2 DNA molecules adsorbed to carbon films as the concentration of each dye is increased. The lowest concentration of quinacrine that will still result in DNA reproducibly bound to carbon films is 3×10^{-6}M. Solution studies of quinacrine binding to DNA under the low ionic conditions employed here indicate that its association constant with DNA is of the order $2\text{–}8 \times 10^{6}\text{M}^{-1}$ (19, 20). Therefore the critical concentration of quinacrine necessary for the entrapment of DNA at the interface is approximately equal to the concentration of quinacrine that can be calculated to saturate the DNA intercalation sites. The lowest concentration of chloroquine that will promote entrapment is 1×10^{-5}M (Fig. 4). Comparison of this concentration to published binding constants of chloroquine for DNA extrapolated to the lower ionic conditions employed here (21) again indicates that this is the concentration necessary to saturate the DNA intercalating sites in solution.

While the reported association constant of ethidium for DNA at low ionic strength levels is similar to that of quinacrine, $2 \times 10^{6}\text{M}^{-1}$ (6, 9, 22), ethidium was not found to entrap DNA at the interface until a concentration of 3×10^{-5}M was reached (Fig. 4). It appears that simple saturation of the DNA intercalation sites with ethidium cannot bring DNA to the surface. However, if a comparison is made of the minimum concentration of ethidium that entraps DNA and the association constant of ethidium for its secondary, ionic type of DNA binding, $4.5 \times 10^{5}\text{M}^{-1}$ (7), a correlation can be found. This would suggest that at least a limited amount of exterior binding of the ethidium to the DNA is required before entrapment at the interface can occur. Extensive secondary binding of ethidium (as well as of quinacrine and chloroquine) at high concentrations of dye (10^{-3}M and above) results in aggregation and eventual precipitation of the DNA.

In summary, the ability of the intercalating dyes with a side arm to support entrapment cannot be correlated with the surfactant properties of the dye itself. Instead, a correlation exists between the concentrations that will support entrapment and those concentrations that will lead to saturation of either the primary (intercalation) or the primary and secondary (ionic) DNA binding sites while in solution. This would suggest that it is not a monolayer of dye at the interface of the solution that brings about entrapment; rather, the binding of the dye to the DNA itself induces the entrapment.

Non-intercalating Ligands that Support Entrapment

The ability of dyes that have side arms and radically different intercalating chromophores to entrap DNA at the interface suggested to us that the side arms alone may be responsible for the actual entrapment of the DNA. Spermidine, $NH_2\text{-}(CH_2)_3\text{-}NH\text{-}(CH_2)_4\text{-}NH_2$, was selected to test this possibility since its structure is gen-

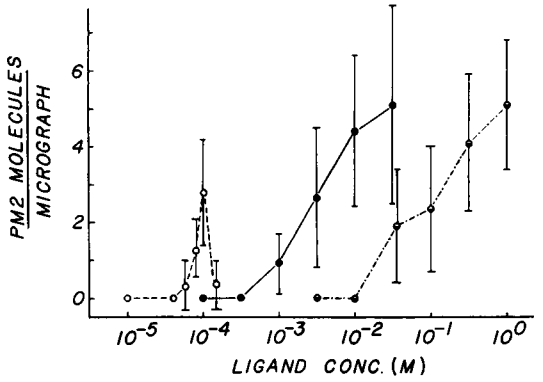

FIGURE 5 DNA adsorption as a function of ligand concentration. Adsorption conditions were 0.3 μg/ml PM 2 DNA, 5 mM Tris (pH 8), and the indicated cation concentrations. Droplets were incubated for 2 min. The greater standard deviation of DNA adsorption in this figure compared to that in the previous figures reflects both the less uniform adsorption of DNA and the smaller number of molecules adsorbed when simple cations are used in the absence of intercalating dyes. (—·—●—·—), ammonium acetate; (——●——), $CaCl_2$; (----○----), spermidine trihydrochloride.

erally similar to the side arms of quinacrine and chloroquine, and it has been postulated to bind tightly to the minor groove of DNA (23). As seen in Fig. 5, spermidine is indeed able to entrap DNA at an air-water interface, indicating that the rigidification and unwinding of the DNA helix brought about by the intercalating chromophores of the dyes are not requirements for the entrapment of the DNA. However, spermidine is able to support this entrapment of DNA only over a very narrow range of concentrations, since precipitation of the DNA occurs (at 1.5×10^{-4}M) before maximum entrapment of DNA at the interface can be reached. While to our knowledge well-characterized binding constants of spermidine for DNA, especially at these low ionic strengths, have not been reported, the narrow concentration range supporting entrapment, slightly below that which precipitates the DNA, indicates that near-saturation of the DNA with spermidine is required for entrapment.

Saturation of the DNA with a simple polyamine like spermidine would result in extensive charge neutralization of the DNA phosphates. Indeed, this is the reason for the precipitation of the DNA before maximum entrapment can be reached (Fig. 5). Therefore, we tested whether such charge neutralization of the phosphates contributes to the entrapment of DNA at the interface of a solution. Monovalent and divalent salts are obviously the simplest ligands that result in DNA phosphate shielding and were consequently tested for their ability to promote the entrapment of DNA. Each of these cations was indeed found to promote a significant level of entrapment, as seen in Fig. 5. While only the results with Ca^{++} and NH_4^{+} are shown, Ba^{++} was quite similar in action to Ca^{++}, as was Na^{+} to NH_4^{+}. As with spermidine, the minimum cation concentration able to support entrapment once again corresponds to the concentration required to shield the DNA phosphate charge (24).

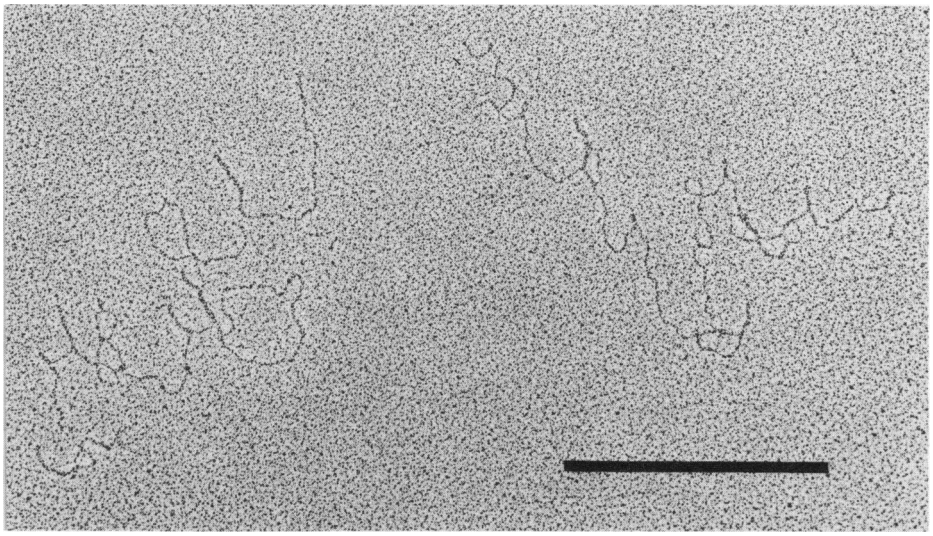

FIGURE 6 DNA adsorbed at high ionic strength. Adsorption conditions were 0.3 μg/ml PM 2 DNA, 0.01 M $CaCl_2$ and 5 mM Tris (pH 8). Scale bar represents 0.5 μm.

It should be mentioned that the entrapment of DNA at the interface by these simple cations is much less efficient than that observed with the intercalating dyes. First, much fewer PM 2 molecules are adsorbed to the carbon films. Fewer molecules were adsorbed to the carbon films in Fig. 5, even though the DNA concentration was twice that shown in Fig. 4. Second, even at very high cation concentrations, most of the molecules adsorbed to the carbon films are "streaked" (see Fig. 6). This orientation of the DNA along a hypothetical common axis can be interpreted as indicating that the DNA was not completely entrapped at the interface the moment the carbon film touched the surface of the solution. As a result, only limited regions of the DNA molecules are initially bound to the carbon films, while the majority of the DNA is swept onto the grid when the film is lifted from the interface.

DISCUSSION

In this study a wide range of DNA ligands has been shown to bring about the entrapment of DNA at an air-water interface. Investigation in this area originated with the screening of a number of intercalating dyes for their ability to entrap DNA at the interface. From this study it was found that the only structural requirement of these compounds, with reference to their use in the entrapment of DNA at the air-water interface, was a side arm extending from the chromophore of the molecule. This is clearly demonstrated by the inability of acridine orange to trap DNA at an air-water interface, while a derivative of acridine orange that contained a pyrrolidinyl-ethyl-thio arm at carbon 9 was entirely capable of bringing DNA to the surface. In addition, the unwinding and rigidification of the DNA helix brought about by the intercalating chromophore of the dye molecules were found to be unnecessary for the entrapment. Spermidine, a simple polyamine with no intercalating chromphore, could entrap DNA

TABLE II

MINIMUM CONCENTRATIONS OF LIGANDS THAT PROMOTE ENTRAPMENT OF DNA AT THE AIR-WATER INTERFACE

Ligand	Minimum concentration*
Quinacrine	3×10^{-6}M
Chloroquine	1×10^{-5}M
Ethidium	3×10^{-5}M
Spermidine	4×10^{-5}M
Ca^{++}, Ba^{++}	1×10^{-3}M
NH_4^+, Na^+	3×10^{-2}M

*Minimum concentration which permits adsorption to carbon films.

at the interface. Even simple mono- and divalent cations in the absence of the above ligands were found to promote a low level of surface entrapment. A summary of the ligands used in this report and of the minimum concentrations that will promote DNA entrapment is given in Table II.

Several lines of evidence presented in the course of this paper suggest that the ability of the various intercalating dyes to entrap DNA is not due to the buildup of a monolayer of dye at the interface. Rather, the association of the dye with the DNA in solution induces the entrapment of the DNA at the interface. Based both on how these dyes bind to DNA in solution and on the physical properties of DNA itself, a mechanism can now be given to explain the ability of these ligands to entrap DNA at the air-water interface.

In low ionic strength solutions the major and minor grooves of the DNA are completely filled with water, while large hydration spheres surround the DNA phosphates (25, 26). When the dye quinacrine (or chloroquine) is intercalated between the base pairs of the DNA, the side arms of the molecules have been assumed to project into one of the grooves of the DNA (17, 27). We believe that these side arms probably extend into the minor groove, at least in the case of quinacrine, because spermine, known to bind in the minor groove of DNA (23, 28), is an effective inhibitor of quinacrine binding (20). With quinacrine bound in this manner, the positively charged amines of the side arms are ionically bound to the DNA phosphate charges, thereby reducing the hydration spheres about the DNA. Meanwhile, the alkyl groups of the side arms replace water from the minor groove, thus increasing the hydrophobic character of this groove. In this state, with partial neutralization of the DNA phosphate charge and increased hydrophobicity of the minor groove, the DNA molecule and its associated ligands can behave as a "surface-active compound." In other words, the minor groove, with its increased hydrophobic character, would repel the polar environment of the aqueous solution and be trapped by the air-water interface. Spermidine binding to DNA would be expected to lead to the same process, since the three amines of the molecule would neutralize the DNA phosphate charge, while the alkyl groups of the molecule could again add hydrophobic character to the minor groove (23, 28). The ability of simple mono- and divalent cations to bring about DNA surface entrapment would suggest that one (or both) of the DNA grooves has sufficient hydrophobic

properties in itself to promote a limited level of entrapment once the large hydration shell about the DNA is reduced through phosphate neutralization.

The necessity of at least partial charge neutralization for the entrapment of DNA at the interface is also demonstrated by the results with ethidium. As was previously mentioned, higher concentrations of ethidium are required to bring about the entrapment of DNA at the interface than would be expected from its association constant. Disagreement exists as to which of the DNA grooves the phenyl and ethyl side groups of ethidium occupy when the dye is bound to DNA. Even though X-ray analysis on the co-crystallization of ethidium and dinucleotides has placed ethidium's side groups in the theoretical minor groove of such crystalline lattices (29), arguments have been presented against extrapolating these results to polynucleotides in solution (30), and in favor of placing the side groups in the major groove of DNA (31–33). While locating ethidium's side arms in the major groove of DNA might indicate why ethidium promotes entrapment of DNA differently from either quinacrine or chloroquine, the major reason for this difference probably lies in the structure of the side arms themselves. Unlike quinacrine or chloroquine, ethidium's side arms do not carry a positively charged group at their distal ends. Consequently, at concentrations of ethidium sufficient only to saturate the intercalation sites of DNA, entrapment of the DNA at the interface may not be possible, since little charge neutralization of the DNA phosphates has occurred. At higher ethidium concentrations, external ionic binding of ethidium molecules to the DNA occurs, resulting in charge shielding of the DNA and its entrapment at the air-water interface. This explains why ethidium is able to support entrapment of DNA at the interface only at concentrations where extensive ionic binding is known to occur. It should also be mentioned that certain of the compounds classified in Table I as not supporting entrapment occasionally gave rise to the adsorption of low numbers of highly aggregated DNA molecules at high dye concentrations (10^{-4}–10^{-3}M). This entrapment, like that of ethidium, is a reflection of the neutralization of the DNA charge brought about by the external ionic binding of the dye molecules. However, in this case, unlike ethidium, the hydrophobicity of the minor groove does not increase, and therefore entrapment at the interface is poor, resulting in extensive aggregation of the DNA.

In light of the above model for the mechanism of DNA surface entrapment, an explanation can now be given for the previously mentioned inability of actinomycin D to promote this phenomenon (Table I). As with the other intercalating dyes, the phenoxazone chromophore of actinomycin D is rapidly intercalated between the DNA bases. This is followed by a slower step, in which the large pentapeptide side-arm rings induce conformational changes in the DNA sugar-phosphate backbone, permitting the cyclic peptides to fit tightly into the minor groove (34, 35). In this state, with the minor groove completely shielded from the external environment, the ability of the DNA-actinomycin D complex to be trapped at an air-water interface is completely dependent upon the hydrophobic properties of these pentapeptide rings. As can be seen in published CPK (Corey-Pauling-Koltun) space-filling models or three-dimensional computer drawings of actinomycin D bound to DNA (35–37), several polar groups (especially the carboxyl groups of L-methyl valine, sarosine, and proline) are ex-

posed to the exterior of the DNA-actinomycin D complex, thus allowing bond formation with water. The DNA-actinomycin D complex is therefore not surface-active, since the minor groove is not repelled by the aqueous solution.

While work on this paper was in progress, an article appeared by Koller et al. (38) describing the use of ethidium for the observation of RNA polymerase molecules bound to DNA. Although these authors made no attempt to understand how ethidium can assist in the adsorption of DNA, and did not even refer to the fact that the DNA was being trapped at the surface of the solution, the clarity with which they demonstrated RNA polymerase molecules bound to DNA clearly supports the usefulness of intercalating dyes in the observation of DNA or DNA-protein complexes.

In summary, the results of this paper indicate that several DNA ligands are capable of changing the hydrophobic properties of DNA so that it will be trapped at an air-water interface. While simple neutralization of the DNA phosphate charge will permit a limited degree of entrapment of the DNA's hydrophobic grooves at the interface, the addition of further hydrophobic character to these grooves greatly increases the ability of the DNA to be entrapped. This entrapment allows easy adsorption of the DNA to carbon films, and hence the electron microscopic observation of DNA. In forthcoming reports the use of this technique in the observation of both DNA and DNA-protein complexes will be presented.

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