DAPI: a DNA-Specific Fluorescent Probe

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ABSTRACT. DAPI (4′,6-diamidino-2-phenylindole) is a DNA-specific probe which forms a fluorescent complex by attaching in the minor grove of A-T rich sequences of DNA. It also forms nonfluorescent intercalative complexes with double-stranded nucleic acids. The physicochemical properties of the dye and its complexes with nucleic acids and history of the development of this dye as a biological stain are described. The application of DAPI as a DNA-specific probe for flow cytometry, chromosome staining, DNA visualization and quantitation in histochemistry and biochemistry is reviewed. The mechanisms of DAPI-nucleic acid complex formation including minor groove binding, intercalation and condensation are discussed.

Key words: 4′,6-diamidino-2-phenylindole nucleic acids, light absorption spectroscopy, fluorescence spectroscopy, flow cytometry, DNA visualization, DNA assay, chromosome staining, intercalation

Development of DAPI

DAPI (4′,6-diamidino-2-phenylindole dichloride) (Fig. 1) was first synthesized in Otto Dann's laboratory at Erlangen as one of many diamidine compounds in the search for new trypanocides related to the drug berenil (Dann et al. 1971). DAPI, to my knowledge, never went to clinical trials as a drug, but in 1975 Williamson and Fennell used it for isolation of mitochondrial DNA in cesium gradient (Williamson and Fennell 1974). They observed enhancement of the dye's fluorescence when it was attached to DNA. This enhancement was particularly good for mitochondrial DNA which is rich in A-T sequences. They also used DAPI for staining DNA of yeast cells and demonstrated that the fluorescence is sensitive to DNase, but not to RNase. These observations established DAPI as a DNA-specific fluorescent stain. At the same time, these authors discredited DAPI as a quantitative measure of DNA because of a mistaken observation that the free dye solution is nearly as fluorescent as the DNA-dye complex. Most likely this phenomenon was a result of the presence of sarcosyl (N-laurosylsarcosine), an anionic detergent in the solution used for DNA separation. Later it was shown that anionic detergents enhanced DAPI fluorescence almost as much as DNA, i.e., about 20 times (Kapuscinski and Skoczylas 1978). Russell et al. (1975) published a simple cytochemical technique for detecting myoplasma infection of cultured HeLa cells treated with DAPI using fluorescence microscopy. These authors published a stunning photograph of vaccinia viruses within DAPI treated cells. These papers attracted the attention of other scientists in the field and during the next two years most of DAPI's application as a DNA-specific fluorochrome was explored. The practicability of DAPI for fluorescent microscopic observation of DNA in bacteria (Grossgebauer et al. 1976, Jagielski et al. 1976), plant (Schweizer 1976a,b, Schweizer and Nagl 1976), protozoa (Hajduk 1976) and mammalian cells (Grossgebauer et al. 1976, Lin and Alfi 1976, Lin et al. 1976, Zworska et al. 1976) was demonstrated. Detection of mycoplasma contamination was explored further by Jagielski et al. (1976) and Grossgebauer et al. (1977). The structure of chromatin and chromosomes stained with DAPI alone (Lin and Alfi 1976, Schweizer 1976a,b, Schweizer and Nagl 1976, Lin et al. 1977) and in combination with actinomycin D or chromomycin (Schweizer 1976a,b), mithramycin (Schnedl et al. 1977) or distamycin D (Schweizer et al. 1978, 1979, Buys et al. 1979a,b) were studied and the high affinity of DAPI for A-T rich DNA sequences was confirmed. In addition, biochemical study revealed that DAPI inhibited EcoRI restriction nuclease activity at A-T rich regions (Kania and Fanning

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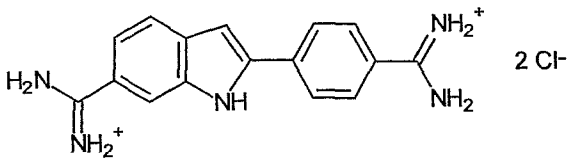

Fig. 1. Chemical structure of 4′-6-diamidine-2-phenylindole (DAPI) dichloride.

1976) and also inhibited the template activity of *P. aurelia* (Skoczylas and Kapuscinski 1977). Several authors pointed out high resistance of DAPI to UV illumination (e.g., Schnedl et al. 1977, Stöhr et al. 1977). These findings established DAPI as a valuable biological stain.

A simple and sensitive quantitative fluorescence method for DNA assay in the presence of RNA and histones was developed by Kapuscinski and Skoczylas (1977). The major advantage of this method is that no preliminary preparations, such as separation or enzymatic RNA degradation, are required. The lowest limit of this assay is 5×10^{-10} g/ml of DNA using a commercially available spectrofluorometer (Kapuscinski and Skoczylas 1977). Later, a similar method was applied to measure nanogram quantities of DNA in cellular homogenates (Brunk et al. 1979). DAPI was also used for visualizing DNA in the presence of proteins (Douglass et al. 1978) and in the presence of large quantities of RNA in electrophoretic gels (Kapuscinski and Yanagi 1979).

Application of DAPI in Histology, Analytical Cytology and Biochemistry

Flow cytometry. The widest application of DAPI is in flow cytometry. The use of the dye as a quantitative DNA assay in cells was reported first by Stöhr et al. (1977), then by Göhde et al. (1978). Since then, hundreds of papers describing the applications of this technique have been published.

Some applications of DAPI for quantitative DNA measurement in individual cells have recently been reviewed in the monograph *Flow Cytometry and Sorting* (Crissman and Steinkamp 1990, Darzynkiewicz 1990, Latt and Langlois 1990, Pallavicini et al. 1990, Waggoner 1990). The dye can be used alone, e.g., for cell cycle studies of drug effects, or in multi-parameter cell analysis in combination with another fluorochrome. One of the most popular stains combines DAPI with sulforhodamine (SR 101) for simultaneous measurement of protein and DNA content in cells (Stöhr and Goerttler 1979). When using DAPI for such measurement, one must remember that DAPI is a base specific stain and that the resulting fluorescence depends not only on the amount of DNA in the cell, but also on A-T the base content. The amount of the DAPI bound in the cell depends on the degree of chromatin condensation, which depends on the method of cell preparation. For instance, removing histone proteins by washing cells with 0.1 N hydrochloric acid nearly doubles the amount of DAPI bound to DNA (Darzynkiewicz et al. 1984, Rundquist 1993). Another DNA-specific stain, Hoechst 33258, has similar spectral properties and binding mechanism as DAPI. There is, however, one important difference between the two fluorochromes. While the bromodeoxyuridine incorporated into DNA at neutral pH has no effect on DAPI emission, Hoechst 33258 fluorescence is quenched by halogenated DNA (Takahama and Kagaya 1988, Latt and Langlois 1990, Hard et al. 1990). Flow cytometry DNA assays were used for many purposes including studies of spermatozoa (Otto et al. 1979, Evenson et al. 1986), DNA analysis of isolated nuclei from a variety of tissues (Thornthwaite et al. 1980, Lee et al. 1984, Otto 1990, Castro et al. 1993), analysis of phytoplankton (Trask et al. 1982), protozoa (Bonaly et al. 1987, Muhlpfordt and Berger 1989), bacteria (Robertson and Button 1989) and plants (Galbraith 1990, Ulrich 1992), analysis of cellular DNA content of paraffin embedded pathological material (Hedley 1990, Heiden et al. 1991), and cancer detection and prognosis (Owainati et al. 1987, Meyer and Coplin 1988, Chi et al. 1990, De Vita et al. 1991, Hatchoh et al. 1992).

Chromosome staining. During the last 25 years, use of DAPI was reported as a biological stain for chromosomes more than 200 times. It was used alone or in combination with other DNA ligands, most frequently with distamycin D (Dhawale and Kessler 1993). Because of the large number of publications in this field, only selected papers are cited here. The dye has been used to study chromosomes of plant (Schweizer 1976a,b, Leemann and Ruch 1978, 1982, 1983, Rawlins and Shaw 1988, Beardsell et al. 1990, Czaban and Forer 1992, Rayburn et al. 1992, Hagemann et al. 1993) and animal cells, and to investigate chromatin structure (Schwarzacher et al. 1984, Schmid et al. 1987, Mills and Massey 1992). Human chromosome aberrations were examined (Fujita et al. 1980, Hasegawa et al. 1984, Mohandas et al. 1985, Sachs et al. 1987, Macera et al. 1989, Merkx et al.

1990, Sago et al. 1991, Smeets et al. 1991, Blennow et al. 1993) in cancer studies and for cancer diagnosis (Macera et al. 1989, Huber et al. 1990, Callahan et al. 1992, Holden et al. 1986).

DNA visualization and quantitation. DAPI has been used frequently for microscopic study of DNA in chloroplasts (James and Jope 1978, Coleman 1979, 1984, Coleman et al. 1981, Hoursiangou Neubrun et al. 1982, Lawrence and Possingham 1986, Ehara et al. 1990) and in mitochondria (Williamson and Fennell 1974, Hyman et al. 1982, Hamada and Fujita 1983, Miyakawa et al. 1984, Van Blerkom and Runner 1984, Yamada et al. 1986, McCarthy et al. 1987, Ito Kuwa et al. 1988, Satoh and Kuroiwa 1991), detection of protozoans including malaria (Hyman and Macinnis 1979, Celada et al. 1983, Kawamoto et al. 1987, Matsumoto et al. 1987), spermatogenesis (Abbott and Gerbi 1981, Sahdev et al. 1989, Bressac and Rousset 1993) and fertilization (Goff and Coleman 1984, Minhas et al. 1984, Coleman and Goff 1985, Hollenbeck and Cande 1985, Mori et al. 1988, Rohloff et al. 1990, Nakanishi et al. 1990, 1991, Perry 1987). The diversity and diagnostic potential of the DAPI staining technique is illustrated by a recent study concerning the detection of both spermatozoa and *Pneumocystis carinii* in the lung of an AIDS patient (Pohle and Grossgebauer 1986).

DAPI and antibody staining techniques. The combination of DAPI and a fluorescent labeled antibody is a powerful cytochemical tool for concurrent visualization of DNA and individual proteins in the cell. Using this technique, DNA and tubulin, microtubules or microfilaments can be identified (Baumstark Khan et al. 1984, Roos et al. 1984, Wang et al. 1988) and malaria parasites (Murakami and Tanabe 1985), bacteria (Hoff 1988), and *Herpes* simplex virus (Randall and Dinwoodie 1986) can be detected. Production of interferon in the cell in relation to cell cycle can also be studied (Tang et al. 1989).

In situ hybridization. In recent years this technique, combined with simultaneous DNA staining with DAPI, has become very popular. The detailed description of its implementation is beyond the scope of this article, but can be found in the following selected recent publications (Fan et al. 1990, Plattner et al. 1991, Smeets et al. 1991, Crolla et al. 1992, Ried et al. 1992, Boyle et al. 1992, Callahan et al. 1992, Najfeld et al. 1992, Heng and Tsui 1993, Trask et al. 1993).

Fluorescent assay of DNA in solution. The fluorescence enhancement of DAPI in the presence of DNA is the basis for a simple and rapid method for DNA microassay in the presence of RNA and proteins (Kapuscinski and Skoczylas 1977) and cellular homogenates (Brunk et al. 1979). The sensitivity of this method reaches 0.5 ng DNA/ml. The assay can not be performed in the presence of an anionic detergent, such as sodium docasyl sulfate (SDS), which enhances the fluorescence of the dye (Kapuscinski and Skoczylas 1978). Also, application of DAPI is limited in the presence of RNA, which binds DAPI to form a nonfluorescent complex. If the nucleic acid mixture contains more than 96% RNA, DNA cannot be measured accurately (Katouzian Safadi et al. 1989). Because the DAPI forms a fluorescent complex only with A-T-sequences, the calibration curves must be made with DNA with a GC:AT ratio similar to the DNA measured in the sample. The other limitation is the presence of large amounts of tubulins or microtubules in the sample, because these are the only proteins known to form fluorescent complexes with DAPI (Bonne et al. 1985). A modification of this technique was also used to assay DNA in epidermis and cultured fibroblasts (Meyer and Grundmann 1984), plant tissue (Lee and Garnett 1993), prokaryotes (whole bacteria treated with toluene without DNA purification) (Legros and Kepes 1985) and for DNA deposited on filters (Wilkins and Kearney 1984). DAPI also has been used for determining cell density in microtiter wells (McCaffrey et al. 1988).

Visualization of DNA in electrophoresis gels. Douglas et al. (1978) described a method for electrophoresis of DNA prestained with DAPI. A method for selective staining of DNA in gels in the presence of double stranded (ds) RNA, which is not visualized, has been proposed (Kapuscinski and Yanagi 1979). This staining method is several times more sensitive than staining with ethidium bromide (Nairn et al. 1982, Buel and Schwartz 1993).

Physicochemical Properties of DAPI and its Complexes with Nucleic Acids

DAPI is commercially available as the dichloride or diacetate salt. In the solid state and kept in the dark, the dye can be stored for several years without decomposition. DAPI is readily soluble in water and concentrated frozen solutions (several mg/ml) are stable for months. The absorp-

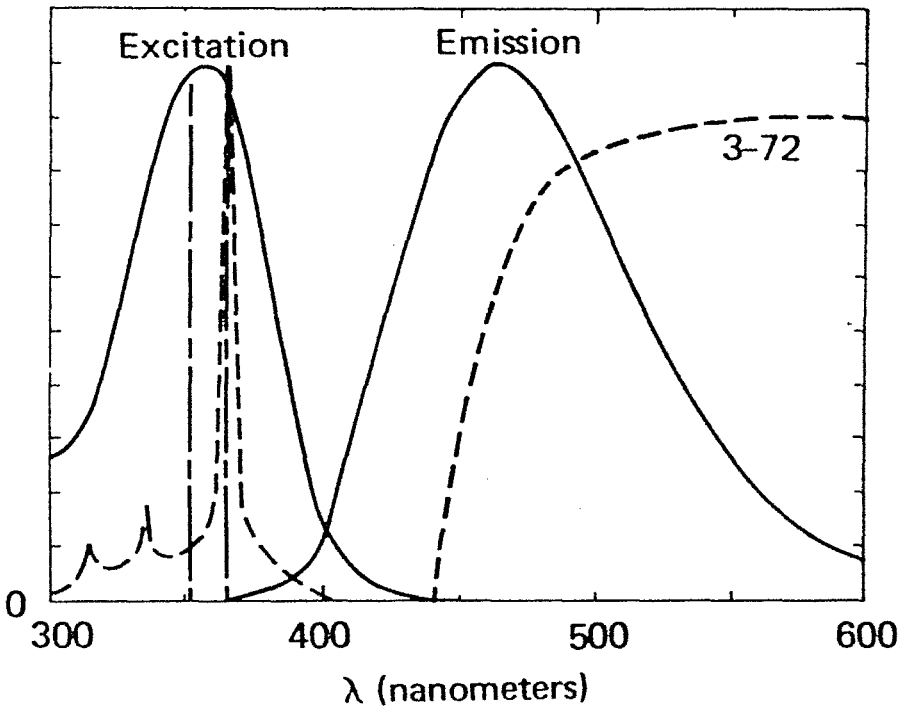

Fig. 2. Excitation and emission spectra of DNA-bound fluorescent dye Hoechst 33258 (or DAPi). Superimposed are the light source spectra of UG 1 filtered mercury arc lamp (---), the laser 351- and 364-nm emission lines (— - —) and the transmission curve of 3-72 high pass barrier filter. Reproduced with permission from Peters (1979).

tion spectrum of DAPI has three maxima, 222, 259 and 340 nm, but only the 340 nm band is important for cytofluorometrics. This band is well positioned for excitation by UG 1 filtered mercury lamp or 351 and 364 nm laser light (Peters 1979) (Fig. 2). The fluorescence quantum yield of the free dye is very low with a maximum of emission at 453 nm; when bound to DNA there is a bathochromic shift of excitation and a hypsochromic shift of emission, and the fluorescence quantum yield increases more than 20-fold (Table 1). The fluorescence decay of the free dye is multi-exponential with the prevalent component at 0.26 nsec; the lifetime of the dye bound to DNA is approximately 4 nsec (Barcellona and Gratton 1991). DAPI is not optically active, but in complexes with nucleic acids, the induced extrinsic positive Cotton effect can be seen. This effect was observed in circular dichroism (CD) spectra, with a maximum above 330 nm, of both fluorescent and nonfluorescent complexes of the dye with nucleic acids (Kapuscinski and Szer 1979). The position of the long wavelength maximum of the

Table 1. Spectral Properties of DAPI and its Complexes with Nucleic Acids

	λ_{mx} nm	E × 10^{-4} cm^{-1} M^{-1}	λ_E nm	λ_F nm	Q	Mode of Binding	K × 10^{-6} M^{-1}
DAPI[a]	340	2.70	347	453	0.04	—	—
DAPI-ds DNA[a]	347	2.36	363	448	0.92	m.g.	20.0
DAPI-poly(dA)·poly(dT)[b]	358	2.21	350	448	0.92	m.g.	7.40
DAPI-poly(rA)·poly(rU)[b]	360	1.93	350	460	0.30	int.	0.05
DAPI-poly[d(G-C)]$_2$[b]	360	1.93		nonfluorescent		int.	0.12
DAPI-poly(rA) 1:2[a]	356	2.04	356	500	0.20	cond.	0.32[c]

λ_{mx}, maximum absorption; E, molar absorption coefficient; λ_E, maximum excitation; λ_F, maximum emission; Q, fluorescence quantum yield; K, association constant; m.g., binding in the minor groove of DNA; int., intercalation into the double helix; cond., condensation of the complex.

[a] Data from reference (Kapuscinski 1990)

[b] Data from reference (Tanious et al. 1992).

[c] Cooperative association constant.

CD spectrum depends on the base composition of the nucleic acid and on the dye/nucleic acid ratio (Kubista et al. 1987, Wilson et al. 1990a, Eriksson et al. 1993).

Mechanism of DAPI Interactions with Nucleic Acids, other Polyanions and Proteins

Despite the extensive use of DAPI as a biological stain and for DNA assay, the mechanism of its interaction with nucleic acids has only recently been clarified. The ability of DAPI to form a highly fluorescent complex with DNA was described in the 1970s (Williamson and Fennell 1974, Russell et al. 1975). The requirements of ds DNA and base specificity (AT-rich sequences) for formation of the fluorescent complex with DAPI were also established early by both biochemical (Willamson and Fennell 1974, Kania and Fanning 1976, Chandra et al. 1977, Kapuscinski and Skoczylas 1977, Kapuscinski and Skoczylas 1978) and histological (Schweizer 1976a,b, Schnedl et al. 1977, Hajduk 1976) methods. Studies using synthetic poly- and oligonucleotides revealed that double stranded DNA containing dA-dT, dA-dU, dA-BrdU and dI-dC (but not dG-dC) sequences enhanced DAPI fluorescence (Kania and Fanning 1976, Lin et al. 1977, Kapuscinski and Szer 1979), and that the binding sites must contain at least three consecutive base pairs (Kapuscinski and Szer 1979). Both single and double stranded RNA form complexes with DAPI, but the fluorescence of these complexes (with the exception of poly(rI)) is much weaker than the fluorescence of DAPI double stranded DNA (Kapuscinski and Szer 1979, Kapuscinski and Yanagi 1979). The affinity of DAPI for double stranded DNA is very high (Kania and Fanning 1976); the apparent association constants (K_{app}) in the range of 10^5–10^7 M^{-1} were reported (Kapuscinski and Skoczylas 1978, Bierzynski et al. 1978, Chandra and Mildner 1980a,b, Morikawa et al. 1981, Masotti et al. 1981, Barcellona et al. 1981, Dall'Asta et al. 1981, Masotti et al. 1982, Manzini et al. 1983, Barcellona et al. 1986, Bumma et al. 1988, Tanious et al. 1992). Most investigators reported two kinds of binding sites for natural DNA. The different K_{app} values reported by these authors can be explained by different experimental and calculation methods.

The Gibbs free energy change (ΔG^0) of DAPI binding to calf thymus DNA is between -9 (Manzini et al. 1983) and -11 kcal/mol (Kapuscinski and Skoczylas 1978), depending on NaCl concentration (0.1 and 0.01 M, respectively). The enthalpy of this interaction is also favorable ($\Delta H < -5$ kcal/mol) (Chandra and Mildner 1980a,b); the binding of DAPI stabilizes double stranded DNA against thermal denaturation (Kapuscinski and Skoczylas 1978). Based on these data and results of a study of solvent effects, intercalation was suggested as a mechanism for formation of the DAPI-double stranded DNA fluorescent complex (Kania and Fanning 1976, Kapuscinski and Skoczylas 1978, Chandra and Mildner 1980a,b, Schweizer et al. 1978, Masotti et al. 1981, Kapuscinski and Szer 1979).

There were also strong arguments against an intercalation mechanism, however (Kubista et al. 1987). The unwinding of supercoiled DNA induced by DAPI (Stepien et al. 1979, Manzini et al. 1983) and the sedimentation of linear DNA bound to DAPI are not consistent with a complex formed by intercalation (Waring 1970). Based on these data and those of linear dichroism, circular dichroism and fluorescence spectroscopy Kubista et al. (1987) rejected the idea that DAPI is bound to DNA by intercalation. They observed that binding geometries and site densities are consistent with DAPI located in the grooves of DNA, with the high-affinity site probably in the minor groove as is the case for netropsin and Hoechst 33258 dye (Portugal and Waring 1988). Earlier CD studies published (Manzini et al. 1983) led to the same conclusion. The location of the binding site in the minor groove of the B-DNA molecule can explain the strong A-T base specificity of the DAPI-DNA fluorescent complex (Manzini et al. 1983, Portugal and Waring 1988, Kubista et al. 1987). This model was confirmed by x-ray diffraction of a single crystal of DAPI bound to the synthetic B-DNA oligonucleotide C-G-C-G-A-A-T-T-C-G-C-G (Larsen et al. 1989). According to these authors, the fluorescent complex is nearly isomorphous with the native DNA molecule with one DAPI and 25 water molecules per DNA double helix. DAPI is inserted edgewise into the narrow minor groove, displacing the ordered spine of hydration. DAPI and a single water molecule together span the four A-T base pairs at the center of the duplex. The indole nitrogen forms a bifurcated hydrogen bond with the thymine oxygen atoms of the two central base pairs, just as netropsin and Hoechst 33258 do. The preference of all three of these drugs for A-T regions of B-DNA is due to three factors: 1) the minor groove associated with A-T regions is narrower

than G-C regions of B-DNA, leading to a snug fit of the flat aromatic rings between the walls of the groove, 2) the more negative electrostatic potential within the minor groove in A-T regions, attributable in part to the absence of electropositive guanine amine groups along the floor of the groove, and 3) the steric advantage of the absence of those amine groups, permitting the dye molecule to sink deeper into the groove. Groove width and electrostatic factors are regional and define the relative receptiveness of a section of DNA since they operate over several contiguous base pairs. The steric factor is local, varying from one base pair to the next, hence it is a means of fine tuning sequence specificity (Larsen et al. 1989).

There presently is little doubt about the structure of the fluorescent complex DAPI forms with double stranded DNA, i.e., that the fluorochrome molecule is bound in the minor grove of consecutive (3–4 base pairs) A-T-rich sequences. It does not mean that DAPI does not bind to other sequences of double stranded DNA, or double stranded RNA. These complexes, however, are much less fluorescent. In the series of elegant papers reporting results of several physicochemical techniques including NMR, light absorption spectroscopy, viscosimetry, stopped-flow kinetics, and molecular modeling methods, Wilson et al. (1989, 1990a,b) and Tanious et al. (1992) provided evidence that DAPI binds to other than the continuous A-T sequences of DNA and to double stranded RNA by intercalation. These authors stated that at continuous dG-dC and mixed (e.g., poly(dA-dC).poly (dG-dT)) sequences, the 2-NH_2 group of guanine sterically inhibits DAPI binding in the minor groove of DNA. Also, in such sequences, the depth of the minor groove is reduced and its width is increased compared to A-T sequences; the energetics of this favors intercalation over minor groove binding (Tanious et al. 1992). The binding strength of DAPI to the dG-dC sequences is in the range typically observed for strong intercalators such as quinacrine, ethidium or propidium. The same authors also provided evidence that DAPI binds strongly to rA-rU sequences by intercalation rather than to the minor groove (Tanious et al. 1992). DAPI should be viewed, therefore, as an intercalator that has unusual and favorable interactions in the minor groove at dA-dT sequences (Wilson et al. 1989).

Most intercalators, including DAPI at high concentrations, condense and precipitate polyanions including both single and double stranded nucleic acids (Kapuscinski and Darzynkiewicz 1990, Kapuscinski 1990). Nucleic acids in solution behave as random coil structures because of repulsive ionic phosphate-phosphate interactions. After neutralization, the polymer collapses and forms compact (condensed) structures. Neutralization of DNA can be achieved by multi-cations with valences ≥ 3, such as $Co[NH_3]_6^{+3}$, $spermidine^{+3}$ or $spermine^{+4}$, simple cations (Widom and Baldwin 1980, Widom and Baldwin 1983), or by aromatic cations (e.g., intercalators) with valences ≥ 1 with an ability to form a complex with nucleic acid bases by stacking interactions (Kapuscinski and Darzynkiewicz 1983, 1984a). The mechanism of nucleic acid condensation induced by these two types of ligand is different. The most significant difference is that the aromatic cation-induced condensation is preceded by denaturation of double helix regions of nucleic acids (Kapuscinski and Darzynkiewicz 1984a,b), while the secondary structure of nucleic acids is preserved in the condensed product with simple cations (Widom and Baldwin 1980). In both cases, however, the electrostatic repulsive forces are reduced, which leads to spontaneous condensation (collapse) of the polymer (Manning 1980, Kapuscinski and Darzynkiewicz 1984a,b). The condensation of the fluorochrome-nucleic acid complexes has profound effects on their spectral properties, resulting in part from limited contact with the solvent. For example, condensation of the acridine-RNA complex is responsible for red luminescence (Kapuscinski and Darzynkiewicz 1983, 1984b, Kapuscinski et al. 1982), and the same process results in the luminescence quenching of the pyronin Y-RNA complexes (Darzynkiewicz and Kapuscinski 1988). Condensed complexes of DAPI-RNA have an emission maximum at approximately 500 nm compared to 448 nm for the complex with double stranded DNA (Fig. 3); the fluorescence quantum yield of this condensed complex is only about ⅕ of the yield of the highly fluorescent DAPI-double stranded DNA complex (Kapuscinski 1990, Skoczylas 1988).

The formation of yellow fluorescence is not limited to DAPI condensed complexes with nucleic acids. Other polyanions including mucopolysaccharides and polyphosphates are also precipitated within cells (e.g., in vacuoles and on the nuclear envelope) and outside the

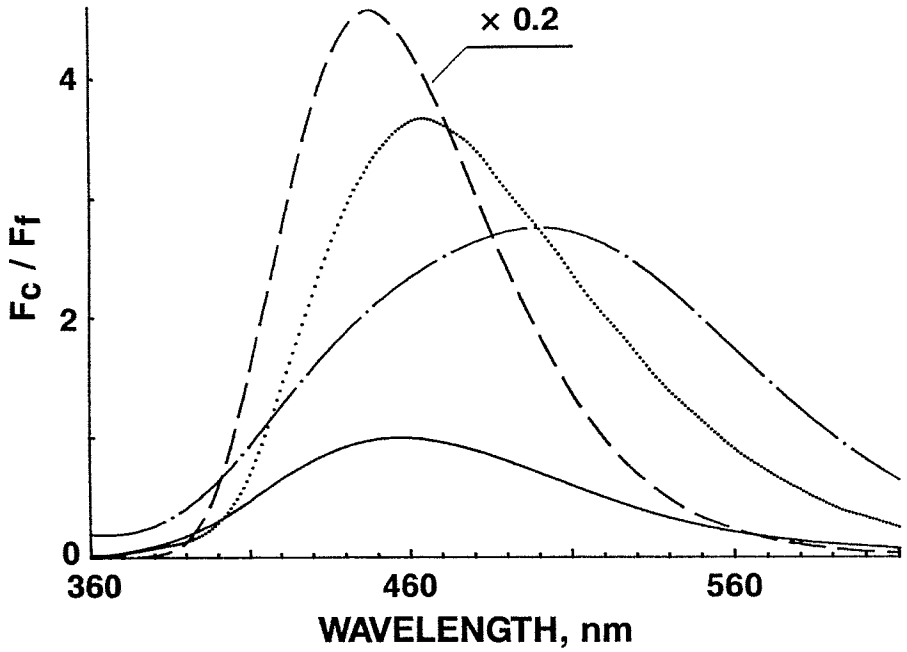

Fig. 3. Fluorescence emission spectrum (excitation 340 nm) of DAPI (2 μM) normalized to 1 (——), its complex with double stranded DNA (D/P = 0.01) (reduced five times from the original amplitude), (---), its complex with poly(rA) at D/P = 0.1 (-·-·-) and at D/P = 0.5 (— - —). F_c and F_f are the fluorescence intensity of the complex and free dye, respectively. Reproduced with permission from Kapuscinski (1990).

plasma membrane (Grossgebauer 1979a,b, 1980, Allan and Miller 1980, Grossgebauer and Kupper 1981, Tijssen et al. 1982). The large bathochromic emission shifts of these non-nucleic acid complexes, similar to and sometimes larger than that observed for condensed DAPI-RNA complex (Kapuscinski 1990) has been reported (Allan and Miller, 1980, Tijssen et al. 1982, Kjeldstad et al. 1991).

Among proteins, only tubulins are known to form fluorescent complexes with DAPI (Bonne et al. 1985, Heusele and Bonne 1985, Heusele et al. 1987, Ortiz et al. 1993). Tubulin is a major protein of microtubules, which are important cytoskeletal constituents of eukaryotic cells. The stoichiometry of the complex is one DAPI molecule per tubulin dimer (Bonne et al. 1985). Bonne et al. (1985) proposed that the DAPI-binding site on tubulin is located partly in the carboxyl-terminal region of tubulin in which the last 40 positions have 19 acidic side chains. These authors also observed energy transfer from tryptophan located close to this binding site to DAPI. The apparent association constants are 2.3×10^4 and 17×10^4 M^{-1} for tubulin and microtubules, respectively (based on data from Bonne et al. (1985)), which indicates a lower affinity of these proteins for DAPI compared to DNA. The changes in the fluorescence spectra of DAPI bound to tubulin (or microtubules) are similar to those observed for changes observed for DAPI vs. DAPI-DNA complex, i.e., a bathochromic shift (+8 nm) for excitation, and a hypsochromic shift (-24 nm) for emission, compared to the spectra of the free dye. The fluorescence quantum yield of DAPI increases several times after binding to these proteins, but it is much lower than that observed for DAPI double stranded DNA complex (Bonne et al. 1985).

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