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ARTICLE

A New Fluorescence Reaction in DNA Cytochemistry: Microscopic and Spectroscopic Studies on the Aromatic Diamidino Compound M&B 938

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SUMMARY We describe the fluorescence properties and cytochemical applications of the aromatic diamidine M&B 938. Treatment of cell smears (chicken blood, Ehrlich ascites tumor, rat bone marrow, mouse mast cells, and *Trypanosoma cruzi* epimastigotes) with aqueous solutions of M&B 938 (0.5–1 μg/ml at pH 6–7; UV excitation) induced bright bluish-white fluorescence in DNA-containing structures (interphase and mitotic chromatin, AT-rich kinetoplast DNA of *T. cruzi*), which was abolished by previous DNA extraction. DNA was the unique fluorescent polyanion after staining with M&B 938 at neutral or alkaline pH, other polyanions such as RNA and heparin showing no emission. M&B 938-stained mouse metaphase chromosomes revealed high fluorescence of the AT-rich centromeric heterochromatin, and strong emission of heterochromatin in human chromosomes 1, 9, 15, 16, and Y was found after distamycin A counterstaining. On agarose gel electrophoresis, M&B 938-stained DNA markers appeared as fluorescent bands. The 1.635-KBP fragment from DNA ladder revealed a higher emission value than that expected from linear regression analysis. Spectroscopic studies showed bathochromic and hyperchromic shifts in the absorption spectrum of M&B 938 complexed with DNA, as well as strong enhancement of fluorescence at 420 nm. In the presence of poly(dA)–poly(dT), the emission of M&B 938 was 4.25-fold higher than with DNA; no fluorescence was observed with poly(dG)–poly(dC). Experimental results and considerations of the chemical structure suggest that the minor groove of AT regions of DNA could be the specific binding site for M&B 938, which shows interesting properties and useful applications as a new DNA fluorochrome.

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KEY WORDS
Diamidines
Cationic fluorochromes
Fluorescence microscopy
DNA cytochemistry
Cell polyanions
DNA minor groove

In the past few years there has been renewed interest in the design and development of aromatic diamidino compounds that exhibit specific DNA-binding modes as well as important biological and therapeutic applications (Krugh, 1994; Wilson, 1990; Zimmer and Wähnert, 1986). The leading drugs, berenil and 2-hydroxystilbamidine (and other similar compounds), have relevant trypanocidal, fungicidal, and anti-tumor activities (Zimmer and Wähnert, 1986; Baguley, 1982; Newton, 1975; Snapper et al., 1952). In addition, some aromatic diamidines have been shown to inhibit the reverse transcriptase from oncogenic RNA viruses (De Clerq and Dann, 1980) and DNA topoisomerase II activity in kinetoplast DNA from trypanosomes (Shapiro and Englund, 1990; Woynarowski et al., 1989). Recently, pentamidine, propamidine, and related diamidine analogues have been found useful in the treatment of *Pneumocystis carinii* pneumonia, the most common opportunistic infection in AIDS patients (Tidwell et al., 1990).

It is well known that aromatic diamidines specifically bind to adenine–thymine (AT) sequences of DNA by a non-intercalative mechanism (Jansen et al., 1993; Luck et al., 1988; Gresh and Pullman, 1984). Both crystallographic and nuclear magnetic resonance

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studies have clearly shown that the specific binding mode of DAPI, berenil, pentamidine, and propamidine involves a close fit between the bowed ligand and the convex floor of the DNA minor groove through van der Waals contacts and hydrogen bonds to consecutive AT base pairs (Nunn et al., 1993; Brown et al., 1992; Edwards et al., 1992; Kopka and Larsen, 1992; Yoshida et al., 1990).

Interestingly, some DNA-binding aromatic diamidines such as berenil, 2-hydroxystilbamidine, and DAPI also exhibit striking fluorescence properties (Bella and Gosálvez, 1994; Stockert et al., 1990b; Murgatroyd, 1982; Kapuscinski and Skoczylas, 1977; Festy and Daune, 1973). Fluorescence microscopy of trypanosomes subjected to berenil treatment (either in culture or isolated from treated animals) revealed a bright blue emission of kinetoplasts and nuclei (Newton, 1975). The same result was obtained with 4,4′-diamidinodiphenylamine, a trypanocidal compound that is the short-chain homologue of berenil (Newton, 1975). The bright nuclear fluorescence of this compound (referred to as M&B 938; Figure 1) was also useful for identifying parakeratosis in human epidermis (Tring and Murgatroyd, 1976).

At present, non-rigid DNA fluorochromes such as DAPI and the bisbenzimidazole Hoechst 33258 (both synthesized as trypanocidal drugs) are widely used in cytochemistry, cytogenetics and cell biology (Bella and Gosálvez, 1991, 1994; Sumner, 1990; Holmquist and Motara, 1987; Latt and Stetten, 1976) because of their specific minor groove binding to AT sequences (Krugh, 1994; Kopka and Larsen, 1992). Other similar non-rigid fluorochromes (thioflavine T, auramines O and G, 2,5-*bis*(4-aminophenyl)-1,3,4-oxadiazole, pyrvinium, and the N-quaternary oxazole scintillator Q4) could also interact with DNA in the same way as indicated above (Stockert, 1992; Stockert et al., 1990b,1991). Taking into account the current interest in studies of new fluorescent probes for DNA and their possible biological activity, the aim of this work was to describe microscopic, electrophoretic, and spectroscopic features of the fluorescence reaction of M&B 938 with DNA, which suggest a highly selective binding mode of this aromatic diamidine to the minor groove of AT-rich DNA regions.

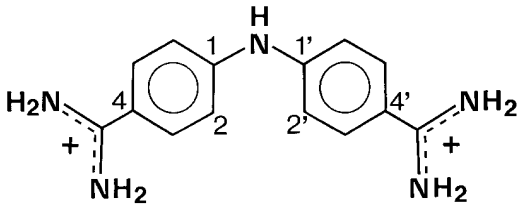

Figure 1 Chemical structure of the compound M&B 938 (4,4′-diamidinodiphenylamine dihydrochloride) shown in the form of the diamidinium cation. Relevant positions on the phenyl rings are numbered.

Material and Methods

Smears of chicken and horse blood, Ehrlich ascites tumor cells, rat bone marrow, mast cells obtained from the peritoneal cavity of Balb/c mice (Espada et al., 1995), and *T. cruzi* epimastigotes growing in culture at 27°C were fixed in methanol for 2 min and air-dried. After fixing, some smears were subjected to DNA extraction with DNAse (Sigma; St Louis, MO) (0.5 mg/ml at 37°C for 2 hr) or 5% trichloroacetic acid (TCA) (Merck; Darmstadt, Germany) at boiling temperature for 20 min. All unstained smears were previously analyzed for autofluorescence.

For metaphase preparations, human lymphocytes were grown at 37°C for 3 days in RPMI 1640 medium (Sigma) supplemented with fetal bovine serum (10%), l-glutamine, antibiotics, and phytohemagglutinin (Sigma) as usual. To arrest cells at metaphase, colcemid (Sigma; final concentration 0.05 μg/ml) was added 3 hr before collection. Cells were centrifuged, resuspended in hypotonic solution (75 mM KCl) at 37°C for 5 min, fixed in methanol–acetic acid (3:1 v/v) for 1 hr, spread on slides, and air-dried. Bone marrow cells from mice previously injected with colcemid solution (0.01% in 0.9% NaCl) for 2 hr were subjected to the same hypotonic treatment, fixation, and spreading as described above.

The compound M&B 938 (4,4′-diamidinodiphenylamine dihydrochloride, batch 7451; Figure 1) was kindly provided by Rhône–Poulenc Rorer (Dagenham Research Centre, Essex, UK). A stock solution (1 mg/ml) was first made in distilled water (pH 6) and then diluted to the appropriate staining concentration (0.5–1 μg/ml) with distilled water. Stock solutions kept in the dark at −20°C for several months also proved to be suitable for the fluorescence reaction. Staining time with M&B 938 solutions at pH 6–7 was 5–15 min at room temperature (RT; ∼20°C).

Smears of bone marrow and peritoneal mast cells were also stained with 1 μg/ml solutions of M&B 938 adjusted to different pH values (from 1 to 9) with 0.1 N HCl or 0.1 N NaOH. All preparations were washed in distilled or tapwater, air-dried, and mounted either in distilled water, 20% or 50% glycerol (Merck), immersion oil (Zeiss; Oberkochen, Germany), or DePeX (Serva; Heidelberg, Germany). According to current methods to enhance fluorescent chromosome banding (Sumner, 1990; Schweizer, 1981), some M&B 938-stained metaphase preparations were counterstained for 15 min with 25 μg/ml distamycin A (DA) (Sigma). In other cases, preparations were first treated for 15 min with 0.2 mg/ml actinomycin D (AMD) (Serva) and then stained with M&B 938. Microscopic observations and photography were performed using either a Zeiss photomicroscope III or a Jenamed fluorescence microscope (Zeiss) equipped with 100 W and 50 W mercury lamps, respectively, and the filter sets for ultraviolet (uv 365 nm), violet (405 nm), and violet-blue (436 nm) exciting light.

To confirm microscopic observations, standard 0.8% agarose gels in 45 mM Tris-borate–1 mM EDTA buffer at pH 8 were loaded with 2 μg of either λ DNA/Hind III fragments or 1 kbp DNA ladder marker (Bethesda Research

Laboratories; Life Technologies, Gaithersburg, MD). After horizontal electrophoresis at 10 mA/cm for 30 min, gels were stained with 30 μg/ml M&B 938 in distilled water for 5 min at RT, washed in distilled water for 20–25 min, and then photographed using an Eagle Eye uv transilluminator (Stratagene; Cambridge, UK). Other DNA samples were run in the same way but incorporating 0.5 μg/ml of M&B 938 into the gel before electrophoresis. For comparative purposes, other gels were also stained with ethidium bromide (EB) as usual. Photographs of M&B 938-stained gels were subjected to densitometry using a color imaging scanner "La Cie" (model G 520 A; Seiko Epson, Tokyo, Japan) and the images were analyzed with the NIH image 1.55 f Macintosh software pack.

Spectroscopic studies were carried out by using freshly made solutions of M&B 938 (1 and 10 μg/ml), calf thymus DNA (Sigma), and the high molecular weight double-stranded homopolymers poly(dA)–poly(dT) and poly(dG)–poly(dC) (Boehringer, Mannheim, Germany; and Sigma). Solutions of DNA and synthetic polynucleotides (10 μg/ml) were made in distilled water to avoid the scattering contribution of ions and buffer co-solutes to the baseline of solvents when excited at uv wavelengths (Stockert et al., 1990a). Spectra of synthetic polynucleotides showed equivalent absorption at 260 nm, indicating a similar degree of double-strandedness. Spectrophotometric measurements were performed with a Perkin–Elmer UV/VIS spectrophotometer 551-S (Perkin–Elmer; Norwalk, CT). Spectrofluorimetric studies were done in a Perkin-Elmer fluorescence spectrophotometer 650-10S equipped with a 150 W xenon lamp, two grating monochromators, an R 372F photomultiplier detector, and 1-cm-wide quartz cuvettes. Fluorescence analysis was made in the low sensitivity range 0.1–1, with the mode switch and gain selector in normal positions and either 4-, 6-, or 10-nm bandpass for the excitation and emission slits.

Results

After treatment of cell smears with 0.5–1 μg/ml solutions of M&B 938 at pH 6, bright bluish-white fluorescence reaction was observed specifically in chromatin from interphase and mitotic cells under uv excitation. Violet and violet-blue excitations were not suitable because of their weaker emission and lower specificity. No appreciable difference was found between 5- and 15-min staining time. Compact chromatin masses in nuclei of chicken erythrocytes, Ehrlich tumor cells, and lymphocytes, as well as kinetoplasts of *T. cruzi* epimastigotes, showed the brightest fluorescence (Figure 2). No emission was found in other cell structures (e.g., heterophil, eosinophil and neutrophil leukocyte granules, erythrocyte cytoplasm, mast cell granules, and basophilic cytoplasm of lymphoblasts, lymphocytes, and Ehrlich tumor cells). Control (unstained) smears did not reveal appreciable fluorescence.

DePeX was the optimal mounting medium for microscopic examination of cell smears stained with M&B 938. In this case, only a very low fading rate was observed, which permitted prolonged analysis as well as photography. Higher fading rates and diffusion artifacts were found with immersion oil or aqueous mounting media, respectively. Previous DNA extraction procedures (DNAse, TCA) abolished the chromatin fluorescence induced by M&B 938. A strong dependence of fluorescence intensity on the pH value was clearly observed. At pH 3, heparin-containing granules from mouse mast cells showed bright emis-

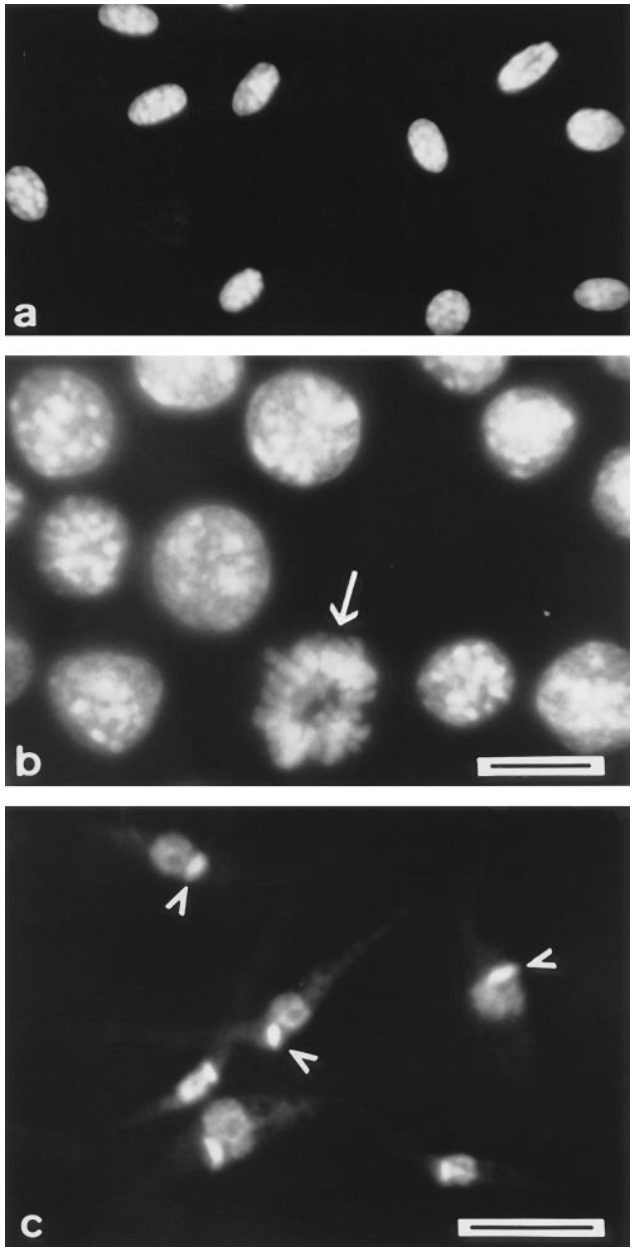

Figure 2 Fluorescence micrographs of cell smears stained with M&B 938 (1 μg/ml, 5 min). (**a**) chicken erythrocytes. (**b**) Ehrlich ascites tumor cells; the arrow points to a metaphase cell. (**c**) *T. cruzi* epimastigotes; kinetoplasts are indicated by arrowheads. UV exciting light. Bars: **a,b** = 10 μm; c = 5 μm.

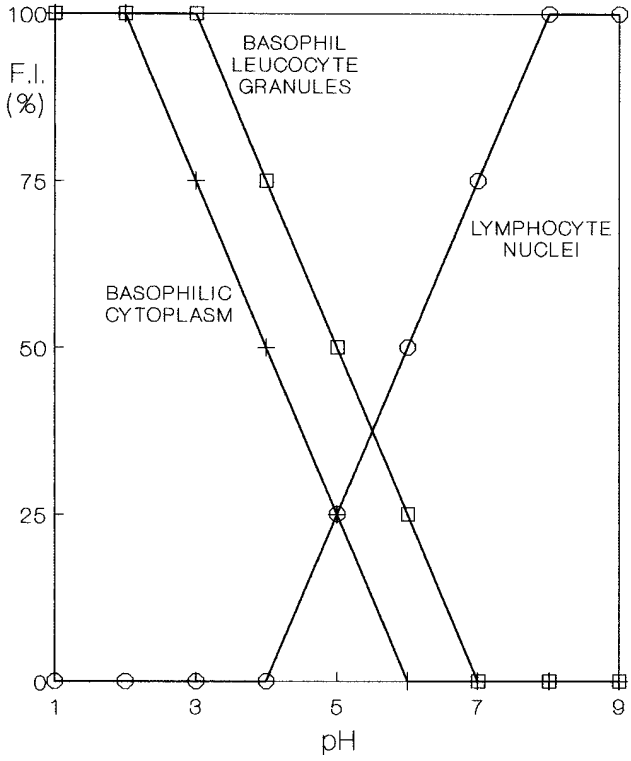

Figure 3 Normalized fluorescence intensity (F.I.) of basophilic cell components from rat bone marrow smears after staining with M&B 938 at different pH values. A minimum of 20 cells was semiquantitatively evaluated in each case, the fluorescence intensity being rated from ++++ (highest) to − (none) and expressed in percent.

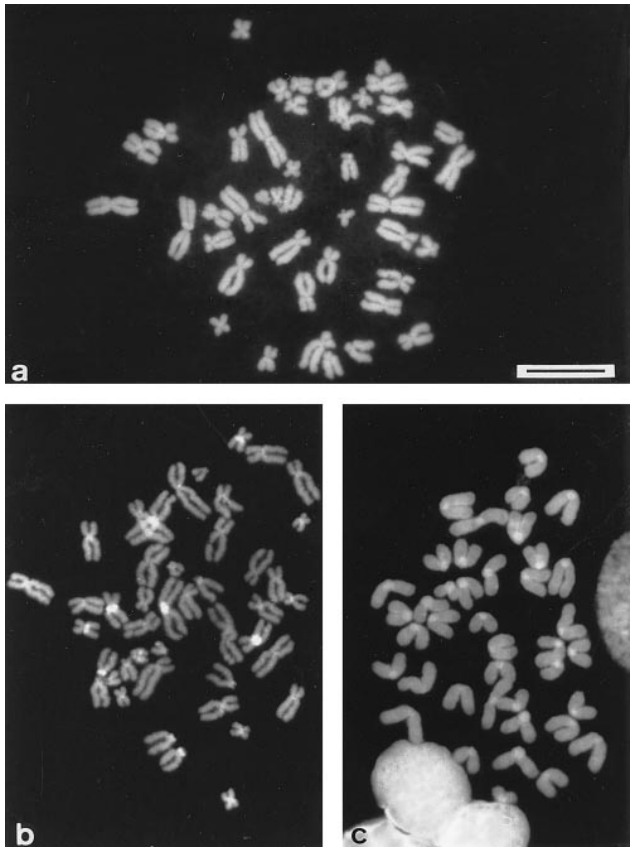

Figure 4 (**a**,**b**) Human metaphase chromosomes (**a**) stained with 1 μg/ml M&B 938 alone or (**b**) counterstained with distamycin A. (**c**) Mouse metaphase chromosomes stained with 0.5 μg/ml M&B 938, showing the higher fluorescence of most centromeric regions. Bar = 10 μm.

sion, whereas nuclei exhibited negligible fluorescence. On the contrary, bright nuclear emission in mast cells and lymphocytes and no fluorescence of mast cell granules occurred at pH 9. The effect of a gradual variation of pH on M&B 938 fluorescence of basophilic structures from rat bone marrow smears is shown in Figure 3, which reveals the striking specificity of M&B 938 for chromatin DNA at neutral and alkaline pH values, whereas no emission is detected in both basophil leukocyte granules and RNA-rich cytoplasm of lymphoblasts and lymphocytes.

After M&B 938 staining, human metaphase chromosomes exhibited bright and rather homogeneous fluorescence (Figure 4a), the remaining cytoplasm showing no emission. In some cases, very weak G/Q banding and somewhat brighter centromeric regions could be detected. When counterstained with DA, human chromosomes revealed a typical fluorescence pattern, with the centromeric heterochromatin of chromosome pairs 1, 9, 15, and 16, and the chromosome Y appearing highly fluorescent (Figure 4b). The same results were obtained when AMD preceded M&B 938 staining. Clearly differentiated centromeric regions could be also observed in mouse chromosomes after staining with M&B 938 (Figure 4c). Centromeric heterochromatin of most chromosomes appeared brighter than the arms, in which weak G/Q banding was found in some cases. DA counterstaining or AMD pretreatment did not improve the fluorescence pattern induced by M&B 938 on mouse chromosomes.

After agarose gel electrophoresis, M&B 938 clearly revealed the DNA markers as fluorescent bands (Figure 5a). Although previous incorporation of M&B 938 into the gel was also suitable, optimal results were obtained when gels were poststained with the compound. In this case, a higher concentration was necessary to achieve adequate penetration of M&B 938 into the bulky agarose matrix. Likewise, thorough washing in distilled water was required to produce the progressive loss of background gel emission and the precise visualization of DNA bands. After densitometric analysis, plotting the molecular length of 1 kbp DNA ladder fragments as a function of their fluorescence intensity revealed a point well above the best-fit regression line (Figure 5b). Preliminary computer anal-

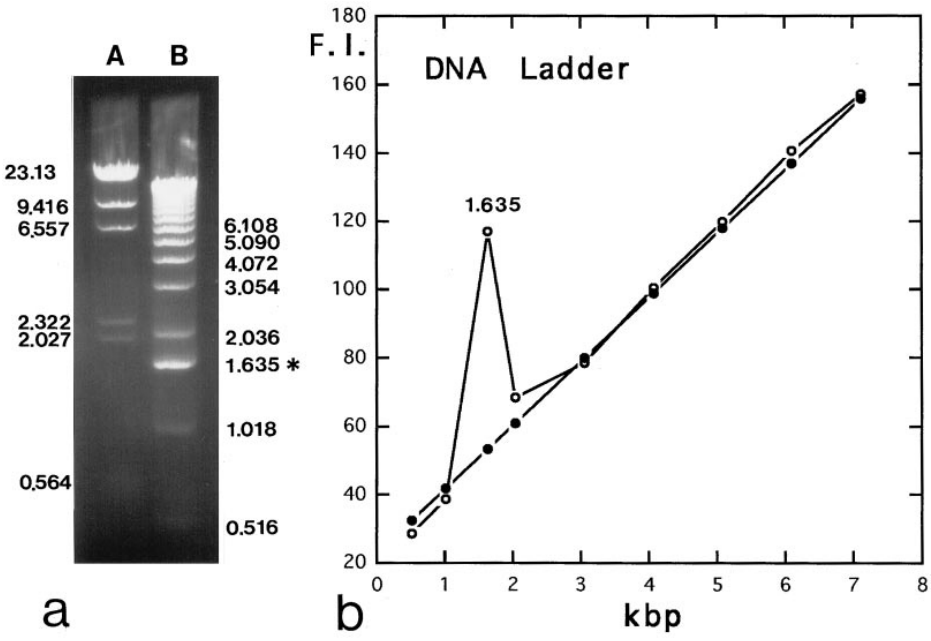

Figure 5 (**a**) Agarose gel electrophoresis of the DNA molecular weight markers, (A) λ DNA/Hind III fragments, and (B) 1 KBP DNA ladder, poststained with M&B 938 and observed under UV transillumination. The molecular length (in KBP) of DNA bands is indicated. Note the highly fluorescent fragment of 1.635 KBP (asterisk). (**b**) Correlation between the mean fluorescence intensity (F.I., in arbitrary units) and the molecular length of DNA ladder bands (open circles). Points corresponding to the best-fit regression line are indicated by black circles.

ysis of the base sequences of the corresponding DNA ladder fragments showed that the band of 1.635 kbp that appears above the regression line contains a higher amount of AT clusters (e.g., four or five consecutive AT base pairs) than other DNA fragments. On the contrary, EB-stained gels showed a more regular fluorescence pattern of DNA bands.

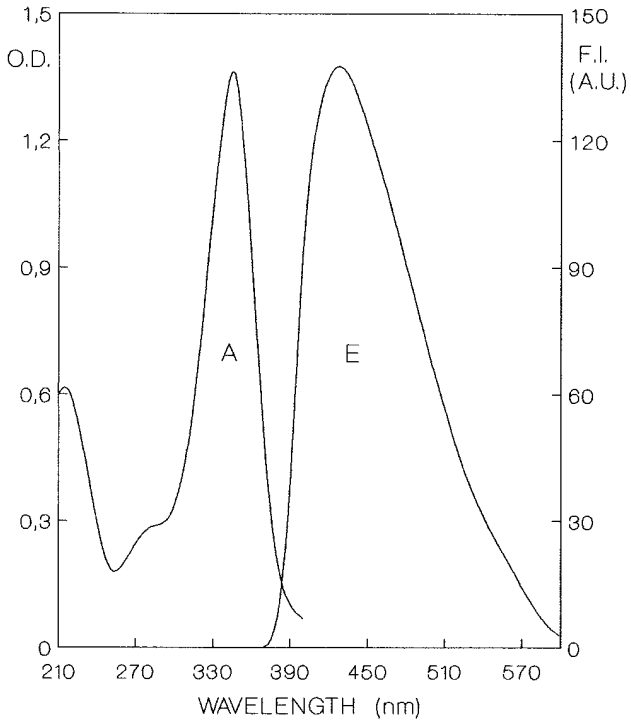

Figure 6 Absorption (A) and fluorescence emission (E) spectra of M&B 938 (10 μg/ml). Exciting wavelength 340 nm. The Raman scattering of the solvent (distilled water) was subtracted from the emission spectrum. O.D., optical density; F.I., fluorescence intensity in arbitrary units.

To analyze the emission characteristics of M&B 938 and to confirm its microscopic fluorescence reaction with DNA-containing structures, spectral studies were performed in vitro. A 10 μg/ml solution of the free compound revealed absorption peaks at 215 and 345 nm, with a shoulder at 270–290 nm; the emission maximum was found at 430 nm (Figure 6). Optimal exciting wavelength was 340 nm, although excitation in the range 340–370 was also suitable. In the presence of 10 μg/ml DNA, the near uv absorption peak of M&B 938 (1 μg/ml) showed bathochromic (345–358 nm) and hyperchromic shifts (Figure 7). Under instrumental conditions of very low sensitivity, strong emission at 420 nm was found for M&B 938 in the presence of DNA, whereas the free compound showed no fluorescence (Figure 7). In the presence of the synthetic polynucleotide poly(dA)–poly(dT), the fluorescence of M&B 938 strikingly increased and shifted from 430 to 415 nm, the emission intensity showing a value 4.25-fold higher than that observed with DNA (Figure 8). At the very low sensitivity level used for these comparative studies, no fluorescence was detected for either M&B 938 alone or in the presence of poly(dG)–poly(dC).

Discussion

With the exception of DAPI, the use of aromatic diamidines has largely been overlooked in DNA cytochemistry, and only very few observations on the microscopic fluorescence induced by berenil (Stockert

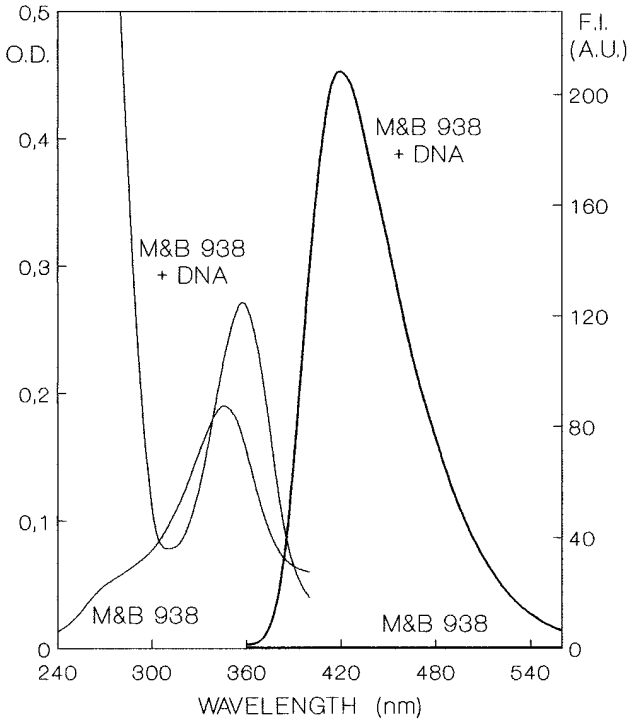

Figure 7 Absorption (thin curves at left) and fluorescence emission (thick curves at right) spectra of M&B 938 (1 μg/ml), either free or in the presence of DNA (10 μg/ml). Exciting wavelength 340 nm. The Raman scattering of the solvent containing DNA alone was subtracted from the emission spectrum of M&B 938 + DNA. O.D., optical density; F.I., fluorescence intensity in arbitrary units.

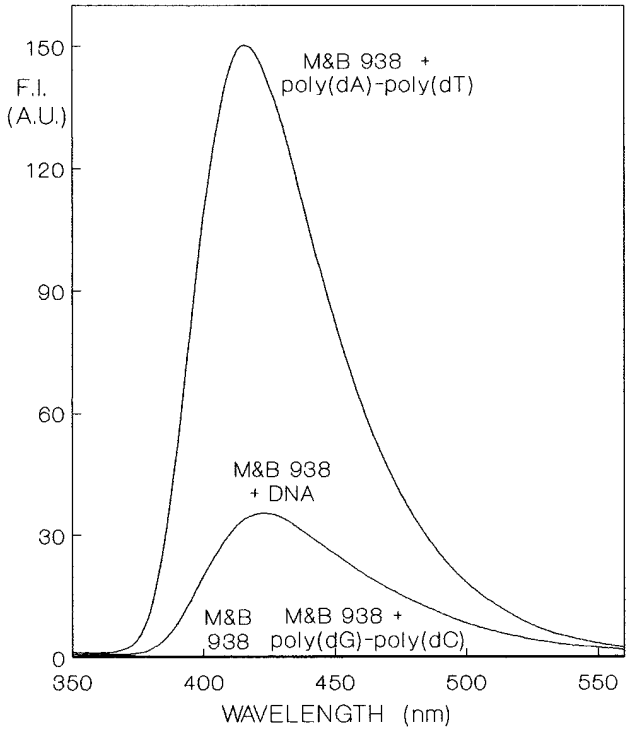

Figure 8 Fluorescence spectra of M&B 938 (1 μg/ml), either free or in the presence of 10 μg/ml DNA, poly(dA)–poly(dT), or poly(dG)–poly(dC). Exciting wavelength 340 nm. Because of its negligible contribution, the Raman scattering of the solvents (containing the polynucleotides alone) was not subtracted from the emission spectra. F.I., fluorescence intensity in arbitrary units.

et al., 1990b; Newton, 1975), 2-hydroxystilbamidine (Murgatroyd, 1982), and M&B 938 (Tring and Murgatroyd, 1976) have been reported. Obvious drawbacks of the described staining techniques using these compounds are the extremely high concentration used (50–100 μg/ml), low specificity, and laborious or time-consuming procedures (Murgatroyd, 1982; Tring and Murgatroyd, 1976).

In this work we show that the aromatic diamidine M&B 938 induces a bright fluorescence emission of chromatin that depends on the presence of DNA. The staining method is very simple and rapid, low fluorochrome concentration is required, and high specificity and stability of the fluorescence reaction can be easily achieved. Results from microscopic observation, DNA extraction, gel electrophoresis, and spectrofluorimetric analysis confirm the specific binding of M&B 938 to DNA. Interestingly, DNA was the unique cell polyanion that proved to be fluorescent at neutral or alkaline pH values. Under these conditions, other polyanions such as RNA (basophilic cytoplasm) and sulfated glycosaminoglycans (heparin-containing granules in mast cells and basophil leukocytes) revealed no fluorescence.

This advantage is not commonly found in fluorescence reactions induced by other non-rigid cationic fluorochromes which, in addition to chromatin DNA, also reveal different polyanionic and/or hydrophobic substrates. In fact, simultaneous orthochromatic (blue-white) and metachromatic (yellow-green) emission of nuclei and mast cell granules, respectively, has been described after staining with DAPI (Grossgebauer, 1979) or the oxazolium compound Q4 (Espada et al., 1995). A pH-dependent selectivity of the DNA fluorescence is known to occur in the case of bisbenzimidazole dyes (Schmued et al., 1982; Hilwig and Gropp, 1975), RNA being also visualized at acid pH values. Likewise, yellow emission of nuclei and mast cell granules and blue-white fluorescence of mucosubstances, glycogen, and elastic fibers were observed with 2-hydroxystilbamidine (Murgatroyd, 1982). In the case of M&B 938 (used at 100 μg/ml), it was claimed that, in addition to DNA, this compound also induced fluorescence in cartilage and goblet cells (Tring and Murgatroyd, 1976). However, our present results show that the ability of M&B 938 to bind specifically to DNA is most remarkable when it is used at very low concentration and neutral pH.

In addition to compact chromatin of interphase nuclei, kinetoplast DNA of *T. cruzi* and centromeric heterochromatin of mouse chromosomes reveal very

bright M&B 938 fluorescence, possibly related to the abundance of AT sequences or AT clusters [e.g., oligo(dA)–oligo(dT) tracts] in these structures (Sumner, 1990; Radic et al., 1987; Diekmann and Zarling, 1987). In this respect, the present results are in agreement with previous data on the increased emission of non-rigid fluorochromes when bound to AT-rich DNA regions (Kapuscinski and Skoczylas, 1977; Stockert et al., 1990a,b,1991), a feature that has proved useful for chromosome characterization by specific banding methods (Bella et al., 1995; Bella and Gosálvez, 1991; Sumner, 1990; Holmquist and Motara, 1987). In the case of human chromosomes, a very weak G/Q banding pattern can be directly observed with M&B 938 staining, and after DA counterstaining or AMD pretreatment, heterochromatic regions of chromosomes 1, 9, 15, 16, and Y show enhanced emission. Although the precise mechanism of this effect is not yet well understood (Stockert et al., 1990a; Schweizer, 1981; Latt et al., 1980), our findings on mouse and human chromosomes using M&B 938 are in full agreement with the banding pattern obtained by similar counterstaining procedures (Bella et al., 1995; Bella and Gosálvez, 1994; Sumner, 1990).

Characteristic spectral changes are observed when cationic dyes bind to polyanionic substrates. In the case of M&B 938, bathochromic and hyperchromic shifts in the absorption spectrum and strong enhancement of fluorescence occur in the presence of DNA. It is known that binding or solvent conditions that increase the molecular rigidity of non-planar (flexible) fluorophores result in a great enhancement of fluorescence (Stockert et al., 1990b; Oster and Nishijima, 1956). This effect is due to the fact that, in the rigid environment of the binding site, the energy of the excited fluorophore cannot be dissipated through intramolecular bond rotation or collision with solvent molecules, and it is released predominantly as fluorescence emission (Oster and Nishijima, 1956). Specific binding of M&B 938 to AT sequences is clearly revealed by the striking increase of emission in the presence of poly(dA)–poly(dT), whereas weaker or no fluorescence occurs with calf thymus DNA or poly(dG)–poly(dC), respectively. Interestingly, the short emission wavelength of M&B 938 appears well correlated with the small size of the resonant fluorophore, as expressed by the "conjugated bond number" (CBN) (Horobin, 1980). Thus, M&B 938 has a CBN value of 20 and fluoresces bluish white, whereas non-rigid and larger bisazo dyes show CBN values of 40-45 and fluoresce red (Espada and Stockert, 1994).

Inspection of the chemical structure of M&B 938 reveals that its molecular geometry is well suited to interact with the DNA minor groove. As illustrated in Figures 1 and 9a, M&B 938 has two cationic amidinium groups with hydrogen bonding capacity and shows a crescent-shaped and non-planar structure. The compound is the shortest-chain homologue of berenil, the triazene bridge (-NH-N=N-) of the latter being replaced by an amino group (-NH-). A torsion angle of about 45° between phenyl rings is necessary to avoid the steric clash that would be produced by the proximity of the C2 and C2′ hydrogens. Although the central amino bridge is rather flexible (allowing rotation and some opening of the C1-N-C1′ bond angle), the normal non-planar conformation of M&B

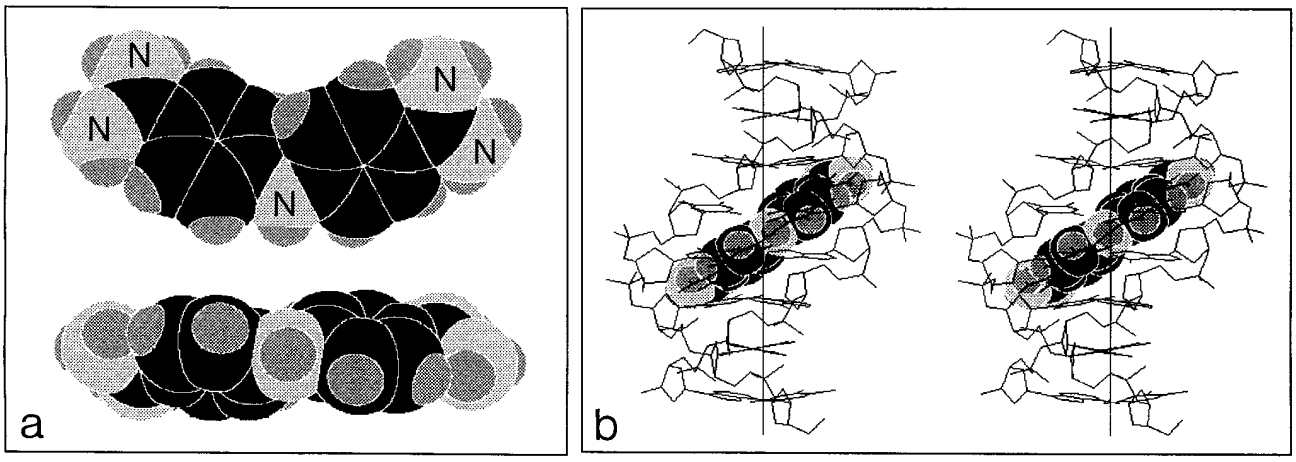

Figure 9 (**a**) Computer-drawn space-filling molecular structure of the M&B 938 cation. Top and bottom: face and edge views, respectively, the former showing the position of nitrogen atoms (N) from the central amine and terminal amidinium groups. The energy-minimized structure was generated by using the Desktop Molecular Modeller program. (**b**) Stereoscopic view of the possible binding mode of M&B 938 (space-filling structure) into the minor groove of the DNA segment $(dA)_8$–$(dT)_8$ (skeletal structure) generated as previously described (Stockert, 1994). The position of amidinium N atoms allows hydrogen bonding with O2 and N3 atoms of T and A, respectively. Vertical line represents the helical axis.

938 must prevent its intercalation between base pairs. This interpretation is also supported by the fact that no fluorescence enhancement of M&B 938 is found in the presence of poly(dG)–poly(dC). On the contrary, DAPI and other non-rigid (unfused) aromatic compounds have a more planar structure and they can also intercalate between GC base pairs of DNA and RNA duplexes (Wilson, 1990; Wilson et al., 1989, 1993).

The diamidino compound used in this work shares some chemical features with AT-specific minor groove binders, such as the bowed shape, cationic status, hydrogen bonding possibilities, and non-rigid bonds between aromatic rings (Krugh, 1994; Kopka and Larsen, 1992; Wilson, 1990). Taking into account the specific interaction of berenil and other aromatic diamidines with AT sequences of B-DNA (Jansen et al., 1993; Nunn et al., 1993; Brown et al., 1992; Edwards et al., 1992), it is tempting to speculate that M&B 938 could also fit tightly into the narrow DNA minor groove (along four AT base pairs; Figure 9b), forming hydrogen bonds with the O2 and N3 atoms of T and A, respectively. Although the possibility that M&B 938 binds to AT regions in the DNA minor groove is in full agreement with results from our microscopic, electrophoretic, and spectroscopic observations, further physicochemical investigations are obviously necessary to support the proposed binding mode.

Studies of the selective interaction of drugs and dyes with DNA minor groove have taken on a new significance in recent years (Kahne, 1995; Krugh, 1994; Kopka and Larsen, 1992). This is in part due to the known potential of such compounds in the chemotherapy of leukemias and tumors (Krugh, 1994; Baguley, 1982) or their use as antiprotozoal, trypanocidal, antiviral, and antibacterial agents (Shapiro and Englund, 1990; Tidwell et al., 1990; De Clerq and Dann, 1980). In this respect, it is noteworthy that M&B 938 shows a trypanocidal activity in vitro that is about 2.5-fold higher than that of berenil (Newton, 1975). AT-specific minor groove binders also have wide use in cytogenetics, cytochemistry, and cell biology (Bella et al., 1995; Stockert et al., 1990b; Holmquist and Motara, 1987; Latt et al., 1980). Therefore, the present results on M&B 938 fluorescence are of considerable interest and suggest further applications in analytic, fluorimetric, and cytochemical studies. The aromatic diamidine M&B 938 has useful characteristics as a new DNA fluorochrome (e.g., brightness, specificity, stability, simple staining procedure, permanent mounting) and it may also be valuable in other fields of biomedical research.

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