The fluorescence properties of a DNA probe*

4'-6-Diamidino-2-phenylindole (DAPI)

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Abstract. Steady-state and dynamic fluorescence measurements have been performed on DAPI in solution and in complexes formed with a number of synthetic and natural polydeoxynucleotides. The decay of DAPI in buffer at pH 7 was decomposed using two exponentials having lifetime values of approximately 2.8 ns and 0.2 ns. The double exponential character of the decay was maintained over a large pH range from 3 to 9. At pH 1 the short component dominated, whereas at pH 12, only the long component was detectable. Two distinct spectra were associated with the two lifetime components; the short component was shifted to the red. The short lifetime component occurs in the presence of water. In water the excitation spectra depended on the emission wavelength and there was no viscosity dependence of the two forms. To explain these results we propose that there is a ground state conformer in which preferential solvation of the indole ring allows proton transfer in the excited state. DAPI complexed with polydeoxynucleotides retained most of the features of the decay of DAPI in solution. However, the complexes with fully AT-containing polymers stabilized the longer lifetime form of DAPI because the stronger binding enhanced solvent shielding. A gradual increase of the short lifetime component, which monitors dye solvent exposure, was obtained as the AT content was decreased. For polyd(GC) the decay was similar to that of free DAPI.

Key words: Fluorescence, DAPI, DNA

Introduction

DAPI, synthesized as an analogue of diarylamin (berenyl) (Dann et al. 1971), is known to bind reversibly with double stranded deoxyribonucleic acids and with various synthetic analogs of different base content and sequence. DAPI exhibits a remarkable increase in fluorescence quantum yield upon binding (Russell et al. 1975; Kapuscinski and Skoczylas 1978; Masotti et al. 1982).

Previous studies have shown that only the binding with AT, AU or IC clusters produces a fluorescent complex (Kapuscinski and Szer 1979). Because of this property, DAPI is currently used in a number of biochemical and cytochemical investigations including a staining procedure for the selective visualization of the paracentromeric constrictions of human chromosomes 1, 9 and 16 and Q-banding-like pattern of the short arm of chromosome 15 and the distal part of the Y and for the assessment of all chromosomal DNA in flow systems (Avitabile et al. 1985; Coleman 1984; Manzini et al. 1983; Masotti et al. 1981; Schweizer 1976; Shinsichi and Setsuya 1983; Rocchi et al. 1980).

DAPI also probes the molecular environment of restriction endonuclease cleavage sites (Kania and Fannin 1976; Palfi et al. 1987).

The spectroscopic properties of DAPI are greatly modified as a consequence of its interaction with nucleic acids. The strong fluorescence enhancement, the
induced CD spectrum and the hypochromism in the ultraviolet absorption following binding to DNA have been interpreted in terms of two different modes of binding by Kapuscinski and Skoczylas (1978). The first mode, in their view, corresponds to a highly energetic and intercalative type of interaction, characterized by a high value of the affinity constant and a high specificity for AT, AU and IC cluster. The second mode, which corresponds to a lower affinity constant, is non-specific, mainly electrostatic in character and shows no increase in fluorescence intensity. However, recent spectroscopic, sedimentation equilibrium, viscometric and calorimetric investigations, have ruled out intercalation as the molecular mechanism for the specific, strong binding and suggested a new model for the interaction (Manzini et al. 1983). This new model assumes the formation, in the narrow helical groove, of two hydrogen bonds between the amido groups of the drug and the adjacent acceptor groups of AT base pairs, in addition to electrostatic interactions of the dye's positively charged ends with the phosphate groups of the polynucleotide backbone. A similar mode of interaction has been proposed and verified for netropsin and distamycin A, two well known antibiotics, non-intercalating and strongly interacting with DNA (Dattagupta et al. 1980; Patel 1982; Gupta et al. 1984). This model of interaction is also supported by the molecular structure of DAPI, which is characterized by three hydrogen donor groups, the two amidino moieties and the indole NH, which, together with the total positive charge of the molecule, provide a strong driving force for the interaction with DNA.

In this work, we have investigated the fluorescence properties of DAPI in solvents and in complexes with natural and synthetic polydeoxynucleotides with the purpose of studying the origins of the photophysical behavior of DAPI and the molecular details of its interaction with nucleic acids. To better understand the different aspects of these interactions, we approached the characterization of the fluorescence of the polydeoxynucleotide-DAPI complexes using frequency domain fluorometry, which has proven to be suitable for studying complex emitting systems (Gratton et al. 1984a and b; Jameson et al. 1984; Lakowicz et al. 1984). The use of frequency domain fluorometry is particularly useful in this case, since this technique has the capability to resolve multiexponential decays rapidly and accurately. An immediate test for lifetime heterogeneity is provided, during the measurement, by the comparison of the apparent phase and modulation lifetime values. The resolution of the spectral emission into two or three components can be obtained in a few minutes, using the method of phase and modulation resolved spectra (Gratton and Jameson 1985). These features have been a determinant factor for studying the spectral properties of DAPI and for recognizing specific molecular mechanisms of interaction with polydeoxynucleotides. Recently Szabo et al. (1986) have performed an accurate study of the decay of DAPI in solutions at different pH values and in different solvents, using correlated single photon counting techniques. Our experimental data are in substantial agreement with these studies, but we propose a molecular mechanism involving solvation to explain the origin of the species observed in the lifetime measurements.

Materials and methods

Calf thymus DNA (Type I, Sigma, St. Louis, Missouri) and Col E1 plasmid DNA (Boehringer, Indianapolis, Indiana) containing no more than 0.5% protein contamination, were used without further purification. pUC8 DNA was a kind gift of Prof. G. Palù (Microbiology, Padova, Italy). Polyd(AT), polyd(A)-polyd(T), and polyd(GC) were from Boehringer. DAPI was purchased from Serva Biochemicals (Heidelberg, FRG) and checked for purity by thin layer chromatography. All organic solvents were of spectroscopic grade and doubly distilled, Millipore filtered water was used throughout. Inorganic chemicals were reagent grade. Highly polymerized linear DNA was sonicated several times at ice-bath temperature to prevent intermolecular aggregation. The concentration of the solutions was determined by using the following molar extinction coefficients: calf thymus DNA (6,600), Col E1 DNA (6,600), pUC8 (6,600), polyd(AT) (6,600), polyd(GC) (8,400), polyd(A)-polyd(T) (6,000). A molar extinction coefficient of 23,000 M⁻¹ cm⁻¹ at 342 nm was used to determine the concentration of DAPI solutions. All polynucleotides were dissolved in aqueous buffered solutions containing 0.01 M NaCl, 0.1 M Tris, pH = 7.2 (buffer A). Depending on the final concentration of DNA, different ratios of deoxyribonucleic acid, as moles of phosphate (P) to dye (D) were used. All the measurements were performed at 24°C, unless otherwise specified. In the measurements pertaining to pH variation effects, a small amount of a concentrated aqueous solution of DAPI was diluted to the desired concentration. For the solvent studies, DAPI powder was directly dissolved in the solvent. For the polydeoxynucleotide/DAPI binding experiments the final concentration was obtained by adding increasing amounts of polymer to give the desired P/D ratio. Constancy of DAPI concentration was achieved by adding a polymer solution containing DAPI at the same molarity as that of the initial polymer free solution. In all experiments each sample was allowed to equilibrate at room temperature for at least 5 min before measurement.