Fluorescence of Chromatin DNA by an Oxazolium Scintillator

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Z. Naturforsch. **47c**, 481–482 (1992);
received October 8, 1991/January 12, 1992

Fluorescence microscopy, Scintillators, DNA Fluorochromes, Chromatin DNA

Aqueous solutions of a yellow and highly fluorescent derivative from dimethyl-POPOP, obtained by treating a chloroform solution of this oxazole scintillator with dimethylsulfate, induce a strong blue-green or yellow-green fluorescence in chromatin DNA under ultraviolet (365 nm) or violet-blue (436 nm) excitation, respectively. It is suggested that this new and selective fluorescence reaction could originate from binding of the oxazolium derivative into the minor groove of DNA.

Most of the commonly used organic scintillators are linear compounds with 2 to 5 aromatic rings which show strong fluorescence in the ultraviolet or violet-blue region [1–3]. Among them, 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-bencene (called dimethyl-POPOP, from "**P**henyl-**O**xazole-**P**henylene-**O**xazole-**P**henyl", see Fig. 1) is widely employed for liquid scintillation counting and as a laser dye in solution or in vapor phase [3, 4]. As far as I know, the fluorescence of ammonium derivatives of oxazole scintillators and their use as cationic fluorochromes have been overlooked. During the course of studies on non-intercalating DNA fluorochromes [5–7] I have realized that a quaternized derivative of dimethyl-POPOP could interact with the minor groove of B-DNA in the same way as other well known drugs and fluorochromes [8–11].

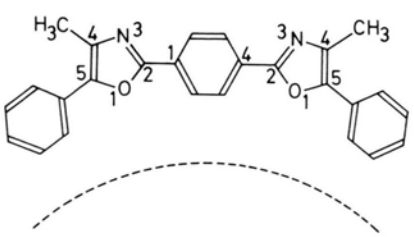

Fig. 1. Chemical structure and atom numbering of the oxazole scintillator dimethyl-POPOP. The corresponding oxazolium derivative results from quaternization of N3. The figure illustrates the possible conformation of the bowed molecule in relation to the convex floor of the B-DNA minor groove, which is schematized by the curved dashed line at bottom.

Dimethyl-POPOP (Merck) was dissolved in chloroform (1 mg/50 ml) and 3 ml dimethylsulfate (DMS, Merck) was added to the solution. A yellowish color with strong blue-green brightness was observed immediately after mixing. Under excitation at 360 nm, the fluorescence peak of dimethyl-POPOP in chloroform (432 nm) shifted to a longer wavelength (480 nm) after addition of DMS. Other solvents (N,N-dimethylformamide, xylene, ether) and reagents (perchloric acid, sulfuric acid) were also tested but chloroform and DMS appeared more suitable to achieve the highest color change of dimethyl-POPOP. The solution was allowed to evaporate at room temperature and then a deep yellow microcrystalline product (assumed to contain the oxazolium derivative of the scintillator) was obtained. In fluorescence microscopy, the dry product showed an intense blue-green or yellow-green emission under 365 or 436 nm exciting light, while that of dimethyl-POPOP was bluish white or blue-green.

Smears of chicken and rat blood, human buccal cells, and *Trypanosoma cruzi* epimastigotes growing in culture (12×10^6 cells/ml) at 27 °C were fixed in methanol for 2 min and air dried. A saturated solution of the yellow product in distilled water (showing an emission peak at 495 nm when excited at 360 nm) was applied on cell smears for 5 min. They were washed in tap water, air dried and observed under immersion oil in an epifluorescence microscope equipped with filter sets for 365, 405 and 436 nm exciting light. Unstained smears were also checked for autofluorescence [12].

Chromatin masses from nucleated chicken erythrocytes and thrombocytes, nuclei of leucocytes, trypanosomes and human epithelial cells, as well as central spots in bacteria from buccal smears showed a high blue-green or yellow-green fluorescence under excitation at 365 or 436 nm, respec-

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D-W-7400 Tübingen
0939–5075/92/0500–0481 $ 01.30/0

tively. A somewhat lower emission was found by exciting at 405 nm. Chromatin fluorescence showed a slow rate of fading under prolonged excitation. Kinetoplast DNA of trypanosomes revealed the brightest fluorescence reaction and the lowest fading rate. The RNA-rich basophilic cytoplasm of lymphocytes only showed a weak fluorescence and no emission was observed in other cells or cell structures. Chromatin fluorescence induced by this dimethyl-POPOP derivative was abolished after removal of DNA with DNase I (Sigma; 0.5 mg/ml in 1 mM $MgCl_2$ at 37 °C for 2 h) or 5% trichloroacetic acid (Merck) at boiling temperature for 15 min, DNA extraction being controlled by staining with 0.1 mM acridine orange (BDH) for 5 min. Simple ionic binding of the product does not occur since chromatin fluorescence is preserved after 5 min washing with 1 M NaCl or $MgCl_2$ solutions.

Inspection of the chemical structure of dimethyl-POPOP reveals that its molecular geometry is well suited to locate on the convex floor of DNA minor groove at the level of adenine thymine (AT) regions (Fig. 1). This narrow binding site would increase the molecular rigidity and therefore the emission yield of the chromophore [2, 6]. The bright fluorescence reaction of kinetoplast DNA could be also related to the abundance of AT regions in this structure (see [5–7] and references therein). A rather planar configuration of the aromatic chain (with helical twist angle [13] of about 36°) and a cationic status could be relevant factors for the interaction of an oxazolium derivative of dimethyl-POPOP with DNA minor groove. In this respect, it is known that the imino ring nitrogen of heterocyclic compounds can accept protons or alkyl groups, thus producing cationic species. Therefore, it is tempting to speculate that as occurs with other structurally similar DNA ligands containing bencene rings and ammonium heterocycles [6–8, 14], a bisquaternary oxazolium derivative of dimethyl-POPOP (probably methylated by means of DMS [15]) could also fit into the helical minor groove from AT regions in B-DNA. Further studies on the chemistry and properties of quaternized scintillators and their use as new DNA fluorochromes are in course.

Acknowledgements

I wish to thank Dr. C. Pelling, Dr. M. F. Braña and Dr. M. Fresno for valuable collaboration. This work was supported in part by a grant from the Cooperation Program Max-Planck-Gesellschaft (Germany) – Consejo Superior de Investigaciones Científicas (Spain).

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