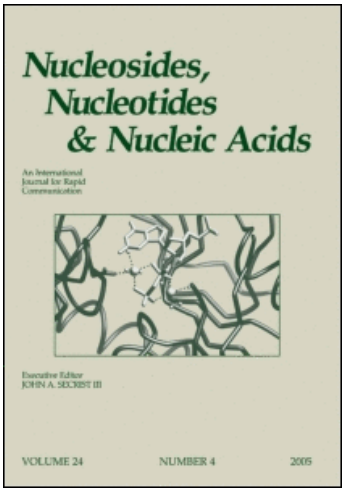

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A New Fluorescent Probe for Studies of Interactions Between Hydrophobic Oligonucleotides and Cellular Membranes

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A NEW FLUORESCENT PROBE FOR STUDIES OF INTERACTIONS BETWEEN HYDROPHOBIC OLIGONUCLEOTIDES AND CELLULAR MEMBRANES

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ABSTRACT: The synthesis of a new fluorescently labeled medium-sensitive lipophilic oligonucleotide is reported. A fluorescent chalcone chromophore was introduced between the 5' end of the nucleic acid and the fatty hydrocarbon chains. A blue shift of both absorption and emission wavelength maxima results from a transfer of the chromophore to a more hydrophobic medium or upon binding of the conjugate to unilamellar vesicles of egg phosphatidyl choline. These conjugates could be used as markers for cell uptake studies of lipophilic nucleic acid derivatives.

Hydrophobization of oligonucleotides has been used as an effective way to increase their cellular uptake for antisense therapy. Certain improvements in biological properties of these oligonucleotides were achieved by their conjugation to lipophilic groups such as cholesterol, fullerene or long hydrocarbon chains (1-3). However, the role of hydrophobic moieties in internalization and their fate inside the cells are still poorly understood. Hydrophobic binding and crossing of the membrane, non-receptor mediated or LDL-receptor mediated endocytosis have been suggested.

In order to study oligonucleotide-membrane interactions and internalization mechanisms we herein report the synthesis of a new fluorescently labeled lipophilic oligonucleotide conjugate. The lipophilic group for conjugation is shown on FIG.1. Fatty hydrocarbon chains serve as a hydrophobic anchor for interaction with the cellular membrane, a chalcone chromophore is introduced as a medium-sensitive fluorescent probe and a tri(ethylene glycol) linker derivatized with iodine is used for further conjugation.

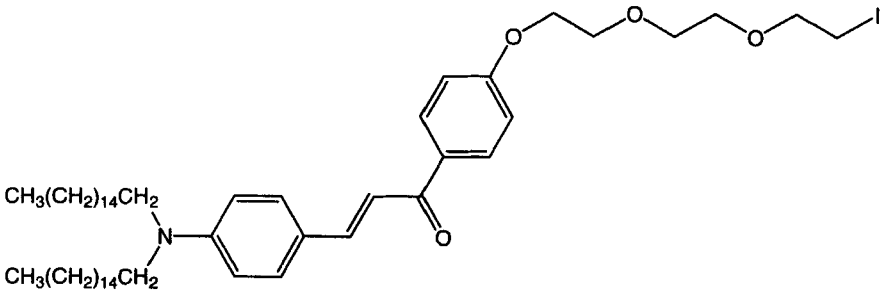

FIG. 1 : Structure of the chalcone iodo-derivative. Anal. Calculated for $C_{53}H_{88}INO_4$: C, 68.44; H, 9.54; N, 1.51. Found : C, 68.39; H, 9.60; N, 1.43. Mass : [M+1] = 930.7.

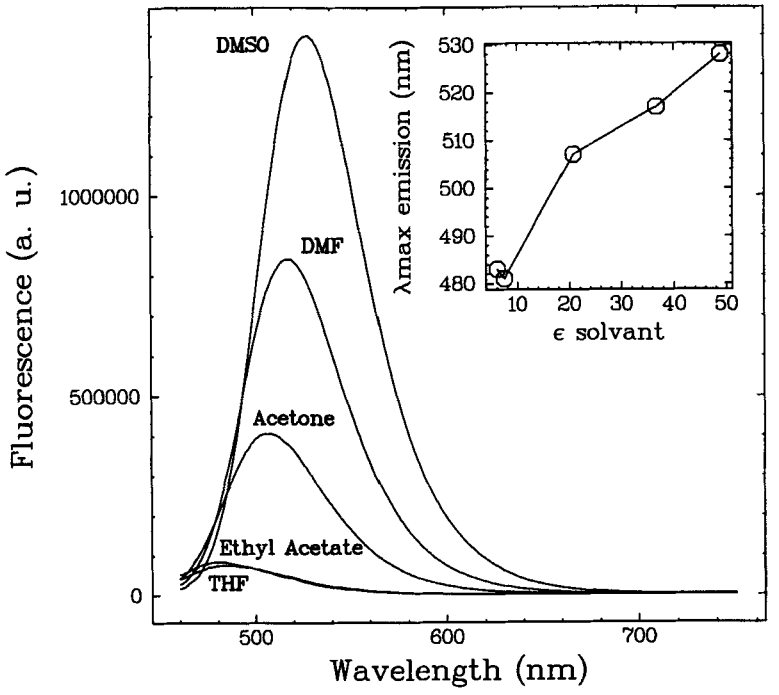

FIG. 2 : Fluorescence spectra of the hydroxyl derivative of the chalcone in various solvents ($\lambda_{ex.}$ = 455 nm, T = 25°C). Insert : Evolution of the wavelength maximun of the emission spectra as a function of the dielectric constant of the solvent.

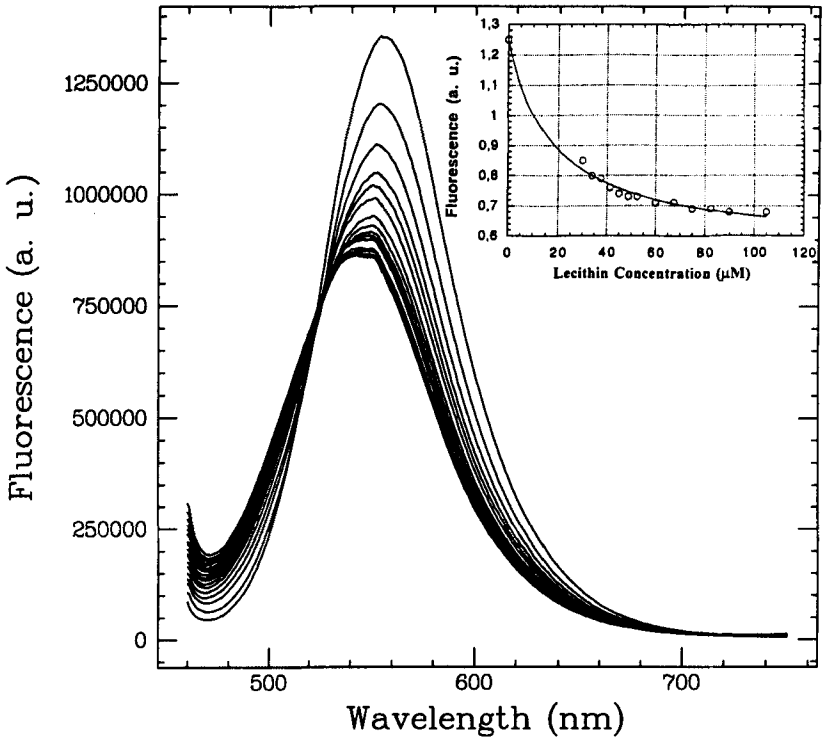

FIG. 3 : Decrease of the fluorescence of a 50 μM 22-mer oligonucleotide conjugate solution (9 mM Tris-borate buffer, pH 8.3, 75 mM NaCl) during the titration by a 3 mM lecithin LUVs suspension ($\lambda_{ex.}$ = 450 nm, T = 25°C). Insert : Fluorescence intensity at 555 nm as a function of the total lipid concentration (markers). The experimental points are fitted as a solid line according to our model.

This group was attached to a variety of oligonucleotides of different lengths and sequences. Sulfhydryl group was generated on the 5'-terminus of oligonucleotides according to the method described earlier (4) using cystamine. The coupling reaction is followed by reduction of the disulfide group by dithiothreitol. Then -SH group was alkylated by the chalcone iodo-derivative and the resulting conjugate was purified by electrophoresis on 1% agarose gel.

Calibrating solvatochromic studies of fluorescence were carried out using the hydroxyl derivative of the chalcone in different solvents with decreasing dielectric constants. A blue shift of both absorption and emission wavelength maxima resulted from a transfer to

a more hydrophobic media (FIG.2). Solvatochromic effect is positive and indicates that in the excited state the chalcone moiety is more polar than in the ground state (5).

Similar solvatochromic effects were observed when the oligonucleotide-chalcone conjugates were mixed with increasing amounts of large (Ø = 100nm) unilamellar vesicles of egg phosphatidyl choline in 75 mM NaCl. The blue shift of fluorescence clearly indicated interaction of the oligonucleotide derivative with the hydrophobic membranes (FIG.3). A simple mathematical treatment of titration data allows us to estimate the association constant K_a of our conjugate with lecithin to be around $5 \cdot 10^4$.

The binding of DNA-lipid conjugate to the vesicles is dependent on the ionic strength (6). Without salt, when the electrostatic repulsion between anchored oligonucleotides on the lipid surface is the largest, the adsorption is low and K_a is lower than 10^2. The increase of the NaCl concentration from 75 to 500 mM leads to a slight increase of the constant K_a from $5 \cdot 10^4$ to $8 \cdot 10^4$. The same effect was observed with oligonucleotide-cholesterol conjugates adsorbed on poly(lactic-co-glycolic) nanoparticles (7).

Since the fluorescence spectra of the chalcone attached to oligonucleotide moiety changes as a function of the medium hydrophobicity, we propose to use these conjugates as markers for cell uptake and intracellular fate studies of lipophilic antisense nucleic acid derivatives by the means of fluorescent and confocal microscopy.

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