

SYNTHESIS AND PROPERTIES OF NEW FLUORESCENCE DYES FOR *IN VIVO* STAINING OF DIATOMS

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Methylated oligopropylamines (MOPA) had been found in diatoms as a part of modified proteins (silaffines [M. Sumper et al. J. Mater. Chem. 14:2059]) and in a free state. It is evident that MOPA take part in silicon metabolism but the exact physiological role of these compounds is not clear. Recently we have elaborated a stepwise method for MOPA synthesis [V.V. Annenkov et al. Arkivoc 2009 xiii:116-130] and this work is devoted to fluorescence derivatives based on MOPA. We have obtained the following new dyes for *in vivo* staining of diatom cells:

- Compounds based on amines with 2 or 3 nitrogen atoms: H-[NMe-(CH₂)₃-NMe]-R and H-[NMe-(CH₂)₃-NMe-(CH₂)₃-NMe]-R where R = H, Me. 7-nitrobenzo-2-oxa-1,3-diazole (NBD), rhodamine B, rhodamine 101 and fluorescein residuals were used as fluorescence groups. NBD was introduced into MOPA molecules using 4-chloro derivative, rhodamines and fluorescein were converted into chloranhydride form before the reaction with amines. Substances bearing one or two fluorescence groups have been obtained, their structure and purity were confirmed with HPLS, HRMS and NMR. Fluorescent properties of the new dyes were studied in water solution depending on pH and association of the tagged MOPA was found at high pH values and in the case of molecules with two fluorescence groups. This association decreases emission of the fluorophores and changes their spectra.
- High-molecular polymers of various charge of the main chain. By the reaction of poly(acryloyl chloride) with NH-containing tagged MOPA or fluoresceine, tagged poly(acrylic acid) was obtained. Positive charged polymers were prepared by modification of poly(vinyl amine) with NBD-Cl or with chloranhydride derivatives of rhodamines or fluorescein. Copolymers of 1-vinylimidazole with vinylamine were used to obtain non-charged (at neutral pH values) tagged polymers. These copolymers were prepared by copolymerization of 1-vinylimidazole with N-vinylformamide followed with alkali hydrolysis of the amide units. The absence of free fluorescence dye in the tagged polymers was confirmed with size-exclusion chromatography.

The obtained fluorescent dyes were used in cultivation experiments with fresh-water diatoms and in synthesis of new fluorescence materials from diatom frustules and by *in vitro* condensation of silicic acid in the presence of tagged MOPA.

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