

CHARACTERIZATION OF 4-NITRO-N-ETHYLENE-N,N'-DIMETHYL, N''-OCTADECYL-1,8-NAPHTHALIMIDE AND 4-NITRO-N-BUTYL-1,8-NAPHTHALIMIDE AS THIOL-REACTIVE FLUORESCENT PROBES

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Introduction and Aims: Selective SH reagents serve for sensitive detection, identification and quantification of thiols. SH groups react with nitronaphthalimides by Nitro/SR substitution. The quantum yield of Nitronaphthalimides is very low but, upon nitro-group displacement by RS-, highly fluorescent products are formed. Nitronaphthalimides are sparingly water-soluble but are easily solubilized in micelles. Our aim was to investigate the possible application of this reaction using both water soluble and hydrophobic thiols in aqueous micellar solutions. Here we present the study and analysis of the reaction of the SH of cysteinyl-n-hexadecylamide, HCys, which has low water solubility, and the water soluble glutathione (?-Glu-Cys-Gly) with 4-nitro-N-ethylene-N,N'-dimethyl,N''-octadecyl-1,8-naphthalimide, 4-NC18N, and 4-nitro-n-butyl-1,8-naphthalimide, 4-NBN, in the presence of hexadecyltrimethylammonium chloride (CTAC) micelles. **Results:** CTAC micelles catalyzed naphthalimides/thiol reactions with accelerations ranging from 10^4 to 10^8 , at pHs lower than 7. Fluorescence emission spectra of 4-NBN and 4-NC18N and their substitution products were determined. Calibration curves of fluorescence intensity vs. GSH and HCys concentration were obtained in excess of 4-NC18N (or 4NBN) with CTAC micelles. The detection limit is ca. 10^{-7} M. With CTAC, both water soluble and water insoluble thiols reacted at high speed with 4-NBN or 4-NC18N. These results indicated that with micelles 4-NC18N and 4-NBN are useful for quantitative determination of thiols and peptides even at pHs below the pKas of the thiol groups. **In conclusion** reaction with nitronaphthalimides may be a convenient tool for targeting of proteins containing Cys residues.

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Keywords: Micelles, thiol, fluorescent probes.