Sensitive and Selective Time-Gated Luminescence Detection of Hydroxyl Radical in Water

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General Considerations

Unless otherwise noted, all experiments were conducted in air at ambient temperature and pressure. Starting materials were obtained from commercial suppliers and used without further purification. Fluorescence measurements were acquired on a Varian Cary Eclipse Fluorescence Spectrophotometer using a quartz cell with a path length of 10 mm, slit width of 10 nm, and temperature of 20 °C. HPLC data were acquired using an Agilent 1100 Series HPLC with a Discovery® RP Amide C_{16} Column (15 cm × 2.4 mm; 5 µm). Water was distilled and further purified by a Millipore Simplicity UV system (Resistivity 18×10^6 Ω). pH measurements were taken using a Thermo Orion 3 Benchtop pH meter.

Photolysis of Trimesate in Sodium Nitrate

All sodium nitrate photolyses (variable time and variable sodium nitrate concentration) were performed in the following manner. The following solutions were combined, made fresh each time: 125 µL 20.0 mM trimesate in mQ water, the required amount of 0.800 M $NaNO_3$ in mQ water, and mQ water to bring total volume to 5 mL. The solutions were mixed, placed in corked test tubes, and photolyzed in a Rayonette reactor with 350 nm bulbs.

Kinetic Competition Reaction with Trimesate and Benzoate

The kinetic competition experiments for determining the rate of reaction of hydroxyl radical with trimesate was performed as follows. The following solutions were combined, made fresh each time: the required volume of 20.0 mM trimesate in mQ water, the required volume of 20.0 mM benzoate, 3020 µL 0.800 M $NaNO_3$ in mQ water, and mQ water to bring total volume to 10 mL. The solutions were mixed, placed in corked test tubes, and photolyzed in a Rayonette reactor with 350 nm bulbs for an hour. Blank samples were removed before the start of the photolysis. Varying ratios of trimesate:benzoate were tested, 1:1, 1:2, 2:1, 1:3, 3:1, 1:5, and 5:1. After the photolysis, the samples were analyzed *via* fluorescence (slit width of 10 nm, PMT voltage of 550, temperature of 20 °C, excitation at 350 nm, and emission from 340-550 nm). The formation of hydroxylated benzoate was monitored *via* HPLC [mobile phase 90:10 mixture of pH 3 phosphate buffer with 10% acetonitrile: methanol, flow rate of 1 mL/min, fluorescence detection with excitation at 225 nm and emission at 400 nm, 20 µL injections, and 24 minute run time].[1] The rate constant of the reaction of hydroxyl radical with benzoate is reported to be 5.9×10^9.[2] The rate of reaction of hydroxyl radical with trimesate was determined by competition to be $7\pm2\times10^9$ M^{-1} s^{-1}.

Screening Factor Determination

To determine the screening correction factor for the fluorescence spectra of hydroxylated trimesate, which is a result of sodium nitrate absorbing the excitation wavelength, the response of a constant concentration of hydroxylated trimesate to an increasing concentration of sodium

nitrate was measured. Hydroxylated trimesate was synthesized according to a published procedure.[3] Two stock solutions were made: (1) 10.7 μM hydroxylated trimesate in mQ water and (2) 10.7 μM hydroxylated trimesate in 795 mM sodium nitrate in mQ water. Increasing amounts of stock (2) were added to stock (1) in a fluorescence cell, and the hydroxylated trimesate fluorescence was monitored, with a slit width of 10 nm, PMT voltage of 550, temperature of 20 °C, excitation at 322 nm, and emission from 340-550 nm. The addition was repeated in triplicate. The screening factor is shown in Figure S1.

General Selectivity Study Considerations

All reactions were run for an hour, unless otherwise noted. The fluorescence spectrum of hydroxylated trimesate was measured, with a slit width of 10 nm, PMT voltage of 550, temperature of 20 °C, excitation at 322 nm, and emission from 340-550 nm, before the addition of 45 μL 1.52 mM Tb-DO3A in mQ water. After two minutes, the time-gated terbium luminescence spectra were acquired, with a slit width of 10 nm, PMT voltage of 800, temperature of 20 °C, time delay 0.2 msec, excitation at 343 nm, and emission from 525-575 nm.

Hydrogen Peroxide Selectivity Study

The following solutions were combined for H_2O_2 selectivity study, made fresh each time: 201 μL 20.0 mM trimesate in mQ water, 800 μL 0.800 M $NaNO_3$ in mQ water, and 6.94 mL mQ water; 75 μL 0.3% H_2O_2 were added.

Superoxide Selectivity Study

The following solutions were combined for the $O_2\bullet^-$ selectivity study, made fresh each time: 6.25 mL 20.0 mM trimesate in mQ water and 24.94 mL 0.800 M $NaNO_3$ in mQ water, which was diluted to 250 mL with mQ water; 18 mg KO_2 were added. Before measuring the fluorescence, the pH of the solution was adjusted to 7.8 with 5 μL 1.0 M HCl.

Hypochlorite Selectivity Study

The following solutions were combined for the $^-\bullet OCl$ selectivity study, made fresh each time: 201 μL 20.0 mM trimesate in mQ water, 800 μL 0.800 M $NaNO_3$ in mQ water, and 6.97 mL mQ water; 47 μL 1% NaOCl in water were added.

Singlet Oxygen Selectivity Study

All solutions for the $^1O_2$ selectivity study were made fresh before photolysis. For the light and dark samples, the following were mixed: 126 μL 20.0 mM trimesate in mQ water and 4356 μL mQ water; 23 μL 100 μM perinaphthanone were added to sensitize $^1O_2$ production during photolysis. Singlet oxygen production was calibrated using the following solutions: 50 μL 10.0 mM FFA and 4.834 mL mQ water; 23 μL 100 μM perinaphthanone were added to sensitize $^1O_2$ production during photolysis. The dark samples were prepared in the same way as the light samples but were wrapped in aluminum foil during the photolysis and used as a reference. The samples were photolyzed for two hours in a Rayonette reactor with 350 nm bulbs; seven samples of 100 μL each were taken during two hours to monitor FFA degradation using HPLC [mobile phase 90:10 pH 5 acetate:acetonitrile buffer, flow rate of 1 mL/min, UV-vis detection at 219 nm, 10 μL injections, and 5 minute run times]. After the photolysis, 495 μL 0.800 M $NaNO_3$ were added to the samples containing trimesate before fluorescence measurements. Special thanks to Kristopher McNeill and the McNeill group for use of their photochemical reactor and HPLC.

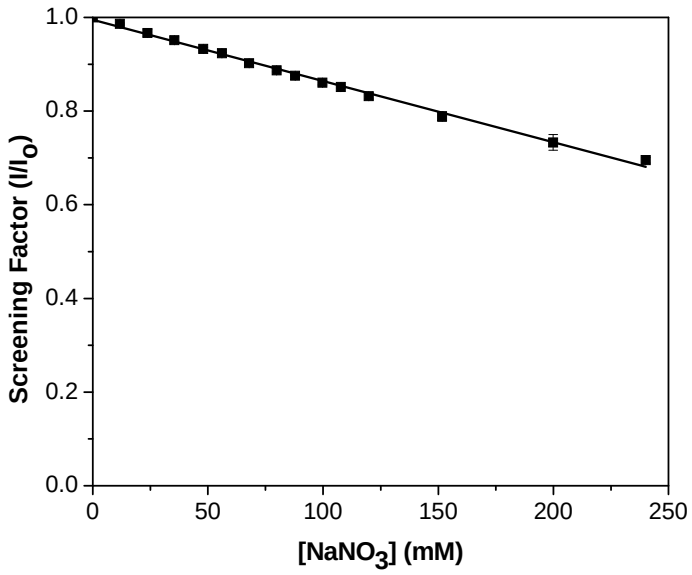
***Alkoxy Radical Selectivity Study*[4]**

The following solutions were combined for the alkoxy radical selectivity study, made fresh each time: 150 µL 13.3 mM trimesate in mQ water, 397 µL 0.800 M $NaNO_3$ in mQ water, 76 µL 0.56 mM $(bpy)_3Fe(ClO_4)_2$, and 3363 µL mQ water; 14 µL 2.95 mM ^tBuOOH were added.

Nitric Oxide Selectivity Study

The following solutions were combined for the NO selectivity study, made fresh each time: 200 µL 20.0 mM trimesate in mQ water, 0.8 mL 0.800 M $NaNO_3$ in mQ water, and 7.01 µL mQ water; 8 µL 6.35 mM DEANO (DEA NONOate; Diethylammonium (Z)-1-(N,N-diethylamino)diazen-1-ium-1,2-diolate) in 0.01 M NaOH were added. To initiate decomposition of the NONOate, 5 µL of 1.0 M HCl were added to bring the solution to approximately pH 4. After an hour, 5 µL 1.0 M NaOH were added to bring the solutions up to approximately pH 7 before addition of the Tb-DO3A.

Sodium Nitrate Screening Factor



S1. Average screening factor (integrated fluorescence intensity/ initial intensity) versus sodium nitrate concentration for hydroxylated trimesate with excitation at 322 nm, emission from 340-550 nm.

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