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Chromogenesis-based Resonance Raman molecular sensor for reactive oxygen species

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ABSTRACT

Here we report a novel design concept for a molecular sensing platform that specifically generates under non-toxic visible laser excitation, resonance Raman signatures of nitrile (~2200 cm^{-1}) in response to reactive oxygen species (ROS). The sensing principle employs a ROS-triggered oxidative chromogenic reaction that activates resonance Raman enhancement through chemical transformation of a non-resonant hydrazo molecule into a resonant azo dye with extended π-conjugation. Experimental studies as well as DFT calculation demonstrate that such a design strategy for activatable resonance Raman sensor is valid for selective detection of highly oxidative ROS, hypochlorite ions in the present case, down to 100 ppm.

1. Introduction

Raman spectroscopy is considered as one of the powerful analytical tools that enables bio-/chemo-detection in label-free as well as labeled fashions. However, techniques based on spontaneous Raman scattering suffer from inherently low sensitivity due to low cross-section of the scattering process. To increase the level of Raman signal, and therefore sensitivity of molecular detection, several techniques such as coherent (coherent anti-Stokes Raman scattering and stimulated Raman scattering) as well as direct resonance (resonance Raman (RR)) or metal plasmonic enhancement (surface enhanced Raman scattering (SERS)) of Raman signal have been successively employed [1–3]. Using exogenous Raman markers with unique vibrational signatures (e.g. deuterium, nitrile or alkyne groups) provides further improvement in detectability for biological applications by generating detection signals in a bio-silent spectral window (1800 – 2800 cm^{-1}) that is far separated from the spectrally crowded regions of biological/environmental backgrounds [4–7].

The resonance Raman (RR) amplification mechanism is based on the local electromagnetic field when RR spectroscopy uses an incident laser frequency that is close to an electronic transition energy of a molecule of interest. One of the critical limitations in this technique is a large fluorescence background produced due to a real electronic transition. For this reason, UV-absorbing small aromatic molecules without fluorescence emission have usually been studied as RR markers [8,9]. Recently, we have reported a new generation of non-UV RR marker based on a non-fluorescent small molecule (azobenzene) that produces Raman enhancement under non-toxic visible light excitation (instead of phototoxic UV irradiation) to enable target-specific imaging in live cells and immunolabeling [10]. In that report, cellular target specificity was endowed by chemical conjugation of the RR-active molecule with organelle-binding ligands or antibodies. It was revealed that the imaging signal from the RR marker, though not as intense as fluorescence, has unique advantages of (i) much higher photo-stability useful for long term sequential measurements and (ii) simultaneous multi-target analyzing feature for intrinsic and extrinsic anlytes with single light source without additional labeling [2,3], encouraging further development of more advanced RR probes.

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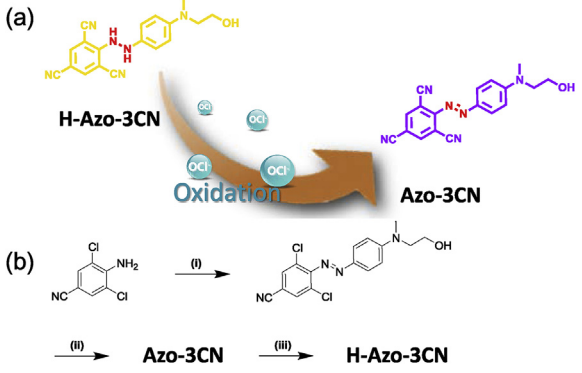
In the present study, we introduce a different approach to target-specific RR signaling, which is based on the reaction of an initially RR-inactive, but chemically activatable sensor molecule. Resonance enhancement requires electronic transition in the Raman at the Raman excitation wavelength. This scheme utilizes a reaction-based design for light absorption modulation in that the initial reactant form of the sensor Raman probe molecule is non-resonant at the excitation wavelength whereas its sensing product with its electronic transition shifted to a longer wavelength is now strongly light-absorbing to present a significantly enhanced RR signal. The resulting target-responsive contrast in RR enhancement allows for recognition of an analyte that causes such a chromogenic sensing reaction. To prove this design concept, we employed an oxidative chromogenic reaction by reactive oxygen species (ROS) as an analyte of biological and environmental significance. ROS are highly oxidizing small molecules that are implicated in normal cell physiology and oxidative damages, as well as in responses to pathological and environmental stresses [11]. As a ROS-sensing RR platform, we here report a hydrazobenzene-based system that is RR-inactive by itself but can be activated by ROS-triggered oxidation. As a proof of concept, we demonstrate that a hydrazobenzene derivative (H-Azo-3CN) is capable of chromogenic oxidation into RR-active azobenzene (Azo-3CN), and thereby RR enhancement of a bio-orthogonal nitrile (−C≡N) signature in response to hypochlorite anion (OCl^-), one of the critical ROS of biological, pathological and environmental importance [5]. This new approach is illustrated schematically in Scheme 1a.

2. Experimental section

2.1. Materials and methods

2-(Methylphenylamino)ethanol, 4-amino-3,5-dichlorobenzonitrile and all other chemicals, such as Hydrogen peroxide (H_2O_2, 30% aqueous solution), sodium hypochlorite (NaOCl, 5% aqueous solution), *tert*-butyl peroxide solution (THBP, 70 wt%), potassium dioxide (KO_2) and iron(II) perchlorate hydrate ($Fe(ClO_4)_2 \cdot xH_2O$) were purchased from Sigma–Aldrich. ^1H and ^{13}C NMR spectra were collected at 25 °C on a Bruker AV-300 spectrometer. The mass spectrum of the final compound was obtained from the fast atom bombardment (FAB) ionization mode with m-NBA matrix (JMS-700, JEOL). Absorption and fluorescence spectra were acquired using a UV–visible spectrometer (8453, Agilent) and a fluorescence spectrophotometer (F-7000, Hitachi), respectively.



Scheme 1. (a) Schematic representation of oxidative chromogenic reaction of H-Azo-3CN ($\lambda_{max.abs}$ = 385 nm) to Azo-3CN ($\lambda_{max.abs}$ = 567 nm) by hypochlorite. (b) Synthetic routes of Azo-3CN and H-Azo-3CN: (i) 2-anilinoethanol, H_2SO_4, $NaNO_2$, 0–4 °C, 6 h, (ii) CuCN, DMF, 130 °C, 6 h, and (iii) hydrazine, THF, 1 h.

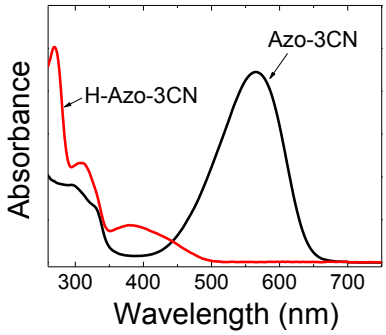


Fig. 1. Absorption spectra of Azo-3CN and H-Azo-3CN in DMSO (10 μM).

The fluorescence quantum yields were determined using a methanol solution of rhodamine B ($\Phi_f = 0.9$) as a reference.

2.2. Synthesis of Azo-3CN

Azo-3CN was synthesized following modified two-step procedures as described in the literature [15]. (1) Diazotization: A solution of 4-amino-3,5-dichlorobenzonitril (3 mmol) in 10 mL ethanol with sulfonic acid (9 mmol) was cooled to 4 °C and a solution of sodium nitrite (4 mmol) in 1 mL water was added dropwise to the solution. The resulting mixture was stirred for 6 h on ice bath. Then the solution of diazonium salt was reacted with 2-(methylphenylamino)ethanol (3 mmol) in ethanol at 4 °C for 12 h. The reaction mixture was poured into water and the precipitate was filtered. The red powder was purified by recrystallization in methanol. Yield: 58%; ^1H NMR (300 MHz, $CDCl_3$): δ = 3.16 (s, 3H), 3.67 (t, 2H, J = 3.2 Hz), 3.91 (t, 2H, J = 3.3 Hz), 6.84 (d, 2H, J = 6.6 Hz), 7.67 (s, 2H), 7.91 (d, 2H, J = 6.6 Hz) ppm. (2) Cyanation: A solution of Azo-3Cl (7 mmol) in 20 mL anhydrous DMF with copper(I) cyanide (CuCN, 15 mmol) was purged with argon gas for 1 h. After purging, the mixture was stirred at 130 °C for 6 h. Then the reaction mixture was extracted with water/chloroform. The organic layer was drying with $MgSO_4$ and evaporated under reduced pressure. The purple solid was purified by silica column with eluent chloroform/ethyl acetate (5:1). Yield: 31%; ^1H NMR (300 MHz, DMSO-d_6): δ = 2.47 (m, 3H), 3.19 (s, 2H), 3.62 (s, 2H), 7.0 (d, 2H, J = 7.2 Hz), 7.82 (d, 2H, J = 6.9 Hz), 8.7 (s, 2H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 54.59, 58.56, 106.89, 110.08, 113.13, 115.68, 116.26, 142.62, 155.64, 157.16 ppm; HR-MS (ESI^+): m/z: calcd for $C_{18}H_{14}N_6O$: 330.12 [M^+]; found: 329.11.

2.3. Synthesis of H-azo-3CN

Hydrazine in THF (4 mL, 1 M) was added dropwise to a solution of Azo-3CN (3 mmol) in 5 mL THF. The resulting mixture was stirred for 30 min until the color faded out. After solvent evaporation, the reaction mixture was washed with water and extracted with chloroform. The organic layer was dried and filtered by silica column with eluent hexane/ethyl acetate (2:1). Yield: 91%; ^1H NMR (300 MHz, $CDCl_3$): δ = 3.05 (s, 3H), 3.59 (t, 2H), 3.87 (t, 2H), 6.88 (d, 2H, J = 6.9 Hz), 7.43 (d, 2H, J = 6.4 Hz), 7.74 (d, 1H, J = 0.9 Hz), 8.10 (d, 1H, J = 0.9 Hz) ppm; ^{13}C NMR (75 MHz, Acetone-d_6): δ = 39.07, 55.09, 59.56, 98.34, 99.84, 110.91, 111.80, 112.48, 126.32, 126.43, 134.30, 135.56, 144.80, 150.50, 154.87 ppm; HR-MS (ESI^+): m/z: calcd for $C_{18}H_{16}N_6O$: 332.14 [M^+]; found: 331.13.

2.4. Preparation of ROS and reactive nitrogen species (RNS)

Various ROS/RNS solutions were prepared for selectivity