ORGANIC LETTERS

2005
Vol. 7, No. 12
2417−2420

A New Fluorogenic Transformation: Development of an Optical Probe for Coenzyme Q

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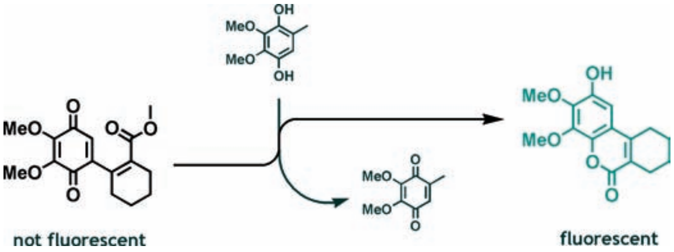
Received April 7, 2005

ABSTRACT



A new fluorogenic transformation based on a quinone reduction/lactonization sequence has been developed and evaluated as a tool for probing redox phenomena in a biochemical context. The probe presented herein is an irreversible redox probe and is reduced selectively by biologically relevant quinols such as ubiquinol but is inert to reduced nicotinamides (e.g., NADH). The ensuing cyclization is fast and quantitative and provides a measurable optical response.

As part of a program aimed at the development of optical probes for visualizing enzymatic redox processes,[1] we became interested in the electron transport chain as an intriguing target for a new type of redox-active fluorogenic molecular probe. Given the central role of redox cofactors such as ubiquinone (Coenzyme Q), plastoquinone, and menaquinone as mobile electron carriers in the electron transport chains of different species, we reasoned that if the reduction of a *p*-quinone core structure could be coupled to a change in fluorescence, it would serve as a basis for the development of such a probe.

Since *p*-quinols (reduced *p*-quinones) are typically unstable to oxygen, we expected that a reasonable fluorogenic mechanism would involve "trapping" of the quinol component of the redox pair. Intramolecular cyclization resulting in the expansion of a π-system has been shown by us[1b,2] and by others[3] to be an effective fluorescence "switching" mechanism. Accordingly, we proposed that reduction of a *p*-quinone bearing a (Z)-α,β-unsaturated ester in the 2-position would result in facile lactonization to form the familiar coumarin fluorophore (Figure 1). This transformation was inspired in part by the biosynthesis[4] and biochemical role of α-tocopherol (vitamin E), whose chromanol ring system is essentially a "trapped" quinol that can undergo ring-opening oxidation to the corresponding quinone as part of its antioxidant mechanism.[5]

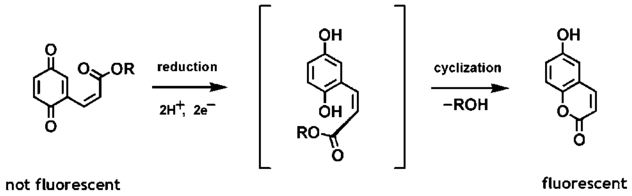


Figure 1. Fluorogenic transformation triggered by quinone reduction.

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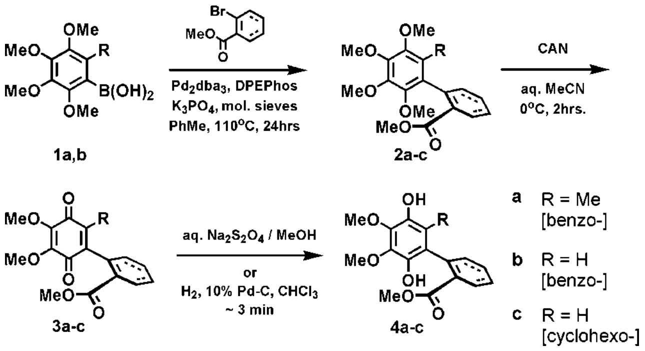
10.1021/ol0507569 CCC: $30.25

Central to the success of this design was the speed of the cyclization reaction. The lactonization of the quinol needed to be fast relative to the quinone reduction so that the optical signal correlated directly with the rate of the reduction event and was not complicated by the kinetics of the cyclization. Also, the tendency for quinols to reoxidize in air required that the trapping occur quickly. Lactonization of a quinol generated in situ by reduction of a quinone has been shown to be spontaneous in aqueous media, but only when facilitated by a trialkyl lock mechanism.[6] This system, however, was not suitable for our design, since the geminal dimethyl group precludes the π-system expansion upon cyclization. Thus, we set out to explore lactonization kinetics of fully conjugated systems.

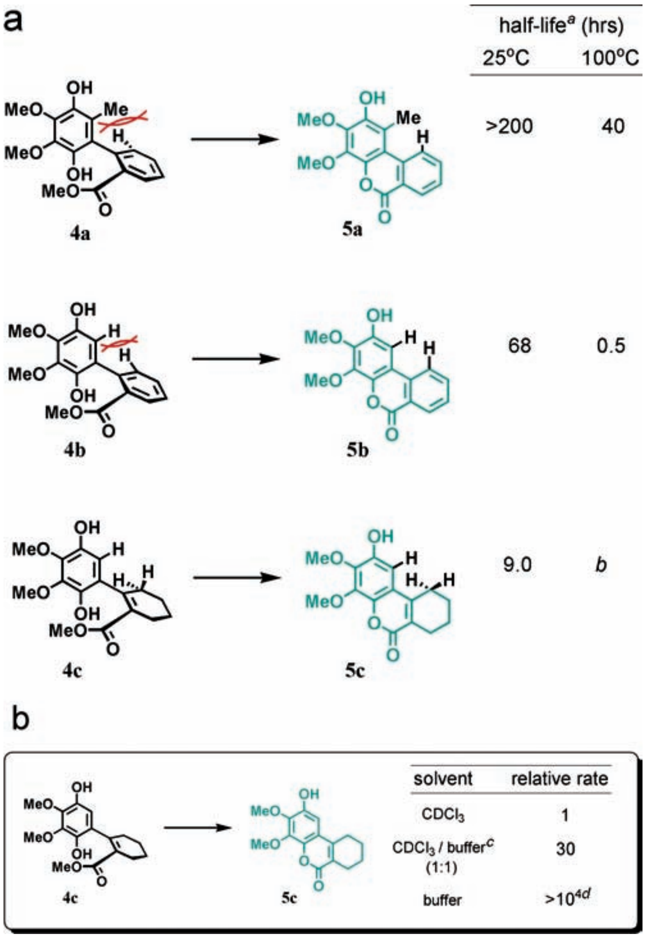
We synthesized a series of probe candidates based on 2,3-dimethoxy-5-methyl-1,4-benzoquinone (the ubiquinone core) since it has been the subject of extensive biological[7] and synthetic[8] study and because we expected that an electron-rich hydroquinone would be sufficiently nucleophilic to effect the lactonization; benzo- and cyclohexo-fused α,β-unsaturated esters were chosen to avoid olefin isomerization during synthesis or under assay conditions (Scheme 1). The sterically demanding Suzuki coupling of electron-rich boronic acids **1a**,**b** with the appropriate aryl or vinyl bromide was carried out using Buchwald's conditions[9] and furnished the adducts **2a**−**c**. Standard oxidative demethylation with cerric ammonium nitrate gave the air-stable quinones **3a**−**c**, which could be reduced quantitatively under mild conditions (aqueous $Na_2S_2O_4$ or brief catalytic hydrogenation in $CHCl_3$) to give the isolable quinols **4a**−**c**.

Scheme 1



Quinols **4a**−**c** underwent clean lactonization to the corresponding coumarins **5a**−**c** at varying rates depending on substitution (Scheme 2a). The cyclization of quinol **4a** in $CDCl_3$ was slow and required elevated temperature. The 5-methyl substituent in **4a** was identified as the chief detriment to cyclization, since the desmethyl derivative **4b** cyclized nearly 2 orders of magnitude faster, now proceeding at room temperature but still slowly. Analogues of **4b** containing activated esters were examined (see Supporting Information), but none proved to be more promising than the cyclohexo- derivative **4c**, which cyclized an order of magnitude faster than **4b** in $CDCl_3$. The cyclization of **4c** was further examined in different solvent systems in order to approximate varying degrees of hydrophobicity and proton availability[10] (Scheme 2b). To our delight, **4c** *cyclized in only seconds at room temperature* in neutral phosphate buffer. The **3c**/**4c** redox pair had thus satisfied the first requirement

Scheme 2



[a] Reaction monitored by ^1H NMR in $CDCl_3$. [b] Not determined. [c] Phosphate buffer (50 mM), pH = 7.4. [d] Reaction was complete upon dissolution (<15 s).

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for a potential probe, namely, that the lactonization reaction occur spontaneously.

Coumarin **5c** showed two absorption bands (271 and 328 nm) and fluoresced weakly, but detectably, with the emission maximum at 550 nm when excited at 330 nm in neutral aqueous media (see Supporting Information). Since quinone **3c** has no absorption bands above 300 nm and no detectable fluorescence, the **3c**/**5c** pair constitutes a competent optical switch.

Having satisfied the criteria for facile cyclization and optical switching, quinone probe **3c** was then screened against various biologically relevant reductants, and a qualitative reduction profile was generated (Figure 2). The relative reduction rate was estimated by measuring the rate of formation of coumarin **5c** by UV−vis absorption in the presence of reductant (1−10 equiv) at rt in pH 7.4 phosphate buffer (Figure 3); formation of **5c** was corroborated by simultaneous HPLC analysis. Coumarin **5c** was detected in the presence of all three *p*-quinols tested (1−3 equiv). The relative rates of reduction by the *p*-quinols were proportional to their reported midpoint potentials[11] (duroquinol, $E^\circ = {\sim}^{(+)}100$ mV; ubiquinol, $E^\circ = {\sim}^{(+)}60$ mV; menaquinol, $E^\circ = {\sim}^{(-)}100$ mV). Since quinol **4c** is immediately and irreversibly converted to coumarin **5c**, the redox couple **3c**/**4c** is unable to equilibrate with other quinone/quinol couples, allowing complete conversion to occur. Despite the significantly more negative midpoint potential of NADH (−320 mV),[11] it did not reduce **3c** even when present in 10-fold excess; it seems that in this case, a significant kinetic (i.e., mechanistic) barrier sufficiently blocks an otherwise thermodynamically favored process. Probe **3c** was not reduced by riboflavin, dopamine, or Fe(II). The notable selectivity of probe **3c** for quinol cofactors over NADH suggests its potential usefulness for detecting local redox potential and electron fluxes in quinone/quinol pools such as those found in membrane-bound electron transport chains. Typical detection limits were in the range of 30−50 μM reductant according to the UV−vis assay and 5−10 μM according to the fluorescence assay, visualized with 1 equiv of probe **3c**. This could be improved by further optimization of coumarin **5c**'s photophysical properties; however, the high local concentration of membrane-confined quinone redox cofactors should render the current detection limit satisfactory for use in intact cells or membrane preparations.

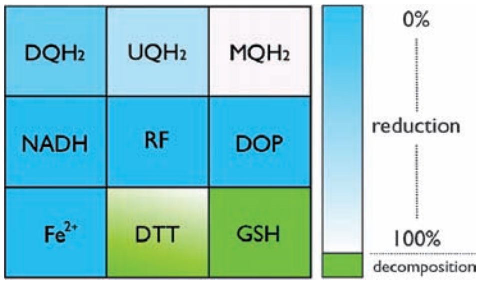

Figure 2. Reduction profile of **3c**. DQH_2 = duroquinol; UQH_2 = ubiquinol; MQH_2 = menaquinol; NADH = nicotinamide adenine dinucleotide hydride; RF = riboflavin; DOP = dopamine; Fe^{2+} = $FeCl_2$; DTT = dithiothreitol; GSH = glutathione. [Conditions: pH 7.4, rt, 10 min, 100 μM **3c**, 1−10 equiv of reductant.]

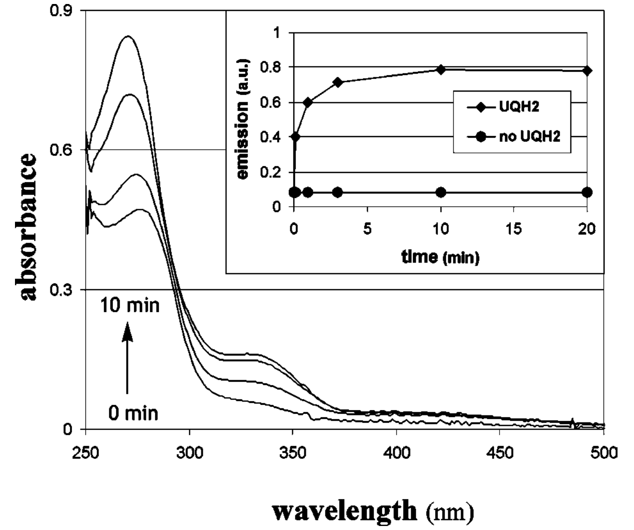

Figure 3. Reduction of probe **3c** monitored by absorbance and fluorescence. Ubiquinol (UQH_2, 3 equiv) was added to **3c** in pH 7.4 phosphate buffer at rt, and absorbance spectra were measured at 0, 1, 5, and 10 min. The absorbance increase at ∼270 nm is due to formation of ubiquinone (UQ) as well as **5c**; the increase at ∼330 nm is due to formation of **5c** only. Inset: the same assay monitored by fluorescent emission at 550 nm.

Probe **3c** was partially decomposed (accompanied by partial reduction to **5c**) by dithiothreitol and completely decomposed by glutathione (Figure 2). The instability of certain quinones to thiols highlights a potential limitation of using the quinone core as a biochemical redox probe, since thiols are important, and abundant, biological reductants. However, preliminary study of the reaction between probe **3c** and thiols indicates that the undesired reaction may be thwarted by rapid localization of the probe into hydrophobic membranes where most quinone redox processes take place.

In summary, a new fluorogenic chemical transformation triggered by *p*-quinone reduction has been developed and incorporated into a probe that responds spontaneously and irreversibly to reduction at room temperature in aqueous media. This process has been coupled to the selective detection of biologically relevant *p*-quinol cofactors, demonstrating its potential usefulness as a molecular redox probe. Although preliminary studies have identified some limitations of this design, we are currently investigating alternative

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applications of this probe, as well as modified designs that circumvent these problems.

Acknowledgment. Generous support for this work was provided by the G. Harold and Leila Y. Mathers Charitable Foundation. D.S. is the recipient of the Pfizer Award for Creativity in Organic Chemistry.

Supporting Information Available: Detailed synthetic procedures, kinetic data, spectral and photophysical characterization, and assay protocol. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0507569