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Caged Q-Rhodamine Dextran: A New Photoactivated Fluorescent Tracer

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Abstract—An amine-reactive caged rhodamine was synthesized and conjugated to aminodextran. The resulting tracer was injected into a single cell zebrafish embryo, and a portion of the tracer was photolyzed in a single cell after development. The resulting fluorescent cell was imaged by fluorescence microscopy through a single round of cell division.

Photoactivatable, or 'caged,' fluorophores[1] are useful tools for benign introduction of fluorescent marks in dynamic systems. After the colorless and nonfluorescent caged fluorophore is homogeneously distributed within the system of interest, a mark is generated by UV photolysis, which can be controlled spatially and temporally with good precision. The resultant fluorescent mark is monitored with time to provide quantitative dynamic information. Caged fluorescence has been used to study cytoskeletal proteins in locomoting cells[2,3] and to measure viscosity and diffusion coefficients in intercellular spaces.[4] Caged fluorophores have been attached to dextrans, which are hydrophilic poly-(α-D-1,6-glucose) polysaccharides, for velocimetry studies in fluid dynamics,[5] and to polyacrylamide to study gel deformation dynamics.[6]

Caged fluorescein covalently linked to dextran has also been used for lineage tracing and fate mapping in developmental biology studies.[7] However, after uncaging, such probes suffer from photoinstability and optical interference from cellular autofluorescence. Therefore, we set out to prepare a caged fluorophore dextran conjugate that would improve on these deficiencies. Rhodamine fluorophores are accessed at longer wavelengths than fluorescein, providing better optical separation from endogenous fluorophores. Rhodamines are also substantially more photostable than fluorescein.[8] Reactive caged versions of a particular rhodamine, carboxy-Q-rhodamine, have been prepared for attachment to proteins.[9] Carboxy-Q-rhodamine wavelengths closely match those of the widely used tetramethylrhodamine fluorophore.[8] Here, we report another version of a reactive caged Q-rhodamine, its attachment to dextran, and use of the resulting probe to monitor cell lineage in a developing embryo.

Bis-acylation of 5- and 6-carboxy-Q-rhodamine with two equivalents of the chloroformate **1** was achieved in moderate yield using potassium *t*-butoxide as base in HMPA/THF[9] (Scheme 1). The carboxylic acid moiety in the resulting nonfluorescent spirolactone **2** was activated as its succinimidyl ester under standard conditions. The caging group *t*-butyl esters in **3** were then selectively cleaved by TFA treatment, giving the water-soluble amine-reactive caged fluorophore **4**.[10] Reaction of **4** with a limited amount of 10,000 average MW amine-functionalized dextran in DMSO–water–$NaHCO_3$ afforded the conjugate **5** as a pale orange solid after chromatography on Sephadex LH-20. The degree of substitution (DOS) was determined to be 2.6 caged fluorophores/dextran by measuring the optical density at λ_{max} (292 nm) of a weighed sample of **5** and using the extinction coefficient that was measured for a pure sample of **4**. UV photolysis of **5** generated a fluorescent

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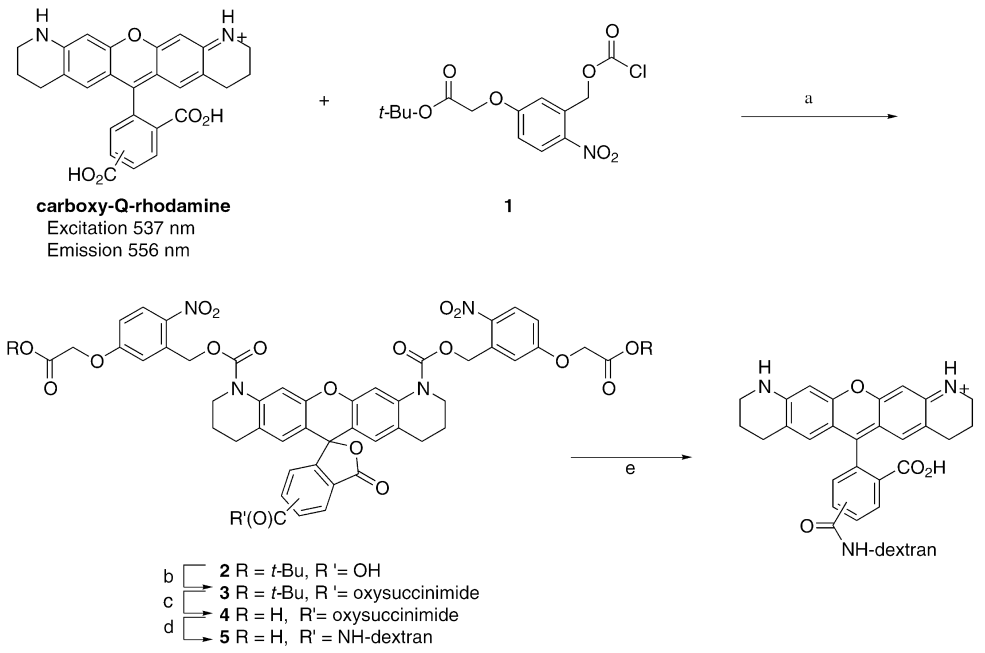

Scheme 1. Reagents: (a) KO-*t*-Bu, HMPA, THF (43%); (b) *N*-hydroxysuccinimide, EDC (59%); (c) TFA (74%); (d) aminodextran, DMSO, H_2O, $NaHCO_3$; (e) hv.

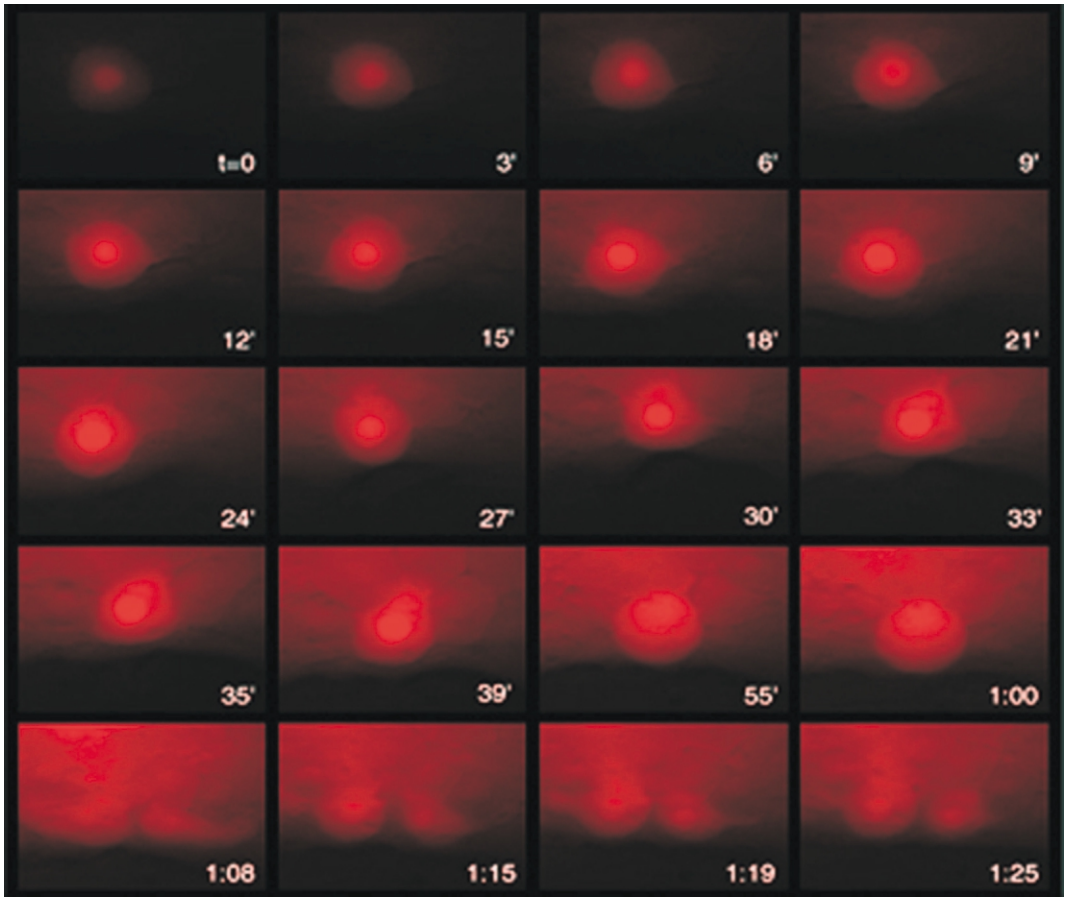

Figure 1. Time course of a cell labeled with uncaged carboxy-Q-rhodamine by single cell photolysis of an embryo containing **5**. A single marginal cell (50% epiboly stage zebrafish embryo) was uncaged ($t=0$) and the rhodamine fluorescence monitored by brightfield illumination using tetramethylrhodamine filters at the indicated times. Nuclear localized fluorescent signal is apparent prior to nuclear envelope breakdown ($t=0$–39 min) and following cell division (1:15 until 1:25).

species with wavelength maxima (537 nm excitation/556 nm emission) identical to uncaged carboxy-Q-rhodamine (Fig. 1).

To determine the effectiveness of caged Q-rhodamine dextran (**5**) as a lineage tracer in a biological system we labeled a single cell by photolysis in an early stage zebrafish embryo and followed the cell through one round of division. A 1% buffered solution of **5** (1–3 nL) was pressure microinjected into the yolk at the one-cell stage. The embryo was allowed to develop and the fluorophore was uncaged in a single marginal cell at 50% epiboly[11] by pinhole illumination with a mercury-arc lamp (>360 nm). Figure 1 shows a time course of the single labeled cell as it undergoes one round of cell division. Throughout most of this time series the uncaged rhodamine fluorescence is predominantly nuclear ($t=0$ until 39 min). This nuclear localization may be a function of the uncapped free amine groups remaining on the dextran portion of **5**. The rhodamine fluorescence becomes more diffuse at 55 min, presumably reflecting breakdown of the nuclear envelope prior to cell division. Following cytokinesis, each daughter cell reestablishes localized Q-rhodamine fluorescence in the nucleus. Brightness of the uncaged Q-rhodamine signal and background fluorescence is similar to that reported for caged fluorescein dextran.[11]

With the emergence of transgenic embryos (zebrafish, worms, *Drosophilia*) expressing tissue-specific green fluorescent protein, development of more stable and longer wavelength caged rhodamine dyes will be an important tool to augment current single and two color lineage tracing methodologies.[12]

References and Notes

1. Krafft, G. A.; Sutton, W. R.; Cummings, R. T. *J. Am. Chem. Soc.* **1988**, *110*, 301.
2. Mitchison, T. J. *J. Cell Biol.* **1989**, *109*, 637.
3. Theriot, J. A.; Mitchison, T. J. *Nature* **1991**, *352*, 126.
4. Xia, P.; Bungay, P. M.; Gibson, C. C.; Kovbasnjuk, O. N.; Spring, K. R. *Biophys. J.* **1998**, *74*, 3302.
5. Lempert, W. R.; Magee, K.; Ronney, P.; Gee, K. R.; Haugland, R. P. *Exp. Fluids* **1995**, *18*, 249.
6. Achilleos, E. C.; Prud'homme, R. K.; Christodoulou, K. N.; Gee, K. R.; Kevrekidis, I. G. *Chem. Eng. Sci.* **2000**, *55*, 3335.
7. Kozlowski, D. J.; Murakami, T.; Ho, R. K.; Weinberg, E. S. *Biochem. Cell Biol.* **1997**, *75*, 551 and references cited therein.
8. Haugland, R. P. *Handbook of Fluorescent Probes and Research Chemicals*, 6th ed.; Molecular Probes: Eugene, OR, 1996.
9. Mitchison, T. J.; Sawin, K. E.; Theriot, J. A.; Gee, K. R.; Mallavarapu, A. *Methods Enzymol.* **1998**, *291*, 63.
10. Melting point 213–216 °C (dec.); ^{1}H NMR (DMSO-d_6) δ 8.60 (s, 1H), 8.41 (t, 1H), 8.19 (d, 2H), 7.85 (s, 2H), 7.59 (d, 1H), 7.16 (br s, 2H), 7.08 (d, 2H), 6.73 (s, 2H), 5.51 (s, 4H), 4.79 (s, 4H), 3.77 (br s, 4H), 2.92 (br s, 4H), 2.62 (m, 4H), 1.82 (m, 4H); ε 27,600 cm^{-1} M^{-1} at λ_{max} (292 nm, MeOH); background fluorescence 34 ppm (MeOH, 537 nm excitation, 556 nm emission); 90% pure by HPLC [254 nm detection, isocratic 5–95% gradient (MeCN/0.1 M TEAA pH 7 buffer) on a Microsorb MV 150×4.6 mm column; t_R 21.1 and 21.4 min for the regioisomers]. Anal. calcd for $C_{51}H_{39}N_5O_{21}\cdot H_2O$: C, 56.90; H, 3.88; N, 6.50. Found: C, 57.19; H, 4.35; N, 5.50.
11. Kozlowski, D. J.; Wienberg, E. S. *Methods Mol. Biol.* **1999**, *135*, 349.
12. Pfeiffer, S.; Alexandre, C.; Calleja, M.; Vincent, J.-P. *Curr. Biol.* **2000**, *10*, 321.