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COMMUNICATION

Chromo-fluorogenic detection of aldehydes with a rhodamine based sensor featuring an intramolecular deoxylactam†

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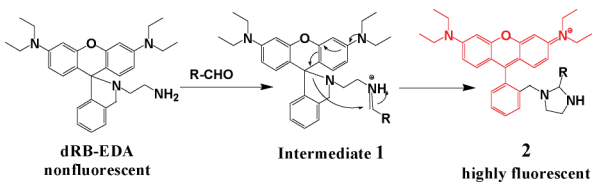
A chromogenic and fluorogenic detection of aldehydes was achieved *via* analyte triggered opening of the deoxylactam of *N*-(rhodamine B)-deoxylactam-ethylenediamine (dRB-EDA). The utility of the sensor was demonstrated by fluorescent labeling of aldehyde-displaying sialoproteins on cell surfaces.

Aldehydes are a group of reactive compounds prone to a number of chemical transformations. Formaldehyde, used in the production of adhesives, textiles, *etc.*, is an allergic and carcinogenic pollutant in the environment.[1] Exposure to formaldehyde can cause detrimental effects on human health and often lead to "sick building" syndrome disease. Apart from being widely utilized in industry, aldehydes are emerging targets in biomedical studies, *e.g.* aldehyde-displaying glycoproteins,[2] formylglycine-containing proteins,[3] occurrence of formyl group in oxidized DNA,[4] production of formaldehyde in cancers,[5] and formaldehyde releasing anticancer drugs.[6] Methods allowing sensitive detection of aldehydes under various conditions, *e.g.* in living cells, are highly desirable for applications ranging from monitoring of indoor gaseous formaldehyde, imaging of cell-surface glycoconjugate[2] and labeling of aldehyde-containing proteins,[7] to quantitation of aldehydes in biological systems.[4–6]

Optical chemosensors are advantageous in the aforementioned applications as they require routine instruments and offer the possibility of detecting aldehydes with the naked eye. Towards this goal, many chromogenic or fluorogenic reagents have been developed for detection of formaldehyde.[8] Prior chemosensors where hydrazones are often used to capture aldehydes required forcing conditions to form colored species (*e.g.* strong base catalysis),[8a–e] and thus are not compatible with applications in living cells. Alternatively, fluorescent reporters have been introduced into living cells by chemoselective ligation for visualization of glycoproteins on the cell surface.[2] We wish to develop sensitive sensors that could detect aldehydes under mild conditions without addition of any catalysts.

Rhodamine-lactams, a group of non-fluorescent rhodamine derivatives, have been extensively explored for fluorogenic sensing of various cations or chemically reactive species, *e.g.* nitric oxide.[9,10] In contrast, the rhodamine-deoxylactams have largely remained unexplored for analyte detection.[11] Herein we report the fluorogenic and chromogenic detection of aldehydes *via* analyte triggered opening of the intramolecular deoxylactam of a rhodamine B derivative.

N-(Rhodamine B)-lactam-ethylenediamine (referred to as RB-EDA) was treated with lithium aluminium hydride in anhydrous tetrahydrofuran to give *N*-(rhodamine B)-deoxylactam-ethylenediamine (referred to as dRB-EDA) in 40% yield (ESI†). The ethylenediamine moiety of dRB-EDA was designed to react with aldehyde to form the Schiff base intermediate **1** which will subsequently promote opening of the intramolecular deoxylactam to form fluorescent and colored imidazolidine-containing rhodamine species **2** (Scheme 1).



Scheme 1 Detection of aldehydes with dRB-EDA *via* tandem imine formation and intramolecular cyclization.

To validate the proposed roles of the ethylenediamine moiety of dRB-EDA in aldehyde recognition, three structurally related analogs of dRB-EDA were synthesized and evaluated for their efficacy at sensing formaldehyde (Fig. 1**A**). The color formation rates of dRB-EDA, RB-EDA, *N*-(rhodamine B)-deoxylactam-2-aminoethanol (dRB-AE), and *N*-(rhodamine B)-deoxylactam-amine (dRB-amine) in DMF solutions containing formaldehyde were respectively monitored by UV–vis absorption at 560 nm as a function of time. It was shown that a red color quickly developed in the solution of dRB-EDA upon addition of formaldehyde while no discernable absorbance was observed for RB-EDA (Fig. 1**B**). The inertness of RB-lactam could be ascribed to the non-nucleophilic nature of the amide moiety which impedes the intramolecular addition to the imine intermediate **3** (ESI†, Scheme S2). dRB-EDA exhibited greatly improved sensing kinetics relative to

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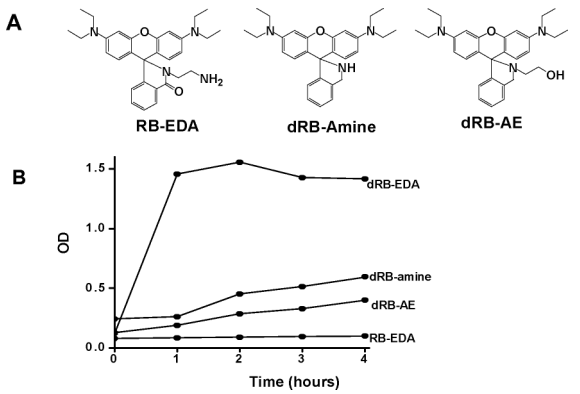

Fig. 1 Comparison of the chromogenic responses of dRB-RDA and its structural analogs with formaldehyde. (**A**) Chemical structures of the analogs of dRB-EDA; (**B**) Time dependant absorption at 560 nm of dRB-EDA and its analogs (1 mg ml^{-1}) with formaldehyde (17.5 mM) in DMF.

dRB-AE, and dRB-amine (Fig. 1**B**), highlighting the essential roles of the deoxylactam-ethylenediamine motif in chromogenic sensing of formaldehyde.

To probe the optimal assay conditions, the color formation rates of dRB-EDA with formaldehyde under a variety of conditions were screened by UV–vis absorbance. Time course studies of the reaction rates showed the assay was efficient in acetonitrile, dimethylformamide (DMF) and aqueous DMF (ESI†, Fig S2, Fig. S4). Changes in relative humidity were problematic in many previous assay systems for formaldehyde. In contrast, dRB-EDA was shown to be effective in aqueous DMF containing 2% (v/v) of water, suggesting its potential application for real-world applications.

To test the assay sensitivity, an aliquot of formaldehyde stock solution was added into DMF containing dRB-EDA to prepare a series of solutions with various amounts of the analyte. The solutions were incubated at room temperature for 90 min and then UV–vis absorption spectra were recorded. Fig. 2 showed the maximum absorption located at 560 nm increased as a function of formaldehyde concentrations. As low as 3.5 mM of formaldehyde can be identified under the assay conditions (Fig. 2; ESI†, Fig. S4). Since the color of the reaction solution was developed in a time dependant manner (ESI†, Fig. S3), the assay limits could be further extended by elongation of the incubation time. The selectivity of dRB-EDA towards a panel of aldehydes was

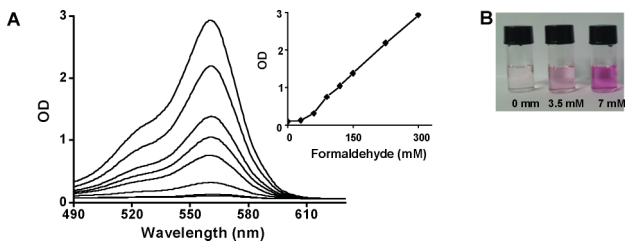

Fig. 2 Chromogenic detection of formaldehyde with dRB-EDA. (A) UV– absorption spectra of dRB-EDA in the presence of formaldehyde (concentration: 0, 3.5, 7, 10.5, 14, 17.5, 35 mM, from bottom to top); The inset shows the titration curve by absorbance at 560 nm; (B) Visual detection of formaldehyde (0–7 mM as indicated) with dRB-EDA in DMF after incubation at room temperature for 90 min.

examined. It was shown that the dRB-EDA could efficiently detect hexanaldehyde and 4-hydroxy-benzaldehyde while it was unable to sense glucose and acetone (ESI†, Fig S5). Taken together, the results showed that dRB-EDA was selective for alkyl or aromatic aldehydes under the assay conditions.

The assay solutions of dRB-EDA with formaldehyde were further examined by fluorometry. The fluorescence emission centered at 590 nm intensified as a function of formaldehyde concentration (Fig. 3), indicating fluorogenic detection of aldehyde. The fluorescence emission spectra of the resultant solutions were shown to be almost identical to that of rhodamine B (ESI†, Fig. S1), indicating the formation of rhodamine fluorophore from ring opening of the deoxylactam in dRB-EDA under the assay conditions (Scheme 1). To access the identity of the possible adduct in the assay conditions, the reaction solution of dRB-EDA with formaldehyde was analyzed by mass spectrometry. A major peak located at 483.3111 was identified, which is consistent with the theoretical molecular weight of the proposed product **2** ($C_{31}H_{39}N_4O^+$; MW: 483.3118) (Fig. 4), confirming formation of imidazolidine in the assay system.

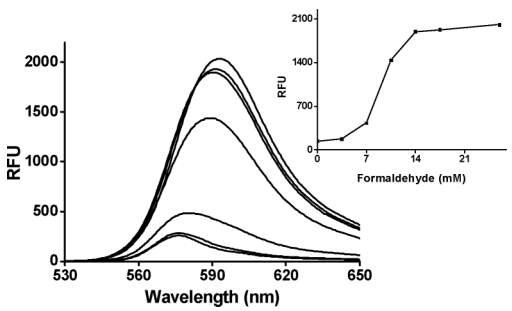

Fig. 3 Fluorescence emission spectra of dRB-EDA (1 mg ml^{-1}) in the presence of formaldehyde (0, 3.5, 7, 10.5, 14, 17.5, 35 mM, from bottom to top). The inset shows the titration curve of formaldehyde with dRB-EDA by fluorescence emission intensity at 590 nm.

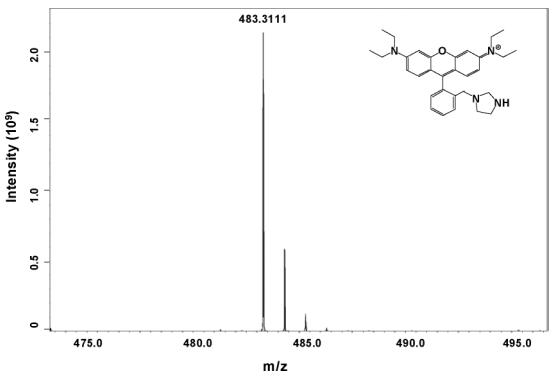

Fig. 4 HRMS confirmation of formation of compound **2** in the reaction of dRB-EDA with formaldehyde.

Chemical tools that allow fluorescent imaging or tracking of glycoconjugates are valuable for exploring their biological functions. Bioorthogonal groups (*e.g.* aldehyde) can be either metabolically incorporated into glycans of the cell surface glycoconjugates[2,12] or generated on the cell surface *via* periodate oxidation of the sialosides on glycoproteins.[13] Demonstrated to be able to detect aldehydes with high sensitivity in solutions, dRB-EDA

was evaluated for its efficacy at labeling cell surface aldehydes. L929 cells with abundantly expressed sialoproteins on the cell surface[14] were subjected to periodate oxidation to introduce an aldehyde moiety at the C-7 position of the sialoside following established procedures (Fig. 5**A**).[13] The resultant cells were further treated with dRB-EDA, and then analyzed with confocal fluorescence microscopy. The images showed that the labeling was exclusively on the surface of cells that have been oxidized with periodate whereas no labeling was observed on the control cells (Fig. 5**B**). The cell-surface restricted staining is in accord with the fact that sialylated proteins are abundantly localized in the plasma membrane. The different labeling patterns of cells treated with or without periodate indicated that the fluorescent labeling of cell-surface glycans with dRB-EDA was aldehyde specific.

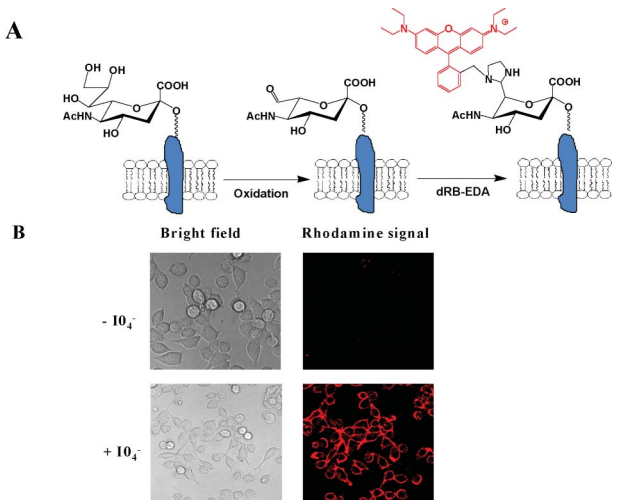

Fig. 5 Fluorogenic labeling of cell surface sialoproteins. (**A**) The labeling was achieved *via* periodate oxidation of the cell surface sialosides followed by chemoselective ligation of the generated aldehyde with dRB-EDA; (**B**) Confocal microscopic images of dRB-EDA labelled L929 cells that were pre-treated with or without sodium periodate.

Given the exceptional fluorescence properties of rhodamines, *e.g.* high fluorescence quantum yields, bioorthogonal fluorescence spectra, and high photo-stability, the chemoselective and fluorogenic labeling of cell-surface aldehydes with dRB-EDA suggests the potential utility of deoxylactam-containing rhodamines in visualization or tracking of cell-surface sialoproteins in living cells.

In summary, dRB-EDA, an ethylenediame containing rhodamine-deoxylactam, was design for fluorogenic and chromogenic sensing of aldehyde under mild conditions *via* analyte mediated opening of the intramolecular deoxylactam. The sensor was capable of labeling cell-surface aldehyde-displaying sialoproteins with stringent selectivity. Given the distinguished fluorescence properties of rhodamines, dRB-EDA might be useful not only for detection of formaldehyde with the aid of instruments or possibly with the "naked eye", but also for a number of biological applications, *e.g.* evaluation of formaldehyde releasing anticancer drugs, and quantitation of endogenous formaldehyde in some cancers.

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References

1 R. L. Maynard, *Occup. Environ. Med.*, 2000, **57**, 502D.
2 Y. Zeng, T. N. Ramya, A. Dirksen, P. E. Dawson and J. C. Paulson, *Nat. Methods*, 2009, **6**, 207.
3 B. Schmidt, T. Selmer, A. Ingendoh and K. von Figura, *Cell*, 1995, **82**, 271.
4 W Hirose, K. Sato and A. Matsuda, *Angew. Chem., Int. Ed.*, 2010, **49**, 8392.
5 (*a*) J. R. Ghilardi, H. Rohrich, T. H. Lindsay, M. A. Sevcik, M. J. Schwei, K. Kubota, K. G. Halvorson, J. Poblete, S. R. Chaplan, A. E. Dubin, N. I. Carruthers, D. Swanson, M. Kuskowski, C. M. Flores, D. Julius and P. W. Mantyh, *J. Neurosci.*, 2005, **25**, 3126; (*b*) M. A. Sabino and P. W. Mantyh, *J. Support. Oncol.*, 2005, **3**, 15; (*c*) S. E. Ebeler, A. J. Clifford and T. Shibamoto, *J. Chromatogr. B Biomed. Sci. Appl.*, 1997, **702**, 211; (*d*) P. Spanel, D. Smith, T. A. Holland, W. Al Singary and J. B. Elder, *Rapid Commun. Mass Spectrom.*, 1999, **13**, 1354.
6 (*a*) L. Bareket, A. Rephaeli, G. Berkovitch, A. Nudelman and J. Rishpon, *Bioelectrochemistry*, 2010, **77**, 94; (*b*) M. Ugarenko, C. K. Chan, A. Nudelman, A. Rephaeli, S. M. Cutts and D. R. Phillips, *Oncol. Res.*, 2009, **17**, 283; (*c*) S. M. Cutts, A. Nudelman, V. Pillay, D. M. Spencer, I. Levovich, A. Rephaeli and D. R. Phillips, *Oncol. Res.*, 2005, **15**, 199; (*d*) I. Levovich, A. Nudelman, G. Berkovitch, L. P. Swift, S. M. Cutts, D. R. Phillips and A. Rephaeli, *Cancer Chemother. Pharmacol.*, 2008, **62**, 471.
7 I. S. Carrico, B. L. Carlson and C. R. Bertozzi, *Nat. Chem. Biol.*, 2007, **3**, 321.
8 (*a*) M. I. Helaleh, M. Kumemura, S. Fujii and T. Korenaga, *Analyst*, 2001, **126**, 104; (*b*) K. Kawamura, K. Kerman, M. Fujihara, N. Nagatani, T. Hashiba and E. Tamiya, *Sens. Actuators, B*, 2005, **105**, 495; (*c*) G. J. Mohr, U. E. Spichiger, W. Jona and H. Langhals, *Anal. Chem.*, 2000, **72**, 1084; (*d*) M. S. Quesenberry and Y. C. Lee, *Anal. Biochem.*, 1996, **234**, 50; (*e*) K. Toda, K. I. Yoshioka, K. Mori and S. Hirata, *Anal. Chim. Acta*, 2005, **531**, 41; (*f*) Y. Y. Maruo, J. Nakamura and M. Uchiyama, *Talanta*, 2008, **74**, 1141; (*g*) T. Nash, *Biochem. J.*, 1953, **55**, 416; (*h*) K. N. Ramachandran and V. K. Gupta, *Chem. Anal.*, 1993, **38**, 513; (*i*) S. Salahuddin, O. Renaudet and J. L. Reymond, *Org. Biomol. Chem.*, 2004, **2**, 1471; (*j*) E. Sawicki, T. R. Hauser and S. McPherson, *Anal. Chem.*, 1962, **34**, 1460; (*k*) Y. Suzuki, N. Nakano and K. Suzuki, *Environ. Sci. Technol.*, 2003, **37**, 5695; (*l*) H. Tsuchiya, S. Ohtani, K. Yamada, M. Akagiri, N. Takagi and M. Sato, *Analyst*, 1994, **119**, 1413.
9 (*a*) X. Chen, X. Wang, S. Wang, W. Shi, K. Wang and H. Ma, *Chemistry*, 2008, **14**, 4719; (*b*) Y. K. Yang, H. J. Cho, J. Lee, I. Shin and J. Tae, *Org. Lett.*, 2009, **11**, 859.
10 (*a*) T. Q. Duong and J. S. Kim, *Chem. Rev.*, 2010, **110**, 6280; (*b*) S. Han, Z. Xue, Z. Wang and T. B. Wen, *Chem. Commun.*, 2010, **46**, 8413; (*c*) H. N. Kim, M. H. Lee, H. J. Kim, J. S. Kim and J. Yoon, *Chem. Soc. Rev.*, 2008, **37**, 1465; (*d*) Z. Li, S. Wu, J. Han and S. Han, *Analyst*, 2011, **136**, 3698–3706; (*e*) W. Zhang, B. Tang, X. Liu, Y. Liu, K. Xu, J. Ma, L. Tong and G. Yang, *Analyst*, 2009, **134**, 367; (*f*) H. Zheng, G. Q. Shang, S. Y. Yang, X. Gao and J. G. Xu, *Org. Lett.*, 2008, **10**, 2357.
11 Q. A. Best, R. Xu, M. E. McCarroll, L. Wang and D. J. Dyer, *Org. Lett.*, 2010, **12**, 3219.
12 (*a*) E. Saxon and C. R. Bertozzi, *Science*, 2000, **287**, 2007; (*b*) L. K. Mahal, K. J. Yarema and C. R. Bertozzi, *Science*, 1997, **276**, 1125.
13 (*a*) P. A. De Bank, B. Kellam, D. A. Kendall and K. M. Shakesheff, *Biotechnol. Bioeng.*, 2003, **81**, 800; (*b*) C. G. Gahmberg and L. C. Andersson, *J. Biol. Chem.*, 1977, **252**, 5888.
14 G. Yogeeswaran, R. Fujinami, R. Kiessling and R. M. Welsh, *Virology*, 1982, **121**, 363.

