ChemComm

COMMUNICATION

View Article Online
View Journal | View Issue

Cite this: *Chem. Commun.*, 2014, 50, 13477

Received 27th July 2014,
Accepted 10th September 2014

DOI: 10.1039/c4cc05831c

www.rsc.org/chemcomm

Bio-inspired enol-degradation for multipurpose oxygen sensing†

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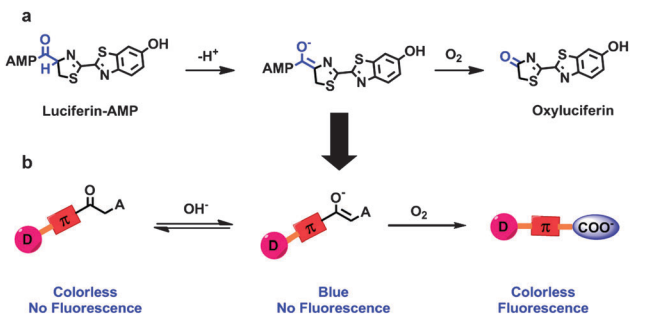
Inspired by the enol-degradation of luciferin, a new oxygen sensor with oppositely changed color and fluorescence has been designed. This new reaction-based dual mode sensor can not only be used as a highly selective instant "fluorescence on" oxygen probe, but also as a freshness indicator of food or food materials by using its property of time-adjustable color fading.

Molecular oxygen (O_2) is of fundamental importance for biological systems and human activities.[1] Precise sensing of O_2 is required in many fields, such as medicine,[2] environmental analysis,[3] industry,[4] *etc.* Even though many admirable technologies, such as titration,[5] colorimetry,[6] electrochemistry,[7] and luminescence,[8] have been developed so far to meet various O_2 sensing needs, intensive research has been performed for decades on redox-based fluorescent/phosphorescent O_2 sensors due to their better sensitivity than dye-based color sensors.[9] However, most of the O_2 sensors so far are "turn-off" type with the intensity of the fluorescence/phosphorescence fading away, and the "turn-on" type sensors are very rare but highly preferred due to their far better visual sensitivity.[10] Recently, Shin *et al.* reported a very good example of a fluorescence "turn-on" oxygen sensor based on a perylene diimide molecule in the reduced form.[10] The sensor can be reused several times after being reactivated electrochemically. The reversibility of a sensor is an important property to ensure its repeatable use, but a reliable process to quickly and conveniently restore the activity of the sensor remains to be a great challenge in the field.[11] Thus, some cheap disposable sensors are also desirable to avoid inconvenient processes of reactivation or a waste of not fully utilized reversible functions in many situations.[6,12]

Inspired by amazing processes in Nature, several fascinating biomimetic systems have been developed to develop turn-on type of oxygen sensors. A successful example that appeared recently is a luciferin based highly sensitive disposable sensor with "turn on" luminescence for sensing oxygen or ATP in the presence of luciferase.[13,14] Since luciferase is too unstable *in vitro*, stability of the luciferin–luciferase-mimic sensing system remains to be a technological challenge. Currently, there is no good way to chemically mimic the entire process of luciferin shining for oxygen sensing as in a firefly, therefore, a highly sensitive disposable chemical sensor partially mimicking the bio-process might be a good choice. Herein, we present a new chemical approach of oxygen sensing based on a process similar to enol-degradation inspired by luciferin. By this method, we have developed an unusual oxygen sensor with instant fluorescence "turn-on", adjustable color fading time, good selectivity, and an attractive prospect to meet various oxygen sensing needs.

This entire work is based on an accepted assumption that the key function of luciferase in the bio-degradation of luciferin is α-H extraction to facilitate the enolization of a luciferin–AMP affixture,[15] and the resulting enolate is very vulnerable to oxygen attack, which subsequently leads to oxyluciferin as a product of enol-degradation and further results in bioluminescence (Scheme 1a). The enol-degradation process has also been speculated to be a major reaction involved in many biological processes, such as food digestion, *etc.*[16] We envision that α-H extraction function of the enzyme could be replaced by a chemical base, and a pre-fluorescent



Scheme 1 Illustration of the design of oxygen sensing by bridging two aromatic motifs with a methyl ketone group, and the color switch mechanism.

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† Electronic supplementary information (ESI) available. CCDC 987215. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c4cc05831c

molecule could substitute for the pre-luminous luciferin (Scheme 1b). If we can chemically mimic the "enol-degradation" process to create a change in color and fluorescence, the scientific insights obtained from this bio-inspired reaction could not only lead to a new class of oxygen sensor, but also help to verify and better understand the hidden mechanism of bio-degradation of luciferin and many other biological processes.

In order to verify our assumptions, we designed a new methyl ketone **TM1** which is based on our previous work[17] and a conjecture that this new nitro-containing methyl ketone molecule with a large conjugate group (or its enolate) should exhibit weak or no fluorescence due to some fatal nonradiative transitions of the nitro (photon-induced electron transfer) and dangling methyl ketone functional groups, and a strong fluorescence could be observed after removal of the nitro and dangling methyl ketone functional groups by enol-degradation.[18] We anticipate that the degradation could occur spontaneously at a suitable temperature upon exposure to oxygen after the molecule being treated with base. Two more molecules (**TM2** and **TM3**) were designed and synthesized to help verify the assumption and prove the reaction mechanism (Fig. 1). Their base induced enolization properties are evaluated first.

To our delight and as per our expectations, the ketone–enol transition of **TM1** was observed in acetonitrile (Fig. S1, ESI†). Upon addition of base to the solution of **TM1**, a new absorption peak at λ_{max} = 585 nm was observed immediately with a noticeable change from colorless (λ_{max} = 376 nm) to reddish-cyan. The enolate of **TM1** can be easily reverted to its original methyl ketone structure by acid.

The anticipated enol-degradation of **TM1** was explored next. Much to our delight, a showy blue-fluorescence turns on instantly after the non-fluorescent **TM1-enolate** was exposed to air at 25 °C, which indicates that the response time of the oxygen sensor is instant. As shown in Fig. 2a, a unique emission peak emerges at 450 nm and its intensity increases with time when the solution of **TM1-enolate** is exposed to oxygen. And the intensity of its visible color with absorption peak at 585 nm decreases synchronously (Fig. 2b). Compared with blue-green fluorescent **TM2** (Fig. S2, ESI†), the oppositely changed color and emission in the solution of **TM1-enolate** is presumably due to an enol-degradation reaction, which subsequently yields a fluorescent product (**TMX**, as shown in Fig. S3, ESI,† fluorescence lifetime, $\tau \approx 1.6$ ns).

To experimentally simplify further investigation of the reaction mechanism, 2-(4-nitrophenyl)-1-phenylethanone (**TM3**) was selected as a proof-of-principle. Both benzoic acid (60%) and 4-nitrobenzoic acid (70%) were isolated after the enol-degradation reaction in which **TM3** was first treated with oxygen in a solution containing 40 eq. of

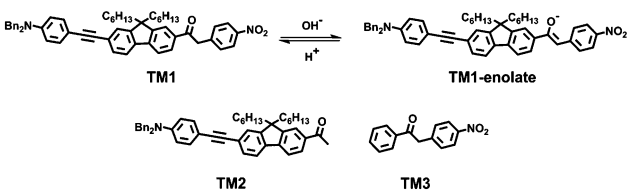

Fig. 1 Structures of the ketone and enolate forms of **TM1** and the model molecules **TM2** and **TM3**.

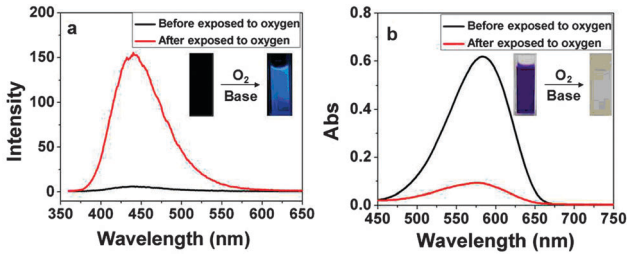

Fig. 2 Fluorescence spectra (a) and absorption spectra (b) of **TM1-enolate** solution (1.0×10^{-5} M, acetonitrile solution, 30 eq. of *t*-BuOK, 25 °C, stirring) before and after exposure to oxygen.

t-BuOK and then acidified. In addition, when the time of treatment with oxygen was shortened to 5 min, nitrobenzaldehyde (20%) was also isolated from the reaction. In contrast, no degradation product was detected and isolated when the same reaction solution of **TM3** was treated with an inert gas (nitrogen). These results demonstrate clearly that oxygen is essential for the degradation of this methyl ketone molecule under alkaline conditions, and 4-nitrobenzaldehyde and benzoate are the initial degradation products. 4-nitrobenzoic acid is presumably a further oxidation product of the 4-nitrobenzaldehyde. Furthermore, a major peak at 674.3917 in HRMS spectrum (Fig. S4, ESI†), which is identical with the theoretical molecular weight of **TMX** (674.3918), is detected from the oxygen treated **TM1-enolate** solution. This is another strong evidence to further prove formation of the degradation product **TMX** during the oxidation process. All the results prompted us to propose a possible mechanism to explain the entire reactions. That is, as depicted in Fig. S3, ESI,† first the **TM1-enolate** is generated, then electrophilic oxygen reacts with the **TM1-enolate** forming a highly unstable intermediate with a possible structure of a four-membered ring,[18] which subsequently decomposes into thermodynamically more favorable 4-nitrobenzaldehyde and a substituted fluorenylcarboxylate.

To evaluate its O_2 probing sensitivity, electrochemical properties of the molecule were examined by using cyclic voltammetry. **TM1-enolate** shows a very low oxidation potential (0.23 V *vs.* NHE, Fig. S5, ESI†), which indicates that it is even more sensitive than the latest "turn-on" probes that use a perylene diimide molecule.[10] The turn-on response of **TM1-enolate** is highly selective towards oxygen. As shown in Fig. 3a, almost no response was observed under N_2, CO_2, H_2, NO_2, SO_2 and NO atmospheres, and the fluorescence was observed only under an oxygen environment. Most importantly, the fluorescence intensities of **TM1-enolate** were found in linear proportional to the concentration of oxygen in the range of 0–100% at the same detection time (Fig. 3b). The linear relationship between the intensity of its fluorescence and the oxygen concentration reveals its good potential for accurate oxygen sensing.

In order to make this new sensing method more convenient for practical applications, a prototype of the sensor strip was developed by integrating **TM1** and an alkaline substrate into paper.[19] Side-by-side testing of the sensor strip under air or nitrogen indicates that the originally non-fluorescent testing strip shows a beautiful blue fluorescence instantly in air, and its fluorescence gets brighter and brighter over time until it reaches a constant intensity (Fig. 3d).

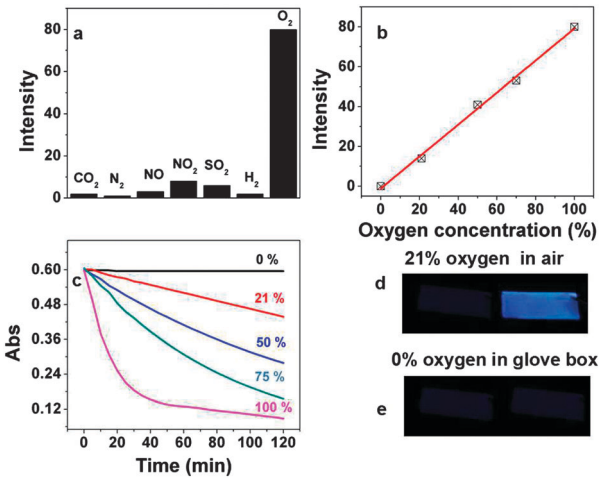

Fig. 3 (a) Fluorescence response of **TM1-enolate** (1.0×10^{-5} M, 30 eq. of *t*-BuOK, 25 °C, stirring) to various common environmental interferents (CO_2, N_2, NO, NO_2, SO_2, H_2, O_2) in acetonitrile solution. (b) Fluorescence intensity changes in **TM1** (1.0×10^{-5} M, acetonitrile solution, 30 eq. of *t*-BuOK, 25 °C, stirring) with the concentration of oxygen recorded at 1 min. (c) Absorbance (at 585 nm) changes in **TM1** (1.0×10^{-5} M, acetonitrile solution, 30 eq. of *t*-BuOK, 25 °C) in the presence of different concentrations of oxygen (0%, 21%, 50%, 75%, 100%). The curves fitted with one-order reaction dynamics $\ln A = -kt$ to calculate the degradation reaction rate constant: $k = 4.25 \times 10^{-2}$ min^{-1} (100%), $k = 1.14 \times 10^{-2}$ min^{-1} (75%), $k = 6.43 \times 10^{-3}$ min^{-1} (50%), $k = 2.36 \times 10^{-3}$ min^{-1} (21%), $k = 0$ min^{-1} (0%). And photographs of testing paper strips showing changes in fluorescence emission before and after 1 min (d) 21% oxygen in air; (e) 0% oxygen in the glove box under 365 nm UV light.

In contrast, no fluorescence can be observed when the testing strip is placed in a nitrogen atmosphere for the same testing period (Fig. 3e). Preliminary results demonstrate that this new testing strip for oxygen sensing is highly efficient with good potential as a commercial portable and disposable testing strip.

In addition to its attractive property of instant fluorescence, the reddish-cyan color of **TM1-enolate** obviously decreases after exposure to oxygen. As illustrated in Fig. 3c and Fig. S6, ESI,† the color fading rate of **TM1-enolate** in solution increased with either the temperature or the concentration of oxygen. In addition, its reaction rate can be tuned from 0 min^{-1} to 4.25×10^{-2} min^{-1} at 25 °C, and the relationship between the oxygen concentration and the color disappearing time of the **TM1-enolate** solution can be described by the function $y = 233.4 - 27.7\ln(t)$, where y is the oxygen concentration at 25 °C (Fig. S7, ESI†).[20] The color fading rate of the sensing strip was also found to be inversely related with the surrounding vacuum level. At a fixed concentration of **TM1** in the strip, the color disappearing time of the strip varied from several hours to several days depending on the vacuum level (Fig. S8, ESI†). Even though this sensor is very sensitive with a very low oxidation potential, for a strip placed in the environment with a fixed oxygen content, the fading time of the sensor could be as short as several minutes or as long as several days by simply adjusting either the concentration of **TM1-enolate** or/and the oxygen diffusion rate in the testing strip. More importantly, a reusable sensing strip can be achieved when one strip contains many individual sensing regions to independently and sequentially detect oxygen (Fig. S9, ESI†).

As regards to potential application of this reaction based oxygen sensing, we can clearly foresee that this very attractive dual-mode feature will enable us to use the different modes for different applications, which is usually lacking in many existing oxygen sensors. Apart from using the unique property of instant fluorescence for highly sensitive oxygen sensing, as a proof-of-principle, we demonstrated another feasible use of the sensing strip for monitoring the food freshness by taking advantage of its another attractive property of time-adjustable color fading in intelligent packaging.[11] In daily life, the freshness of meat cannot be distinguished normally when it is stored in a sealed bag. In addition, both the quality guarantee period of fresh meat and the fading rate of our visual testing strip are dependent on the oxygen concentration under the same testing conditions.[11] Thus, it is easy to monitor the freshness of the meat visually, based on the correlation between the freshness of the meat and the color fading degree of the testing strips. When the O_2 testing strips as freshness indicators are isolated and sealed with meat in the same packet, customers or store-workers can easily determine the quality and freshness of the meat by observing the color of the strip against the reference provided in the package (as shown in Fig. 4a and b).

In summary, we have, for the first time, developed a novel O_2 sensor with good selectivity by bio-mimicking the enol-degradation process. The fluorescence "turn-on" and color-fading dual-mode feature of our sensor facilitates both instant (fluorescence mode) and long-time tracking (color mode) of the oxygen concentration. The sensing precursor of **TM1** is stable in air, and a ketone–enol isomerization, which is easily triggered by chemical base, is a perquisite for the oxidative degradation. Therefore, such a two-step reaction will avoid the storage problem that is usually associated with other reduced dyes, and will make the sensor more practical for applications. In addition, the underlying reaction mechanism was fully proved by spectroscopy studies. Scientific insights obtained from this research will not only stimulate and accelerate further development of a new class of outstanding oxygen sensors, but will also help to verify and better understand the hidden mechanism of bio-degradation of luciferin and many other biological processes, and help to better understand how the Nature works, which would be fundamentally very important to science.

This work was supported by the National Science Foundation of China (Grant No. 51373068).

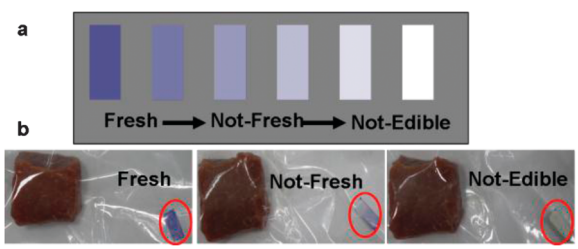

Fig. 4 (a) A photograph of reference strips for judging freshness of meat. (b) Photographs of freshness monitoring **TM1** visual strips before and after the expiry date of fresh meat.

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