Photostabilization of Rhodamine 6G in acetone by β-carotene and *t*-butyl hydroxyanisole

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The photostability of Rhodamine 6G is of crucial importance for the organic light-emitting display of mobile phones. Photobleaching of Rhodamine 6G in acetone was not protected by β-carotene, but was efficiently photostabilized by *t*-butyl hydroxyanisole. The results indicate that photobleaching of Rhodamine 6G in acetone was not caused by singlet oxygen but some radical species.

Introduction

Recently, the organic light-emitting display is widely used for mobile phones, television, etc. One of the crucial problems for the organic light-emitting display is the short life of fluorescent dyes.

In many cases, photoinduced bleaching, photobleaching, of a fluorescent dye is the irreversible conversion of the dye. The photobleaching mechanisms of organic dyes are complex and mostly unknown (Weber 1973; Yamashita ans Kashiwagi 1976; Dunne and Quinn 1976; Rosenthal 1978; Korobov and Chibisov 1978; Soper *et al*. 1991; Song *et al.* 1996; Widengren and Rigler 1996; Eggeling *et al*. 1998; George *et al*. 1999; Wennmalm and Rigler 1999; Patterson and Piston 2000; Deschenes and Bout 2002). They probably follow different pathways involving various intermediates. Some of these bleaching reactions need oxygen, and others require the optical excitation of an intermediate. It is therefore difficult to present a general description of photobleaching.

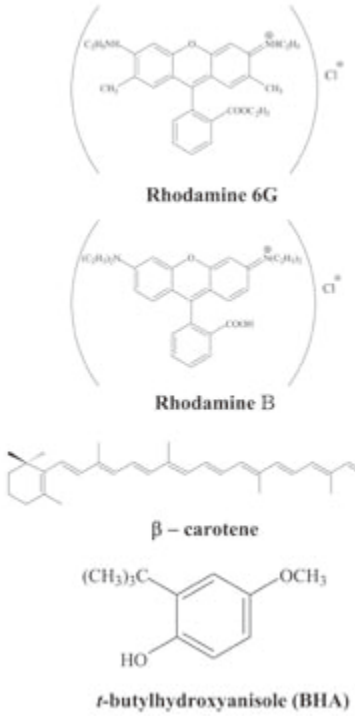

Fig. 1 Molecular structures of Rhodamine 6G (Rh6G), Rhodamine B, β -carotene and *t*-butyl hydroxyanisole (BHA).

Rhodamine 6G (Rh6G) is one of the excellent organic fluorescent dyes possessing high fluoresce yield. Oxygen is generally regarded as the most important reagent in photobleaching, and is believed to react in its singlet excited state, singlet oxygen, generated by reaction with the triplet state of Rh6G, even though the oxidation mechanisms are poorly known. If the photobleaching of Rh6G was brought about by singlet oxygen, Rh6G could be stabilized by singlet oxygen scavengers. However, Zondervan *et al*. (2004) reported that the main species involved in the photobleaching of Rh6G was its radical anion, formed through the triplet. If so, Rh6G can be photostabilized by radical scavengers.

In this paper, we examined the photostabilization of Rh6G in acetone by a radical scavenger, *t*-butyl hydroxyanisole (BHA) as well as a singlet oxygen scavenger, β-carotene. BHA exhibited the effective photo-stabilization of Rh6G, but no effect was seen in β-carotene.

Materials and methods

Rhodamine 6G (Rh6G: Wako 183-00622), *t*-butyl hydroxyanisole (BHA: Wako 021-07612), and acetone (Wako 014-00347) were used without further purification. β-carotene was extracted with acetone/methanol (7/3, *v/v*) mixture at 277 K from parsley (Petroselinum crispum Nym.). The extract was applied to a preparative-scale HPLC (Senshupak 5251-N, 250 mm x 20 mm i.d.) and eluted with hexane/2-propanol/methanol (100/2/0.4, *v/v/v*) at a flow rate of 7 mL min^{-1} at 277 K, as described elsewhere (Kobayashi *et al*. 1991).

A 10 mL of acetone solution in a 10ml of glass vial (SV-10, 24 mm i.d. x H45 mm, NICHIDEN-RIKA GLASS CO.,LTD.) containing Rh6G was illuminated on a blue LED flat light unit

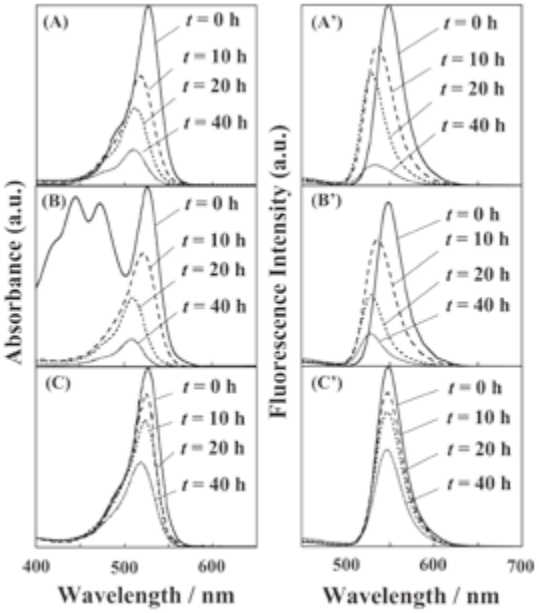

Fig. 2 Changes in the absorption (left) and fluorescence (right) spectra of Rh6G in acetone with time of illumination without scavengers (A and A'), in the presence of β-carotene (B and B') and BHA (C and C'). Initial dye concentration, 1.0 x 10^{-4} M; β-carotene, 7 x 10^{-5} M; BHA, 1.0 x 10^{-3} M. Illumination, λ = 470 nm. λ_{ex} = 345.6 nm for A' at 0 - 40 h, and for B' and C' at 0 h; 337.6 nm for B' and C' at 10, 20, 40 h.

(λ=470nm, LDL-60x60-BL, CCS Inc.) with a power supply (PD-3024, CCS Inc.; coarse 7 and fine 8) at room temperature. The experiments were carried out under aerated condition.

Absorption and fluorescence spectra were measured by a JASCO UV/VIS spectrophotometer (Model V-550) and a Shimadzu spectrofluorometer (Model RF-5000) at room temperature. A 0.4 mL aliquot of the solution was diluted with 3.6 mL acetone for absorption spectra measurement, and 0.04 mL with 3.96 mL for fluorescence measurements, respectively.

Results and Discussion

Change in the absorption and fluorescence spectra of Rh6G in acetone with time of illumination was illustrated in Fig. 2. By illumination Rh6G showed a photobleaching, accompanying a gradual hypsochromic shift from 527 to 509 nm (Fig. 2A). Similar spectral change was reported for Rhodamine B (Fig. 1), which was due to a highly efficient photochemical N-deethylation accompanying acetaldehyde formation (Watanabe *et al.* 1977). From the viewpoint of similarity of the absorption spectral change futures and molecular structures of both dyes, first step of photobleaching of Rh6G was most probably due to the N-deethylation.

Even in the presence of β-carotene, photobleaching of Rh6G in acetone was not controlled. As seen in Fig. 2B, by illumination for 40 h, the absorbance at 527 nm quickly decreased down to 0.09 % compared to the initial one. Note that β-carotene it self was also photobleached immediately by illumination (Fig. 2B); no β-carotene remained after 10 h irradiation. As expected from the very similar absorption spectral changes in the absence (Fig. 2A) and in the presence of β-carotene (Fig. 2B), the corresponding fluorescence spectral changes induced by illumination were also the same (Figs. 2A' and B'), although by 20 h illumination absorbance and fluorescence intensity in the presence of β-carotene (Figs. 2B and B') were smaller than those in the absence of β-carotene (Figs. 2A and A'). Conclusively, β-carotene cannot be used as a photostabilizer for Rh6G, and that contribution of singlet oxygen to the Rh6G photobleaching in the present study is slight.

In contrast, BHA exhibited the effective photostabilization. Even by 40 h illumination, more than 50% of Rh6G remained (Fig. 2C), and retained strong fluorescence (Fig. 2C').

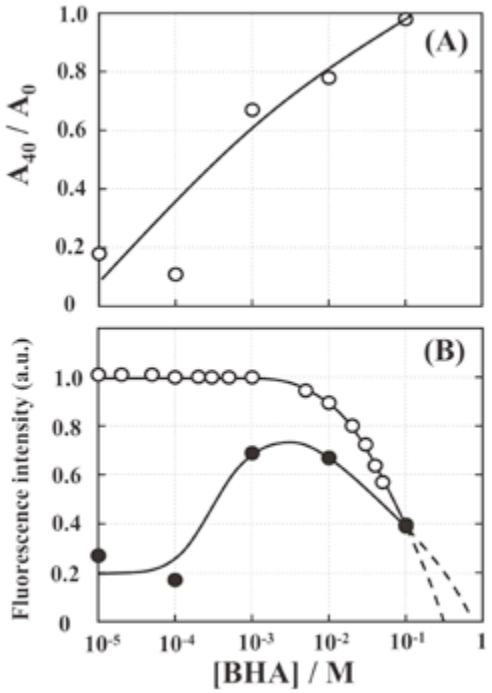

Fig. 3 (A) Relative absorbance of Rh6G in acetone after 40 h illumination to the initial absorbance, A_{40}/A_0, monitored at 527 nm, and (B) (○) initial and (●) 40 h illumination fluorescence intensity of Rh6G at the various concentrations of BHA ranging from 10^{-1} ～ 10^{-5} M. Initial dye concentration, 1.0 x 10^{-4} M. λ_{ex} = 345.6 nm except for [BHA] = 10^{-5} and 10^{-4} M at 40 h; λ_{ex} = 337.6 nm at 40 h for [BHA] = 10^{-5} and 10^{-4} M. λ_{em} = 548.8 nm at 0 h. At 40 h, λ_{em} = 548.8 nm for 10^{-1} M, 545.6 nm for 10^{-3} M and 10^{-2} M, 528.0 nm for 10^{-5} and 10^{-4} M.

At various concentrations of BHA ranging from 10^{-5} to 10^{-1} M, the fluorescence intensity of Rh6G after 40 h illumination was displayed in Fig. 3B. It is of interest to note that initial fluorescence (○) was not quenched at the concentration of BHA below 10^{-3} M; at the higher concentration, the initial fluorescence was gradually quenched. At the concentrations of 10^{-3} and 10^{-2} M, ca. 70% fluorescence intensity relative to the initial one was kept even after 40 h illumination (●), while at the lower concentrations of BHA drastic decrease of fluorescence down to ca. 20% was observed (Fig. 3B).

At the BHA concentrations of 10^{-3}～10^{-2} M, the remaining absorbance of Rh6G at 527 nm (Fig. 3A) and the fluorescence intensity after 40 h illumination (Fig. 3B) were almost the same. Note that as seen in Fig. 3 at the BHA concentration of 10^{-1} M neither absorbance nor fluorescence intensity changed by illumination, although the initial fluorescence was largely quenched (Fig. 3B). Consequently, efficient photostabilization of Rh6G in acetone at room temperature was performed at the BHA concentrations ranging from 10^{-3} to 10^{-2} M.

Taking into account the well-documented fact that most of the oxidative N-dealkylations are preceded by radical cation formation (Watanabe *et al*. 1977; Zondervan *et al*. 2004), and considering the radical scavenger function of BHA, observed Rh6G photostabilization by BHA in acetone is supposed to arise from the scavenging the photoinduced Rh6G radical by BHA.

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