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Invited feature article

Using photochemistry to understand and control the production of reactive oxygen species in biological environments

Nicolas Macia, Belinda Heyne *

Chemistry Department, University of Calgary, AB T2N 1N4, Canada

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ABSTRACT

Reactive oxygen species (ROS) originating from the metabolism of molecular oxygen are a double-edge sword. Their involvement in various diseases is well documented, while they also serve essential signaling functions. In order to shed light on their biochemical role, chemical tools have been designed for ROS detection and production. This featured article describes and illustrates different photochemical processes, which have been exploited to engineer new probes for the detection and the site-specific production of ROS.

1. Introduction

With the exception of some anaerobic organisms, molecular oxygen (O_2) is vital for all animals, plants, and bacteria. However, its necessity conceals often the fact that it is a toxic and strongly oxidizing gas [1]. Interestingly, under physiological conditions, O_2 is fairly inert towards most organic and biological molecules, as it cannot combine immediately with them [1]. This lack of spontaneous reactivity originates from the particular O_2 electronic configuration. Indeed, in the ground state, O_2 is a bi-radical, as it possesses two unpaired electrons occupying separate orbitals, granting it a triplet multiplicity [1,2]. Since most organic and biological molecules are found with all their electrons paired, *i.e.*, in the singlet manifold, direct interaction with O_2 is thus forbidden due to the spin conservation rule [3]. This begs the question, why is O_2 toxic if it is inert? It is now understood that O_2 toxicity is linked to its metabolism, allowing for its reduction and generation of a variety of reactive oxygen species (ROS) [1]. In other words, these ROS are key in O_2 toxicity.

The first culprit in ROS intermediates is the superoxide anion ($O_2^{\bullet-}$), which originates from O_2 reduction by a single electron [1,4]. This species is primarily produced *in vivo* by the mitochondrial electron transport chain [4,5]. However, its anionic nature results in fairly low reactivity towards biomolecules. The occurrence of superoxide dismutases enzymes within mitochondria allows for its catalytic dismutation into hydrogen peroxide (H_2O_2) [1,4,5]. H_2O_2, a non-radical species, also achieved by two-electron reduction of O_2 by oxidases, is considered to be pernicious [1]. Its main toxicity is associated with its ability to generate the highly reactive hydroxyl radical ($^{\bullet}OH$) in presence of metal ions (Fe^{2+} or Cu^{+}) found in complex with different proteins (Fenton reaction) [1,4,5].

Since the seminal works of Gerschman et al. in 1954 and Harman in 1956, [6,7] there has been an ever-increasing interest in studying the role of ROS in biology. To date, the connection of ROS with many diseases has been proven beyond any reasonable doubt [1,5,8]. In fact, pathological conditions have been associated with over production of ROS, drop of antioxidant mechanisms, or a combination of both [1].

While the deleterious effects of ROS on living organisms have been identified for over half a century, the discovery of their beneficial involvement in cell signaling is much more contemporary. To date, a large body of work supports ROS serving essential functions in cell proliferation and differentiation as well as in the immune response system [5,9–14].

Therefore, current opinions point to a more complex role of ROS in biological systems, as a lack of ROS results in improper signaling functions, while their uncontrolled production lead to pathological conditions [5]. To date, a detailed understanding regarding the architecture of ROS-producing systems remains elusive and critical debates exist on the role of various cell components in the production of ROS, which is complicated by the diversity in ROS types, level, and activity when comparing different cells [5].

* Corresponding author. Tel.: +1 403 220 3887; fax: +1 403 289 9488.
E-mail address: bjmheyne@ucalgary.ca (B. Heyne).

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In order to achieve a more accurate picture of the biochemical role of ROS *in vivo*, numerous chemical tools have been engineered allowing for the selective detection and production of ROS in living organisms. The focus of this feature article is twofold: first to illustrate some fluorescent probes that achieved the selective detection of ROS, second, to characterize the sensitizers developed for site specific generation of ROS, in particular singlet oxygen ($^1O_2$). The present work is not meant to be an exhaustive review on both topics. In fact, our goal is rather to describe some of the various photochemical processes, which have been exploited for the design of numerous chemical tools. By reviewing theoretical concepts and offering concrete examples, we believe this feature article will enable an inexperienced reader in the field of photochemistry to understand the ideas behind the probes, and interest the expert photochemists by surveying recent work in the field of ROS detection and production.

2. Fluorescent probes for ROS detection

Because of their inherent reactivity and their transient nature, ROS are difficult species to detect, and various methodologies have been established over the years to tackle this problem. Owing to its high sensitivity, non-invasive character and ease of application, fluorescence has gained tremendous popularity in the field of ROS sensing. To date, a wide array of fluorescent probes have been engineered and numerous publications have already reviewed these probes, their advantages and pitfalls [15–21]. Based on some of our research along with others, two important photochemical concepts enabling the design of fluorescent probes will be described herein.

2.1. Photoinduced electron transfer (PeT)

PeT probes are fluorogenic in nature, which means that they switch from an initial dark or "*off*" state to a fluorescent or "*on*" state upon oxidation by ROS [21]. These type of probes are very popular as they afford high detection sensitivity in biological environments [15,18,21].

The concept of PeT is based on the fact that an excited state is always a better oxidizing and reducing agent compared to the ground state [22,23]. In his pioneer work, Weller established that PeT is a common reaction for organic excited states [24], and since then numerous scientists have taken advantage of this photochemical process to design various fluorescent probes, particularly for ion recognition [25–27].

Typically, a PeT probe is composed of three distinct moieties: a reporter, a receptor, and a spacer whose function is to covalently link the two first entities (Fig. 1) [26,27]. This construct allows for the reporter and the receptor to be close but still separated. The receptor is the moiety responsible for the interaction with the ROS of interest, whereas the reporter corresponding to a fluorophore is the site of both excitation and emission [21,26,27]. PeT probes are designed to ensure that deactivation of the reporter excited state by PeT outcompetes fluorescence, resulting thus in the probe being in its "*off*" state. Fig. 1 illustrates a PeT probe where the reporter excited state acts as an electron acceptor from the highest occupied molecular orbital (HOMO) of the receptor, generating then a pair of radicals [21,27]. The thermal back electron transfer (BeT) is the self-repair mechanism, guaranteeing the ground state restoration of both the reporter and the receptor [26,27]. An alternate scenario exists in which the reporter is the electron donor thus injecting an electron in the lowest unoccupied molecular orbital (LUMO) of the receptor. However, these type of PeT probes are less common than the one described in Fig. 1 [18,21].

Upon chemical reaction with ROS, the receptor is modified in such a way that PeT is not an option, and deactivation of the

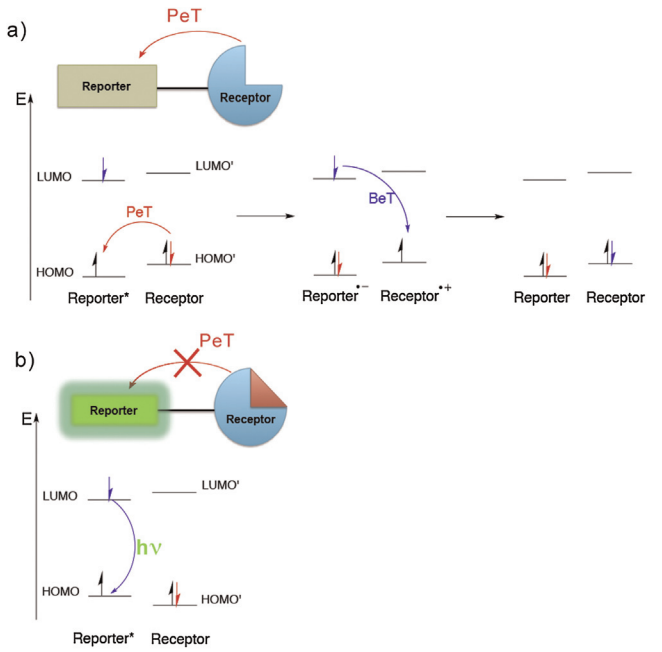

Fig. 1. Format of a PeT probes composed of a receptor, a reporter and a spacer (black line). Initially, the fluorescence of the reporter is quenched by PeT (a), followed by a back electron transfer (BeT) yielding both the receptor and reporter in the ground state. Upon chemical reaction of the receptor with a ROS (triangle) PeT is canceled (b).

reporter occurs predominantly by radiative decay (Fig. 1) [21,26,27]. Thermodynamic aspects are critical in designing PeT probes, and the redox potentials of the reporter and the receptor must be taken into consideration (Fig. 2) [21,27]. In fact, design can be achieved by the use of the Weller equation [28,29]. However, kinetics of the photochemical reactions also play a significant role, in order to ensure PeT is the fastest deactivation pathway. For this reason, short spacers are preferred [25–27].

Using the strategy of Fig. 1, we designed a new PeT probe, **NBFhd**, which took inspiration from previous work [30,31]. As shown in Fig. 2, the probe links a phenolic receptor to a 4-amino-7-nitrobenzofurazan (NBF) fluorophore reporter. NBF was chosen as it is a known fluorescent molecule, presenting little overlap with biological auto-fluorescence, and has been used exhaustively as a lipid and protein labeling agent [32–35]. In addition, the synthesis of **NBFhd** was straightforward, affording the probe in a single step with high yield [30].

When designing the probe, we assessed the possibility for electron transfer between phenol and the NBF fluorophore experimentally by a simple Stern–Volmer experiment, where quenching of NBF fluorescence was monitored as a function of phenol concentration. [30] Electron transfer between the two

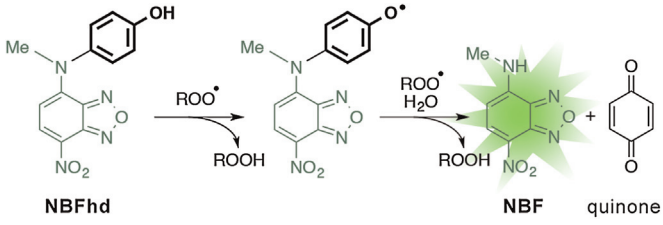

Fig. 2. Mechanism of reaction of the PeT probe **NBFhd** with peroxyl radical. The phenolic group (bold) is the receptor interacting with ROS, while the nitrobenzofurazan moiety (green) is the reporter, being the site of both excitation and emission. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)