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Selective C–H bond functionalization with light-driven P450 biocatalysts

Fonctionnalisation sélective de liaisons C–H par voie photochimique en utilisant des biocatalyseurs de cytochromes P450

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ABSTRACT

The unique photochemical properties of Ru(II)-diimine photosensitizers have enabled light-induced electron transfers in hybrid P450 heme domain enzymes. Rapid quenching of the excited state by soluble molecules generates either a highly oxidative or reductive species depending on the nature of the quencher. Under flash quench oxidative conditions, the heme cofactor of the P450 BM3 enzyme is oxidized to a high-valent ferryl species. Meanwhile, a photogenerated reductive species is able to deliver the necessary electrons to P450 heme active sites and sustain photocatalytic activity in the selective hydroxylation of substrate C–H bonds.

RÉSUMÉ

Les remarquables propriétés photochimiques des photosensibilisateurs à base de ruthénium(II) et de bipyridine ont rendu possible des transferts d'électrons induits par la lumière dans les domaines héminiques hybrides de l'enzyme P450 BM3. La désactivation rapide de leurs états excités par des composés solubles génère, soit un oxydant, soit un réducteur, selon la nature du composé. En conditions oxydantes, le cofacteur hème est oxydé en une espèce ferryle. Un composé très reducteur peut aussi être photogénéré ; il est alors en mesure de délivrer les électrons nécessaires au cytochrome P450 et de maintenir l'activité photocatalytique dans l'hydroxylation sélective des liaisons C–H de nombreux composés organiques.

1. Introduction

Sustainability challenges of today and the future have drawn considerable efforts toward the development of greener alternatives for synthetic chemical reactions. As we celebrate the year of light in 2015, the emphasis on sunlight to drive chemical reactions is receiving greater attention [1–5]. Already at the turn of the 20th century, Prof. Ciamician envisioned the use of sunlight energy to meet global needs with his pioneering work on photochemical organic chemistry [6]. In nature, plants have long mastered the ability to harness light and efficiently convert it into

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chemical energy through photosynthesis [7]. In the current context of light-driven alternatives, our research group has focused on hybrid P450 enzymes to harness the synthetic potential of P450 enzymes upon visible light excitation [8–13].

The large superfamily of cytochrome P450 heme-thiolate enzymes utilizes two reducing equivalents and molecular dioxygen to perform regio- and stereoselective functionalizations of unactivated C–H bonds in a wide range of substrates [14–16]. They have attracted a lot of interest from deciphering their mechanism to their importance in pharmaceutical and biotechnological applications [17]. The necessary electrons are typically supplied in rate-limiting steps by redox-partner proteins, reductases, from the NAD(P)H cofactor [18, 19]. Due to the limitations associated with the reductase and its cofactor [20], alternative approaches are being developed to power P450 enzymes.

With the hybrid P450 enzymes, we proposed that a Ru(II)-diimine photosensitizer covalently attached to P450 heme domain enzymes will be able to deliver the necessary electrons to the heme active site upon visible light irradiation and sustain photocatalytic activity [11]. This approach stemmed from the wealth of electron transfer studies in Ru(II) functionalized metalloproteins from the Gray group [21–23] and the recent examples of Ru(II) complexes used to power various metalloenzymes leading to the activation of small molecules [24–29]. The first Ru(II)-diimine functionalized heme domain was used to investigate intramolecular electron transfer and the formation of elusive reactive intermediates in the P450 catalytic cycle [8]. We showed that a photogenerated Ru(III) species is able to rapidly oxidize the ferric aquo heme center and generate a high-valent ferryl species, namely Compound II. We have since then used a photoreductive approach to inject electrons into the heme active site and perform P450 reactions upon visible light irradiation.

The first part of this report will introduce the hybrid P450 enzymes with some background on cytochromes P450 and Ru(II)-diimine complexes. We will then highlight the work to date on the optimization of their photocatalytic activity in the hydroxylation of long chain fatty acids and on the expansion of the light-driven approach to another member of the cytochrome P450 superfamily.

2. Cytochrome P450 enzymes

This superfamily of heme-thiolate enzymes utilizes molecular dioxygen and two electrons to perform the selective oxidation of unactivated C–H bonds in a variety of organic substrates. The consensus mechanism involves two successive one-electron transfer steps from the electron-providing reductase to the heme center with the formation of several well-characterized intermediates [30]. Activation of molecular dioxygen at the heme center generates a high-valent Fe(IV)-oxo porphyrin radical species, namely Compound I, capable of performing the desired C–H bond functionalization via a rebound mechanism [31, 32].

To date, the superfamily of P450 enzymes comprises more than 18,000 identified genes with a conserved tertiary fold of their heme domain despite low sequence homology [17]. The cytochrome P450s are often categorized in four major classes depending on their electron-providing reductases [18]. One unique class includes systems where the reductase is fused to the heme domain in a single polypeptide chain, of which cytochrome P450 BM3 or CYP102A1 from *Bacillus megaterium* is the most well known member [33]. P450 BM3 displays the highest catalytic hydroxylation rate ever measured for P450 enzymes. This high catalytic activity is attributed to the fused reductase, which leads to efficient coupling between the delivery of electrons and activation of oxygen at the heme center.

The dependence on the reductase domain and the use of NAD(P)H to initiate the ET cascade are still limitations in the development of P450 enzymes as biocatalysts [20]. To address these limitations, alternative approaches to deliver electrons have emerged, which include the use of artificial fusion P450 proteins to mimic the P450 BM3 system [34, 35], NAD(P)H regeneration systems [36], terminal oxidants [37], direct chemical [38] and electrochemical [39] reductions, as well as light-activated approaches [40–43].

3. Hybrid Ru(II)-diimine functionalized P450 enzymes

As an alternative approach to perform light-driven P450 reactions, we have developed hybrid P450 BM3 enzymes consisting of a Ru(II)-diimine photosensitizer covalently attached to P450 BM3 heme domain mutants (Fig. 1B,C) [8–13]. This approach takes the advantage of the unique photochemical properties of Ru(II)-diimine complexes, which have been extensively reviewed elsewhere [44–46]. Briefly, these complexes of general formula $[Ru(LL)_{3-n}(LL')_n]^{2+}$, with LL,LL′ = diimine ligands such as bipyridine or phenanthroline and their derivatives, possess valuable photochemical properties amenable to light-induced electron transfers. They often display a broad absorption band in the visible range centered around 450 nm attributed to a metal-to-ligand charge transfer transition (MLCT). Excitation into this band followed by rapid intersystem crossing due to the heavy atom effect leads to a long-lived triplet excited state with unusual redox properties [46]. This $[Ru]^{2+*}$ excited state can decay back to the ground state via a radiative process or encounter other solute molecules called quenchers. Participation in these bimolecular processes results in energy transfer and either reductive or oxidative electron transfer. Depending on the nature of the quencher, a highly reductive, $[Ru(LL)_{3-n}(LL')_n]^{+}$ abbreviated $[Ru]^{+}$ ($E_{1/2} = -1.28$ V vs NHE), or oxidative, $[Ru(LL)_{3-n}(LL')_n]^{3+}$ abbreviated $[Ru]^{3+}$ ($E_{1/2} = +1.26$ V vs NHE), species can hence be photogenerated (Fig. 1).

Covalent attachment of the photosensitizer was established early on to be crucial for electronic communication with the buried heme center in P450 enzymes [8] and for photocatalytic activity of the hybrid enzymes [9]. Introduction of an iodoacetamide-1,10-phentanthroline (PheniA) [47] or more recently of the 5,6-epoxy-5,6-dihydro-1,10-phenanthroline [12] ligands into the $Ru(bpy)_2(LL)$ framework leads to the selective covalent attachment of the photosensitizers to non-native single cysteine mutants of the P450 BM3 heme domain.