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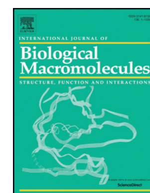
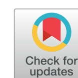


Recombinant lignin peroxidase-catalyzed decolorization of melanin using *in-situ* generated H_2O_2 for application in whitening cosmetics



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Lignin peroxidase has high potential as ingredient in skin whitening cosmetics due to its high redox potential to oxidize recalcitrant melanin. Currently crude mixtures of lignin peroxidase from fungal fermentation are usually applied to cosmetics due to the intrinsic difficulties of expression and purification. However, the present study focused on heterologous expression and purification of lignin peroxidase isozyme H8 (LiPH8) from *Phanerochaete chrysosporium* and was further used for melanin decolorization. Results revealed that the optimum pH for melanin decolorization using LiPH8 was obtained at pH 4.0. The intermittent feeding of hydrogen peroxide (H_2O_2) was effectively elevating melanin decolorization efficiency up to 73%, since excessive H_2O_2 inactivated LiPH8. For cosmetic application, intermittent feeding of H_2O_2 is not feasible, thus glucose oxidase (GOx) from *Aspergillus niger* was employed for *in-situ* generation of H_2O_2. By optimizing the GOx and glucose concentrations, a melanin decolorization efficiency up to 63.3 ± 2.4% was obtained within 1 h and continued to 84.0 ± 1.8% in 8 h. Conclusively, lignin peroxidase-catalyzed decolorization of melanin with *in-situ* generated H_2O_2 revealed a promising approach for whitening cosmetics applications.

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1. Introduction

The skin color is mainly determined by the presence of a pigmented polymer called melanin and their production is regulated by multiple genes in the specialized cells of skin epidermis called melanocytes [1]. Melanin also plays an important role in protecting the skin from ultraviolet radiations by diverting it away from the healthy cells and therefore it determines the skin color as it present in different forms and ratios in the skin epidermis [2]. There are two forms of melanin: eumelanin and pheomelanin. Eumelanin shows a black or brown color; however, pheomelanin exhibits a red or yellow color [3].

Recently, as demand for skin whitening has increased worldwide, various studies on melanin decolorization are under way. Usually the whitening agents are derived from natural sources as the active ingredients in cosmetic products which prevent melanin production in the skin epidermis through tyrosinase inhibition, inhibition of pigment synthesis, or other alternative mechanisms [4]. Nevertheless, the inhibitors of tyrosinase are most actively studied to reduce melanin production in cells [5,6]. For instance, rhododenol is a tyrosinase inhibitor which interfere with conversion of L-DOPA (L-3,4-dihydroxyphenylalanine) to DOPAquinone that results in the obstruction of melanin biosynthesis. Other examples include kojic acid [7], azelaic acid [8], aloesin [9], arbutin [9], *etc.* are also often used as skin whitening agents. However, other alternative mechanisms to inhibit the melanogenesis include use of antioxidants to prevent oxidative damage to the skin, inhibition of tyrosinase related proteins (such as TRP-1 and TRP-2), inhibition of melanosomal transfer, *etc.* Like, butin from Chinese herb *Spatholobus suberectus* is a most effective compound that is frequently used in whitening cosmetics for inhibition of tyrosinase related proteins TRP-1 and TRP-2 [10]. These inhibitors of melanogenesis have revealed many potential side effects on skin and may cause disease like vitiligo [11]. Correspondingly, Tain et al. (2009) evaluated the whitening efficacy of cosmetic products containing arbutin on a human skin pigmentation spot models and revealed that the 3% arbutin has a certain level of whitening effect but further increase in arbutin concentrations can cause cytotoxicity [12]. Thus, percentage composition of such cytotoxic whitening agents in the cosmetic products is also a major concern. Therefore, the use of these inhibitors as active ingredient in skin whitener cosmetics should not be a favorable approach. For this reason, studies are underway to decolorize melanin using enzymes instead of chemicals since enzymes holds great promise to the selective melanin decolorization with less toxicity. Some oxidases and peroxidases have been previously tested for melanin decolorization such as laccase, dye-decolorizing peroxidase, manganese peroxidase and lignin peroxidase [13–17]. Out of them, lignin peroxidase is a promising catalyst for efficient decolorization of melanin since it shows a high redox potential to oxidize veratryl alcohol. Thus, improved oxidation of melanin has been noticed with lignin peroxidase rather than other peroxidases as they exhibited less sufficient redox potential to oxidize melanin [18].

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However, despite its high potential as a melanin decolorizing enzyme, lignin peroxidase has only been studied as an unpurified crude enzyme due to the difficulties in expression and purification since up to 17 different isozymes are biosynthesized from white-rot fungi *Phanerochaete chrysosporium* [19]. In addition, lignin peroxidase can be easily inactivated by excessive amounts of hydrogen peroxide (H_2O_2), which is crucially necessary to oxidize melanin [20]. Although H_2O_2 used as the fuel in the process of melanin decolorization, nevertheless it inactivates the enzyme and it is a major obstacle to increase the efficiency of melanin decolorization.

In present study, recombinant lignin peroxidase isozyme H8 (LiPH8) was expressed, purified and used for decolorization of melanin, rather than using crude enzyme mixtures from white-rot fungi as previously used for decolorization of melanin by Woo et al. [17]. In addition, *in-situ* generation and supply of H_2O_2 by glucose oxidase (GOx) was combined with LiPH8-catalyzed melanin decolorization to suppress the inactivation of LiPH8 by excessive H_2O_2. Since GOx can generate and feed low concentration of H_2O_2 continuously by utilizing glucose and air-borne molecular oxygen, maximized melanin decolorization was anticipated. Therefore, this method can provide a useful basis for application in whitening cosmetics.

2. Materials and methods

2.1. Materials

Trizma hydrochloride (T5941), trizma base (T1503), sodium hydroxide (NaOH; S5881), urea (U5378), L-glutathione oxidized (G4376), hemin (51280), calcium chloride ($CaCl_2$; C1016), potassium chloride (KCl; P9333), boric acid (H_3BO_3; B6768), synthetic melanin (M8631), β-D-glucose (G8270) and glucose oxidase from *Aspergillus niger* (GOx; G6766) were purchased from Sigma-Aldrich. Hydrogen peroxide (4104-44) was procured from Daejung Chemicals & Metals, South Korea. Acetic acid (CH_3COOH; 31010-0330) was obtained from Junsei, Japan. Guanidine chloride (G0162), sodium acetate (CH_3COONa; S0559), phosphoric acid (H_3PO_4; P1745) and veratryl alcohol (VA or 3,4-dimethoxybenzyl alcohol; V0020) were purchased from Tokyo Chemical Industry, Japan. *Escherichia coli* BL21(DE3) (Real Biotech Corporation, Taiwan) and pET-21b( ) expression vector (Novogene, USA) were used for protein overexpression. Isopropyl β-D-1-thiogalactopyranoside (IPTG; I1401) for induction of gene expression was purchased from Duchefa Biochemie, Netherlands. BugBuster® Master Mix (71456-4) was used for cell lysis which was obtained from EMD Millipore, USA. HiTrap Q HP anion exchange column was used for protein purification which was procured from GE Healthcare Bio-Sciences, USA.

2.2. Production and purification of recombinant LiPH8

The synthetic gene encoding LiPH8 from *Phanerochaete chrysosporium* was prepared by the Bioneer Company (South Korea). Gene sequencing (UniPortKB entry: P06181), expression, refolding and purification method were adapted from a previously published report [21]. Briefly, the synthesized LiPH8 gene sequence was cloned in pet21b( ) vector which was transformed *E. coli* BL21(DE3) host system for heterologous expression of LiPH8 [22]. The induced cultured cells (1 mM IPTG) were harvested for lysis using BugBuster® Master Mix. The lysed cells were sonicated (at 1 s pulse on and 2 s pulse off, 20% amplitude, 2 mins) and then centrifuged (at 11000 rpm, 4 °C, 10 min) for the three time to get the purified inclusion bodies. Further, the inclusion bodies were solubilized in 0.1 M Tris-buffer (pH 8.5), 8 M urea and 6 M guanidine hydrochloride. The refolding of LiPH8 was carried out in 0.1 M Tris-buffer (pH 8.5) containing 1.8 mM $CaCl_2$, 0.71 mM L-glutathione oxidized and 25 μM hemin. Finally, the refolded proteins were purified using HiTrap Q HP column through ÄKTA FPLC chromatography system (GE Healthcare Life Sciences, USA). The purified LiPH8 was dialyzed in 10 mM sodium acetate buffer (pH 6) for enzyme storage.

2.3. Specific activity of LiPH8 for VA

Initially the enzyme activity was determined using 0.1 M Britton-Robinson buffer (BR buffer), pH 3.0 (1.043 g L^{-1} NaOH, 2.089 g L^{-1} CH_3COOH, 3.409 g L^{-1} H_3PO_4, 2.151 g L^{-1} H_3BO_3 and 5.42 g L^{-1} KCl [23]), 0.02 μM LiPH8, 2 mM VA and 250 μM H_2O_2 which was analyzed at 310 nm using extinction coefficient of produced veratraldehyde ($\varepsilon_{310\ nm} = 9.3\ mM^{-1}\ cm^{-1}$) within 1 min on spectrophotometer (UV-1650PC, Shimadzu, Japan) [24]. For pH-dependent specific activity study, the 0.1 M BR buffer ranging pH 3.0–6.0 (for achieving particular pH, the varying amount of 0.2 M NaOH was added and the rest chemical composition of BR buffer remained same as explained above) were used with aforementioned concentration of LiPH8, VA, and H_2O_2. Specific activity with varying H_2O_2 concentrations ranging from 0 μM to 550 μM was further analyzed. One unit of LiPH8 is defined as the amount of enzyme that can oxidize 1 μmol of VA per minute.

2.4. LiPH8-catalyzed melanin decolorization

pH-dependent melanin decolorizing reaction was conducted using 0.06 U mL^{-1} LiPH8, 2 mM VA, 250 μM H_2O_2, and 50 mg L^{-1} synthetic melanin in BR buffer ranging pH 3.0–6.0 under 100 rpm stirring at room temperature for 1 h. Furthermore, the effect of ionic strength of BR buffer ranging 0.0 M – 1.0 M on melanin decolorization was analyzed by varying the amount of KCl in the buffer. The reaction was initiated by adding H_2O_2 into reaction mixture with final volume of 2 mL. The enzyme unit was decided based on initially measured specific activity for VA at pH 3.0.

The decolorization efficiency of melanin was measured by spectrophotometer at 540 nm after 1 h using Eq. (1). The control experiment was conducted under the same conditions as the melanin decolorizing reaction without the addition of H_2O_2. All the experiments were reported as mean ± S.D. and performed in duplicates.

$$\text{Decolorization efficiency } (\%) = \frac{A_{540\ control} - A_{540\ after\ 1\ h\ reaction}}{A_{540\ control}} \times 100 \quad (1)$$

The inhibition study of LiPH8 caused by excessive H_2O_2 concentrations was carried out using fixed concentration of 0.02 μM LiPH8, 2 mM VA and 50 mg L^{-1} synthetic melanin in pH 4.0 BR buffer with varying H_2O_2 concentrations ranging 0–550 μM under 100 rpm stirring at room temperature for 1 h.

2.5. LiPH8-catalyzed melanin decolorization with intermittent addition of H_2O_2

To confirm that instantaneously added excessive H_2O_2 concentration is the main inhibition factor for LiPH8, a melanin decolorizing reaction was conducted with pH 4.0 BR buffer as mentioned in the previously section but H_2O_2 was added intermittently up to 1400 μM in 12 min, 15 min, 20 min, 30 min and 60 min intervals within an hour of experiment.

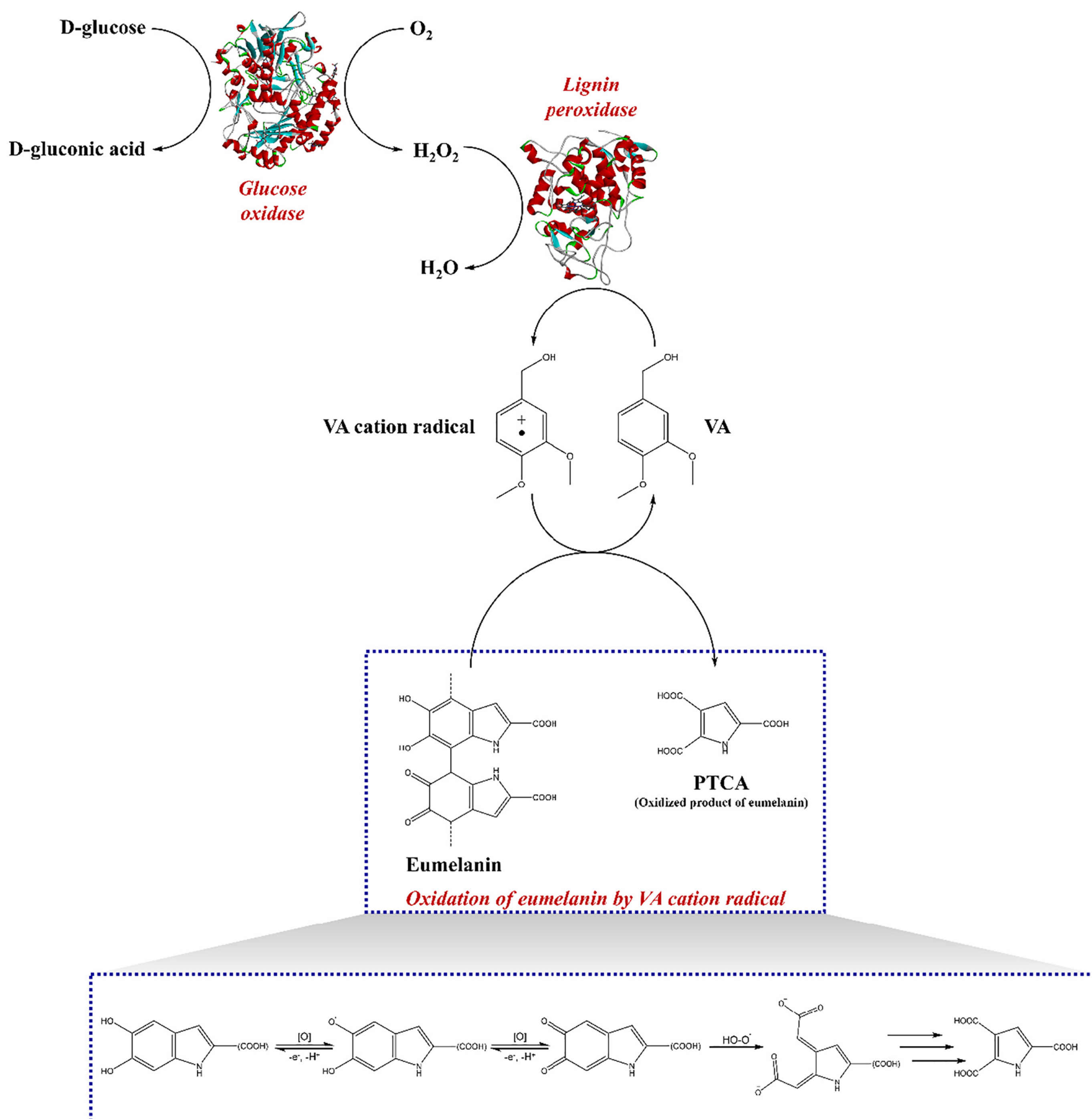
2.6. LiPH8-catalyzed melanin decolorization utilizing in-situ generated H_2O_2 by GOx

Instead of adding H_2O_2 directly, *in-situ* generated H_2O_2 by glucose oxidase was utilized for melanin decolorization catalyzed by LiPH8. Melanin decolorizing reactions were performed for 1 h with aforementioned condition with pH 4.0 BR buffer, 300 mM β-D-glucose, and glucose oxidase ranging 0–0.24 U mL^{-1} instead of adding H_2O_2. The reaction was initiated by adding GOx in final volume of 2 mL reaction mixture. The GOx unit was decided based on specific activity for H_2O_2

production which is labeled on the product. One unit of glucose oxidase is defined as the amount of enzyme that can produce 1 μmol of H_2O_2 per minute. For studying the effect of glucose concentration on melanin decolorization, the reaction was conducted with varying β-D-glucose concentration ranging 0–300 mM under 100 rpm stirring at room temperature for 1 h. The control experiment was carried out under the same conditions as the melanin decolorizing reaction without the addition of glucose oxidase. However, the other control experiments were also performed to analyze the effect of individual component of melanin decolorization reaction such as glucose, LiPH8, VA and H_2O_2 (Table S1). All the experiments were reported as mean ± S.D. and performed in duplicates.

2.7. Time profile of LiPH8-catalyzed melanin decolorization using in-situ generated H_2O_2

For the time profile of melanin decolorization efficiency, the reaction was performed with the condition as stated above in pH 4.0 BR buffer, 0.24 U mL^{-1} glucose oxidase, and 300 mM β-D-glucose under 100 rpm stirring at room temperature for 24 h. The reaction was initiated by



Scheme 1. Decolorization scheme of melanin through co-catalyzed reaction of glucose oxidase and lignin peroxidase. The produced redox mediator VA cation radical from the lignin peroxidase catalyzed VA causes native eumelanin decolorization and change to its possible oxidized form *i.e.* PTCA (pyrrole-2,3,5-tricarboxylic acid) through series of oxidation reactions.

adding optimized concentration of GOx. The reaction solution was collected every 10 min and the decolorization efficiency was determined immediately using spectrophotometer at 540 nm.

3. Results and discussion

In order to meet the increased demand for skin whitening agents in the cosmetic formulations, the cosmetic industries have started incorporating the chemical additives namely hydroquinone [25,26], cysteine [27], glutathione [28], topical retinoids [29], topical corticosteroids [30], tyrosinase inhibitors [31] and other agents [32,33]. These active chemical ingredients are either natural or synthetic substances which interfere the biosynthetic pathways of melanogenesis in epidermal melanocytes [4]. However, most of the active ingredients have proven to be cytotoxic and thus, they are questionable for their use in the cosmetics [11]. Therefore, the present research was an effort to introduce enzyme as the active ingredients which is green alternative replacing harsh chemical-based whitening agents in the cosmetics. Also, the enzyme-based formulations have the advantages of high specificity and selectivity with no toxic effects [34]. Thus, the present study aims to exploit the recombinant lignin peroxidase for decolorization of melanin which may act as the active enzyme-based whitening cosmetic agent.

Accordingly, the work was initiated with determination of pH, ionic strength and H_2O_2 effect on melanin decolorization using lignin peroxidase. The determination of H_2O_2 effect is important since their excessive concentration can lead to lignin peroxidase inactivation [20]. Therefore, continuously supplied low concentration of H_2O_2 has been required to achieve the maximum decolorization efficiency of melanin. For that reason, glucose oxidase was used for *in-situ* generation of H_2O_2 in the co-catalyzed system of lignin peroxidase.

3.1. Decolorization efficiency of melanin on varying pH and ionic strength

Lignin peroxidase oxidizes VA to the VA cation radical (VA•), which acts as a redox mediator to decolorize melanin (Scheme 1). VA also has the advantage that it is a natural compound produced from white-rot fungi which implies less side effects than synthetic mediators [35]. In this experiment, the effect of pH 3.0–6.0 on the LiPH8-catalyzed decolorization of melanin for 1 h was investigated. Similar analysis of pH effect on melanin decolorization using crude laccases of *Lentinus polychrous* was carried out by Khammuang and Sarnthima [13], they revealed that the melanin decolorization was significantly efficient at pH 4.0–6.5 since the laccases are stable and active in this pH range [13]. However, LiPH8 had the highest specific activity for VA under more acidic conditions that is pH 3.0 whereas, the melanin precipitation was observed in the pH 3.0 buffer which resulted in no decolorization of melanin as shown in Fig. 1. Similarly, the melanin precipitation was also observed at ionic strength 0.5 M and 1.0 M while the optimum melanin decolorization has been noticed in lower ionic strengths ranging 0.0 to 0.1 M (Table S2). The formation of melanin precipitate was accelerated with the addition of enzyme which led to complexation between solid melanin and enzyme was occurred. For the proper decolorization of melanin, direct contact between melanin and a redox mediator is crucial. Otherwise there will be a lower probability of decolorizing melanin by VA cation radicals since the VA cation radical is known to have a very short life-time [36,37]. The declined melanin decolorization efficiency was observed at pH 5.0 and 6.0 whereas it was increased to 30% at pH 4.0 within 1 h. since the specific activity of LiPH8 was significantly reduced at pH 5.0 and 6.0 compared to the specific activity at pH 4.0. Fig. 1 clearly suggests that the optimum pH for maintaining higher specific activity of LiPH8 is a prerequisite for efficient melanin decolorization. Therefore, in the subsequent experiments, all the melanin decolorizing reactions were performed in pH 4.0 buffer.

3.2. Inhibition effect of excess H_2O_2 concentration on melanin decolorization

Even though H_2O_2 is an essential substrate as a final electron acceptor during the melanin decolorization, lignin peroxidase is easily inactivated in their excess concentration. Excessive H_2O_2 causes rupturing of heme structure or formation of inactive compound III in lignin peroxidase [20]. It is reported that this inhibitory effect can be subdued by oxidation of VA to VA cation radical [38] which is crucially used for oxidization of melanin in the present study (Scheme 1).

The results showed that melanin decolorization efficiency was approached to the highest level of 31.9 ± 0.9% at 250 μM H_2O_2, it further decreased gradually with increase in H_2O_2 concentration (Fig. 2). However, the initial specific activity for VA steadily increased with higher H_2O_2 concentrations, which clearly indicates that H_2O_2 is a crucial substrate in the short-term specific activity (within 1 min) and is an inhibitor of decolorization of melanin in the long term (within 1 h). For

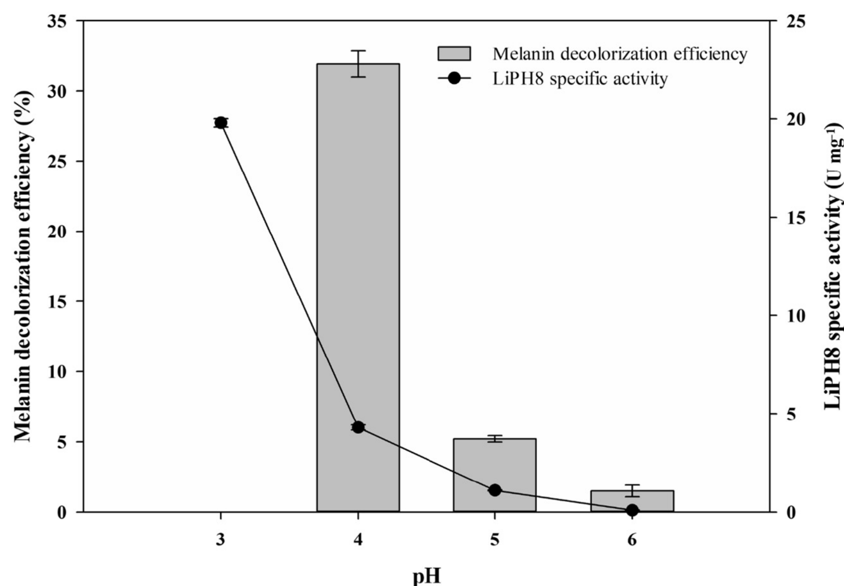


Fig. 1. Estimation of pH-dependent melanin decolorization efficiency and specific activity of LiPH8 using veratryl alcohol. Decolorization of melanin was carried out with 50 mg L^{-1} synthetic melanin, 2 mM veratryl alcohol, 250 μM H_2O_2, and 0.06 U mL^{-1} LiPH8 in BR buffer for 1 h. Specific activity was measured using 2 mM veratryl alcohol, 250 μM H_2O_2 in BR buffer.

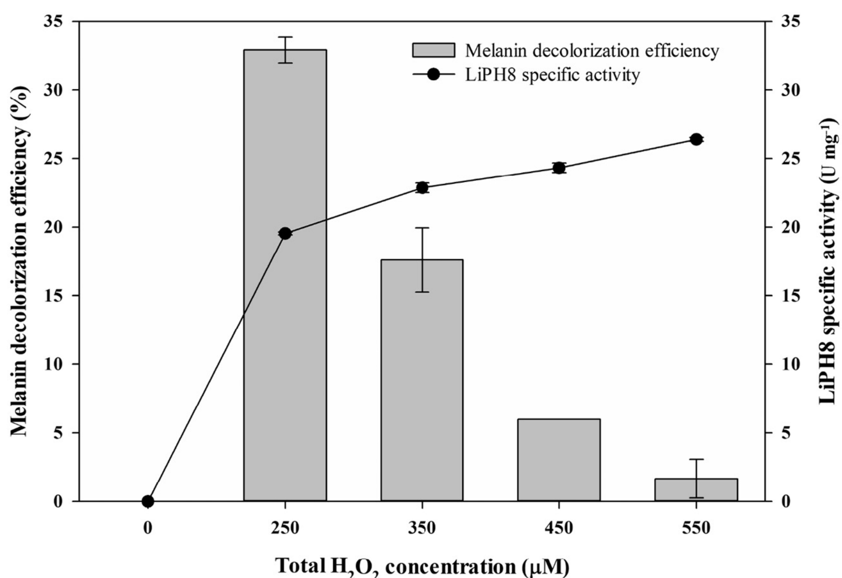


Fig. 2. The effect of H_2O_2 concentration on melanin decolorization efficiency and specific activity of LiPH8 for veratryl alcohol. Decolorization of melanin was carried out with 50 mg L^{-1} synthetic melanin, 2 mM veratryl alcohol, and 0.06 U mL^{-1} LiPH8 in BR buffer, pH 4.0 for 1 h. Specific activity was measured with 2 mM veratryl alcohol in BR buffer, pH 3.0.

higher melanin decolorization efficiency, higher concentration of H_2O_2 will be required even though it can act as an inhibitor of the enzyme simultaneously. Thus, the present study attempted the continuous supply of a low concentration of H_2O_2 which improves the overall melanin decolorization efficiency.

3.3. Effect of intermittently added H_2O_2 on LiPH8-catalyzed melanin decolorization

To confirm the hypothesis that maintaining a low level of H_2O_2 by their continuous feeding will improve the melanin decolorization efficiency, the intermittent addition of H_2O_2 was performed. Based on the results shown in Fig. 2 and Fig. 3, a critically excessive concentration of H_2O_2 capable of inactivating LiPH8 was assumed to be in between 300 μM and 350 μM. However, in the case of a H_2O_2 concentration of 1400 μM with a time interval of 12 min, an inhibitory effect was observed even though the instantaneous H_2O_2 concentration was only 280 μM (Fig. 3). It was thought that the added LiPH8 in this experiment may not have been sufficient to consume 280 μM of H_2O_2 within 12 min. Nevertheless, since melanin decolorization efficiency seemed saturated around 1000 μM H_2O_2 and it approached to 73%, adding more enzyme was not effective even it could consume 280 μM of H_2O_2 per 12 min. Except for this case, an instantaneous concentration of H_2O_2 lower than 300 μM did not result in any inhibition effect on melanin decolorization. This result strongly supported the hypothesis that intermittent feeding of H_2O_2 over certain time intervals is very effective for improved melanin decolorization by repressing inactivation of LiPH8 caused by excessive H_2O_2. However, for whitening cosmetic applications, it is almost

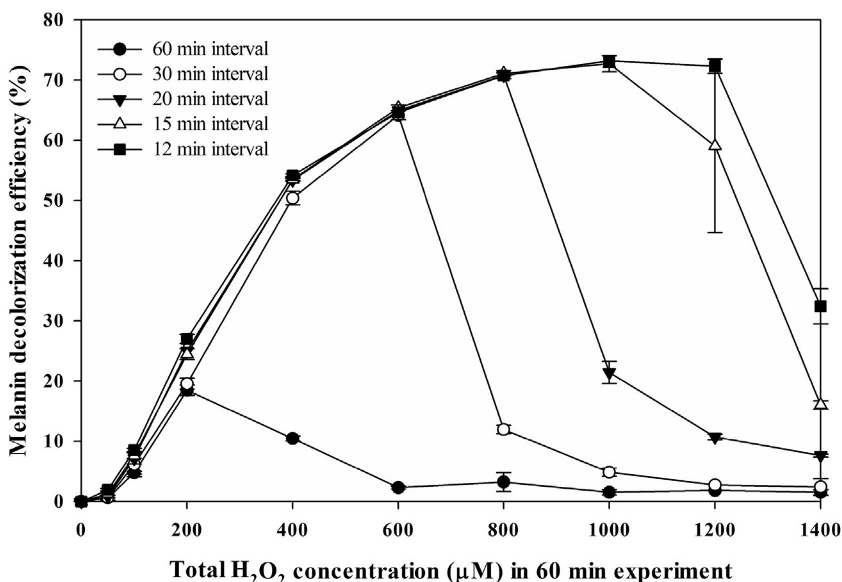


Fig. 3. Melanin decolorizing reaction by adding H_2O_2 intermittently. Decolorization of melanin was carried out with 50 mg L^{-1} synthetic melanin, 2 mM veratryl alcohol, and 0.06 U mL^{-1} LiPH8 in BR buffer, pH 4.0. The total H_2O_2 was equally divided at time intervals of 60, 30, 20, 15, and 12 min within 1 h.

Table 1
Melanin decolorization with varying glucose oxidase units.

Glucose oxidase unit (U mL^{-1})	Melanin decolorization efficiency (%)
0	0
0.03	31.94 ± 0.42
0.06	49.70 ± 0.63
0.12	57.01 ± 2.96
0.24	63.43 ± 1.06

Reaction condition: 50 mg L^{-1} synthetic melanin, 2 mM VA, 0.06 U mL^{-1} LiPH8 and 300 mM β-D-glucose in BR buffer, pH 4.0 with glucose oxidase ranging from 0 to 0.24 U mL^{-1}.

Table 2
Melanin decolorization with varying glucose concentrations.

Glucose concentration (mM)	Melanin decolorization efficiency (%)
0	0
0.1	−2.35 ± 0.44
0.5	−0.78 ± 0.44
1	−0.78 ± 0.44
5	21.35 ± 1.11
10	47.10 ± 0.67
20	57.30 ± 0.44
100	62.48 ± 0.22
300	62.17 ± 4.22

Reaction condition: 50 mg L^{-1} synthetic melanin, 2 mM VA, 0.06 U mL^{-1} LiPH8 and 0.24 U mL^{-1} glucose oxidase in BR buffer, pH 4.0 with varying β-D-glucose concentration ranging from 0 to 300 mM.

impossible to supply H_2O_2 intermittently over time intervals, which implies that there is the need to generate low concentrations of H_2O_2 continuously *in-situ* basis for LiPH8.

3.4. Melanin decolorization using LiPH8 co-catalyzed with GOx for in-situ generation of H_2O_2

For the *in-situ* generation of H_2O_2, glucose oxidase (GOx) from *Aspergillus niger* was employed, since it catalyzes the oxidation of β-D-glucose using molecular O_2 to produce D-gluconic acid and H_2O_2 [39]. This produced H_2O_2 was utilized by lignin peroxidase to decolorize the melanin as an electron acceptor (Scheme 1). GOx also retained its activity from pH 4.0 to pH 7.0 which means it is applicable to decolorization of melanin at pH 4.0 [40]. The optimal amount of GOx required was determined by melanin decolorization efficiency with varying GOx units as shown in Table 1. As the unit of GOx increased, the decolorization efficiency proportionally increased. However, without the addition of GOx, LiPH8 did not show any activity for melanin decolorization (Table 1). This result clearly demonstrated that GOx was supplying indispensable H_2O_2 for LiPH8-catalyzed melanin decolorization.

Melanin decolorization was further carried out with varying initial glucose concentrations since the generated H_2O_2 amount is dependent on the glucose concentration used by fixed GOx concentration. Melanin decolorization efficiency increased as the concentration of glucose increased and seemed saturated at approximately 100 mM of glucose (Table 2). However, at a very low concentration of glucose less than 1 mM, melanin decolorization efficiency showed a slightly negative value, which implies that the color of melanin was slightly intensified due to insufficient oxidation of melanin [41,42]. The results also revealed that the optimal GOx and glucose concentration was obtained at 0.24 U mL^{-1} and 300 mM, respectively (Tables 1 and 2), since sufficient levels of GOx and glucose did not yield any side effects on melanin decolorization.

Melanin decolorization efficiency was gradually increased and it led to lighting of the color of melanin solution with time (Fig. 4). A total of 43.5 ± 1.2% melanin decolorization efficiency was achieved within 20 min and the overall efficiency approached up to 63.3 ± 2.4% in 1 h as shown in the inset of Fig. 4. Although the decolorization rate was decelerated after 20 min due to less available melanin concentration, enzymes appeared to harbor sufficient activity to achieve 84.0 ± 1.8% after 8 h and remained constant maximally to 86.9 ± 1.8% till 24 h. This strongly implies that the *in-situ* generation of H_2O_2 by GOx is very effective in maintain the activity of LiPH8 during overnight application. However, considering the cytotoxicity and genotoxicity issues of LiPH8 and other substrates (VA and GOx) is the future aspects of the present work. Thus, the human skin pigmentation model can be applied to study the toxicity issues of these proposed ingredients.

4. Conclusion

The present research was an effort to prove that recombinant LiPH8 expressed in *E. coli* can decolorize melanin very efficiently and

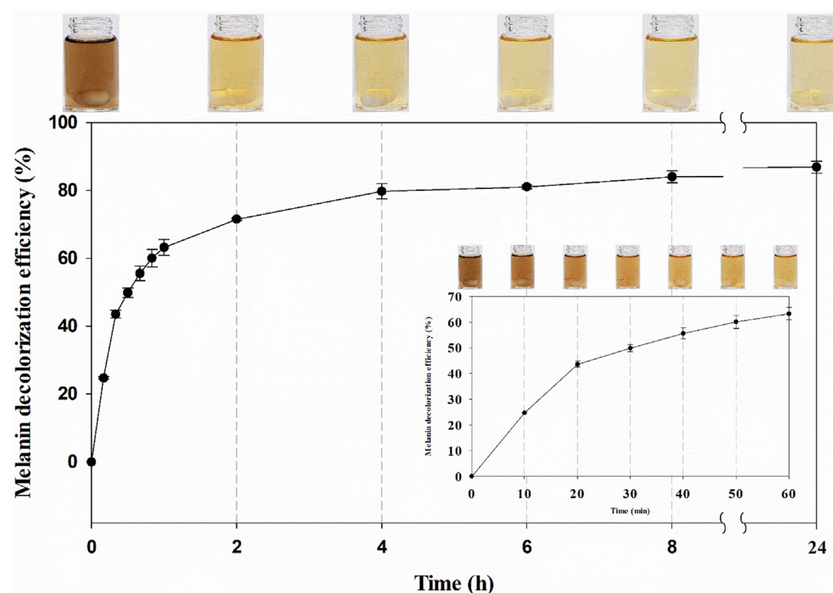


Fig. 4. Time profile of melanin decolorization from 0 h to 24 h by LiPH8 and GOx co-catalyzed reaction. The inset shows the time profile of melanin decolorization within 1 h. Decolorization of melanin was carried out with 50 mg L^{-1} synthetic melanin, 2 mM veratryl alcohol, 0.06 U mL^{-1} LiPH8, 300 mM β-D-glucose, and 0.24 U mL^{-1} glucose oxidase in BR buffer, pH 4.0. The pictures on the graph and its inset are the melanin solutions after decolorization.

effectively. Co-catalysis of LiPH8 and GOx was performed to achieve the improved decolorization efficiency of melanin. Utilizing GOx enabled us to overcome the inhibitory effect caused by excessive H_2O_2 concentrations by continuously maintaining a low level of H_2O_2. Within the short time of 1 h, melanin decolorization efficiency up to 63.3 ± 2.4% was achieved and later to 84.0 ± 1.8% in 8 h which proved to be a promising approach in formulation of whitening cosmetics as additive of lignin peroxidase and glucose oxidase.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijbiomac.2019.06.026.

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