Pheomelanin

Subjects: Biochemistry & Molecular Biology

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Pheomelanin is a natural yellow-reddish sulfur-containing pigment derived from tyrosinase-catalyzed oxidation of tyrosine in presence of cysteine. It is one of the existing forms of the natural pigment melanin, which is present in the skin in two forms: eumelanin and pheomelanin. Generally, the formation of melanin pigments is a protective response against the damaging effects of UV radiation in skin.

pheomelanin nitrotyrosine dityrosine photooxidation

1. Introduction

Pheomelanin is one of the existing forms of the natural pigment melanin. Melanin is present in the skin in two forms: eumelanin and pheomelanin. Eumelanin is a heterogeneous polymer composed mainly of dihydroxyindole units derived from tyrosinase-catalyzed oxidation of tyrosine or 3,4-dihydroxyphenylalanine (DOPA) to dopaquinone. Compared to eumelanin, pheomelanin structure differs due to non-enzymatic addition of cysteine to dopaquinone during the pathway of pigment biosynthesis. DOPA-derivatives with cysteine, such as 5′-S-cysteinyldopa and in minor amount 2′-S-cystenyldopa, are incorporated into the pigment in the form of 1,4-benzothiazine units (**Figure 1**) [1]. Before being incorporated into pheomelanin, a minor part of 1,4-benzothiazine units may undergo further structural modifications with formation of benzothiazole moiety which copolymerizes with benzothiazine units [2][3][4]. Interestingly, slight variations in the monomer composition of pigment polymer skeleton have been shown to determine significant differences in light absorption, antioxidant activity, redox behavior, and metal chelation [5].

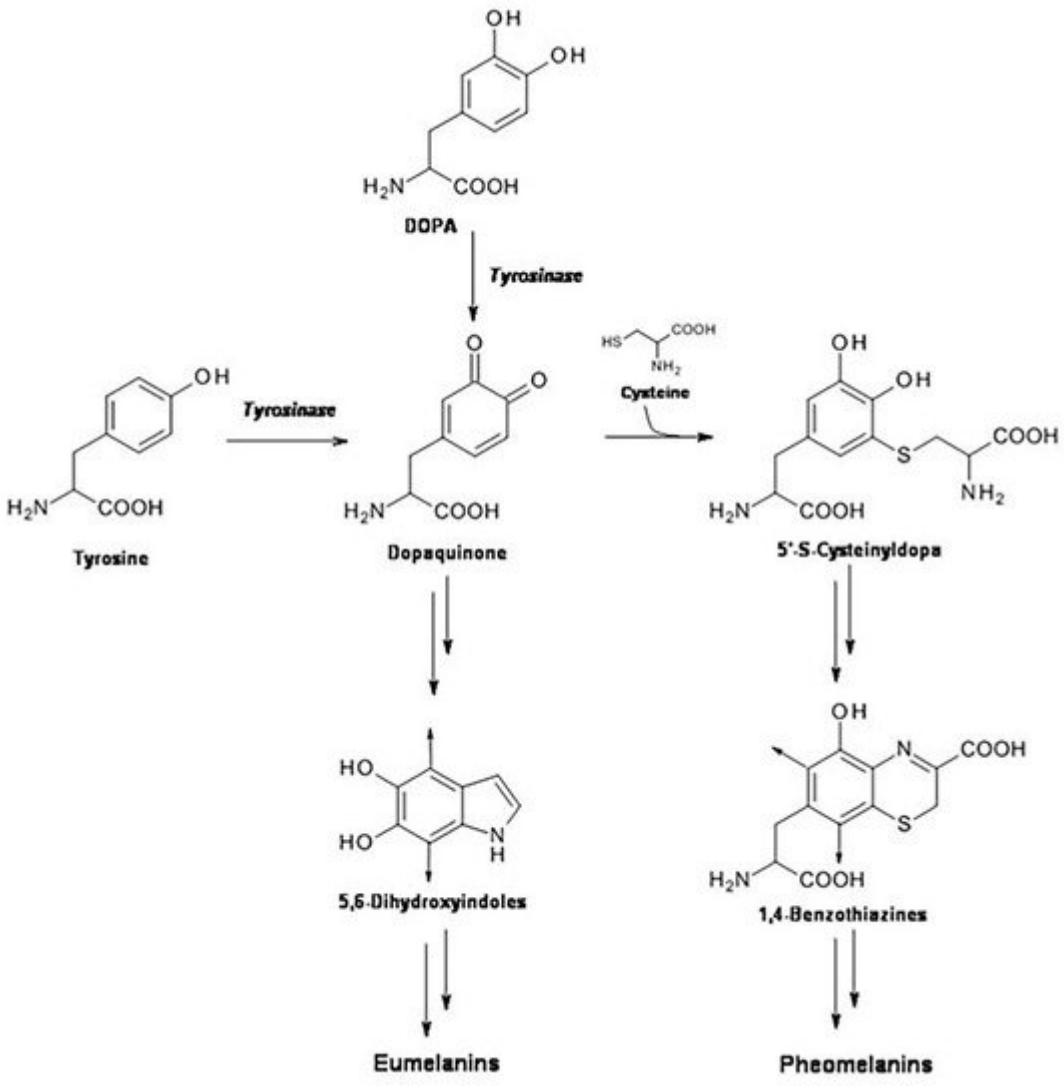

Figure 1. Schematic pathway of eumelanin and pheomelanin biosynthesis.

It is commonly believed that melanin plays an important role in the modulation of the photochemical reactions that occur in the skin. Numerous experimental and clinical evidences have shown a protective role of eumelanin on the damage triggered by UV irradiation on the skin [6]. Notably, pheomelanin has the capacity to act as photosensitizer by inducing the generation of reactive oxygen species (ROS) upon irradiation with UV light [7][8][9][10][11][12]. Pheomelanin has been observed to increase lipid peroxidation following exposure of liposomes to UV irradiation, suggesting that pheomelanin may act as a pro-oxidant [13]. Analogously to other photosensitizing substances, pheomelanin is able to trigger, following exposure to UV radiation, photochemical reactions capable of modifying and damaging cellular components [14][15].

In particular, by exposure to UV radiation, aromatic rings present in the pheomelanin (Pheo) are excited to the singlet state (^{1}Pheo*) and rapidly converted to the excited triplet state (^{3}Pheo*) [16][17].

The triplet state of pheomelanin can act as photosensitizer triggering photooxidative events by radical-mediated (type I) and singlet oxygen-mediated (type II) mechanisms. The type I mechanism involves free radical formation

through the hydrogen atom or electron transfer by interaction of the triplet excited state of the sensitizer with target molecules (S) or molecular oxygen.

$$^3Pheo^* + S \rightarrow Pheo^{\bullet -} + S^{\bullet +}$$

Superoxide anion ($O_2^{\bullet -}$) is generated when the pigment in the excited triplet state transfers electrons to molecular oxygen by type I mechanism [8][18].

$$^3Pheo^* + O_2 \rightarrow Pheo^{\bullet +} + O_2^{\bullet -}$$

The type II process involves the generation of singlet oxygen ($^1O_2$) by energy transfer from the excited triplet sensitizer to a ground state oxygen molecule [8][10].

$$^3Pheo^* + O_2 \rightarrow Pheo + {}^1O_2$$

In this study, the photoproperties of this natural pigment were studied by analyzing the effect of pheomelanin on the oxidation/nitration of tyrosine induced by UVB radiation under different pH values and in presence of iron ions. In particular, pheomelanin effect on UVB-induced oxidation/nitration of tyrosine has been studied at physiological pH and at a weakly acid pH. Under pathophysiological situations, such as inflammation, tissue pH close to 5.5–6 can be found. Moreover, recent studies have shown that acid melanosomal pH suppress melanogenesis, especially eumelanin formation, in melanocytes [19]. Notably, it has been observed that, at pH 5.8, eumelanin biosynthesis is suppressed, while pheomelanin production is enhanced [20].

Following UVB radiation of l-Tyrosine, tyrosyl radical that is generated dimerizes with the formation of 3,3′-dityrosine; in presence of nitrite the photochemical reaction produces tyrosyl radical and reactive nitrogen species which combine to form 3-nitrotyrosine as a further product [21][22][23] (**Figure 2**).

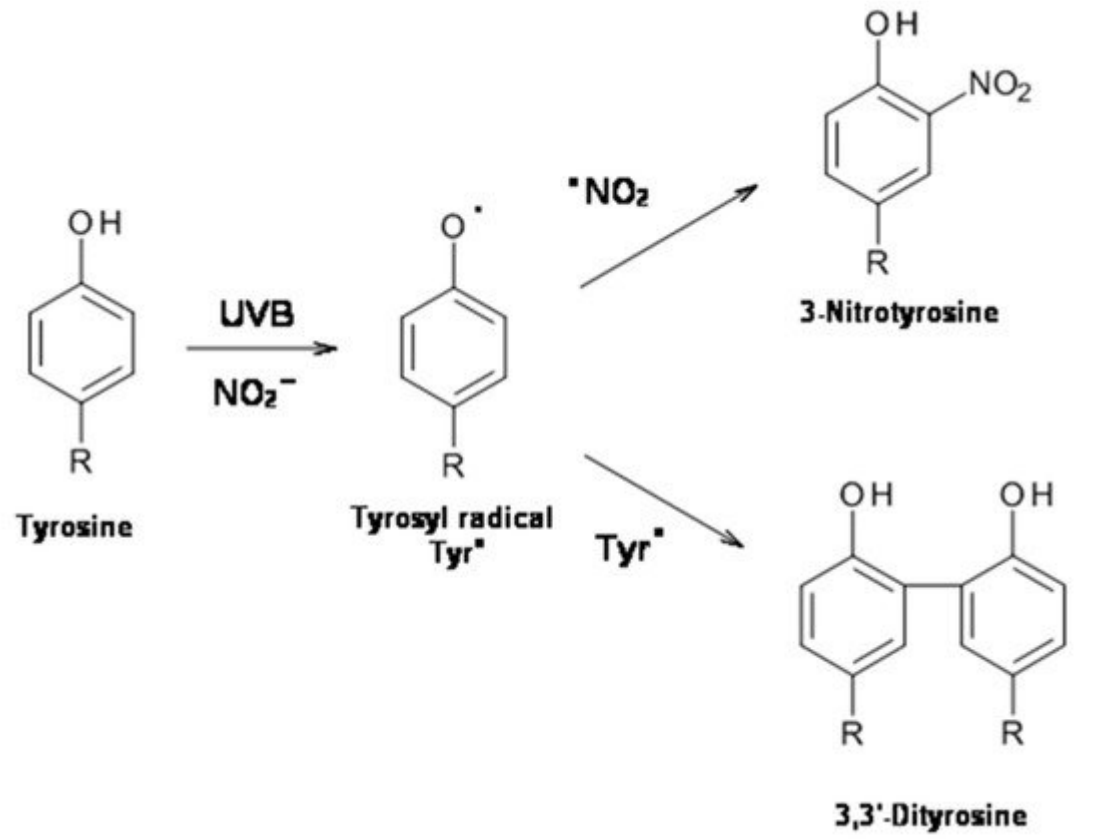

Figure 2. UVB-induced 3-nitrotyrosine and 3,3′-dityrosine formation.

Both 3,3′-dityrosine and 3-nitrotyrosine are considered diagnostic markers of the in vivo production of reactive oxygen and nitrogen species [23][24][25]. At this regard, free tyrosine and tyrosine protein residue nitration can be achieved through mechanisms involving peroxidase/H_2O_2-dependent oxidation of nitrite to nitrogen dioxide radical ($^{\bullet}NO_2$) [26][27][28]. In inflammation, myeloperoxidase from activated leukocytes catalyzes tyrosine nitration at high levels [29][30][31][32]. Photonitration of tyrosine to 3-nitrotyorosine has been already shown by methylene blue dye and riboflavin as sensitizers [33][34]. Methylene blue-sensitized photomodification of tyrosine in the presence of nitrite occurs mainly through a process which involves singlet oxygen (type II mechanism). Conversely, singlet oxygen plays a minor role in the tyrosine photooxidation/photonitration mediated by riboflavin as sensitizer [34][35][36]. Interestingly, the oxidation and nitration of tyrosine residues in proteins are considered important post-translational modifications with consequences on the function of proteins and therefore on cellular homeostasis [37][38][39][40].

2. UVB Radiation-Induced Photooxidation/Photonitration of l-Tyrosine

The exposure to ultraviolet light (UVB), at room temperature, of a solution containing 1 mM tyrosine leads to the formation of 0.25 ± 0.07 μM at pH 5.5 and 0.13 ± 0.02 μM at pH 7.4 of 3.3'-dityrosine after 30 min of exposure. Tyrosine dimerization was not observed in controls kept in the dark. The exposure of 1 mM tyrosine solution to UVB radiation in the presence of 10 mM nitrite, under the same experimental conditions reported above, leads to

3-nitrotyrosine as a further product in addition to 3,3'-dityrosine (**Figure 3**). When nitrite is present, 3,3′-dityrosine is 0.08 ± 0.02 μM and 3.60 ± 0.46 μM, at pH 5.5 and pH 7.4, respectively. The amount of 3-nitrotyrosine formed is 2.37 ± 0.4 μM and 1.89 ± 0.15 μM, at pH 5.5 and pH 7.4 respectively, after 30 min of exposure. At low pH values nitrite generates nitrating species which, in the presence of tyrosine, lead to the formation of 3-nitrotyrosine [41]. Control experiments, in which tyrosine and nitrite are incubated in the dark, indicate that, under our experimental conditions, this reaction pathway can contribute minimally to the production of 3-nitrotyrosine only at pH below 3.3.

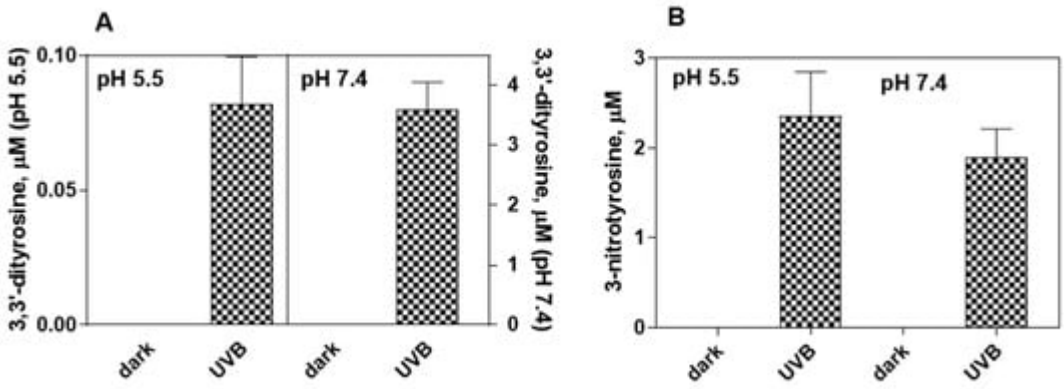

Figure 3. UVB-induced photooxidation/photonitration of tyrosine. A reaction mix containing 1 mM tyrosine in 0.2 M K-phosphate buffer at pH 5.5 or pH 7.4, 0.1 mM DTPA, 10 mM K-nitrite is exposed to UVB radiation. After 30 min of exposure, the reaction is stopped by placing the mixture in the dark and the solution is analyzed by HPLC, to determine the formation of 3,3'-dityrosine (**A**) and 3-nitrotyrosine (**B**), as reported in Materials and Methods. Controls in the dark correspond to the unexposed solution.

3. Effect of Pheomelanin on UVB Radiation-Induced Photooxidation/Photonitration of l-Tyrosine

In order to evaluate the photoproperties of pheomelanin on the oxidative/nitrative modifications of tyrosine induced by UVB rays, 1 mM tyrosine and 10 mM nitrite were exposed to UVB radiation in the presence of 4.2 μg/mL synthetic pheomelanin at physiological pH 7.4 and pH 5.5. Pheomelanin was enzymatically prepared from l-Dopa and cysteine as reported in the experimental section. After an exposure of 30 min both the formation of 3,3'-dityrosine and the conversion of tyrosine to 3-nitrotyrosine was assayed. Overall, pheomelanin exerts a photoprotective effect (antioxidant) on the oxidation/nitration of tyrosine induced by UVB radiation (**Figure 4**). However, at pH 5.5 pheomelanin acts as photosensitizer (prooxidant) in the nitrative modification of tyrosine. As shown in the **Figure 5**B, pheomelanin does not inhibit the nitration of tyrosine but there is a 60% increase in the formation of 3-nitrotyrosine compared to the control exposed to UVB radiation in the absence of pheomelanin. In control experiments in which pheomelanin alone and nitrite were exposed to UVB radiation, neither nitrotyrosine nor dityrosine were detectable.

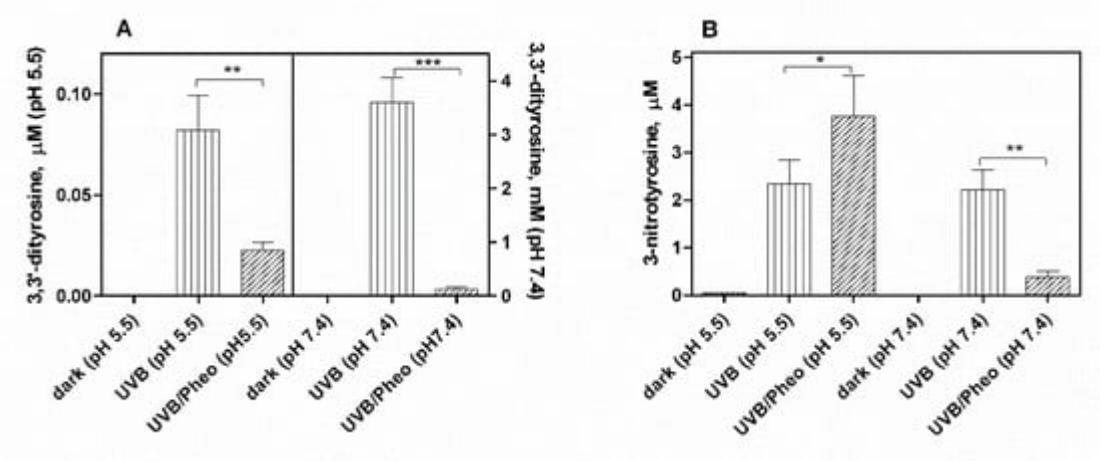

Figure 4. Photooxidation/photonitration of tyrosine by the nitrite/pheomelanin/UVB system. Pheomelanin 4.2 µg/mL is added to reaction mixture containing 1 mM tyrosine, 10 mM K-nitrite, 0.1 mM DTPA in 0.2 M K-phosphate buffer at pH 5.5 or pH 7.4. The solution is exposed to UVB rays for 30 min. The reaction is stopped by placing the mixture in the dark and the supernatant, obtained after centrifugation, is analyzed by HPLC to measure 3,3'-dityrosine (**A**) and 3-nitrotyrosine (**B**), as reported in Materials and Methods. Controls in the dark correspond to unexposed reaction mixtures (pheomelanin/nitrite/tyrosine system).*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

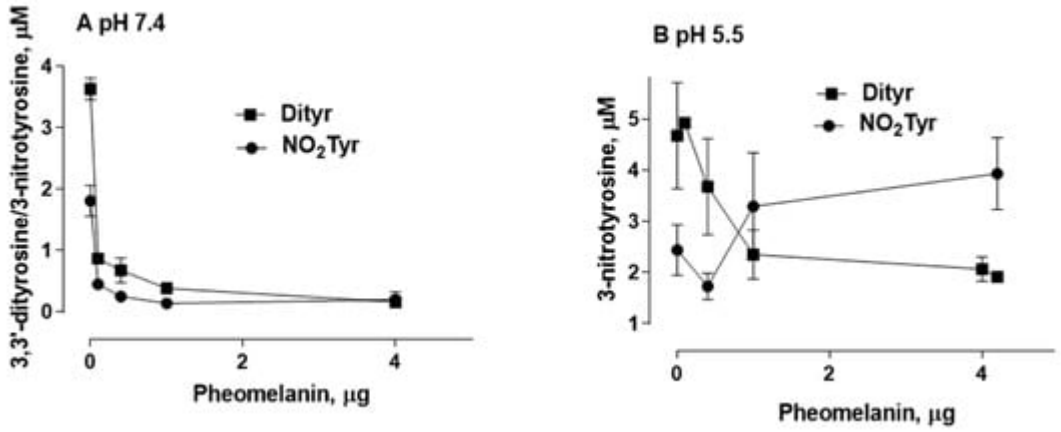

Figure 5. Photooxidation of tyrosine by the nitrite/pheomelanin/UVB system at various synthetic pheomelanin concentrations. Pheomelanin (0.1–4 µg/mL) is added to a reaction mixture containing 1 mM tyrosine, 10 mM K-nitrite, 0.1 mM DTPA in 0.2 M K-phosphate buffer at pH 7.4 (**A**) or pH 5.5 (**B**). The solution is exposed to UVB rays for 30 min. The reaction is stopped by placing the mixture in the dark and the supernatant, obtained after centrifugation, is analyzed by HPLC to determine the formation of 3-nitrotyrosine (●) and 3,3′-dityrosine (■) as reported in Materials and Methods.

Figure 5 shows the formation of 3,3'-dityrosine and 3-nitrotyrosine at various concentrations of pheomelanin (0.1–4 µg/mL) at pH 7.4 and pH 5.5. At all concentrations used, pheomelanin has a dose-dependent photoprotective action on the formation of 3,3′-dityrosine at both pH 5.5 and pH 7.4. The photosensitizing action on the formation of 3-nitrotyrosine at pH 5.5 is observed in the range 0.4–4 µg/mL.

4. Photoproperties of Pheomelanin on UVB-Induced Oxidative/Nitrative Modifications of l-Tyrosine: Effect of Fe(III)

It is known that melanins have the ability to bind various metals with the result of modifying their photoproperties [42][43][44][45]. In order to evaluate how the presence of metals can influence the oxidative/nitrative modifications of tyrosine, exposure to UVB rays was performed with the addition of Fe(III) to the reaction mixture. Experiments performed in the absence of metal chelator DTPA showed analogous results (data not shown). At pH 5.5, it is observed that the presence of metals influences the photoproperties of pheomelanin by reducing its antioxidant activity against dityrosine formation (**Figure 6**). Regarding the formation of 3-nitrotyrosine, the photosensitizer effect of pheomelanin is not affected either by the absence of the chelator or by the addition of Fe(III).

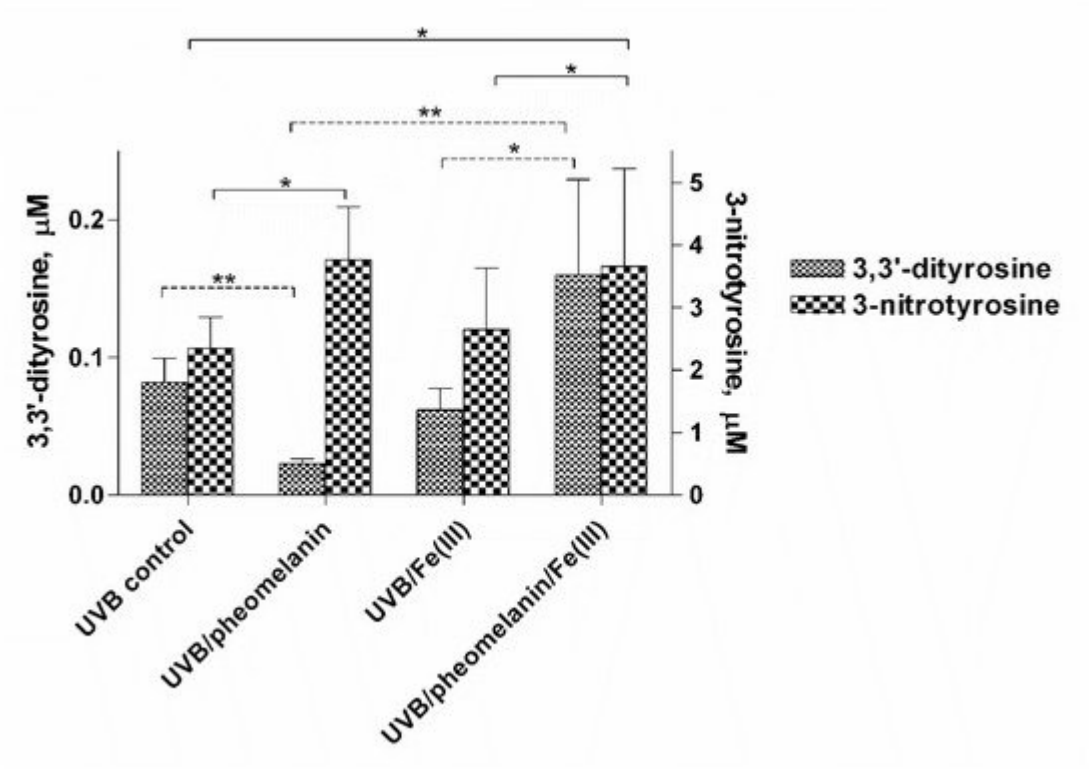

Figure 6. Photooxidation of tyrosine by the nitrite/pheomelanin/UVB system: effect of Fe(III). Pheomelanin 4.2 µg/mL is added to the reaction mixture containing 1 mM tyrosine, 10 mM K-nitrite, 0.1 mM DTPA in 0.2 M K-phosphate buffer at pH 5.5. The UVB control is pheomelanin free. Fe (III) is added as $FeCl_3$ at a concentration of 0.1 mM. The solution is exposed to UVB rays for 30 min. The reaction is stopped by placing the mixture in the dark and the supernatant, obtained after centrifugation, is analyzed by HPLC to determine the formation of 3-nitrotyrosine and 3,3′-dityrosine, as reported in Materials and Methods. ** $p < 0.01$, * $p < 0.05$.

5. Pheomelanin Effect on Oxidative/Nitrative Modifications of l-Tyrosine Induced by UVB Radiation: Role of Singlet Oxygen

The photooxidative reactions can be the result of radical type processes (type I) or of processes mediated by singlet oxygen (type II). Both mechanisms can contribute to the photooxidative reactions at the same time. In order to evaluate the role of singlet oxygen ($^1O_2$) in pheomelanin-sensitized nitration reaction of tyrosine at pH 5.5, the yields of 3-nitrotyrosine in H_2O and D_2O as solvent were compared. Replacement of H_2O by D_2O increases the lifetime of singlet oxygen by about 15 times [46] and, consequently, stimulates $^1O_2$-dependent reactions. As shown in **Figure 7**, the production of 3-nitrotyrosine is approximately 8.4 times greater in D_2O than in H_2O. This effect is indicative of the participation of singlet oxygen in the reaction. The formation of 3,3'-dityrosine is not affected by D_2O.

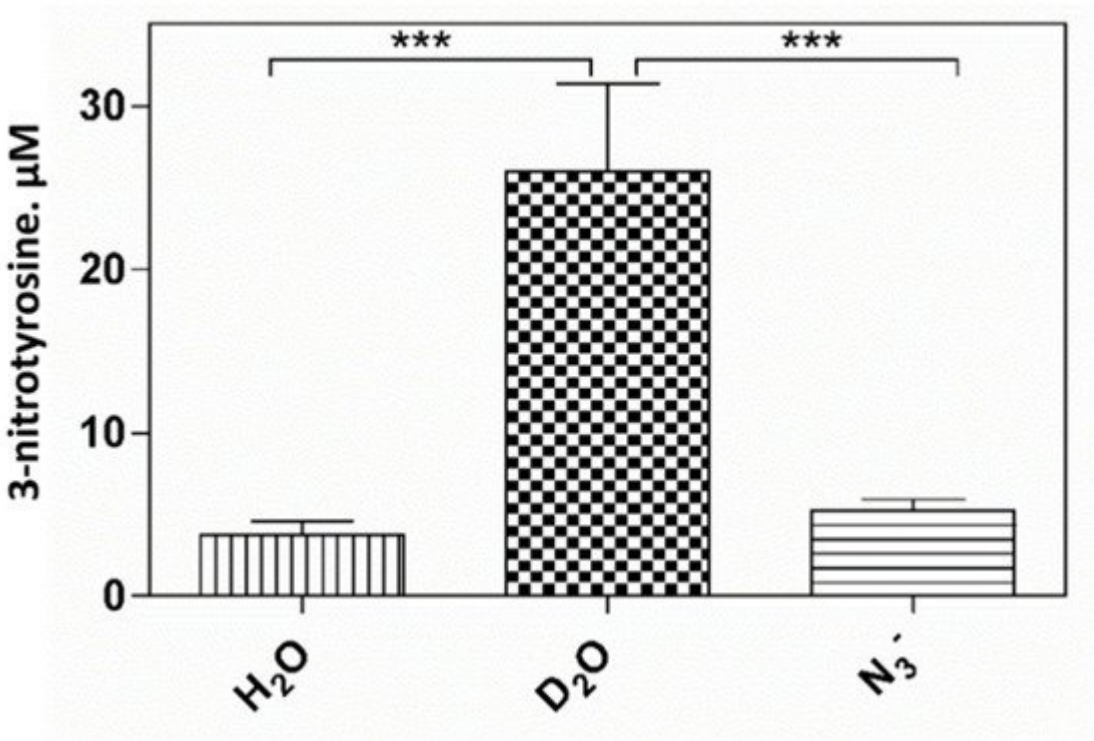

Figure 7. Photooxidation of tyrosine by the nitrite/pheomelanin/UVB system: effect of D_2O and NaN_3. Pheomelanin 4.2 µg/mL is added to the solution, containing 1 mM tyrosine, 10 mM K-nitrite in 0.2 M K-phosphate buffer at pH 5.5 and 0.1 mM DTPA. The solution is exposed to UVB rays for 30 min. The reaction is stopped by placing the mixture in the dark and the supernatant, obtained after centrifugation, is analyzed by HPLC to determine the formation of 3-nitrotyrosine, as reported in Materials and Methods. In D_2O, the pD (5.5) was taken as the measured pH + 0.4. NaN_3 is added to a final concentration of 1 mM. *** $p < 0.001$.

It has been also observed that the formation of 3-nitrotyrosine is significantly reduced in the presence of sodium azide (NaN_3), a known quencer of singlet oxygen (**Figure 8**). The inhibitory effect of azide confirms intermediacy of type II mechanism in the pheomelanin-sensitized formation of 3-nitrotyrosine.

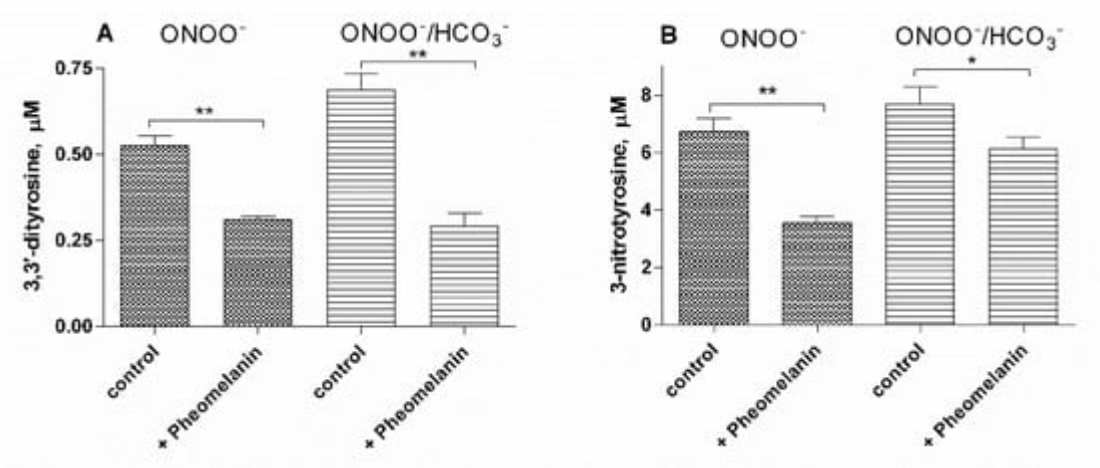

Figure 8. Pheomelanin effect on peroxynitrite-induced oxidation/nitration of tyrosine. To the reaction mix containing 100 µM tyrosine, 4.2 µg/mL pheomelanin, 0.1 mM DTPA in 0.2 M K-phosphate buffer, 100 µM peroxynitrite is added, Na-bicarbonate when present is at a concentration of 25 mM. After 5 min at room temperature, the reaction mixture is analyzed by HPLC to measure 3,3'-dityrosine (**A**) and 3-nitrotyrosine (**B**), as reported in Materials and Methods. ** $p < 0.01$, * $p < 0.05$.

6. Pheomelanin Effect on Oxidative/Nitrative Modifications of l-Tyrosine Induced by Peroxyitrite

Peroxynitrite induces both tyrosine oxidation to 3,3'-dityrosine and tyrosine nitration to 3-nitrotyrosine. Under our experimental conditions, peroxynitrite (100 µM, final concentration) added to a solution containing 100 µM of tyrosine generates 0.53 ± 0.02 µM of 3,3'-dityrosine and 6.75 ± 0.27 µM of 3-nitrotyrosine, respectively. As shown in **Figure 8**, pheomelanin, at a concentration of 4.2 µg/mL, is able to inhibit both the formation of 3,3'-dityrosine (~42%) and that of 3-nitrotyrosine (~47%). As reported [47][48], peroxynitrite reacts, in vivo, mainly with carbon dioxide, forming a peroxynitrite-CO_2 adduct which decomposes generating the nitrogen dioxide radicals ($^{\bullet}NO_2$) and carbonate radical anion ($CO_3^{\bullet-}$). In the presence of bicarbonate, tyrosine nitration mediated by peroxynitrite is generally increased due to high oxidative/nitrative properties of radicals generated by the decomposition of the peroxynitrite-CO_2 adduct. The results shown in **Figure 8** indicate that pheomelanin is equally effective in protecting tyrosine from the nitrative and oxidative action of peroxynitrite also in the presence of 25 mM bicarbonate.

7. Discussion and Conclusions

The results of this study show that the photoproperties of pheomelanin can be modulated by various experimental conditions. These properties were studied by analyzing the effect of pheomelanin on UVB radiation-induced oxidation/nitration of tyrosine. UVB irradiation leads to the dimerization of tyrosine with the formation of 3,3′-dityrosine and in the presence of nitrite the photochemical reaction forms 3-nitrotyrosine as an additional product. In the presence of pheomelanin, tyrosine is dose-dependently protected from oxidation to 3,3′-dityrosine both at pH 5.5 and physiological pH (pH 7.4). Pheomelanin can perform a protective function on the conversion of tyrosine to

3-nitrotyrosine at pH 7.4. The experiments conducted on the formation of 3,3′-dityrosine and 3-nitrotyrosine induced by peroxynitrite ($ONOO^-$) confirm this hypothesis. Peroxynitrite, which is generated in vivo from the reaction of nitric oxide ($^{\bullet}NO$) with the superoxide anion ($O_2^{\bullet-}$), is a very reactive species capable of nitrating and oxidizing tyrosine. Pheomelanin showed protective properties both on the formation of 3,3′-dityrosine and on the conversion of tyrosine to 3-nitrotyrosine induced both by peroxynitrite and peroxynitrite-CO_2 adduct. These results indicate that pheomelanin can act as free radical scavenger and the observed protective action of the pigment on UVB-induced tyrosine modifications can be attributed to this property.

An interesting result that emerged from our investigations is that pheomelanin can have pro-oxidant properties under some experimental conditions. We observed that the nitration of tyrosine to 3-nitrotyrosine induced by UVB radiation in presence of nitrite at pH 5.5 is increased when carried out in the presence of pheomelanin. These results indicate that the properties of pheomelanin can be significantly influenced by the pH during UVB irradiation, switching from antioxidant (pH 7.4) to pro-oxidant (pH 5.5). Furthermore, pheomelanin has a remarkable ability to bind metals and this property leads often to a modification of the photoprotective capabilities of the pigment. In our experimental conditions by adding Fe(III), we observed a reduced ability to inhibit the oxidative reaction. The pigment bond with iron induces an increase in the production of highly oxidizing reactive species whose action can only be partially counteracted by the antioxidant activity of the pigment itself. The investigations carried out to obtain information on the mechanism through which the nitrite/pheomelanin/UVB system induces the nitration of tyrosine at pH 5.5 indicate that, in our experimental conditions, the process can involve singlet oxygen, indeed, in the presence of D_2O, the production of 3-nitrotyrosine is considerably higher than that formed in H_2O.

The result presented herein indicates that photoproperties of pheomelanin can be modulated by various experimental conditions. It is well-known that pheomelanin undergoes structural modifications by UV rays. In the course of the biosynthetic pathways, modification involves benzothiazine units which are gradually converted to benzothiazole [4]. The relative ratio of these two types of pheomelanin moieties appears important in determining whether pheomelanin acts as a pro-oxidant [5]. Under our experimental conditions, UVB radiation and reactive nitrogen species could similarly influence pigment photoreactivity and induce structural modifications of pheomelanin worth to be further explored.

In conclusion pheomelanin is able to perform a protective function both on the tyrosine oxidation to 3,3′-dityrosine and on the conversion of tyrosine to 3-nitrotyrosine when the exposure is conducted at physiological pH; conversely at pH 5.5, the presence of pheomelanin induces a 60% increase in the formation of 3-nitrotyrosine. The addition of Fe(III) during the irradiation of tyrosine in presence of nitrite provokes a decrease of the antioxidant activity of pheomelanin also against the formation of 3,3’-dityrosine, so the photoproperties of pheomelanin may be affected by the presence of metal ions. Finally, pheomelanin showed protective properties on oxidation/nitration of tyrosine induced by peroxynitrite and by the decomposition of the peroxynitrite-CO_2 adduct.

References

1. Prota, G. The Chemistry of Melanins and Melanogenesis. Progress in the Chemistry of Organic Natural Products; Springer: Berlin/Heidelberg, Germany, 1995; Volume 64, pp. 93–148.
2. Simon, J.D.; Peles, D.; Wakamatsu, K.; Ito, S. Current challenges in understanding melanogenesis: Bridging chemistry, biological control, morphology, and function. Pigment Cell Melanoma Res. 2009, 22, 563–579.
3. Napolitano, A.; De Lucia, M.; Panzella, L.; D'Ischia, M. The "Benzothiazine" Chromophore of Pheomelanins: A Reassessment. Photochem. Photobiol. 2008, 84, 593–599.
4. Wakamatsu, K.; Ohtara, K.; Ito, S. Chemical analysis of late stages of pheomelanogenesis: Conversion of dihydrobenzothiazine to a benzothiazole structure. Pigment Cell Melanoma Res. 2009, 22, 474–486.
5. Micillo, R.; Panzella, L.; Koike, K.; Monfrecola, G.; Napolitano, A.; D'Ischia, M. "Fifty Shades" of Black and Red or How Carboxyl Groups Fine Tune Eumelanin and Pheomelanin Properties. Int. J. Mol. Sci. 2016, 17, 746.
6. Brenner, M.; Hearing, V.J. The Protective Role of Melanin Against UV Damage in Human Skin†. Photochem. Photobiol. 2008, 84, 539–549.
7. Rouzaud, F.; Kadekaro, A.L.; Abdel-Malek, Z.A.; Hearing, V.J. MC1R and the response of melanocytes to ultraviolet radiation. Mutat. Res. Mol. Mech. Mutagenes. 2005, 571, 133–152.
8. Chedekel, M.R.; Smith, S.K.; Post, P.W.; Pokora, A.; Vessell, D.L. Photodestruction of pheomelanin: Role of oxygen. Proc. Natl. Acad. Sci. USA 1978, 75, 5395–5399.
9. Panzella, L.; Szewczyk, G.; D'Ischia, M.; Napolitano, A.; Sarna, T. Zinc-induced Structural Effects Enhance Oxygen Consumption and Superoxide Generation in Synthetic Pheomelanins on UVA/Visible Light Irradiation†. Photochem. Photobiol. 2010, 86, 757–764.
10. Takeuchi, S.; Zhang, W.; Wakamatsu, K.; Ito, S.; Hearing, V.J.; Kraemer, K.H.; Brash, D.E. Melanin acts as a potent UVB photosensitizer to cause an atypical mode of cell death in murine skin. Proc. Natl. Acad. Sci. USA 2004, 101, 15076–15081.
11. Wenczl, E.; Van Der Schans, G.P.; Roza, L.; Kolb, R.M.; Timmerman, A.J.; Smit, N.P.M.; Pavel, S.; Schothorst, A.A. (Pheo)melanin photosensitizes UVA-induced DNA damage in cultured human melanocytes. J. Investig. Dermatol. 1998, 111, 678–682.
12. Sarna, T.; Menon, I.A.; Sealy, R.C. Photoinduced oxygen consumption in melanin systems—II. Action spectra and quantum yields for pheomelanins. Photochem. Photobiol. 1984, 39, 805–809.
13. Ezzahir, A. The influence of melanins on the photoperoxidation of lipids. J. Photochem. Photobiol. B Biol. 1989, 3, 341–349.
14. Menon, I.A.; Persad, S.; Haberman, H.F.; Kurian, C.J. A comparative study of the physical and chemical properties of melanins isolated from human black and red hair. J. Investig. Dermatol.

1983, 80, 202–206.

15. Menon, I.A.; Persad, S.; Ranadive, N.S.; Haberman, H.F. Role of superoxide and hydrogen peroxide in cell lysis during irradiation in vitro of Ehrlich ascitic carcinoma cells in the presence of melanin. Can. J. Biochem. Cell Biol. 1985, 63, 278–283.
16. Felix, C.C.; Hyde, J.S.; Sealy, R.C. Photoreactions of melanin: A new transient species and evidence for triplet state involvement. Biochem. Biophys. Res. Commun. 1979, 88, 456–461.
17. Sarna, T.; Menon, I.A.; Sealy, R.C. Photosensitization of melanins: A comparative study. Photochem. Photobiol. 1985, 42, 529–532.
18. Foote, C.S. Photosensitized Oxidation and Singlet Oxygen: Consequences in Biological Systems. In Free Radicals in Biology; Elsevier: Amsterdam, The Netherlands, 1976; pp. 85–133.
19. Ambrosio, A.L.; Boyle, J.A.; Aradi, A.E.; Christian, K.A.; Di Pietro, S.M. TPC2 controls pigmentation by regulating melanosome pH and size. Proc. Natl. Acad. Sci. USA 2016, 113, 5622–5627.
20. Wakamatsu, K.; Nagao, A.; Watanabe, M.; Nakao, K.; Ito, S. Pheomelanogenesis is promoted at a weakly acidic pH. Pigment Cell Melanoma Res. 2017, 30, 372–377.
21. Berlett, B.S.; Stadtman, E.R. Protein oxidation in aging, disease, and oxidative stress. J. Biol. Chem. 1997, 272, 20313–20316.
22. Cai, S.; Saito, T.; Fujii, N. Simultaneous ultraviolet B-induced photo-oxidation of tryptophan/tyrosine and racemization of neighboring aspartyl residues in peptides. Free Radic. Biol. Med. 2013, 65, 1037–1046.
23. Giulivi, C.; Traaseth, N.J.; Davies, K.J.A. Tyrosine oxidation products: Analysis and biological relevance. Amino Acids 2003, 25, 227–232.
24. Giulivi, C.; Davies, K.J. Dityrosine and tyrosine oxidation products are endogenous markers for the selective proteolysis of oxidatively modified red blood cell hemoglobin by (the 19 S) proteasome. J. Biol. Chem. 1993, 268, 8752–8759.
25. Ischiropoulos, H. Biological Tyrosine Nitration: A Pathophysiological Function of Nitric Oxide and Reactive Oxygen Species. Arch. Biochem. Biophys. 1998, 356, 1–11.
26. Van der Vliet, A.; Eiserich, J.P.; Halliwell, B.; Cross, C.E. Formation of Reactive Nitrogen Species during Peroxidase-catalyzed Oxidation of Nitrite. J. Biol. Chem. 1997, 272, 7617–7625.
27. Sampson, J.B.; Ye, Y.; Rosen, H.; Beckman, J.S. Myeloperoxidase and horseradish peroxidase catalyze tyrosine nitration in proteins from nitrite and hydrogen peroxide. Arch. Biochem. Biophys. 1998, 356, 207–213.

28. Brennan, M.L.; Wu, W.; Fu, X.; Shen, Z.; Song, W.; Frost, H.; Vadseth, C.; Narine, L.; Lenkiewicz, E.; Borchers, M.T.; et al. A tale of two controversies. Defining both the role of peroxidases in nitrotyrosine formation in vivo using eosinophil peroxidase and myeloperoxidase-deficient mice, and the nature of peroxidase-generated reactive nitrogen species. J. Biol. Chem. 2002, 277, 17415–17427.
29. Eiserich, J.P.; Hristova, M.; Cross, C.E.; Jones, A.D.; Freeman, B.A.; Halliwell, B.; Van Der Vliet, A. Formation of nitric oxide-derived inflammatory oxidants by myeloperoxidase in neutrophils. Nature 1998, 391, 393–397.
30. Burner, U.; Furtmüller, P.G.; Kettle, A.J.; Koppenol, W.H.; Obinger, C. Mechanism of reaction of myeloperoxidase with nitrite. J. Biol. Chem. 2000, 275, 20597–20601.
31. Gaut, J.P.; Byun, J.; Tran, H.D.; Lauber, W.M.; Carroll, J.A.; Hotchkiss, R.S.; Belaaouaj, A.; Heinecke, J.W. Myeloperoxidase produces nitrating oxidants in vivo. J. Clin. Investig. 2002, 109, 1311–1319.
32. Capuozzo, E.; Pecci, L.; Giovannitti, F.; Baseggio Conrado, A.; Fontana, M. Oxidative and nitrative modifications of enkephalins by human neutrophils: Effect of nitroenkephalin on leukocyte functional responses. Amino Acids 2012, 43, 875–884.
33. Pecci, L.; Montefoschi, G.; Antonucci, A.; Costa, M.; Fontana, M.; Cavallini, D. Formation of nitrotyrosine by methylene blue photosensitized oxidation of tyrosine in the presence of nitrite. Biochem. Biophys. Res. Commun. 2001, 289, 305–309.
34. Fontana, M.; Blarzino, C.; Pecci, L. Formation of 3-nitrotyrosine by riboflavin photosensitized oxidation of tyrosine in the presence of nitrite. Amino Acids 2012, 42, 1857–1865.
35. Silva, E.; Godoy, J. Riboflavin sensitized photooxidation of tyrosine. Int. J. Vitam. Nutr. Res. 1994, 64, 253–256.
36. Lu, C.Y.; Liu, Y.Y. Electron transfer oxidation of tryptophan and tyrosine by triplet states and oxidized radicals of flavin sensitizers: A laser flash photolysis study. Biochim. Biophys. Acta Gen. Subj. 2002, 1571, 71–76.
37. Schopfer, F. NO-dependent protein nitration: A cell signaling event or an oxidative inflammatory response? Trends Biochem. Sci. 2003, 28, 646–654.
38. Gow, A.J.; Farkouh, C.R.; Munson, D.A.; Posencheg, M.A.; Ischiropoulos, H. Biological significance of nitric oxide-mediated protein modifications. Am. J. Physiol. Cell. Mol. Physiol. 2004, 287, L262–L268.
39. Atwood, C.S.; Perry, G.; Zeng, H.; Kato, Y.; Jones, W.D.; Ling, K.Q.; Huang, X.; Moir, R.D.; Wang, D.; Sayre, L.M.; et al. Copper Mediates Dityrosine Cross-Linking of Alzheimer’s Amyloid-β. Biochemistry 2004, 43, 560–568.

40. Monteiro, H.P. Signal transduction by protein tyrosine nitration: Competition or cooperation with tyrosine phosphorylation-dependent signaling events? Free Radic. Biol. Med. 2002, 33, 765–773.
41. Park, J.Y.; Lee, Y.N. Solubility and decomposition kinetics of nitrous acid in aqueous solution. J. Phys. Chem. 1988, 92, 6294–6302.
42. Schmitz, S.; Thomas, P.D.; Allen, T.M.; Poznansky, M.J.; Jimbow, K. Dual role of melanins and melanin precursors as photoprotective and phototoxic agents: Inhibition of ultraviolet radiation-induced lipid peroxidation. Photochem. Photobiol. 1995, 61, 650–655.
43. Sealy, R.C.; Felix, C.C.; Hyde, J.S.; Swartz, H.M. Structure and Reactivity of Melanins: Influence of Free Radicals and Metal Ions. In Free Radicals in Biology; Elsevier: Amsterdam, The Netherlands, 1980; pp. 209–259.
44. Palumbo, A.; D'Ischia, M.; Misuraca, G.; Prota, G.; Schultz, T.M. Structural modifications in biosynthetic melanins induced by metal ions. Biochim. Biophys. Acta Gen. Subj. 1988, 964, 193–199.
45. Zadlo, A.; Mokrzyński, K.; Ito, S.; Wakamatsu, K.; Sarna, T. The influence of iron on selected properties of synthetic pheomelanin. Cell Biochem. Biophys. 2020, 78, 181–189.
46. Rodgers, M.A.J.; Snowden, P.T. Lifetime of oxygen (O2(1.DELTA.g)) in liquid water as determined by time-resolved infrared luminescence measurements. J. Am. Chem. Soc. 1982, 104, 5541–5543.
47. Lymar, S.V.; Jiang, Q.; Hurst, J.K. Mechanism of carbon dioxide-catalyzed oxidation of tyrosine by peroxynitrite. Biochemistry 1996, 35, 7855–7861.
48. Goldstein, S.; Czapski, G.; Lind, J.; Merényi, G. Tyrosine nitration by simultaneous generation of NO and O2/-under physiological conditions. How the radicals do the job. J. Biol. Chem. 2000, 275, 3031–3036.
49. Napolitano, A.; Panzella, L.; Monfrecola, G.; D'Ischia, M. . Pheomelanin-induced oxidative stress: Bright and dark chemistry bridging red hair phenotype and melanoma.; Pigment Cell Melanoma Res.: Italy , 2014; pp. 721–733.