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Mechanistic insights into the bleaching of melanin by alkaline hydrogen peroxide

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ABSTRACT

This work aims to determine the roles of reactive oxygen species HO· and HO_2^- in the bleaching of melanins by alkaline hydrogen peroxide. Experiments using melanosomes isolated from human hair indicated that the HO· radical generated in the outside solution does not contribute significantly to bleaching. However, studies using soluble *Sepia* melanin demonstrated that both HO_2^- and HO· will individually bleach melanin. Additionally, when both oxidants are present, bleaching is increased dramatically in both rate and extent. Careful experimental design enabled the separation of the roles and effects of these key reactive species, HO· and HO_2^-. Rationalisation of the results presented, and review of previous literature, allowed the postulation of a simplified general scheme whereby the strong oxidant HO· is able to pre-oxidise melanin units to *o*-quinones enabling more facile ring opening by the more nucleophilic HO_2^-. In this manner the efficiency of the roles of both species is maximised.

1. Introduction

Human hair colour is due to the presence of melanin granules (melanosomes) within hair. These melanosomes contain melanin pigments that are believed to have antioxidant and photoprotective roles [1,2]. The precise structure of this highly insoluble class of compounds has not been elucidated. However, in the case of eumelanins (responsible for brown/black hair colours) they are proposed to consist of oligomeric 5,6-dihydroxyindole (DHI) derivatives. These derivatives are aggregated into nanoparticles and further encapsulated into melanosomes (Fig. 1). These DHI derivatives are synthesised in the body from tyrosine in a process known as melanogenesis [3].

Cosmetic hair bleaching relies on destructive oxidation of melanin pigments, usually as a result of treatment by hydrogen peroxide under alkaline conditions (ammonia or monoethanolamine). Currently the roles of the base and reactive oxygen species (ROS) in the bleaching of melanin by hydrogen peroxide are not well understood. Mechanistic insights into this melanin oxidation are of interest to the cosmetic industry and may inform the development of hair bleaching and colouring products.

Conceptually there are at least three distinct steps that need to take place for the bleaching of melanin within the hair to occur: 1) Diffusion of oxidants/base into the hair to access the melanosomes. 2) Rupture of the melanosomal membrane and the solubilisation of the released melanin nanoparticles and 3) Action of the oxidant/oxidants on the melanin pigments.

A body of literature exists which investigate the processes involved in bleaching/decolourisation of melanin within hair [4–6], isolated melanosomes [4,6–8], or synthetic melanin pigments [9–12]. Elucidating precise mechanisms is complicated by the fact that several processes are happening at once and that the reaction constituents may also play different roles at different stages.

1.1. Bleaching of melanin

Metal ions in the hair fibre, such as Fe^{3+} and Cu^{2+}, may lead to Fenton [14] or Fenton-like reactions, which decompose hydrogen peroxide during bleaching. This decomposition results in the production of reactive oxygen species (ROS), such as hydroxyl radicals (HO·), perhydroxyl radicals (HO_2·) and superoxide anions (O_2^{-}·).

There has been considerable debate in the literature over the precise mechanism of the deceptively simple Fenton reaction, predominantly as to whether the reactive intermediate is a hydroxyl radical or a hypervalent iron complex (such as ferryl ion $L_xFe{=}O$) [15,16]. For

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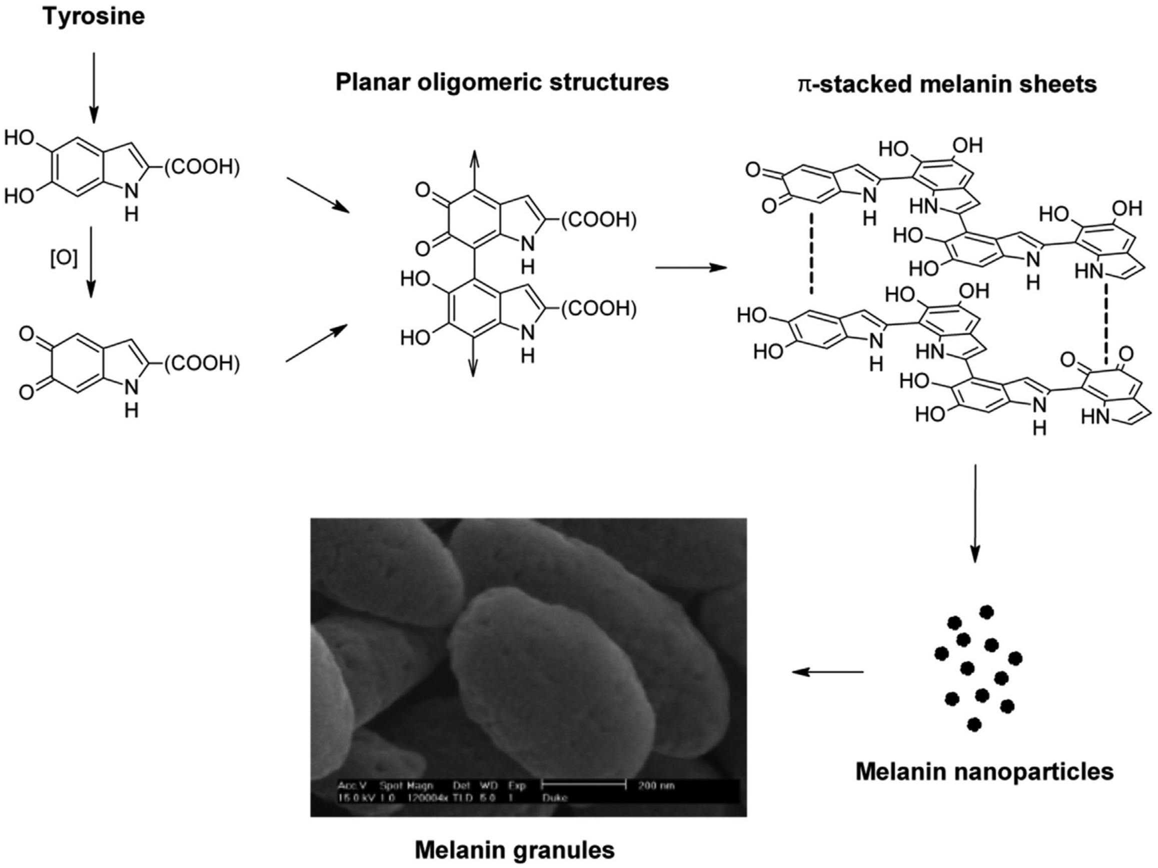

Fig. 1. Schematic overview of eumelanin formation, including the structures of the monomeric units 5,6-dihydroxyindole (DHI) and 5,6-dihydroxyindole carboxylic acid (DHICA) and the hierarchical aggregation of melanin sheets into nanoparticles and granules.

this work the distinction between the reactive intermediates was not made, as the reactivity of these two intermediates is likely to be similar. Instead HO• is used to represent a reactive intermediate.

Despite the fact that the precise structural details of melanin are unknown, there is evidence that the main substructure is based upon 5,6-dihydroxyindole (DHI) derivatives. This evidence comes from studies of the breakdown of various melanins to pyrrolic acids *via* bleaching processes [9,10,17–19]. Several complex mechanisms for this process have been proposed [9–11]. A general consensus is that a melanin subunit must first be oxidised from its *o*-hydroquinone form through a semiquinone radical to the *o*-quinone form. This step is a reversible equilibrium that occurs naturally in melanin and is proposed to be the origin of its antioxidant activity [1]. The *o*-quinone can then be irreversibly oxidised ultimately to a pyrrolic acid. A simplified mechanism to represent this is shown in Fig. 2.

The identities of the oxidising species for the reversible and irreversible oxidation stages are difficult to determine. HO· radicals have been suggested to be involved in the breakdown of melanin, though the stages at which they are involved have not been postulated. These radicals are presumed to be produced *via* site-specific Fenton (-like) reactions between melanin bound metals and hydrogen peroxide [11]. It has also been shown that radiolytically generated hydroxyl radicals can lead to both reversible and irreversible oxidation of melanin [20].

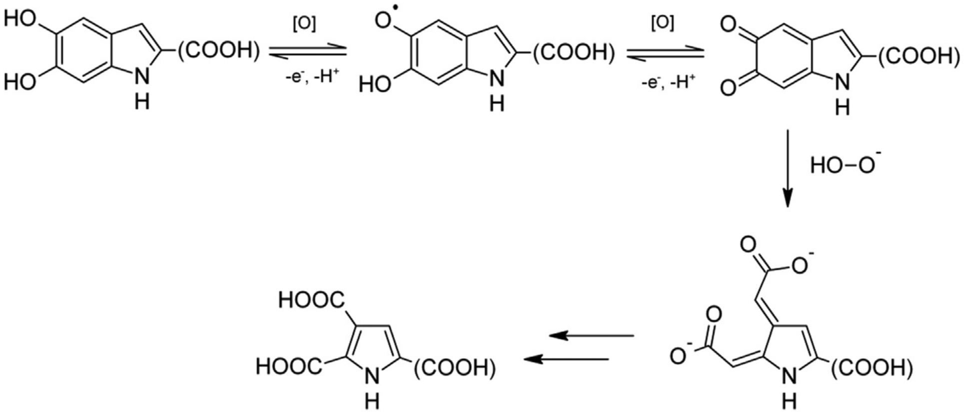

Fig. 2. A generalised and simplified melanin bleaching scheme showing how a monomeric 5,6-dihydroxyindole (DHI) unit that comprises melanin can be oxidised ultimately to a pyrrolic acid [11].