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Short Communication

Control of Melanogenesis in the Human Epidermis by the Redox-Status of Tetrahydrobiopterins

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Summary

Activity of tyrosinase directly controls melanogenesis in the human epidermis. Recently, it has been shown that the biosynthesis and recycling of (6R)L-erythro 5,6,7,8 tetrahydrobiopterin (6-BH_4) plays a central role in regulating the supply of L-tyrosine, the substrate for tyrosinase. In this report, we present evidence that 6-BH_4 and other tetrahydropterins, have the capacity to regulate tyrosinase activity directly by a specific uncompetitive mechanism. This fine control of tyrosinase activity/melanogenesis in the human epidermis depends on the redox equilibrium 6-BH_4 ⇔ dihydropterin ⇔ 6-biopterin. The accumulation of 6-biopterin is cytotoxic to normal human melanocytes.

Introduction

The human skin represents with 1.8 m^2 one of the largest organs of the body surface, where the epidermis forms the outer most layer. The epidermal unit consists of 36 keratinocytes and one pigment producing melanocyte. In the latter dendritic cell, melanization takes place in their specific organelles i.e., the melanosomes. Several maturation stages of these melanosomes and the number of these organelles correlate with different constitutional photo skin types I-VI (Fitzpatrick classification) (1). However, the number of melanocytes are not different in the different skin types (1).

Tyrosinase (EC 1,14,18,1) presents the key enzyme for melanogenesis. The functional enzyme is located on the melanosomal membranes and catalyzes the oxidation of L-tyrosine via L-dopa to the highly reactive product L-dopaquinone. This orthoquinone enters a series of free radical coupling reactions to produce the black pigment eumelanin. It can also react with L-cysteine, by Michael addition, yielding L-cysteinyl-dopa which subsequently polymerizes to the red pigment pheomelanin (2). The activity of tyrosinase depends on three major events: (a) the supply of the substrate L-tyrosine, (b) the generation of superoxide anion radical (3,4) (O_2^-), and (c) the transcription of the tyrosinase gene (5). L-tyrosine not only presents the substrate for tyrosinase in the melanocytes, it also is central to catecholamine biosynthesis in undifferentiated keratinocytes (4). Recently it has been shown that both cell types have the capacity for *de novo* synthesis and recycling of (6R)L-erythro-5,6,7,8-tetrahydrobiopterin (6-BH_4) associated with levels of phenylalanine hydroxylase (EC 1,14,16,1) (PAH) comparable to those in liver (3,4). PAH activities correlate with the constitutional skin colour of the six different photo skin types (1). These results strongly suggest that L-tyrosine as the central substrate for catecholamine biosynthesis in keratinocytes and melanin biosynthesis in melanocytes, can be independently produced via PAH-activity in both cell types. Very recently, it has been

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recognized that the promoter of the tyrosinase gene contains a putative 16-base inverted palindrome with sequence homology to the hepatocyte nuclear factor 1 (HNF1-α) homodimer binding site (6).

It has been shown that 4a-hydroxy-tetrahydropterin dehydratase (a) catalyzes the dehydradation of 4a-OH-tetrahydrobiopterin, the immediate product of PAH-activity to form quinonoid dihydrobiopterin, and (b) serves in the regulation of dimerization of the transcription factor HNF1-α (5, 6). Based on computer analysis using the UK human genome mapping project, a putative binding site with significant sequence homology for HNF1-α homodimer has been located in the promoter region of the tyrosinase gene in human, mouse and frog (4, 5). Taken together, these results suggested that the *de novo* synthesis and recycling of $6BH_4$ in the melanocyte not only controls the L-tyrosine supply for melanogenesis, but could also influence the transcription of the tyrosinase gene. As a consequence, it has been of great interest to examine the possibility that tetrahydropterins could directly regulate tyrosinase activity.

Material and Methods

Pterins were obtained from B. Schirks, Jona, Switzerland. Other reagents and enzymes were purchased from Sigma Chemical Company, St. Louis, MO, U.S.A. Human tyrosinase and human thioredoxin reductase were purified from metastatic melanoma tissue by the modified methods described in references 7 and 8. Tyrosinase was assayed by measuring the formation of dopaquinone at 475 nm (9). Human melanocytes were grown in MCDB 153 culture medium according to the conditions described by Pittelkow and Shipley (10). IC_{50} values for 6-biopterin to human melanocytes were determined by viable cell counting. The conversion of 6-biopterin to quinonoid dihydropterin was followed spectrophotometrically by the change in extinction at 370 nm (9).

Results

1. The specific inhibition of tyrosinase was demonstrated in the presence with saturating levels of L-tyrosine and tetrahydropterins (i.e., $6BH_4$, $7BH_4$ and 6,7 dimethyl-BH_4). There was no inhibition when L-dopa was used as substrate. The oxidized pterins, dihydropterin and 6-biopterin, did not inhibit the enzyme in the presence of L-tyrosine under the same experimental conditions (9). Both Lineweaver-Burk and Hanes plot analyses confirmed that tetrahydropterins regulate tyrosinase by an uncompetitive specific mechanism. Kinetic parameters for the inhibition of tyrosinase by the tetrahydropterins are presented in Table 1.

Table 1. Kinetic parameters for the inhibition of tyrosinase by tetrahydropterins

Tyrosinase	$K_m \times 10^{-6}$	$K_i \times 10^{-6}$ M			
		$6BH_4$	$7BH_4$	6,7-diCH_3	BH_4
Mushroom	43	29	18	30	
Human	24	13	8	n.d.	

2. Recently, it has been realized that the human epidermis can accumulate oxidized pterins (3, 4). One reason for this result could be e.g., the permanent oxidative stress via UV-radiation. Since the tetrahydropterin structure was essential for the down regulation of tyrosinase, it was anticipated that oxidative stress to the melanocyte could activate tyrosinase by conversion of $6BH_4$ to dihydropterin and ultimately, to 6-biopterin. The questions remained whether the epidermis contains an effective reducing system for the reversal of this process. One excellent candidate was the strong nucleophilic thioprotein thioredoxin reductase with its electron acceptor thioredoxin (8). Both thioredoxin reductase and reduced thioredoxin catalyzed a slow reduction of 6-biopterin to quinonoid dihydropterin (11).

3. It has been shown that 6-biopterin is extremely cytotoxic to normal human melanocytes with an IC_{50} value of 10^{-7} M under *in vitro* conditions (11).

Discussion

The biosynthesis, recycling and redox-equilibria for $6BH_4$ appear to be of critical importance to the regulation of melanogenesis in the human epidermis. Our results showed for the first time that the redox equilibrium $6BH_4 \Leftrightarrow$ dihydropterin $\Leftrightarrow$ 6-biopterin directly regulates melanogenesis (a) by control of the substrate supply (L-tyrosine), (b) by a direct uncompetitive inhibition of tyrosinase, and (c) by the survival of the melanocyte population. Furthermore, these results gained additional support by a putative 13-amino acid binding site for tetrahydropterins on tyrosinases conserved in human, mouse, frog and neurospora (9). However, a complete understanding of the tetrahydropterin binding site on tyrosinase awaits the x-ray cristallographic structure of the enzyme. Significant evidence for the importance of pterins in the pigmentation process has been obtained upon examination of the depigmentation disorder vitiligo (3, 4). In these patients, both 6- and $7BH_4$ as well as their oxidation products, are

increased up to 20-fold compared to controls. The presence of $7BH_4$ inhibits PAH, thus interfering with the supply of L-tyrosine from L-phenylalanine (12). The high levels of 6- and $7BH_4$ lead to direct inhibition of tyrosinase. However, examination by WOODs light and fluorescence spectroscopy identified the presence of oxidized pterins in the epidermis of these patients (3, 4). These results could explain also the decreased functioning melanocytes due to 6-biopterin cytotoxicity (11). Since tyrosinase levels in the affected epidermis are reduced to 40% of the normal enzyme activity, defective transcription via decreased 4a-OH-tetrahydropterin dehydratase/HNF1-α activity could provide a reasonable hypothesis to explain these results (6, 13, 14).

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