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Quantitative Analysis of Eumelanin and Pheomelanin in Hair and Melanomas

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In this study, a method is provided for analyzing quantitatively the content and the class of melanin pigments in the tissues, e.g., hair and melanoma. The method is simple and rapid because it does not require the isolation of melanins from the tissues. The rationale was that permanganate oxidation of eumelanin yields pyrrole-2,-3,5-tricarboxylic acid (PTCA) as its major pyrrolic product, which may serve as a quantitatively significant indicator of eumelanin, while hydriodic acid hydrolysis of pheomelanin yields amino-hydroxyphenylalanine (AHP) as a specific indicator of pheomelanin. The degradation products, PTCA and AHP, were determined by high-performance liquid chromatography. Sepia melanosome-melanin and synthetic 5-S-cysteinyldopa-melanin served as reference standards of eumelanin and pheomelanin, respectively. Our method provided data that corresponded well to the content and class of melanins in normal hair. Based on this control study, it was found that the melanins in the melanosomes of both B16 and Harding-Passey (HP) melanomas were eumelanic and that the melanin content in B16 melanosomes was more than 10 times higher than that in HP melanosomes, though these two melanosomes revealed distinct colors and ultrastructures, i.e., brown-black, eumelanosome-like granules in B16 and reddish- or light-brown, pheomelanosome-like granules in HP.

In animals, there are 2 major classes of melanin pigments, eumelanin and pheomelanin [1]. The classification of the 2 pigments depends mostly on the color, solubility properties, and sulfur content of isolated melanin. Eumelanin is a dark-brown to black pigment which is insoluble in acid and alkali, and contains nitrogen (6–9%) but no sulfur (0–1%). On the other hand, pheomelanin is a yellow to reddish-brown pigment which is soluble in alkali, and possesses both nitrogen (8–11%) and sulfur (9–12%) [2]. The 2 classes of melanins are also chemically distinct; eumelanin is composed mainly of monomer units of 5,6-dihydroxyindole and 5,6-dihydroxyindole-2-carboxylic acid, while pheomelanin is made up of benzothiazine units derived from cysteinyldopas (Fig 1) [1]. The synthesis of melanins occurs in melanosomes, but it is difficult, with the exception of normal hair, to identify even by ultrastructural observations which class of melanin pigments is produced in them (type of melanogenesis) [3].

The isolation of melanins from the melanogenic tissues is time consuming and may involve the risk of structural alteration due to the drastic isolation procedures employed [2,4–6]. Many attempts have been made to correlate the type of melanogenesis with the tissue level of dopa and 5-S-cysteinyldopa [7–9]. Correlation, however, has not always been achieved [9]; melanin contents in melanogenic tissues are some 10^3 times higher than the levels of these melanin precursors [5,9] and cysteinyldopas are found in any type of active melanocyte [1]. Furthermore, recent findings that eumelanic pigments often contain high amounts of sulfur (as much as 6%) [4,5,10] have raised the need for more quantitative criteria for the classification of melanin pigments.

We now report a simple and rapid method for quantitatively analyzing eumelanin and pheomelanin in the tissues, e.g., hair and melanomas, which makes unnecessary the isolation of melanin. This method is based on the formation of pyrrole-2,3,5-tricarboxylic acid (PTCA) and an isomeric mixture of 4(3)-amino-3(4)-hydroxyphenylalanines (AHP) by permanganate oxidation [10,11] of eumelanin and hydriodic acid (HI) hydrolysis of pheomelanin [10,12]. The products were determined by high-performance liquid chromatography (HPLC).

MATERIALS AND METHODS

Chromatography

A Yanaco Model L-2000 liquid chromatograph was employed with the detectors of a Yanaco Model U-213 ultraviolet detector (254 nm) and a Yanaco Model VMD-101 electrochemical detector (a glassy carbon electrode) for the determination of PTCA and AHP, respectively. The electrochemical detector was set at +500 mV vs. Ag/AgCl reference electrode. All analyses were performed on a C_{18} reversed-phase column (Yanaco ODS-T, 0.4 × 25 cm) maintained either at 60°C (for PTCA) or at 40°C (for AHP). The mobile phase was (a) 0.1 M potassium phosphate buffer (pH 2.1) for PTCA, and (b) a mixture of 97 vol of 0.1 M sodium citrate buffer (pH 4.0) containing 1 mM sodium octanesulfonate and 0.1 mM EDTA-Na_2, and 3 vol of methanol for AHP. The flow rate was 0.7 ml/min.

Chemicals

L-3,4-Dihydroxyphenylalanine (dopa), 3-amino-4-hydroxyphenylalanine (3-aminotyrosine), and mushroom tyrosinase (2230 units/mg) were purchased from Sigma Chemical Co. and 5-hydroxyindole-2-carboxylic acid was obtained from Aldrich Chemical Co. 4-Amino-3-hydroxyphenylalanine was prepared by HI hydrolysis of 5-S-cysteinyldopa-melanin [12], and PTCA by $KMnO_4$ oxidation of 5-hydroxyindole-2-carboxylic acid [13]. 5-S-Cysteinyldopa was chemically prepared by us [14].

Melanins

Dopa-melanin, 5-S-cysteinyldopa-melanin, and a mixed-type melanin were prepared essentially as described by Ito et al [15]. The reaction mixture contained 1 mmol of L-dopa or 5-S-cysteinyldopa, or a mixture of 0.5 mmol of each of these catechols, and 8 mg of mushroom tyrosinase in 80 ml of 0.05 M sodium phosphate buffer, pH 6.8. For the preparation of 5-S-cysteinyldopa-melanin, 0.05 mmol of L-dopa was added as catalyst; most of the L-dopa (more than 80%) was found unchanged in the supernatant of the reaction mixture. The yields were 133 mg, 150 mg, and 185 mg for dopa-melanin, 5-S-cysteinyldopa-melanin, and the mixed-type melanin, respectively. By elemental analysis the mixed-

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Abbreviations:
AHP: amino-hydroxyphenylalanine
dopa: dihydroxyphenylalanine
HI: hydriodic acid
HP: Harding-Passey
HPLC: high-performance liquid chromatography (chromatograph)
PTCA: pyrrole-2,3,5-tricarboxylic acid

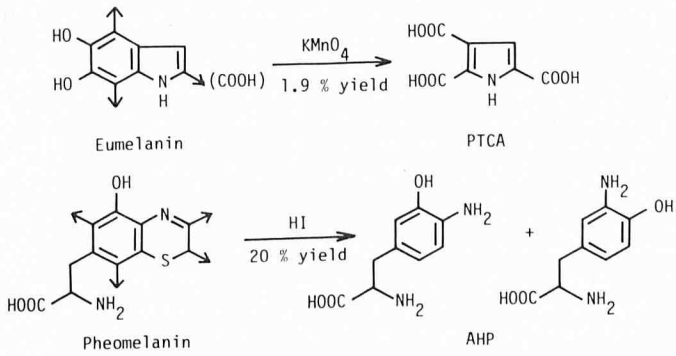

FIG 1. Chemical degradations of eumelanin and pheomelanin. The *arrows* represent the points of connections with adjacent monomer units.

type melanin was found to be composed of C (48.85%), H (3.76%), N (8.90%), and S (7.88%).

Melanosomes

The melanosomes were prepared from B16 and Harding-Passey (HP) mouse melanomas and from the ink sac of a sepia (cuttlefish). The purification procedure of melanoma melanosomes has been reported previously [9]. Briefly, they were purified by 2 steps of sucrose density gradient ultracentrifugation with elimination of surface membranous components by detergent. They were then washed extensively with distilled water and lyophilized. The sepia melanosomes were obtained from the ink sac of a cuttlefish and kindly supplied by Dr. Y. Fujinuma, Research Laboratory of Shiseido Cosmetic Company (Yokohama, Japan). Similar to the melanoma melanosomes, the lyophilized sepia melanosomes did not contain any external contaminants nor were they found to possess the other membrane under electron microscopy. The melanins from these melanosomes were isolated by our previously described method [9].

Permanganate Oxidation of Melanins and Tissues

The amounts of samples used for each oxidation were: 20 mg of melanin, 50 mg of lyophilized melanosomes, 50–100 mg of intact hair, and the acid-insoluble fraction [9] prepared from about 0.5–1.0 g of tissue. The method for the oxidation was similar to those described by Nicolaus et al [6] and Hackman and Goldberg [11]. A sample was dissolved or homogenized in 5 ml of 1 M K_2CO_3; to this, 3% $KMnO_4$ was added in portions of 0.2 ml with vigorous stirring until the purple color of $KMnO_4$ persisted for at least 10 min. The reaction required 20–60 min. Solid Na_2SO_3 was added to decompose the residual $KMnO_4$, and the mixture was kept in a hot-water bath for 5 min and centrifuged. The MnO_2 precipitate was washed with 3 ml of hot water. The supernatants were combined, acidified to pH 1–2 with 6 M HCl, and extracted 5 times with 10 ml of alumina-treated, peroxide-free ether. The combined ether extract was dried over $MgSO_4$, filtered, and evaporated to dryness in a rotory evaporator. The residue was dissolved in 2 ml of water and centrifuged. Usually, 10–100 μl of the supernatant was injected into the HPLC for the analysis of PTCA.

HI Hydrolysis of Melanins and Tissues

The amounts of samples for each hydrolysis were the same as described for the $KMnO_4$ oxidation. A sample was suspended in 10 ml of 57% HI containing 200 mg of H_3PO_3 and heated under reflux for 20 h. The hydrolysate was evaporated to dryness in a rotory evaporator at 70°C and the residue was taken up in 10 ml of 0.1 M HCl and centrifuged. Usually, 10–100 μl of the supernatant was injected into the HPLC for the analysis of AHP, but appropriate dilution was necessary for pheomelanic samples. 3-Aminotyrosine was used as the standard.

RESULTS

Analytical Conditions

Reaction conditions for the permanganate oxidation of melanins have been elaborated upon by others [6,11] and therefore were adapted with minor modifications. Fig 2 demonstrates typical chromatograms of the oxidation products of synthetic eumelanin and eumelanic tissue; PTCA was the major UV-absorbing product and was easily determined. Reproducibility of PTCA determination was moderate. The coefficients of variation for 3 determinations ranged from 5–20%.

HI hydrolysis of pheomelanic pigment and tissue gave high yields of AHP. HPLC analysis of AHP, however, presented some difficulties because of poor separation and rapid elution of 2 AHP isomers. The difficulties were overcome by using, as the mobile phase, a buffer containing octanesulfonate to retard basic AHP. Typical chromatograms of the hydrolysis products of synthetic pheomelanin and pheomelanic tissue are shown in Fig 3. AHP was quantified with the electrochemical detector by taking advantage of its reducing property. AHP isomers gave a sharp, single peak which was well separated from peaks of other hydrolysis products such as dopa. Inasmuch as the 2 isomers of AHP had nearly identical responses to the detector,

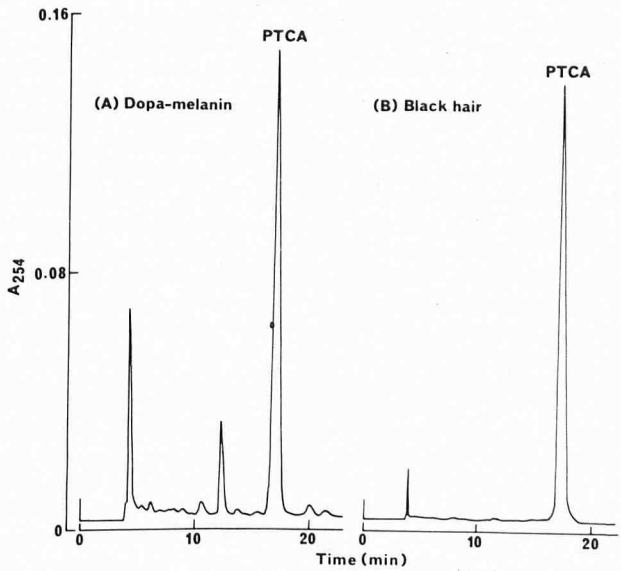

FIG 2. Chromatograms of permanganate oxidation products of (*A*) dopa-melanin and of (*B*) black mouse hair. Injected amounts were equivalent to (*A*) 400 μg of dopa-melanin and to (*B*) 400 μg of black mouse hair. Chromatographic conditions are described in *Materials and Methods.*

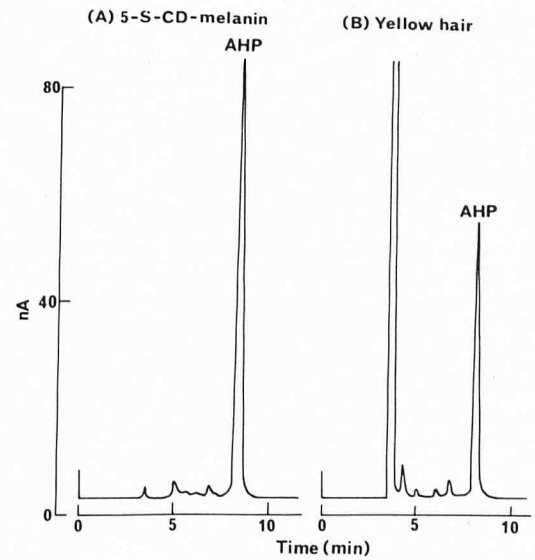

FIG 3. Chromatograms of HI hydrolysis products of (*A*) 5-S-cysteinyldopa-melanin (5-S-CD-melanin) and of (*B*) yellow mouse hair. Injected amounts were equivalent to (*A*) 200 μg of 5-S-cysteinyldopa-melanin and to (*B*) 10 μg of yellow mouse hair. Chromatographic conditions are described in *Materials and Methods.* AHP and dopa appeared at 8.2 and 6.8 min, respectively.

we chose as the reference standard the 3-amino-4-hydroxy isomer (3-aminotyrosine), a commercially available isomer.

The optimum reaction time for the HI hydrolysis of melanins was determined by using 5-S-cysteinyldopa-melanin. As the yields of AHP became constant after 20 h of reflux, this reaction time was adapted throughout the study. Reproducibility of AHP determination was excellent because of the simple procedure of hydrolysis.

Permanganate Oxidation and HI Hydrolysis of Melanins and Related Compounds

The results of permanganate oxidation and HI hydrolysis of melanins and related compounds are summarized in Table I. Oxidation of synthetic dopa-melanin gave a 0.3% yield of PTCA. Melanin in sepia melanosomes, a natural eumelanin, afforded a 1.9% yield of PTCA. However, PTCA is not a specific indicator of eumelanin; synthetic 5-S-cysteinyldopa-melanin also gave a 0.08% yield of PTCA. Mixed-type melanins gave PTCA in a yield intermediate between the synthetic eu- and pheomelanin.

There seemed to be 3 reasons for the low yields of PTCA from synthetic as well as natural eumelanins: (1) The insoluble property of eumelanin may be a factor unfavorable for higher yields: this was in agreement with the fact that dopa itself gave a higher yield of PTCA than dopa-melanin. (2) Permanganate may have a low selectivity with respect to the site of attack: this view was confirmed by the relatively low yield of PTCA from 5-hydroxyindole-2-carboxylic acid which possesses the skeleton of eumelanin monomer (Fig 1). (3) The product PTCA itself is unstable under the oxidation conditions: treatment of PTCA with $KMnO_4$ for 60 min resulted in only a 10% recovery of PTCA.

The yield of PTCA from dopa-melanin was much lower than those from natural melanins. This fact seemed to suggest that synthetic dopa-melanin is much more heterogeneous with respect to the monomer unit than are natural eumelanins. This possibility was supported by the fact that the chromatogram of the oxidation products of dopa-melanin was much more complex than those from natural eumelanins, as exemplified in Fig 2.

Table I also indicates that acid hydrolysis of melanins with HCl resulted in a sharp decrease in the yields of PTCA, e.g., 0.3–0.08% in dopa-melanin and 1.9–0.8% in sepia melanosome-melanin. Thus, the treatment with hot HCl appeared to alter the structural properties of eumelanin to a great extent.

TABLE I. *Permanganate oxidation and HI hydrolysis of melanins and related compounds*

Material	Percent yield, wt/wt (n)[a]	
	PTCA ($KMnO_4$ oxidation)	AHP (HI hydrolysis)
Synthetic melanin		
Dopa-melanin	0.29 (3)	0.000
5-S-Cysteinyldopa-melanin	0.079 (3)	20.0 (2)
Mixed-type melanin[b]	0.14 (3)	12.0 (2)
Dopa-melanin[c]	0.081	—
Natural melanin		
Sepia melanosome-melanin[d]	1.9 (3)	<0.001
Sepia melanosome-melanin[c]	0.77 (2)	—
B16 melanosome-melanin[c]	0.26 (2)	0.018
HP melanosome-melanin[c]	0.21	—
Black-hair melanin (C57BL mouse)[c]	0.47	—
Related compound		
Dopa	0.46	—
5-S-Cysteinyldopa	0.14	0.000
5-Hydroxyindole-2-carboxylic acid	4.8 (3)	—

[a] Averages and the numbers (n) of determinations.

[b] Prepared from a mixture of dopa and 5-S-cysteinyldopa in a ratio of 1:1 (M) or 1:1.6 (weight).

[c] Insoluble material left after HCl hydrolysis [9].

[d] Lyophilized sepia melanosomes were used and the yield was corrected for the melanin content of 59% (see Table II).

HI hydrolysis of 5-S-cysteinyldopa-melanin gave a very high yield (20%) of AHP while dopa-melanin gave no trace of the compounds. Mixed-type melanins gave AHP in a yield intermediate between the synthetic eu- and pheomelanin. Only trace amounts of AHP were detected in the HI hydrolysates of melanosome-melanins from sepia ink and B16 melanoma. Thus, AHP appeared to be a specific, excellent indicator of pheomelanin.

Eu- and Pheomelanin Contents in Tissue

To delineate the concentrations of eu- and pheomelanins with the levels of PTCA and AHP, melanogenic tissues such as hair, and melanoma and nonmelanogenic tissues were analyzed after $KMnO_4$ oxidation and HI hydrolysis. The tissue contents of eu- and pheomelanins, shown in Table II, were obtained by multiplying those of PTCA and AHP by factors of 50 and 5, respectively. The 2% yield of PTCA from sepia melanosome-melanin and the 20% yield of AHP from 5-S-cysteinyldopa-melanin served as the basis for the calculation. The eumelanin levels thus obtained cannot be applied to pheomelanic tissues. However, this did not pose a serious problem, inasmuch as the yields of PTCA from synthetic and natural pheomelanins were only trace amounts.

Melanin Contents in Hair

Black mouse hair contained a high level of eumelanin but only a trace level of pheomelanin, while yellow mouse hair contained a high level of pheomelanin. White mouse hair contained no trace of eumelanin but a trace level of pheomelanin. These results were in accord with the melanogenesis responsible for each type of hair coloration, i.e., eumelanic in black mouse hair, pheomelanic in yellow, and amelanotic in white. Acid-soluble and -insoluble fractions of yellow hair contained 0.55% and 0.33% of pheomelanin, respectively. AHP in the acid-soluble fraction may be derived from trichochromes which are acid-soluble pheomelanic pigments with dimeric structure [2]. Human black hair contained eumelanin at a level much lower than that of mouse black hair. In accord with this finding it was found that acid hydrolysis of human black hair yielded only a trace amount of insoluble material.

Melanin Contents in Lyophilized Melanosomes

Melanin in sepia melanosomes was found to be exclusively eumelanic [16]. Eumelanin content in B16 melanosomes was more than 10 times higher than in HP melanosomes, while pheomelanin content, although negligible, was higher in HP than in B16 melanosomes. The eumelanin levels calculated from PTCA values corresponded well with the amounts of insoluble material obtained by HCl hydrolysis.

Melanin Contents in Melanoma Tissues and Their Fractions

The eumelanin level in B16 melanoma was more than 10 times higher than that in HP melanoma. Most of melanins in B16 and HP melanomas were found in the melanosome fractions. Both B16 and HP melanomas were found to be eumelanic, but the contribution of pheomelanin was much greater in HP than in B16 melanoma. It is interesting that in the soluble fractions of B16 and HP melanomas, especially in those of the latter, small amounts of both eu- and pheomelanins were found. However, the possibility cannot be ruled out that the formation of melanin took place during the fractionation procedure. Although most of the oxidation procedures were done with acid-insoluble fractions, the fractionation with 0.4 M $HClO_4$ can be omitted, as indicated in Table II.

Melanin Contents in Normal Nonmelanogenic Tissues

Eumelanin contents in the liver and kidney of mice and in human serum were negligible. Also, these normal tissues contained only trace amounts of pheomelanin.

TABLE II. *Estimation of relative contents[a] of eumelanin and pheomelanin in hair, melanosomes, melanomas, and normal tissues*

Tissue	Percent content, wt/wet wt (n)[b]		
	Eumelanin[a] (PTCA of $KMnO_4$ oxidation)	Pheomelanin[a] (AHP of HI hydrolysis)	Melanin[c] (HCl hydrolysis)
Hair			
Black (C57BL mouse)[d]	6.5 (2)[c]	0.02	9.3
Yellow (C57BL, A^y mouse)[d]	0.18	1.3 (2)	—
White (A/J mouse)	nd[e] (<0.001)	0.02	—
Black (Japanese)	0.67 (3)	0.02	—
Melanosome (lyophilized)			
Sepia melanosome	57 (3)	<0.01	59
B16 melanosome	32 (2)	0.07	30
HP melanosome	2.3 (2)	0.20	3.0
Melanoma and its fractions			
B16 melanoma	2.6, 0.95, [1.7][f]	0.003	—
HP melanoma	0.1, 0.075, [0.14][f]	0.008	—
B16 melanosome fraction	0.88 (2)	0.0008	—
HP melanosome fraction	0.06 (2)	0.003	—
B16 soluble fraction	0.006	0.0005	—
HP soluble fraction	0.02	0.002	—
Normal tissue			
Liver (A/J mouse)	nd (<0.001)	0.0002	—
Kidney (A/J mouse)	nd (<0.001)	0.0004	—
Serum (human)	nd (<0.001)	0.0002	—

[a] Obtained by multiplying the PTCA concentration by a factor of 50 and the AHP concentration by a factor of 5.
[b] Averages and the numbers (n) of determinations.
[c] Insoluble material left after HCl hydrolysis [9].
[d] The genetic background of yellow and black mice was reported previously [3].
[e] Not detected.
[f] Values in brackets were obtained with intact melanoma by omitting the fractionation with 0.4 M $KMnO_4$.

DISCUSSION

The isolation of melanins from the tissues has been required to determine the content and the class of melanin pigments, and their degradation products have been analyzed qualitatively or semiquantitatively. Eumelanin has also been identified by the formation of 5,6-dihydroxyindole on alkali fusion [6,10,-11,15]. This method, however, did not appear to be suitably applicable to melanin quantification in the tissues.

This study provided a new method for analyzing quantitatively the content and class of melanin pigments in the tissues. The method is simple and rapid, inasmuch as it does not require the isolation of melanins from the tissues. The rationale of the method is that permanganate oxidation of eumelanin gives PTCA as the major pyrrolic product [6,11], while HI hydrolysis of pheomelanin yields AHP in addition to other phenolic amino acids [2,12]. Thus, PTCA is a quantitatively significant indicator of eumelanin, while AHP is a specific indicator of pheomelanin. The degradation products of PTCA and AHP were determined by HPLC. Sepia melanosome-melanin and synthetic 5-S-cysteinyldopa-melanin served as reference standards for eumelanin [16] and pheomelanin [12], respectively.

In this study, synthetic dopa-melanin was found to be much more heterogeneous with respect to the monomer units than natural eumelanins. The fact that the yields of PTCA from eumelanins were low (2%) does not mean that the contents of the units of 5,6-dihydroxyindole and 5,6-dihydroxyindole-2-carboxylic acid in eumelanins were also low. Ito and Nicol [13] reported that permanganate oxidation of 5,6-dihydroxyindole-2-carboxylic acid and its oligomers also gave low yields (5–6%) of PTCA. This study also revealed that acid treatment of melanin to remove proteins causes alteration in the structural properties of melanins.

The tissue contents of eu- and pheomelanins were estimated by multiplying those of PTCA and AHP by 50 and 5, respectively. A good correlation was observed between the eu- and pheomelanin contents with the type of melanogenesis in normal mouse hair. Eumelanin was found in black hair at a high level whereas pheomelanin was found in yellow hair at a high level. Based on this control study of normal hair, the melanosomes of both B16 and HP melanomas were found to be eumelanic. The melanin content in B16 melanosomes appeared to be more than 10 times higher than that in HP. Thus, a striking difference in the color of melanosomes between B16 and HP melanomas seems largely to be related not to the class but to the content of melanin pigments, although B16 and HP melanosomes reveal quite distinct ultrastructures, i.e., brown-black, eumelanosome-like granules in B16 and reddish- or light-brown, pheomelanosome-like granules in HP melanosomes. While we are confident that the eumelanin/pheomelanin ratio obtained by this method could serve as an index for the classification of various melanins, further work is necessary to confirm whether the method can be applied well to natural melanins whose chemical and physical properties may often be intermediate between those of typical eumelanin and pheomelanin [1,5,10]. It may also be necessary to find an indicator other than PTCA for eumelanin calculation inasmuch as the yield of PTCA after $KMnO_4$ oxidation is approximately 2%, thus making it necessary to multiply it by 50 for estimation of tissue content.

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Identical Lectin Binding Patterns of Human Melanocytes and Melanoma Cells In Vitro

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Cell surface glycoconjugate patterns of human epidermal cells and of melanoma cells (MC) in primary culture derived from 11 primary and metastatic melanomas were investigated using fluorescent and horseradish peroxidase conjugated lectins for visualization at the light and electron microscopic level. The lectin labeling profiles of human melanocytes (M) and MC were found to be identical. According to their binding patterns, the lectins tested were grouped into three categories: (1) lectins binding to both keratinocytes (K) and M/MC, irrespective of neuraminidase pretreatment (concanavalin-A, wheatgerm agglutinin, succinylated wheatgerm agglutinin); (2) lectins binding to K but not to M/MC, irrespective of neuraminidase pretreatment (*Ulex europaeus* agglutinin I); (3) lectins binding to K, but to M/MC only after neuraminidase pretreatment (soybean, *Helix pomatia*, and peanut agglutinins). Untreated M were reactive for soybean and peanut agglutinins only at contact sites with K. Since the lectins from soybean, *Helix*, and peanut bind specifically to D-galactose and N-acetyl-D-galactosamine residues, we conclude that these particular glycoconjugates are normally masked by sialic acid on M/MC surfaces and can be unmasked by neuraminidase. These features, which have been previously observed in guinea pig M, appear to be interspecies surface markers of melanocytic cells which remain unaltered in the course of malignant transformation.

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Abbreviations:
α-D-GalNAc: N-acetyl-alpha-D-galactosaminyl
α-D-Glc: alpha-D-glucosyl
α-D-Man: alpha-D-mannosyl
α-L-Fuc: alpha-L-fucosyl
β-D-Gal: β-D-galactosyl
β-D-GlcNAc: N-acetyl-β-D-glucosaminyl
Con-A: concanavalin A
HPA: *Helix pomatia* agglutinin
K: keratinocyte(s)
M: melanocyte(s)
MC: melanoma cell(s)
NANA: sialic acid
PBS: phosphate-buffered saline
PNA: peanut agglutinin
SBA: sobyean agglutinin
UEA I: *Ulex europaeus* agglutinin I
WGA: wheatgerm agglutinin

Melanocytes (M) differ from keratinocytes (K) in vivo as well as in vitro by a number of important behavioral parameters, which may be related to differences in surface organization (shape, proliferation, motility, substrate adhesion). In previous studies on guinea pig epidermal cell cultures, we have shown that such differences can in fact be demonstrated for the distribution and lateral mobility of anionic surface sites [1]. As a further distinguishing feature, terminal D-galactose (D-Gal) and N-acetyl-galactosamine (GalNAc) residues were found masked by sialic acid on guinea pig M surfaces in lectin binding experiments; unmasking could be achieved by neuraminidase [2].

In this study we demonstrate that human M and K display very similar lectin binding patterns to guinea pig cells. Furthermore, the lectin binding patterns of normal human M and their response to neuraminidase were found to be identical to that of cultured melanoma cells (MC). The masking of terminal D-Gal and GalNAc by sialic acid therefore appears to be an interspecies surface marker of melanocytic cells and to remain remarkably stable in the course of malignant transformation.