Melanin Chemistry and Melanin Precursors in Melanoma

Shosuke Ito, Ph.D., and Kazumasa Wakamatsu, Ph.D.
School of Hygiene, Fujita-Gakuen Health University, Toyoake, Aichi, Japan

Two basic classes of melanin pigments are synthesized in mammals: brown-to-black eumelanin and yellow-to-reddish pheomelanin (Fig 1) [1]. In melanocytes, a specific enzyme tyrosinase converts tyrosine to dopa and then to dopaquinone, which is cyclized to leucodopachrome in a rapid, nonenzymatic reaction. This compound is oxidized to dopachrome, a red pigment, which in turn gives 5,6-dihydroxyindole (5,6DHI) by decarboxylation and to some extent 5,6-dihydroxyindole-2-carboxylic acid (5,6DHI2C) by rearrangement [2]. These dihydroxyindoles are highly reactive and are further oxidized to give rise to a eumelanin polymer. If dopaquinone encounters cysteine or glutathione, pheomelanin is formed via cysteinyldopas. Among these melanin precursors, 5-S-cysteinyldopa (5-S-CD), a major isomer of cysteinyldopas, has been shown to reflect the metastasis of melanoma [3].

Until recently, it was generally accepted that eumelanin is made mostly from 5,6DHI, but not from 5,6DHI2C. However, the following evidence has accumulated in recent years indicating the significance of 5,6DHI2C as an alternative precursor of eumelanin: 1) urine from melanoma patients contains high levels of the O-methyl derivatives of 5,6DHI2C in addition to the derivatives of 5,6DHI [4]; 2) divalent cations such as Cu^{2+} catalyze the conversion of dopachrome to 5,6DHI2C but not to 5,6DHI [5], and these ions are rich in melanocytes [6]; 3) the dopachrome conversion factor found by Pawelek's group also catalyzes the conversion of dopachrome to 5,6DHI2C [7] but not to 5,6DHI, as previously proposed [8].

This review describes the results of our recent studies dealing with the melanin chemistry and melanin precursors in melanoma.

Re-examination of the Structure of Eumelanin Eumelanins are highly heterogenous polymers consisting of various monomer units [9,10]. These units include 5,6DHI (I), 5,6DHI2C (II), pyrrole (III), and pyrrole-2-carboxylic acid (IV) (Fig 2); the latter two units are derived from the former two units by peroxidative cleavage of o-quinone form. The purpose of this study [11] was to estimate the degree of incorporation of the 5,6DHI2C-derived units (II and IV) in intact natural eumelanins. The analytical methods used were elemental analysis, acid treatment to liberate CO_2, and permanganate oxidation to form pyrrole-2,3,5-tricarboxylic acid (PTCA) [12].

Melanins were prepared either by tyrosinase oxidation at pH 6.8 or by air oxidation at pH 8.0 or 10.0. Table I summarizes the yields and C/N ratios of synthetic and natural melanins and the results of acid treatment and permanganate oxidation of synthetic melanins, eumelanic tissues, and related compounds.

The C/N ratios of synthetic dopa-melanins indicate that one to nearly two carbon atoms per monomer unit were lost in the course of oxidation, and the carbon loss was more pronounced at higher pH. With respect to the C/N ratio, enzymatically prepared dopa-melanins, tyrosine-melanin, and dopamine-melanins were similar to each other and also to 5,6DHI-melanin. The C/N ratios of 5,6DHI-melanin and 5,6DHI2C-melanin suggest significant degrees of decomposition of the indole ring. Table I also shows that acid treatment of synthetic melanins resulted in considerable loss of nitrogen atom, suggesting the decomposition of the indole or pyrrole ring.

On acid treatment, 90% of the monomer units of 5,6DHI2C-melanin lost CO_2. Furthermore, 5,6DHI2C, 5-hydroxyindole-2-carboxylic acid, and pyrrole-2-carboxylic acid liberated amounts of CO_2 that were equal to or slightly more than that of the theoretical value. These results indicate that the amount of CO_2 liberated by acid treatment reflects the content of the carboxyl group attached to the indole or pyrrole ring in eumelanin.

The CO_2 liberation from dopa-melanins increased with pH of the buffer, indicating that oxidation at higher pH favors the retention of the CO_2 carboxyl group or the cleavage of the indole ring to form the carboxyl group. Approximately 10% of the monomer units of enzymatically prepared dopa-melanins were shown to liberate CO_2 when corrected for the CO_2 liberation from dopamine-melanins. This fact suggests that ca. 10% of the carboxyl group of dopa was incorporated into dopa-melanins in the forms of the monomer units II and IV.

The yields of PTCA from enzymatically prepared dopa-melanins were $< 10\%$ than that from 5,6DHI2C-melanin, indicating that these melanins consist mostly of units I and III. Dopa-melanin prepared at pH 10.0 gave a high yield of PTCA, a low C/N ratio, and a high CO_2 liberation. These results suggest that the pyrrole-2-carboxylic acid unit IV comprises a significant fraction of this melanin.

Acid treatment of melanosome preparations and black mouse hair yielded ca. 60% of CO_2 per monomer unit of melanin. The yields were intermediate values between those from 5,6DHI-melanin and 5,6DHI2C-melanin. Furthermore, the yields of PTCA from eumelanic tissues were 30% - 40% of that from 5,6DHI2C-melanin. From these results, it is concluded that 5,6DHI2C-derived units II and IV may comprise one-third to one-half of the monomer units of intact, natural eumelanins.

The present study shows that natural eumelanins are not homopolymers of 5,6DHI, but rather copolymers of 5,6DHI and 5,6DHI2C in various ratios. It appears that 5,6DHI2C could play a

Reprint requests to: Dr. Shosuke Ito, Ph.D., School of Hygiene, Fujita-Gakuen Health University, Toyoake, Aichi 470-11, Japan.

Abbreviations:
5B6MI: 5-benzyloxy-6-methoxyindole
6B5MI2C: 6-benzyloxy-5-methoxyindole-2-carboxylic acid
5,6DHI: 5,6-dihydroxyindole
5,6DHI2C: 5,6-dihydroxyindole-2-carboxylic acid
5H6MI: 5-hydroxy-6-methoxyindole
6H5MI: 6-hydroxy-5-methoxyindole
5H6MI2C: 5-hydroxy-6-methoxyindole-2-carboxylic acid
6H5MI2C: 6-hydroxy-5-methoxyindole-2-carboxylic acid
HPLC: high-performance liquid chromatography
PTCA: pyrrole-2,3,5-tricarboxylic acid

0022-202X/89/$03.50

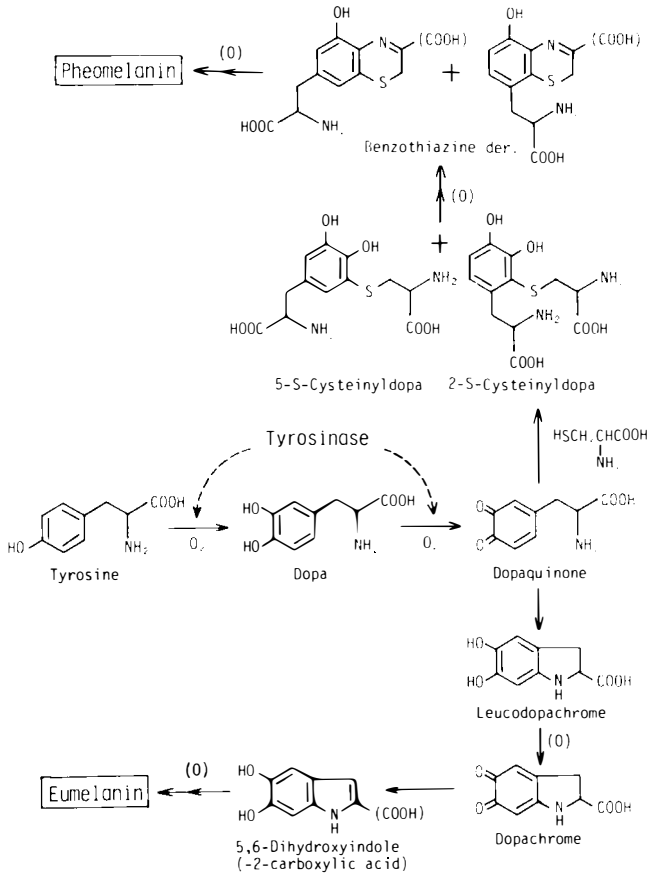

Figure 1. Outlines of eumelanogenesis and pheomelanogenesis in melanocytes.

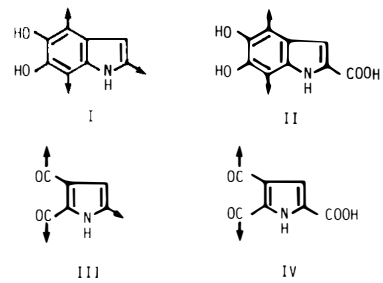

Figure 2. Various monomer units present in eumelanins. *I:* 5,6DHI unit, C/N ratio = 8; *II:* 5,6DHI2C unit, C/N ratio = 9; *III:* pyrrole unit, C/N ratio = 6; *IV:* pyrrole-2-carboxylic acid unit, C/N ratio = 7. Units *I* and *II* may be present in both reduced, o-diphenolic form and oxidized, o-quinone form.

more important role in melanogenesis than previously believed. It is also shown that 1) enzymatically prepared dopa-melanins are quite different from natural eumelanins in terms of the content of carboxyl group; 2) dopa-melanins prepared at higher pH retain higher percentages of the carboxyl group of dopa and contain higher percentages of pyrrole units; and 3) melanins are decomposed to a significant extent on acid treatment, the method commonly used to isolate eumelanins from natural sources.

Preparation of Eumelanin-Related Metabolites In the course of the previous study, we found it necessary to prepare the eumelanin precursors 5,6DHI and 5,6DHI2C in subgram quantities. However, reported methods [13] require several steps of tedious chemical reactions. We describe here one-step synthesis of 5,6DHI and 5,6DHI2C based on the conversion of dopachrome generated in situ by ferricyanide oxidation of dopa (Fig 3) [14].

5,6DHI and 5,6DHI2C are formed and partly O-methylated in melanocytes. These metabolites are excreted in urine either free or

Table I. Preparation and Analysis of Synthetic Melanins and Eumelanic Tissues

Material	Yield (mg)	C/N ratio[a]	CO_2 (mol%)[b]	PTCA (μg/mg)[c]
Synthetic melanin[d]				
Dopa-melanin, 12.5 mM, tyrosinase	149	8.00 (8.36)	21	1.7
Dopa-melanin, 2.5 mM, tyrosinase	114	7.91	28	1.3
Dopa-melanin, 2.5 mM, tyrosinase[e]	141	7.89	27	1.3
Dopa-melanin, 12.5 mM, pH 8.0	120	7.92	30	1.3
Dopa-melanin, 2.5 mM, pH 8.0	120	7.70	40	2.1
Dopa-melanin, 12.5 mM, pH 10.0	110	7.24 (7.86)	46	3.1
Dopa-melanin, 2.5 mM, pH 10.0	68	7.21	54	7.7
Dopa-melanin, 2.5 mM, pH 10.0[e]	146	7.12	36	4.4
Tyrosine-melanin, 2.5 mM, tyrosinase	89	7.75	16	1.3
Dopamine-melanin, 12.5 mM, tyrosinase	75	7.54 (8.71)	11	0.7
Dopamine-melanin, 2.5 mM, tyrosinase	61	7.67	16	0.9
5,6DHI-melanin, 12.5 mM, tyrosinase[f,g]	172	7.76 (8.10)	24	1.3
5,6DHI2C-melanin, 12.5 mM, tyrosinase[g]	212	8.37 (8.31)	90	30
Eumelanic tissue				
Sepia melanosomes	—	— (7.73)	62[h]	10[h]
B16 melanosomes	—	— (8.39)	56[h]	8.5[h]
C57BL black mouse hair	—	— (8.66)	65[h]	12[h]
Related compounds				
5,6DHI2C	—	—	106	10
5-hydroxyindole-2-carboxylic acid	—	—	98	32
Pyrrole-2-carboxylic acid	—	—	116	—

[a] In parentheses are the C/N ratios for melanins obtained after acid treatment (110°C, 24 h).

[b] 20 mg of a melanin or a related compound or 30–200 mg of a tissue sample was heated in 20 ml of 6 M HCl under reflux for 24 h. The CO_2 gas liberated was determined as $BaCO_3$.

[c] Determined by the method of Ito & Fujita [12].

[d] 1 mM of precursor was dissolved in 80 ml (12.5 mM) or 400 ml (2.5 mM) of a buffer of pH 6.8, 8.0, or 10.0. The mixture was incubated at 37°C for 4 h in the presence of mushroom tyrosinase (8 mg) under oxygen current or for 24 h under air. After acidification to pH 1, the black melanin was collected by centrifugation and washed 3 times with 0.1 M HCl.

[e] Prepared in the presence of 5 mg of catalase.

[f] Prepared from 5,6DHI containing 9% of 5,6DHI2C.

[g] Prepared in the presence of 0.05 mmol of L-dopa.

[h] Calculated on the basis of melanin recovered after acid treatment.

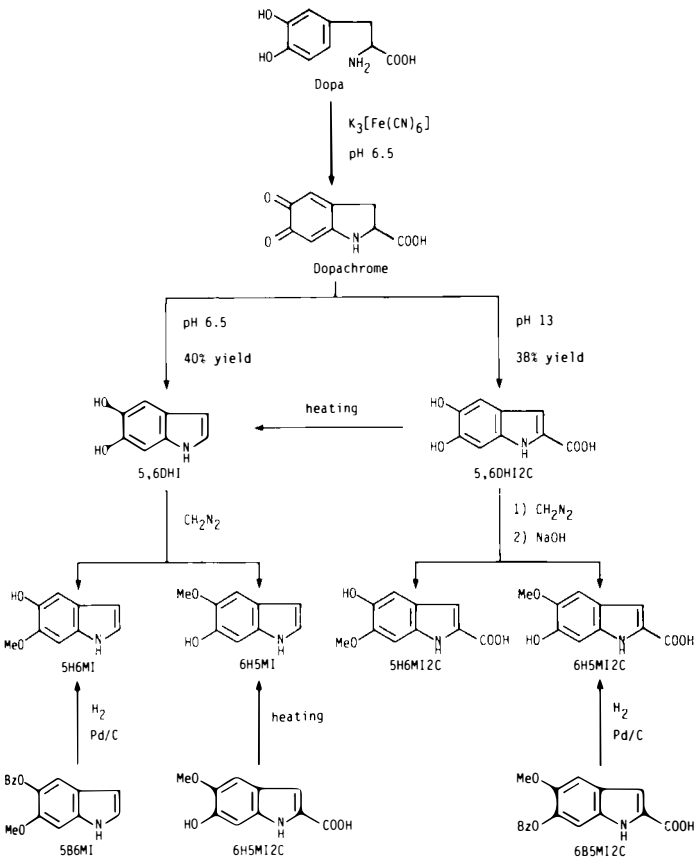

Figure 3. An outline of preparation of 5,6DHI, 5,6DHI2C, and their O-methyl derivatives.

conjugated with glucuronic or sulfuric acid [15]. The eumelanin precursors and their O-methyl derivatives are found in urine of melanoma patients at high levels and in healthy subjects at low levels [4,16]. The O-methyl derivatives are 5-hydroxy-6-methoxyindole (5H6MI), 6-hydroxy-5-methoxyindole (6H5MI), 5-hydroxy-6-methoxyindole-2-carboxylic acid (5H6MI2C), and 6-hydroxy-5-methoxyindole-2-carboxylic acid (6H5MI2C). Methods for preparing the O-methyl derivatives of 5,6DHI and 5,6DHI2C have been reported, but they are also tedious, multi-step methods [13]. Therefore, we developed simplified methods to prepare these O-methyl metabolites in small quantities.

When dopa is oxidized either enzymatically by tyrosinase [2] or chemically by ferricyanide [17] or silver oxide [2], a wine red solution of dopachrome is formed. Dopachrome undergoes either decarboxylation to form 5,6DHI or rearrangement to form 5,6DHI2C [2]. We successfully applied these reactions to prepare the two eumelanin precursors. 5,6DHI was prepared in 40% isolation yield by keeping the dopachrome solution at pH 6.5 for 3 h, followed by extraction and recrystallization. High-performance liquid chromatography HPLC analysis of the reaction mixture showed the ratio of 5,6DHI to 5,6DHI2C to be ca. 70:1. 5,6DHI could also be obtained in 20% yield by heating 5,6DHI2C in decalin.

Stravs-Mombelli and Wyler [18] have recently reported that the rearrangement of dopachrome to 5,6DHI2C is accelerated at pH 13. We applied this reaction for preparing 5,6DHI2C in 38% isolation yield. Although the ratio of 5,6DHI2C to 5,6DHI in the reaction mixture was ca. 12:1, the isolated material was found to be homogeneous.

The two isomeric O-methyl derivatives of 5,6DHI were prepared by treatment with diazomethane [19]; 5H6MI and 6H5MI were obtained, after separation and purification, in 12% and 2.6% yield, respectively. These O-methyl derivatives could be prepared more easily through other routes (Fig 3). Decarboxylation of 6H5MI2C by heating in decalin gave 6H5MI in 38% yield. Debenzylation of commercially available 5-benzyloxy-6-methoxyindole (5B6MI) by catalytic hydrogenation on palladium afforded 5H6MI in 61% yield.

The two isomeric O-methyl derivatives of 5,6DHI2C were prepared by treatment with diazomethane followed by alkaline hydrolysis of the individual methyl esters; 5H6MI2C and 6H5MI2C were obtained in 46% and 11% yields, respectively. 6H5MI2C could also be prepared in 78% yield by debenzylation of commercially available 6-benzyloxy-5-methoxyindole-2-carboxylic acid (6B5MI2C).

In conclusion, eumelanin precursors 5,6DHI and 5,6DHI2C could be prepared in subgram quantities based on the conversion of dopachrome generated in situ by ferricyanide oxidation of dopa. We also described the methods for preparing the two isomeric O-methyl derivatives of these eumelanin precursors.

Melanin-Related Metabolites in Urine of B16 Melanoma-Bearing Mice Among melanin-related metabolites, 5-S-CD has been most extensively studied as a biochemical marker of melanoma progression [3]. However, recent studies by Pavel's group have indicated that the O-methyl derivatives of 5,6DHI and 5,6DHI2C may also serve the same purpose [4]. They have also shown that among dopa, 5-S-CD, 5H6MI, and 5H6MI2C, 5H6MI2C is the best urinary marker of melanin formation in normal skin pigmentary system [16]. In order to determine which of the two markers, 5H6MI2C or 5-S-CD, reflects better the progression of melanoma, we studied the urinary excretion of these metabolites in mice bearing B16 melanoma [20].

Experiments were performed on 8 C57BL/6 mice and two mice were used for control. Five million cells of B16 melanoma were inoculated s.c., and urine samples were collected 3 d before inoculation and every 3 d after inoculation into a beaker containing 1 ml of 20% acetic acid and 20 mg of sodium metabisulfite. 5H6MI2C and 6H5MI2C were determined by HPLC with fluorescent detection [21] and 5-S-CD by HPLC with electrochemical detection [22].

In the control urine 5H6MI2C and 6H5MI2C were hardly detectable. On the other hand, melanoma-bearing mice excreted large amounts of 5H6MI2C and 6H5MI2C. Figure 4 shows the changes in the urinary excretion of 5H6MI2C and 5-S-CD and the tumor volume in melanoma-bearing mice. Tumors became palpable 9 d after tumor inoculation and grew exponentially for 6 d thereafter.

The excretion values of 5H6MI2C and 5-S-CD on day 15 were 3 orders of magnitude higher than the control values. The excretion value of 5H6MI2C started to increase on as early as day 6 and increased rapidly in parallel with the tumor volume until day 15. The decline after day 15 may be related to the onset of tumor necrosis. The excretion level of 6H5MI2C was one order of magnitude lower than that of 5H6MI2C.

The excretion value of 5-S-CD showed a pattern similar to that of 5H6MI2C. However, some differences were noted: a temporary increase on day 3 and a slight increase even after day 15. These increases may be ascribed to the lysis of melanoma cells. Of the two markers 5H6MI2C and 5-S-CD, the former appears to reflect better the progression of melanoma at the early stage: 5H6MI2C had a lower control level and a higher excretion on day 9, when the tumor was barely detectable.

The present study has demonstrated that 5H6MI2C is a better marker of melanoma progression in a model system of B16 mouse melanoma. On the other hand, 5-S-CD appears to reflect better the lysis of melanoma cells. The analytical method employed is simple and reproducible, because the urine sample is directly injected into the HPLC system without any pretreatment. Furthermore, 5H6MI2C was found to be fairly stable: when stored at −30°C, 5H6MI2C in melanoma urine decomposed by only 19% in 2 mo.

Melanin-Related Metabolites in Healthy Japanese The previous study using a B16 melanoma model suggested the potential usefulness of 5H6MI2C as a biochemical marker of melanoma progression. Normal values for urinary excretion of 5H6MI2C and its isomer 6H5MI2C have been reported [16,23]. However, great variations were observed among people of different skin colors. It appeared necessary to establish normal values for urinary excretion of 5H6MI2C and 6H5MI2C in healthy Japanese.

In our previous study as well as in the studies by other groups [16,23,24], HPLC with fluorescent detection has been used to determine the eumelanin-related indolic metabolites. We noticed, however, that HPLC with electrochemical detection gave lower, more reliable values for 5H6MI2C. A typical example is shown in Fig 5.

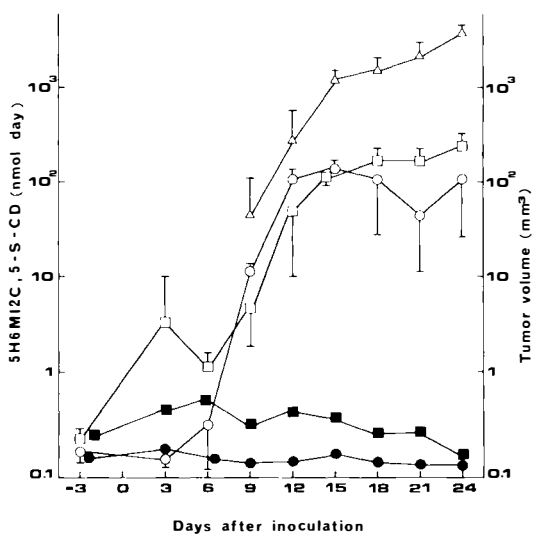

Figure 4. Changes in the urinary excretion of 5H6MI2C and 5-S-CD and the tumor volume in B16 melanoma-bearing mice. Eight C57BL/6 mice were used for experiment and 2 were for control. *Open circles:* 5H6MI2C in the experimental group; *solid circles:* 5H6MI2C in the control group; *open squares:* 5-S-CD in the experimental group; *solid squares:* 5-S-CD in the control group; *open triangles:* tumor volume. Data for the experimental group were mean ± SD, calculated for the log values. Tumor volume was calculated by the formula: long axis × (short axis)² × 1/2.

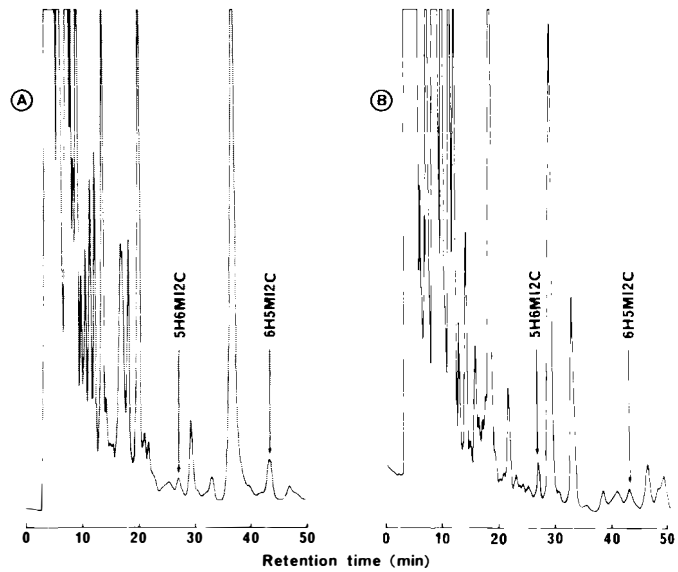

Figure 5. HPLC chromatograms of a urine sample from a healthy Japanese. *A:* monitored with a fluorescent detector (excitation and emission wavelengths were 315 and 390 nm, respectively); 5H6MI2C 0.98 μmol/day, 6H5MI2C 0.41 μmol/day. *B:* monitored with an electrochemical detector (applied potential, 750 mV vs an Ag/AgCl electrode), 5H6MI2C 0.52 μmol/day, 6H5MI2C 0.42 μmol/day. A Yanaco ODS-A reversed-phase column (4.6 × 250 mM) was used at 45°C. The mobile phase was 0.1 M potassium phosphate buffer, pH 2.1, containing 1 mM Na_2EDTA : methanol, 88 : 12 (v/v). The flow rate was 1.0 ml/min.

Table II compares the excretion values of 5H6MI2C and 6H5MI2C determined with fluorescent and electrochemical detectors. The fluorescent detection gave nearly two times higher value for 5H6MI2C than the electrochemical detection, although the values for 6H5MI2C were almost identical. On the basis of these results, we recommend electrochemical detection for 5H6MI2C determination. We also noticed that attempts to shorten the retention times by increasing the methanol concentration in the mobile phase or by raising the column temperature resulted in artificially higher values.

Table II also compares the urinary excretion of 5H6MI2C, 6H5MI2C, and 5-S-CD. Of the two eumelanin-related metabolites, 5H6MI2C was the major isomer, although the difference was not as great as in B16 melanoma-bearing mice (see above). The excretion ratio 6H5MI2C/5-S-CD was found to be 0.47, which was close to the ratios reported for people with red or blond hair and fair skin (0.36 and 0.50, respectively), but not to the ratio for people with black hair and dark skin (1.85) [23].

The present study has shown that the electrochemical detection is preferable to the fluorescent detection and that the excretion level of 5H6MI2C is comparable to that of 5-S-CD in healthy Japanese.

Table II. Urinary Excretion of 5H6MI2C, 6H5MI2C, and 5-S-CD in Healthy Japanese

Detection	(μmol/day)[a] 5H6MI2C	6H5MI2C	5-S-CD
Fluorescent	1.06 ± 0.77	0.34 ± 0.17	—
Electrochemical	0.60 ± 0.24	0.35 ± 0.20	0.74 ± 0.32

[a] Mean ± SD for 20 healthy subjects (10 male and 10 female, mean age: 29).

It was recently found that the HPLC conditions should be changed to the following in order to obtain higher selectivity for both 5H6MI2C and 6H5MI2C (see the legend to Fig. 5): emission wavelength, 420 nm; applied potential, 600 mV.

REFERENCES

1. Prota G: Recent advances in the chemistry of melanogenesis in mammals. J Invest Dermatol 75:122–127, 1980
2. Mason H: The chemistry of melanin. III. Mechanism of the oxidation of dihydroxyphenylalanine by tyrosinase. J Biol Chem 172:83–99, 1948
3. Rorsman H, Agrup G, Hansson C, Rosengren E: Biochemical Recorders of Malignant Melanoma. In: MacKie RM (ed.). Pigment Cell, Vol 6. Basel, Karger, 1983, pp 93–115
4. Pavel S, Elzinga H, Muskiet FAJ, Smit JM, Mulder NH, Koops H: Eumelanin-related indolic compounds in the urine of treated melanoma patients. J Clin Chem Clin Biochem 24:167–173, 1986
5. Palumbo A, d'Ischia M, Musuraca G, Prota G: Effect of metal ions on the rearrangement of dopachrome. Biochem Biophys Acta 925:203–209, 1987
6. Flesch P: The role of copper in mammalian pigmentation. Proc Soc Exp Biol Med 70:79–83, 1949
7. Korner AM, Gettins P: Synthesis in vitro of 5,6-dihydroxyindole-2-carboxylic acid by dopachrome conversion factor from Cloudman S91 melanoma cells. J Invest Dermatol 85:229–231, 1985
8. Korner AM, Pawelek J: Dopachrome conversion: a possible control point in melanin biosynthesis. J Invest Dermatol 75:192–195, 1980
9. Nicolaus RA: In: Lederer E (ed.). Chemistry of Natural Products. Melanins. Paris, Hermann, 1968, pp 68–91
10. Swan GA, Waggott A: Studies related to the chemistry of melanins. Part X. Quantitative assessment of different types of units present in dopa-melanin. J Chem Soc C:1409–1418,1970
11. Ito S: Re-examination of the structure of eumelanin. Biochem Biophys Acta 883:155–161, 1986
12. Ito S, Fujita K: Microanalysis of eumelanin and pheomelanin in hair and melanomas by chemical degradation and liquid chromatography. Anal Biochem 144:527–536, 1985
13. Benigni JD, Minnis RL: The synthesis of 5,6-dihydroxyindole and some of its derivatives (1). J Heterocyclic Chem 2:387–392, 1965
14. Wakamatsu K, Ito S: Preparation of eumelanin-related metabolites 5,6-dihydroxyindole, 5,6-dihydroxyindole-2-carboxylic acid, and their O-methyl derivatives. Anal Biochem 170:335–340, 1988
15. Pavel S, Muskiet FAJ, de Ley L, The TH, van der Slik W: Identification of three indolic compounds in a pigmented-melanoma cell-culture supernatant by gas chromatography-mass spectrometry. J Cancer Res Clin Oncol 105:275–279, 1983
16. Westerhof W, Pavel S, Kammeyer A, Beusenberg FD, Cormane R: Melanin-related metabolites as markers of the skin pigmentary system. J Invest Dermatol 89:78–82, 1987
17. Bu'Lock JD, Harley-Mason J: Melanin and its precursors. Part III. New synthesis of 5,6-dihydroxyindole and its derivatives. J Chem Soc C:2248–2252, 1951
18. Stravs-Mombelli L, Wyler H: Reinvestigation of the formation of dopa-melanin: new aspects of the autoxidation of dopa. In: Bagnara J, Klaus SN, Paul E, Schartl M (eds.). Pigment Cell 1985. Tokyo, University of Tokyo Press, 1985, pp 69–76
19. Pavel S, Muskiet FAJ: Synthesis of deuterium labelled eumelanin (precursor) metabolites. J Labelled Comp and Radiopharm 20:101–110, 1983
20. Wakamatsu K, Ito S, Fujita K: Melanin-related metabolites in urine of melanoma-bearing mice. Acta Derm Venereol (Stockh) 68:385–389, 1988
21. Pavel S, Slik WV: Analysis of eumelanin-related indolic compounds in urine by high-performance liquid chromatography with fluorimetric detection. J Chromatogr 311:154–159, 1984
22. Ito S, Kato T, Maruta K, Fujita K, Kurahashi T: Determination of DOPA, dopamine, and 5-S-cysteinyl-DOPA in plasma, urine, and tissue samples by high-performance liquid chromatography with electrochemical detection. J Chromatogr 311:154–159, 1984
23. Wirestrand LE, Hansson C, Rosengren E, Rorsman H: Melanocyte metabolites in the urine of people of different skin color. Acta Derm Venereol (Stockh) 65:345–348, 1985
24. Hansson C: 6-Hydroxy-5-methoxyindole-2-carboxylic acid in normal human urine. Acta Derm Venereol (Stockh) 64:185–190, 1984