Adsorption of molybdenum by melanin

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Abstract

Background: Melanin is detectable in various sense organs including the skin in animals. It has been reported that melanin adsorbs toxic elements such as mercury, cadmium, and lead. In this study, we investigated the adsorption of molybdenum, which is widely recognized as a toxic element, by melanin.

Methods: Molybdenum level of the mouse skin was measured by inductively coupled plasma mass spectrometry. The pigmentation level of murine skin was digitalized as the \( L^* \) value by using a reflectance spectrophotometer. An in vitro adsorption assay was performed to confirm the interaction between molybdenum and melanin.

Results: Our analysis of hairless mice with different levels of skin pigmentation showed that the level of molybdenum increased with an increase in the level of skin pigmentation (\( L^* \) value). Moreover, our analysis by Spearman’s correlation coefficient test showed a strong correlation (\( r = -0.9441, p < 0.0001 \)) between \( L^* \) value and molybdenum level. Our cell-free experiment using the Langmuir isotherm provided evidence for the adsorption of molybdenum by melanin. The maximum adsorption capacity of 1 mg of synthetic melanin for molybdenum was 131 \( \mu \)g in theory.

Conclusion: Our in vivo and in vitro results showed a new aspect of melanin as an adsorbent of molybdenum.

Keywords: Melanin, Molybdenum (Mo), Molybdate, Langmuir

Introduction

Melanin is a stable pigment that is widely found in various animals and plants [1]. Melanin is synthesized from melanocytes in various sense organs including the skin in animals [2]. Previous studies have shown the protective effects of melanin in the skin against ultraviolet light irradiation [3, 4]. Previous studies have also shown adsorption of toxic elements such as mercury, lead [5], cadmium [6], and barium [7] by melanin. However, there is a lack of evidence for interactions of melanin with various toxic elements strictly based on chemical theory.

We previously established constitutively activated RET, a receptor-type tyrosine kinase [8–10], hairless transgenic mice (HL-RET-mice) of lines 242 and 304, which have different levels of skin pigmentation [7]. We objectively evaluated the skin pigmentation levels for HL-RET-mice of lines 242 and 304 and wild-type hairless mice as \( L^* \) values by using a reflectance spectrophotometer [7, 11].

Moreover, we newly showed the interaction of melanin with barium after showing that the level of barium increased with increases in the levels of pigmentation in the skin in the HL-RET-mice of lines 242 and 304 and wild-type hairless mice [7].

Molybdenum is widely recognized as a toxic element. In fact, previous studies showed that dysregulation of molybdenum was associated with neurological abnormalities [12], osteoporosis [13], and liver dysfunction [14]. At present, however, there is no chemical evidence directly showing an interaction between molybdenum and melanin.

In this study, we tried to clarify the association between melanin and molybdenum using the skin of hairless mice with different pigmentation levels. We then tried to strictly elucidate the interaction between molybdenum and melanin based on chemical adsorption theory.

Materials and methods

Mice

RET-transgenic hairless mice (HL-RET-mice) with constitutively different pigmentation were developed by crossing hairless mice (Hos:HRM) with RET-transgenic...
mice of lines 242 [3] and 304 [2], respectively, following the method previously described [11]. All of the mice were kept in the Animal Research Center of Nagoya University under the conditions of controlled temperature and humidity. Mice were used for digitalization of pigmentation levels in the skin and also for measurement of levels of molybdenum that had spontaneously accumulated in the skin.

Digitalization for pigmentation levels of mouse skin
Previous studies showed that a reflectance spectrophotometer (CR-400; Konica Minolta Sensing Inc., Japan) can be used to evaluate the levels of skin pigmentation in mice as well as in humans [11, 15]. Following the methods, the pigmentation level of murine skin was digitalized as the $L^*$ value.

**Measurement of molybdenum level in mouse skin**
Molybdenum level of the mouse skin was measured by inductively coupled plasma mass spectrometry (ICP-MS; 7500cx, Agilent Technologies) following the method previously described [16]. In brief, to measure the level of molybdenum that had spontaneously accumulated in the skin, dorsal skin was obtained and digested by the wet ashing method for ICP-MS.

**Batch adsorption assay using synthetic melanin**
An adsorption assay using synthetic melanin (CAS # 8049-97-6, Nacalai Tesque Inc., Japan) was performed following the method described previously [17]. The stock solution of molybdenum was prepared by dissolving ammonium molybdate (Wako Pure Chemical Industries Ltd., Japan). To determine the contact time for equilibrium adsorption, 1 mg synthetic melanin was suspended in 400 μL molybdenum solution with the concentration of 115 μg molybdenum/mL. After incubation for 0, 5, 20, 60, 90, and 120 min, the suspension was centrifuged at 13,200 rpm for 10 min for solid-liquid separation. For adsorption kinetics and isotherm studies, 1 mg of synthetic melanin was incubated with 0.1, 1, 40, 150, 300, 600, 900, and 1200 μg of molybdenum/ml for 60 min followed by solid-liquid separation. The concentrations of molybdenum in the supernatant and the precipitate were measured by ICP-MS after ashing the samples.

**Langmuir model analysis of an equilibrium adsorption isotherm**
The amount of molybdenum adsorbed by synthetic melanin was calculated by plotting $C_e$ on the x-axis and $Q_e$ on the y-axis after confirming that the Langmuir adsorption isotherm model was suitable as shown previously [18]. To calculate the maximum adsorption capacity, $C_e$ on the x-axis and $C_e/Q_e$ on the y-axis were plotted to draw Langmuir linear graph, and the predicting equation was obtained according the method previously described [19].

$$QE = V(Co - Ce)/M$$

$Q_e$: molybdenum adsorbed by melanin (μg/mg)
$V$: volume of molybdenum solution (0.4 mL)
$Co$: initial molybdenum concentration (μg/mL)
$Ce$: molybdenum concentration in the supernatant (μg/mL)

$$Ce/Qe = (1/a + Ce)/Qmax$$

$a$: adsorption equilibrium constant
$Qmax$: maximum adsorption capacity (μg/mg)
$M$: weight of melanin (1 mg)

**Statistical analysis**
Multiple comparisons were performed using one-way analysis of variance (ANOVA) with Tukey’s test. The correlations were performed using Spearman coefficient. All statistical analyses were performed using SPSS 25.0 (IBM Corp., Armonk, NY, USA). $p < 0.05$ was considered statistically significant.

**Results**
**Hairless mice with different skin pigmentation levels**
Skin pigmentation levels of wild-type hairless mice and HL-RET-mice of lines 242 and 304 (Fig. 1a) were digitalized by using a reflectance spectrophotometer as the $L^*$ value according to the method previously shown [11]. As shown in Fig. 1b, the $L^*$ values in HL-RET-mice of lines 242 and 304 were decreased by 28% and 38%, respectively, compared to the $L^*$ value in wild-type mice, suggesting that the pigmentation levels in the skin of HL-RET-mice are increased.

**Correlation between levels of dorsal skin pigmentation and molybdenum**
The levels of molybdenum that had spontaneously accumulated in the dorsal skin of HL-RET-mice of lines 242 and 304 were 1.9-fold and 9.8-fold higher, respectively, than the level in wild-type mice (Fig. 2a). Moreover, there was a significant correlation ($r = -0.9441$, $p < 0.0001$) between the $L^*$ values of wild-type hairless mice and HL-RET-mice of lines 242 and 304, and the levels of molybdenum that had spontaneously accumulated in the dorsal skin by Spearman’s correlation coefficient test (Fig. 2b).

**Adsorption of molybdenum by synthetic melanin**
Interaction between molybdenum and melanin was then examined on the basis of the chemical adsorption theory. Concentrations of melanin-bound molybdenum and unbound molybdenum are shown with time courses in Fig. 3a. The equilibrium adsorption isotherm was obtained
after 60-min incubation and was analyzed by the Langmuir adsorption isotherm model (Fig. 3b, c). The maximum adsorption capacity of synthetic melanin for molybdenum was 131 μg/mg in theory (Fig. 3c).

Discussion

We demonstrated that molybdenum levels spontaneously accumulated in the strains of mice with different levels of skin pigmentation were correlated with levels of digitalized skin pigmentation ($L^*$ values). Our previous study showed a correlation ($r = -0.54$) between barium levels and levels of digitalized skin pigmentation expressed as $L^*$ values after preparing genetically modified hairless mice with different pigmentation levels [7]. We then proved an interaction between synthetic melanin and barium in vitro according to the chemical adsorption theory using the Langmuir isotherm [7]. Since Spearman’s correlation coefficient ($r = -0.9441$) between molybdenum levels and $L^*$ values was stronger than that between barium concentrations and $L^*$ values, direct evidence of an interaction between synthetic melanin and molybdenum in vitro was again obtained in this study. Our results suggest that correlations between $L^*$ values and elements might be a useful screening system for detecting elements adsorbed by melanin as a primary screening.

Previous studies showed that the maximum adsorption capacities (Qmax) of mercury and lead to synthetic melanin synthesized from *Pseudomonas stutzeri* were 82.4 μg/mg and 147.5 μg/mg, respectively [5]. A previous study showed that melanin purified from squid ink maximumly adsorbed 19.6 μg of chromium/mg [20]. Indole-5, 6-quinone unit-based synthetic melanin maximumly adsorbed 38.5 μg of chromium/mg.
Further study is needed to clarify the biological significance of adsorption of molybdenum by melanin.

**Conclusion**
This study demonstrated a strong correlation between levels of skin pigmentation and molybdenum in murine skin. Our cell-free analysis then showed adsorbed molybdenum by melanin using the Langmuir isotherm. Thus, this study chemically showed a new aspect of melanin as an adsorbent of molybdenum.

**Additional file**

**Additional file 1:** Maximum adsorption capacities of melanin species for metal elements. (PPTX 39 kb)

**Abbreviations**
HL: Hairless; ICP-MS: Inductively coupled plasma mass spectrometry

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**Availability of data and materials**
All data generated or analyzed during this study are available from the corresponding author on reasonable request.

**Authors' contributions**
WC, KH, YO, and MK conceived and designed the experiments. WC, YO, AI, and LKI performed the experiments. WC, AT, NO, and YQD analyzed and interpreted the data. WC, KH, and MK wrote the manuscript. All authors read and approved the final manuscript.

**Ethics approval**
Animal Care and Use Committee (approval number 30258) and Use Committee and Recombination DNA Advisory Committee (approval numbers 18-57) in Nagoya University authorized all murine experiments.

**Consent for publication**
Not applicable

**Competing interests**
The authors declare that they have no competing interests.

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