主論文の要旨

Adsorption of molybdenum by melanin

〔 メラニンによるモリブデンの吸着 〕

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【Background】

Melanin is a stable pigment that is widely found in various animals and plants. Previous studies have reported the adsorption of toxic elements such as mercury, cadmium, and lead by melanin. Molybdenum is widely recognized as a toxic element, and it has been shown that dysregulation of molybdenum was associated with neurological abnormalities, osteoporosis, and liver dysfunction. However, there is limited information about the interaction of melanin with molybdenum strictly based on chemical adsorption theory. In this study, we tried to clarify the association between melanin and molybdenum using the skin of hairless mice with different pigmentation levels and elucidate the interaction between molybdenum and melanin based on chemical adsorption theory.

【Methods】

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

The pigmentation level of murine skin was digitalized as the L* value using a reflectance spectrophotometer following the methods shown by previous studies.

The molybdenum level of the mouse skin was measured by inductively coupled plasma mass spectrometry (ICP-MS). To measure the level of molybdenum that had spontaneously accumulated in the skin, the dorsal skin was obtained and digested by the wet ashing method for ICP-MS.

An adsorption assay was performed using synthetic melanin. We prepared the stock solution of molybdenum by dissolving ammonium molybdate. 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 wet ashing the samples.

The amount of molybdenum adsorbed by synthetic melanin was calculated by plotting Ce on the x-axis and Qe on the y-axis after confirming that the Langmuir adsorption isotherm model was suitable as shown previously. To calculate the maximum adsorption capacity, Ce on the x-axis and Ce/Qe on the y-axis were plotted to draw Langmuir linear graph and the predicting equation was obtained.

【Results】

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. 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.

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).

The Interaction between molybdenum and melanin was then examined based on 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】

In this study, we showed that the levels of molybdenum spontaneously accumulated in the strains of mice with different levels of skin pigmentation were correlated with levels of digitalized skin pigmentation (L* values). In our previous study, we found a correlation (r=-0.54) between barium levels and levels of digitalized skin pigmentation and then proved an interaction between synthetic melanin and barium *in vitro* according to the chemical adsorption theory using the Langmuir isotherm. Since the Spearman's correlation coefficient (r=-0.9441) between molybdenum levels and L* values was stronger than that between barium levels and L* values, the 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 of mercury and lead to synthetic melanin synthesized from Pseudomonas stutzeri were 82.4 μg/mg and 147.5 μg/mg, respectively, melanin purified from squid ink maximumly adsorbed 19.6 μg of chromium/mg, and Indole-5, 6-quinone unit-based synthetic melanin maximumly adsorbed 38.5 μg of barium/mg. Our results showing 131 μg of molybdenum/mg as the maximum adsorption capacity of synthetic melanin may be reasonable compared with the other adsorbents previously reported. Thus, synthetic melanin is a potential candidate as an adsorbent of

molybdenum. It remains unclear whether the adsorption of molybdenum by melanin biologically plays a beneficial role or not. Further study is needed to clarify the biological significance of the adsorption of molybdenum by melanin.

【Conclusion】

Our 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.