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# Further Analysis of the Relation between Oxidative Damage and Ca Accumulation Examined by X-ray Contact Spectromicroscopy with Electronic Zooming Tube

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## 1 Introduction

We have been comparing the distributions between Ca content and oxidative damage in human hair in order to discriminate Ca accumulation by external stimuli from the one supplied from blood flow around hair matrix cells at the hair root [1]. The information about the distribution of the latter Ca may be worth for applications to medical diagnosis such as forecasting human breast cancer [2]. Recently we found that increase in Ca caused by bleach treating oxidative damage mainly distributes in outer region called cuticle and cortex, while Ca distribution in medulla, a central part of hair, seems to be independent of oxidative damage [3, 4], suggesting that Ca in medulla may be useful for the medical purpose. In the present study, we extended this observation to the samples of age-related positions of a hair from its root.

#### 2 Materials and Methods

For the mapping of cysitne, cysteic acid and Ca X-ray contact microscopy with an electronic zooming tube with a resolution of about 0.5  $\mu$ m was employed at the S-K edge and Ca-K edge at BL-11B. Since highly sensitive Ca mapping with X-ray fluorescence analysis has not unfortunately been available any more at BL-4A, this study was also aimed to study the applicability of the electronic zooming tube system having lower sensitivity than that of X-ray fluorescence analysis.

Human hair specimens with medulla were selected using an IR scope. For the bleach treatment in the laboratory, hair specimens were soaked twice in a solution containing 1.2 % ammonia and 3.5 % hydrogen peroxide for 30 min. Ca soaking was performed with 10 mM CaCl<sub>2</sub> for 2 days with a daily change of the solution. These treatments were done for different specimens from the same lot. At the positions of around 1 cm and 10 cm from the root side, they were cut at the thickness of about 20  $\mu$ m, and then placed on a SiN membrane with 100 nm thickness. The opposite side of the membrane was coated with Au as a photocathode of the zooming tube.

## 3 Results and Discussion

Fig. 1 shows X-ray images of cystine, cysteic acid and Ca in human hair specimens at the position of 10 cm from the root. Bleach treatment with subsequent Ca soaking increased Ca content particularly in the cuticle and cortex regions (panel c) by comparing image of untreated specimen (panel a), although the Ca images did not have a good contrast and sensitivity compared with X-ray fluorescence images. In the untreated hair little Ca was detected in cuticle and cortex regions, while medulla had detectable amount of Ca. These results were also obtained for specimens of 1cm from the root. However the results cannot exclude the possibility that Ca in medulla also increased with bleach treatment in addition to the intrinsic Ca. Determination of chemical form of Ca in medulla and other areas is definitely useful. Based on our measurement of XANES of various Ca compounds including CaCl<sub>2</sub> [1], comparison of XANES profiles in hair with that of CaCl<sub>2</sub> is planned.



Fig. 1: Cystine, cysteic acid and Ca distributions in human hair with medulla. (a) control, (b) bleaching, (c) soaking in CaCl<sub>2</sub> solution after bleaching.

## References

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