NANO LETTERS

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Vol. 0, No. 0
A–E

Early Use of PbS Nanotechnology for an Ancient Hair Dyeing Formula

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Received June 28, 2006; Revised Manuscript Received August 4, 2006

ABSTRACT

Lead-based chemistry was initiated in ancient Egypt for cosmetic preparation more than 4000 years ago. Here, we study a hair-dyeing recipe using lead salts described in text since Greco-Roman times. We report direct evidence about the shape and distribution of PbS nanocrystals that form within the hair during blackening. It is remarkable that the composition and supramolecular organization of keratins can control PbS nanocrystal growth inside a hair.

For thousands of years, cosmetics have been used and were made by the judicious combination of naturally available minerals with oils, various creams, or water. Expertise included the chemistry that was developed to prepare natural or synthetic cosmetic materials such as lead white (lead carbonates) as foundation cream and galena for eye makeup.[1,2] Since the Greco-Roman period, organic hair dyes obtained from plants such as henna have been used, but other unusual formulas based on lead compounds, such as the recipes describing several methods to dye hair and wool black, were also common. It is remarkable that these Greco-Roman techniques have been used up to modern times: related recipes were described by Arabian authors during the medieval period,[3] during the Renaissance as practical application of alchemical knowledge,[4] and by modern chemists, from the *Encyclopedie* of Diderot and d'Alembert[5] through to the present day.[6] In these cases, the same specific formula is provided: a mixture of lead oxide, PbO, and slaked lime, $Ca(OH)_2$, with a small amount of water to form a paste, is applied on the hair. Successive applications on gray or light hair give rise to the black color. It is known that the blackening of hair is due to the precipitation of galena (PbS) crystals during the chemical treatment: the lead is provided from the paste deposited on the hair shafts, and the sulfur involved in the reaction comes from the amino acids of hair keratins.[6]

Here, we show that a consequence of these practices consists of synthesizing galena (lead sulfide) nanocrystals to dye hair black. With a size of about 5 nm, their appearance is quite similar to PbS quantum dots synthesized by recent materials science techniques.[7,8] In contrast to modern nanotechnology, the dyeing process is characterized by basic chemistry methods and has been developed more than 2000 years ago with low-cost natural materials. Although the presence of PbS particles in the cuticle and the cortex modifies the optical aspect of the hair shaft irreversibly, the hair mechanical properties are essentially unaffected because of the extremely small size and volume fraction of the crystals. This opened the application of the dye formula for 2000 years. What is particularly surprising in this reaction is that, despite the structural complexity of hair and its relative chemical inertness, metal sulfide nanoparticles easily crystallize and get organized inside this biomaterial. Finally, sulfur-rich peptides in the matrix surrounding supramolecular-organized keratin proteins may serve as nanoscale reactors.

The hair shaft contains three principal concentric histological regions: the cuticle, the cortex, and the medulla. The cylindrical cortex, enveloped in the cuticle, consists of macrofibrils, which can be considered as biological composites of α-helix-containing microfibrils roughly 7 nm in diameter embedded in an amorphous, sulfur-rich interfilamentary matrix. The large amount of cysteinyl residues in

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10.1021/nl061493u CCC: $33.50
Published on Web 09/01/2006

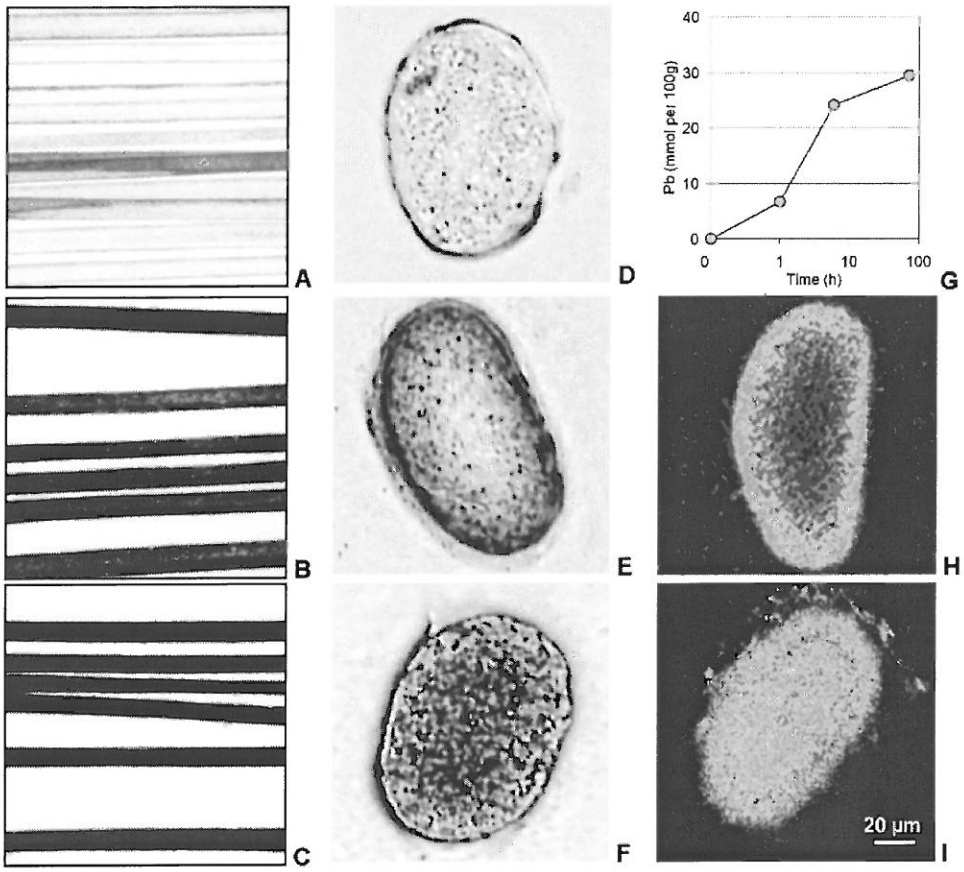

Figure 1. Optical macrophotographs of hair (A−C) and microphotographs (D−F) of the corresponding transverse cross sections (thickness = 10 μm), showing progressive blackening during treatment with lime and lead oxide in water (25 °C, pH = 12.5). (A and D) nontreated, (B and E) 6h, (C and F) 72h. (G) time dependence of total adsorbed lead concentration in the bulk sample measured by X-ray fluorescence spectroscopy; these analytical methods are nonspecific to galena but reveal all Pb species (mainly lead soaps and PbS). We assume that Pb uptake on lipids is limited to about 15 mmol per 100 g of hair according to ref 12. Pb maps of the respective treated samples are obtained by SEM-EDX, (H) 6 h, (I) 72 h, and show a progressive radial fixation from the cuticle to the center of the hair (concentration increasing from dark blue to green)

the amorphous matrix is responsible for very strong intra- and interchain bonds that confer highly unusual physical properties to the hair but also a high degree of chemical inertness.[9,10] Alternatively, it has been shown that various ions have the ability to penetrate the hair matrix, in as little as 30 min, for example, in the case of a dye in aqueous solution.[11]

We used an ancient recipe by immersing 50 mg of Caucasian blonde human hair in 50 mL ultrapure water with two reagents as described in the literature, that is, equal weights of $Ca(OH)_2$ (technical grade, Mercier, France) and PbO (analytical grade, Interchim, France). The solution thus obtained had a pH controlled by the solubility of slaked lime (pH = 12.5 at 25 °C) and the lead oxide provided a source of Pb ions. The dyeing, carried out for up to 3 days, resulted in a progressive blackening of the hair (Figure 1A−C) with a simultaneous fixing of lead, visible on cross sections by optical microscopy (Figure 1D−F) and Pb elemental mapping as shown in Figure 1H and I. X-ray fluorescence analyses allowed quantification of the Pb uptake with the internal standard method, as illustrated in Figure 1G. In addition, powder X-ray microdiffraction analysis of a dozen of treated hairs put in a 300-μm-diameter glass capillary shows the presence of galena crystals (Figure 2A). These crystals are nanoparticles, and their average size (4.8 nm) is found from the experimental patterns by the simulation shown in Figure 2B. Interestingly, we can note that the α-helical coiled structures in keratin proteins are preserved after the blackening reaction. The characteristic spots observed on the diffraction diagram correspond to the distance between the centers of two helices (equatorial repetition k_1, perpendicular to the fiber axis, of 0.98 nm) and to the helix pitch (meridian repetition k_2, parallel to the fiber axis, of 0.515 nm).

The three-dimensional distribution of Pb in hair and its speciation are fundamental to the final appearance of the fiber, and this was investigated in transmission through the observation of cross sections by scanning confocal electron microscopy (SCEM)[12,13] and of longitudinal sections by high-resolution transmission electron microscopy (HRTEM). Usually, a staining procedure including various heavy atoms is required on the thin cross sections in order to contrast with the electronic images. These coloring techniques reveal specific histological regions in the hair. In our case, because

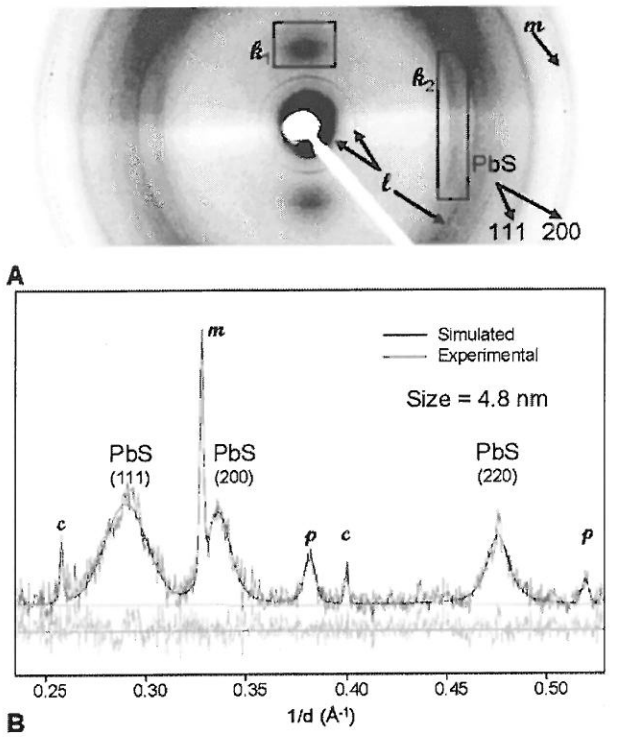

Figure 2. X-ray diffraction analysis of hairs after treatment with lime and lead oxide in water (25 °C, pH = 12.5) during 6 h. (A) Two-dimensionnal pattern showing the features of α-helical coiled structures in keratin proteins (k_1 and k_2) and the rings of Pb-soaps (l), β-PbO massicot (m), PbS Bragg lines (111) and (200). (B) Pattern of the same hairs showing remains of calcite (c) and portlandite $Ca(OH)_2$ (p). The Scherrer crystallite size of galena is estimated from the experimental pattern by the simulation shown.

of the affinity of lead for various groups (thiols, carboxyls), the blackening treatment allows similar observations without staining.

The Pb accumulations in the cuticle and cortex are revealed in Figure 3A: globular 200 nm aggregates are seen clearly in the cortex. We attribute these accumulations to lipid-associated compounds resulting from the interaction of Pb ions with the lipid components of the hair, for instance, Pb soaps of free fatty acids.[14] The characteristic rings of these soaps are also observed by X-ray diffraction (Figure 2A). Pb is also present between macrofibrils and inside macrofibrils, as shown in a higher magnification view of the cortex. In Figure 3B, we illustrate how these nanoparticles decorate the contour of macrofibrils. These regions correspond to a cell membrane complex containing mainly a lipid mixture.[15] The presence of Pb was checked by X-ray microanalysis in the SCEM.

Small particles are accumulated in the macrofibrils (Figure 3B). High-resolution images and electron diffraction patterns have established the crystalline nature of these particles: they are PbS nanocrystals (Figure 4A and B). The particles size in Figure 4B suggests an average particle size around 5 nm, although particles as small as 4 nm and as large as 15 nm have been observed. These observations are consistent with the average size of galena crystallites estimated from XRD (Figure 2B).

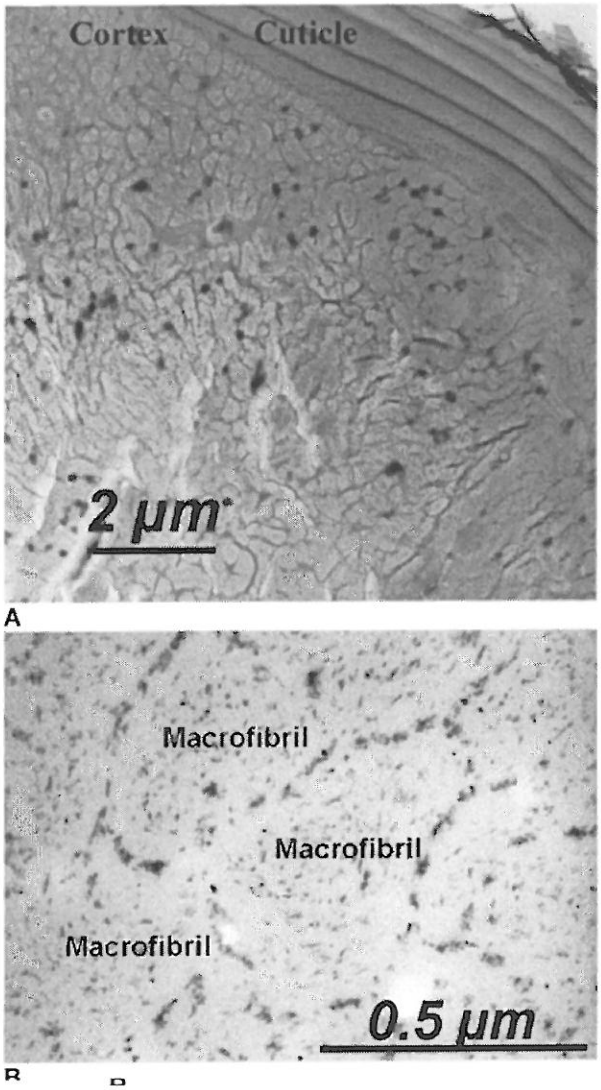

Figure 3. Observation by electron microscopy (SCEM) of a 2-μm-thick transverse cross section of 6-h-treated hair. The contrast is due to the Pb fixation. (A) Pb accumulations located in the cortex and cuticle. (B) At higher magnification, small Pb particles are observed inside macrofibrils and at their interfaces.

Observations of longitudinal sections by TEM show that the PbS particles tend to be arranged in lines along the axis of the hair fiber (Figure 4C). A striking fact is that the distances between these lines is about 8−10 nm and correspond roughly to the distance between the microfibrils. We assume that lead ions react to form PbS in the sulfur-rich material in which the microfibrils are embedded. Lead-based hair-dying chemicals generate a kind of melanin substitute produced by synthesis of PbS inside hair. Natural black hair color is due to melanin clusters of ca. 300 nm dispersed within the colorless keratin-based cortex of hair. Blackening PbS particles are much smaller than melanin clusters by 4−5 orders of magnitude in volume. Moreover, unlike natural melanin, these in situ crystallized nanomaterials display a specific distribution pattern: at the scale of

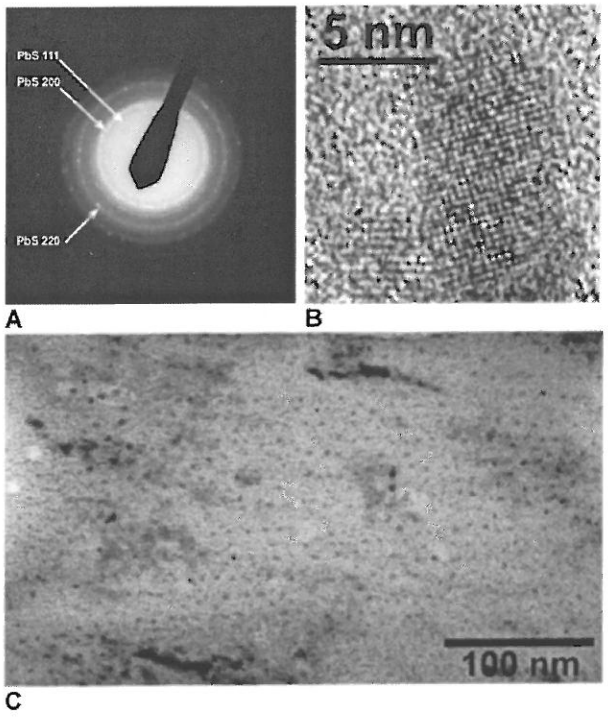

Figure 4. Observation and identification by electron microscopy (HRTEM) of PbS crystallites inside the cortex. (A) Electron diffraction pattern: the *d* spacing is consistent with the unit cell of PbS. (B) High-resolution micrograph of a representative PbS nanocrystal. (C) Longitudinal section of 3-day-treated hair.

10 nm, they are aligned in a way induced by the supramolecular organization of keratin microfibrils.

Three natural amino acids (cystine, cysteine, and methionine) can provide the sulfur needed for the formation of galena. We analyzed the amino acids in treated hair samples using acid hydrolysis (HCl 9N) of peptide links and separation by ion exchange chromatography (Hitachi L-8500 amino acid analyzer, column 60 mm, diameter 4,6 mm filled with 2622SC resin). Only one natural amino acid showed significant changes in a lead-free solution containing lime (Figure 5A) or during the dyeing process (Figure 5B): the amount of cystine decreases and is accompanied by the appearance of two unusual abiogenic amino acids, lanthionine and lysino-alanine. The lanthionine contains two alanyl residues bridged by a thioether linkage and was first detected in 1941 in alkaline hydrolysates of wool.[14] The degradation of cystinyl residues results in the formation of dehydroalanyl and S-thiocysteinyl residues by reaction with alkali, according to a β-elimination mechanism.[17,18] Upon addition of cysteinyl and lysyl residues to very reactive dehydroalanyl residues, lanthionyl and lysino-alanyl residues are formed. This mechanism assumes the release of sulfur, which can subsequently participate in the growth of galena crystals in the hair shaft. We have also determined that a solution with lead oxide and without lime (pH = 10.5) provides neither cystine degradation products nor blackening of the hair. In fact, for the dyeing process to occur, high alkalinity of the solution is required.

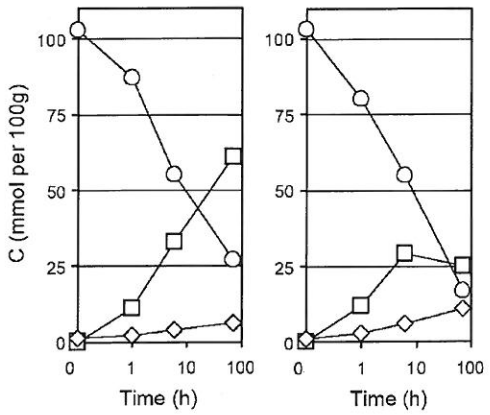

Figure 5. Amount of sulfur-containing amino acids during the treatment of human hair immersed in water with (left) calcium hydroxide, (right) calcium hydroxide, and lead monoxide. The treatment was carried out from 1 h to 3 days, under moderate stirring, and induced the transformation of cystine (○) into lanthionine (□) and lysino-alanine (◇).

We note that a significant amount of sulfur-rich amino acids is lacking after 3 days of dyeing treatment (Figure 5B); the lanthionine amount is lower than that in the case of lime treatment (Figure 5A). We can assume that side reactions for cystine degradation occurred and other complexes were formed but were not detected with our analytical method. For instance, the formation of a complex between cysteinyl residues, Pb ions, and other residues (cysteinyl, carboxyl, etc.) is possible.

Hair blackening has been shown to be due to the formation of PbS nanocrystals. Interestingly, the dyeing process discussed here is a remarkable illustration of synthetic nanoscale biomineralization using a formula dating from antiquity. This work has provided independent information on the supramolecular organization of hair, resulting from the decoration of specific areas in which Pb is fixed. Further studies with other metal ions are in progress to decipher the mechanisms of crystal growth and ion diffusion through the hair and to assess the ability of the matrix to act as a nanoreactor. Better control of the conditions for growth and organization of nanoparticles in organic matrix could open new perspectives in the development of nanocomposites.

Acknowledgment. We thank the French Ministry of Research for the ACI Grant "Chimie, Santé et Beauté dans l'Antiquité". This work was supported in part by the U.S. DoE under BES-MS W-31-109-Eng-38 at ANL and the SESAME microdiffraction project of the Région Ile de France. C.D. acknowledges the NSF for funding this research under grant INT-0205039. We thank Professor Arthur H. Heuer at Case Western Reserve University for providing access to the Tecnai F30 for high-resolution observations.

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NL061493U