An Enzymatic Method for Harvesting Functional Melanosomes After Keratin Extraction – Maximizing Resource Recovery from Human Hair

Nan Zhang (nanzhang@vip.jiangnan.edu.cn)  
Jiangnan University  https://orcid.org/0000-0003-4719-7771

Hui Ying Lai  
Nanyang Technological University - Jurong Campus: Nanyang Technological University

Archana Gautam  
Nanyang Technological University - Jurong Campus: Nanyang Technological University

Darien Yu De Kwek  
Nanyang Technological University - Jurong Campus: Nanyang Technological University

Yibing Dong  
Nanyang Technological University - Jurong Campus: Nanyang Technological University

Qiang Wang  
Jiangnan University

Kee Woei Ng  
Nanyang Technological University - Jurong Campus: Nanyang Technological University

Research Article

Keywords: Human hair, resource recovery, keratin, melanin, melanosome, sustainability

DOI: https://doi.org/10.21203/rs.3.rs-388421/v1

License: ☛ Ⓒ This work is licensed under a Creative Commons Attribution 4.0 International License.  
Read Full License
Abstract

Hair contains about 80% keratins and 1–3% melanin packaged in melanosomes. Both of which are high-value and functional raw materials that have potential applications in wide ranging fields. While keratin extraction has been widely refined, efficient methods of melanosome extraction are limited. The extraction of melanosomes requires complete removal of keratin, thus combined keratin extraction and melanosome isolation is logical. Herein, a successive process to harvest melanosomes after keratin extraction from human hair waste was developed. The yield of melanosome was about 1.3% of total hair mass. The structure of harvested melanosomes is well preserved based on surface morphology and interior ultrastructural observations using electron microscopy. The chemical structure, UV-filtering ability, and thermal stability of the melanosomes are examined to demonstrate preservation of native functions. Our strategy of combining melanosome isolation with keratin extraction is shown to be effective and significantly improves the total resource recovery efficiency from human hair waste.

1 Introduction

Human hair waste accumulated in waste streams causes environmental problems due to its poor degradability [1]. Direct burning or disposal in landfills cause secondary environmental pollution and wastage of resources[2]. Recently, complete utilization of hair resources is becoming the mainstream in waste hair resource management to convert human hair waste into high-value products [3].

Proteins constitute the major component of human hair fibers, representing about 95% of the total fiber by weight. Of these, keratins make up 85% of the total protein content of the fiber [4]. Human hair contains about 1-3% by weight of melanin packaged in melanosomes, and is one of the main source of natural melanin [5]. Melanin essentially determines structural coloration in organisms and has ultraviolet (UV) protective functions [6]. Besides, studies show that natural melanin also has unique physicochemical properties like intrinsic free radical quenching, broadband light absorption, humidity-dependent electronic semiconductivity, and radiative energy transferring ability [7]. Both keratins and melanin are high-value and functional materials. Extracted keratins from human hair have great potential in a myriad of applications in biomedical sciences, alternative plastics, functional composites and even electronic devices [8-10]; and melanin can be used in the areas of catalysis, biomedicine, energy storage, nano-composites, structural coloration and UV-filtering[11, 12].

Extracting both keratin and melanin requires degradation or dissolution of hair. However, because of the highly cross-linked disulfide and hydrogen bonds between keratins, hair is highly resistant to digestion or solubilization [13]. Initially melanin isolation processes involved using concentrated mineral acids and alkalis [14, 15], hydrazine/ethanol mixtures [16], or thioglycolic acid/phenol mixture [17] to remove the protein components. However, these isolated melanin suffered profound structural disruption like extensive decarboxylation [18, 19]. These thus necessitate a milder but efficient procedure for non-destructive isolation of melanin. Liu et al. concluded that melanin extracted with an enzymatic method remained intact and had comparable spectroscopic and morphological characteristics as those extracted
with acid-base procedures[5]. Subsequently, in most research about melanosome structure analysis in human hair, dithiothreitol (DTT) was used to cleave disulfide bonds in human hair, followed by proteinase K, papain, or trypsin either in combination with DTT or individually to hydrolyze the keratins to release the melanosomes [7, 13, 20-23]. However, this melanin isolation procedure is not only tedious and costly, but also causes severe hair keratin resource wastage if large-scale melanosome isolation is to be realized. Thus, acids or bases extraction methods are still used for hair melanin investigations [11, 24].

The general keratin extraction methods use acid, alkalis, ionic liquids, and combined urea and reducing regents to dissolve keratins [25-28]. Those methods dissolve both KAP (which located in matrix) and keratin together, thus the extracted keratins are mixers of KAP and keratins. Particularly, Fujii et al used DTT-urea system to extract KAP and keratins from hair separately and successively, inspiring us to combine keratins and melanin extraction together. Herein, a facile and continuous process combining keratins extraction using DTT-urea system and hydrolysis using esperase was developed to obtain both functional keratins and melanosomes for downstream application. Then the structure and performance of melanosomes were characterized in detail.

2 Materials And Methods

2.1 Preparation of hair residues after KAP and keratin extraction

Human hair was pretreated with an extraction protocol reported by Fuji et al [28]. Briefly, delipidized hair was first incubated in a KAP extraction solution [8 M urea (Chem-Impex), 200 mM DTT (Gold Biotechnology) and 25% ethanol in 25 mM Tris-HCl buffer at pH 9.5] at 50 °C for 72 hours. The extracted KAP fraction was filtered, and the remaining KAP-free hair residues were washed thoroughly with DI water and air-dried prior to the subsequent keratin extraction. KAP-free hair was incubated in keratin extraction solution [2.6 M thiourea (Sigma), 5 M urea, and 200 mM DTT in 25 mM Tris-HCl buffer at pH 8.5] for 24 hours at 50 °C. The keratins fraction was then filtered, while the residue was collected and used to isolate melanosome using the enzymatic treatment. The molecular weight of the KAP and keratins were characterized with sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

2.2 Enzymatic hydrolysis of hair residues and isolation of melanosomes

Hair residues (generated from 10 g KAP-free hair) were immersed in 25 mM Tris-HCl at pH 9.5 (total volume was 100 mL), and 200 µL esperase (Sigma, Product number. P5860, activity: 8 KNPU E/mL) was added. The whole system was incubated at 50 °C for 4 h, then the hydrolysate was filtered through a nylon cloth (800 mesh). The collected filtrate was centrifuged at 8,586 g for 10 min twice. Then the pellets were resuspended in DI water and centrifuged at 8,586 g for 5 min, with supernatant discarded.
This process was repeated to completely remove contaminants. Finally, the pellet was freeze-dried into melanosome powder. The schematic of melanosome isolation procedures is shown in Scheme 1.

2.3 Preparation of polyacrylamide-melanosome (PAM-M) film

PAM-M film was prepared based on a method previously reported [29]. Briefly, 2.5 g acrylamide (AM), 50 mg ammonium persulphate (APS), and 3 mg N,N methylenebisacrylamide (BIS) were dissolved in 10 mL distilled water then the required amount of hair extracted melanosomes was mixed in an ice bath under stirring. Then 20 µL TMEDA was added in the solution for 5 min before the ice bath and stirring were removed. The mixed solution was cast between two glass panels with 1 mm gap for 30 min to form a hydrogel. The PAM-M hydrogel was subsequently removed and air dried by placing it in between two pieces of cellophane.

2.4 Characterization of isolated melanosome

2.4.1 Morphology of isolated melanosome

Scanning electron microscopy (SEM): Each melanosome suspension was dropped on tinfoil and dried, then attached onto aluminum stubs and gold-sputtered before viewing using a JEOL-7401 SEM (JEOL Ltd). The sizes of 50 melanosomes were measured for each sample, using Image J.

Transmission electron microscopy (TEM): (1) the morphology observation: Each melanosome suspension was diluted, and drop-cast onto copper grids and air-dried. Samples were imaged using a JEM-1230 TEM (JEOL Ltd). (2) the interior structure of isolated melanosomes: Samples were fixed with a mixture of paraformaldehyde (4%) and glutaraldehyde (2.5%) in PBS overnight and post-fixed in osmium tetroxide (2%, OsO4, Sigma Aldrich) for 2 hours. Then the samples were dehydrated in an ascending ethanol concentration series before embedded in resin (Araldite 502 kit, Ted Pella) within polyurethane molds at 60°C for 2 hours. Afterwards, the resin capsules were sectioned at 100 nm thickness with a diamond knife in an ultramicrotome and collected on copper grids. The sections were stained with 2% uranyl acetate and lead citrate prior to imaging using a JEM-1230 TEM (JEOL Ltd).

2.4.2 Fourier transform infrared spectroscopy (FTIR)

Melanosome powder and virgin hair fibers were deposited on the ATR plate of the FTIR spectroscope (Perkin Elmer Frontier), and spectra were recorded in the range of 650-4000 cm⁻¹ at a resolution of 4 cm⁻¹. Each sample was subjected to 16 scans.
2.4.3 Solid state $^{13}$C nuclear magnetic resonance (NMR)

The one-dimensional $^{13}$C CP-MAS solid-state NMR experiments were operated using a 600 MHz Bruker AVANCE III spectrometer equipped with a 4 mm HX probe. The MAS spinning frequency was 13 kHz. The recycle delay was 3 s and $^1$H decoupling rf strength was 72 kHz. $^{13}$C chemical shifts were referenced with adamantane (downfield signal at 38.4 ppm).

2.4.4 Thermogravimetric analysis (TGA)

TGA was carried out using a thermogravimetric analyzer (TGA, Q500 TA instrument) using 5 mg samples heated from 30 to 800°C at 10 °C/min.

2.4.5 UV-vis absorption and transmittance

The UV-vis absorption and transmittance spectra of melanosome suspension and PAM-M film were obtained using UV-visible spectrophotometer (SHIMADZU, UV-2450). The scanning range was from 200-800 nm. The UV-vis transmittance curves were used to calculate the filtering percentage of UVA (320-400 nm), UVB (280-320 nm), UVC (220 -280nm) and the transparency of melanosome suspensions ($T_{660}$ nm) [30, 31].

$$ UVA \text{ filtering} \% = \left(1 - \frac{\int_{320}^{400} T(\lambda)d(\lambda)}{\int_{320}^{400} d(\lambda)} \right) \times 100 \quad \text{(Eq 1)} $$

$$ UVB \text{ filtering} \% = \left(1 - \frac{\int_{280}^{320} T(\lambda)d(\lambda)}{\int_{280}^{320} d(\lambda)} \right) \times 100 \quad \text{(Eq 2)} $$

$$ UVC \text{ filtering} \% = \left(1 - \frac{\int_{220}^{280} T(\lambda)d(\lambda)}{\int_{220}^{280} d(\lambda)} \right) \times 100 \quad \text{(Eq 3)} $$

Where $\lambda$ is the wavelength, $d(\lambda)$ is bandwidth, and $T(\lambda)$ is the average spectral transmittance of samples.

3 Result And Discussion

The quantity of eumelanins (within melanosomes) determined the colour of human hair. Melanosomes are tightly embedded in the inter-macrofibrillar matrix of cortex [32]. To remove those keratin, we extracted KAP and keratins from human hair adequately in advance according to Fujii et al’s protocol[33]. the KAP and keratin had molecular weight distribution of about 10-28 kDa and 45-65 kDa, respectively (Fig. Fig. 1 b). The KAP-free hair and hair residues remained black (Fig. Fig. 1 a), indicating retention of the melanosomes. Then an enzyme esperase was used to hydrolyze the hair residues to release the melanosomes. The enzymatic hydrolysis process was highly efficient due to DTT and urea disrupted disulfide bonds and hydrogen bonds in hair residues during keratin extraction process [34]. Therefore,
melanosome isolation can also be achieved through enzymatic hydrolysis of human hair residues after keratin extraction with urea and other reducing regents like mercaptoethanol, mercaptoacetic acid, and L-cysteine, etc.

3.1 Morphology of the melanosomes

Pure, dispersed melanosomes with yields of about 1.3% (by weight of original hair fibers) were isolated successfully with centrifugation procedures as all protein components in the hair residues were degraded into soluble products. Morphological observations of the melanosomes showed the melanosomes were individually isolated and maintained their structural integrity. The melanosomes were well-defined rod-shaped and ellipsoidal particles with lengths of 904±171 nm and diameters of 341±75 nm (Fig. 2a, c). High magnification SEM and TEM images showed rough surfaces of the hair melanosomes, and melanosomes containing 10-60 nm secondary nanoparticles (Fig. 2b, d). These results are consistent with published work about human hair melanosomes, suggesting the integrity of the melanosomes was well preserved [13].

Takehito et al. investigated the ultrastructure of intact hair melanosomes and concluded that they were formed from a membrane-like outer structure (MS) and an inner matrix. The MS included numerous spherical vesicles (V) while the inner matrix showed a stripe-like structure (MT) in the elongated direction and a sheet-like arrays structure (SA) in the cross section[32]. As shown in Fig. 2e, spherical vesicles with a diameter of 10-20 nm were evident, along with the MS in the longitudinal section of the melanosome. In the cross-section view, a series of concentric ring structures could be observed (Fig. 2f). All these morphological observations in the isolated melanosomes corresponded to earlier published results of intact melanosomes in human hair, suggesting that the structure of melanosomes isolated as described herein was preserved.

3.2 Structural characterization of the melanosomes

Hair fibers are mainly composed of proteins (90%) and minor components including lipids (5%) and melanin (1-3%) [4], while melanin mainly comprise of indole structures (Fig. 3 a. The FTIR and $^{13}$C NMR spectra of hair and melanosome reflect these differences. In Fig. 3 b, the FTIR spectra of hair shows representative absorption bands related to peptide bonds at 3,268 cm$^{-1}$ (amide A), 3,068 and 3,073 cm$^{-1}$ (amide B), 1,629 cm$^{-1}$ (amide I), 1,523 cm$^{-1}$ (amide II) and 1,240 cm$^{-1}$ (amide III). The intense peaks at 1,040 cm$^{-1}$ were related to the S-O stretching vibrations of the cysteic acid [35]. In the FTIR spectra of isolated hair melanosome the absorption peaks of indolic and pyrrolic groups can be observed. The intense absorption peak at 3,248 cm$^{-1}$ was ascribed as the N-H and O-H stretching vibrations in the indolic and pyrrolic structures [36]. In the range of 2,950-2,850 cm$^{-1}$, very weak peaks at 2,967, 2,940, 2,885 cm$^{-1}$ were related to the C-H stretching vibration in aliphatic groups.[7]. Aromatic C=C, C=N bending, amide C=O stretching, and N-H bending from indole, pyrrole in melanin overlapped with an absorption
plateau at the region of 1700-1480 cm\(^{-1}\). The peaks at 1,446 cm\(^{-1}\), 1,375 cm\(^{-1}\) and 1,240 cm\(^{-1}\) were ascribed as aliphatic C-C bending, C-N stretching, and phenolic C-OH stretching in melanin, respectively [5, 22].

The solid-state \(^{13}\text{C}\) NMR spectra of intact hair and the isolated melanosomes are given in Fig. 3c. The solid state \(^{13}\text{C}\) NMR signals of hair proteins were composed of four typical spectral ranges including the carbonyls region mainly due to backbone carbonyls in peptide bonds and side-chain carboxyl (190-160 ppm), the aromatic region from the bulk of the aromatic residues (160-90 ppm), backbone \(\alpha\) and \(\beta\)-carbons (80-45 ppm), and aliphatic region (45-10 ppm) [23]. The solid state \(^{13}\text{C}\) NMR spectra of the isolated melanosome also show complex but different spectra. The intense signals centered at 170 ppm and aliphatic region (90-10 ppm) were mostly due to residual proteinous components, whereas the signals at 160-90 ppm were related to both the melanin and the proteinous components. As melanin mainly resulted in the aromatic signals while proteins mainly contributed to the aliphatic and carboxyl signals, the ratio of the aromatic to carbonyl signal area could be used to evaluate qualitatively the relative content of melanin in the melanosomes extracted from hair [23]. As shown in Table 1, Simona Ghiani et al had reported the ratio of aromatic-to-carbonyl signal area for intact hair as 0.15, while that for melanosomes isolated with concentrated HCl and enzymes (papain and DTT) were 2.63 and 0.75, respectively [23]. The values were 1.88 and 0.27 for our isolated melanosomes and intact hair, respectively, suggesting a higher melanin content in our isolated melanosome due to complete removal of hair protein contaminations. In addition, the melanin-bound proteins in melanosomes from human hair have a high content of arginine, which has signal at 157 ppm [22]. Our result also demonstrated higher intensity at 157 ppm, indicating the isolated melanosomes kept the melanin-bound proteins.

### 3.3 Functional properties of the melanosomes

The TGA mass loss and derivative thermogravimetric (DTG) curves of intact hair and melanosomes were shown in Fig. 3d and 3e, respectively. The mass loss process of melanin was mainly divided into two stages (30°C-150°C, 150°C-500°C), and the weight loss rates were larger at the temperatures of 65.5 °C, 346°C. The first weight loss stage was due to the evaporation of weakly and/or strongly bound water [37, 38]. In the second stage, the percent weight loss of about 48.8% was due to the loss of carbon dioxide, water, and ammonia during the heating of melanins at elevated temperatures [38-40]. This suggests that the hair melanosomes can be used in polymer blends such as melt extrusion and injection molding where processing temperature is lower than 150°C [37, 41].

As shown in Fig. 4a and b, melanosomes at different concentrations showed broad absorption regions at 200-800 nm. The absorption intensities at 200-400 nm (UV region) are higher than that at 400-800 nm (visible-light region), and the absorption intensity increased with concentration of melanosomes. The UV filtering percentage of melanosome suspensions increased with concentration of melanosome, while the transparency showed an opposite trend. At concentrations up to 0.1 mg/mL, the filtering percentages of UVA, UVB, and UVC were above 99%, with a transparency of 12.8%. After that, increasing the
The concentration of melanosome suspensions did not improve filtering capacity further, but transparency decreased sharply (Table 2). Therefore, human hair melanosomes isolated based on our protocol retained highly effective UV filtering ability, although transparency is compromised. Regardless of transparency, the UV barrier property of the melanosomes can potentially be used as a sustainable photo-protection agent in wide ranging applications including cosmetics and packaging [24, 41].

The UV-vis transmittance spectra of PAM and PAM-M films with various concentrations of melanosome and their percentages of UV filtering were shown in Fig. 4c and Table 3, respectively. The filtering percentages of UVA and UVB in PAM film were very low (about 23.2% and 38.7%, while a higher UVC filtering percentage of 79.0% was recorded. With increasing human hair melanosome loading from 0.1% to 0.6%, the UVC filtering percentage increased to 90% and 99%. Increasing the melanosome loading concentrations to 0.4% and 1% resulted in UVA and UVB filtering percentage increase to over 90% and 99%, with the transparency sharply decreasing to 48% and 14.0% consequently. These results indicate that human hair melanosomes enhance UV-filtering performance of polymeric films such as those made with PAM.

4 Conclusions

Combining effective keratin extraction and melanosome isolation is logical to maximize resource recovery from human hair and is achieved successfully herein. After keratin extraction, corresponding hair residue is hydrolyzed using esperase to thoroughly release intact melanosomes into suspension. The isolated melanosomes retain their structural integrity and UV filtering ability with a high yield of about 1.3% by mass of total hair. This strategy upscales melanosome isolation and is proposed as a significant improvement of utilization efficiency of human hair compared to previous methods.

Declarations

Funding

This research was supported by the Agency for Science, Technology and Research (A*STAR) under its Acne and Sebaceous Gland Program & Wound Care Innovation for the Tropics IAF-PP (H17/01/a0/008 & H17/01/a0/0L9), the National Natural Science Foundation of China (51673087), and the China Scholarship Council (Grant No.201906790039 to N.Z.).

Conflicts of interest

We declare we have no competing interests.

Availability of data and material

All data are presented in the main text.

Acknowledgment
The authors would like to acknowledge the NTU Center of High Field NMR Spectroscopy and Imaging and the Facility for Analysis, Characterization, Testing and Simulation (FACTS), for their support in ss $^{13}$C NMR experiments and electron microscopy analysis.

**References**

30. Yang W, Jing Su, Ting, Li et al. A Novel UV-Shielding and Transparent Polymer Film: When Bioinspired Dopamine–Melanin Hollow Nanoparticles Join Polymers. ACS Applied Materials & Interfaces. 2017;9(41)

Tables

Table 1 Normalized signal areas in solid state $^{13}$C-NMR spectra of human hair and the isolated melanosomes.

<table>
<thead>
<tr>
<th></th>
<th>Aromatic (90-160 ppm)</th>
<th>Aliphatic (0-90 ppm)</th>
<th>Carboxyl (160-180 ppm)</th>
<th>Aromatic/carboxyl</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact hairs</td>
<td>3.6</td>
<td>72.7</td>
<td>23.7</td>
<td>0.15</td>
<td>[23]</td>
</tr>
<tr>
<td>CH-MGs&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.4</td>
<td>60.8</td>
<td>10.8</td>
<td>2.63</td>
<td></td>
</tr>
<tr>
<td>CP-MGs&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.6</td>
<td>70.7</td>
<td>16.7</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td>Hair</td>
<td>4.2</td>
<td>62.6</td>
<td>15.2</td>
<td>0.27</td>
<td>Current result</td>
</tr>
<tr>
<td>Melanosome</td>
<td>51.7</td>
<td>128.7</td>
<td>27.4</td>
<td>1.88</td>
<td></td>
</tr>
</tbody>
</table>
CH-MGs: melanin granules isolated from human hair using concentrated HCl.

CP-MGs: melanine granules isolated from human hair using protease (Papain).

**Table 2** Percentage of UVA, UVB and UVC filtering ability of melanosome suspensions at various concentrations.

<table>
<thead>
<tr>
<th>Melanosome Con. (mg/mL)</th>
<th>Blocking (%)</th>
<th>Transparency (T&lt;sub&gt;660 nm&lt;/sub&gt;(%))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UV-A</td>
<td>UV-B</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.025</td>
<td>55.3</td>
<td>57.2</td>
</tr>
<tr>
<td>0.05</td>
<td>85.9</td>
<td>87.1</td>
</tr>
<tr>
<td>0.1</td>
<td>97.3</td>
<td>97.7</td>
</tr>
<tr>
<td>0.25</td>
<td>99.6</td>
<td>99.7</td>
</tr>
<tr>
<td>0.5</td>
<td>99.6</td>
<td>99.7</td>
</tr>
</tbody>
</table>

**Table 3** Percentage of UVA, UVB and UVC filtering ability of PAM and PAM-M films at various concentrations of melanosomes.

<table>
<thead>
<tr>
<th>Con. of M in PAM-M film</th>
<th>Blocking (%)</th>
<th>Transparency (T&lt;sub&gt;660 nm&lt;/sub&gt;(%))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UV-A</td>
<td>UV-B</td>
</tr>
<tr>
<td>Baseline</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>23.2</td>
<td>38.7</td>
</tr>
<tr>
<td>0.10%</td>
<td>57.6</td>
<td>68.8</td>
</tr>
<tr>
<td>0.20%</td>
<td>74.4</td>
<td>81.8</td>
</tr>
<tr>
<td>0.40%</td>
<td>90.8</td>
<td>94.2</td>
</tr>
<tr>
<td>0.60%</td>
<td>94.8</td>
<td>96.7</td>
</tr>
<tr>
<td>1.00%</td>
<td>99.2</td>
<td>99.5</td>
</tr>
<tr>
<td>1.50%</td>
<td>99.4</td>
<td>99.6</td>
</tr>
<tr>
<td>2.00%</td>
<td>99.9</td>
<td>99.9</td>
</tr>
</tbody>
</table>
Figures

(a) Human hair → KAP extraction → KAP-free hair → Keratin extraction → Hair residues

(b) KAP Keratin

<table>
<thead>
<tr>
<th>Molecular Weight (kDa)</th>
<th>Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>250 kDa</td>
<td></td>
</tr>
<tr>
<td>150 kDa</td>
<td></td>
</tr>
<tr>
<td>100 kDa</td>
<td></td>
</tr>
<tr>
<td>75 kDa</td>
<td></td>
</tr>
<tr>
<td>50 kDa</td>
<td></td>
</tr>
<tr>
<td>37 kDa</td>
<td></td>
</tr>
<tr>
<td>25 kDa</td>
<td></td>
</tr>
<tr>
<td>20 kDa</td>
<td></td>
</tr>
<tr>
<td>15 kDa</td>
<td></td>
</tr>
<tr>
<td>10 kDa</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1

Photos of virgin human hair, hair after KAP extraction (KAP-free hair), and hair after KAP and keratin extraction (hair residues).
Figure 2

Morphology of melanosomes isolated from hair fiber using esperase. (a, b) SEM images of hair melanosomes; (c, d) TEM images of hair melanosomes; TEM images of (e) longitudinal and (f) transverse sections of melanosomes (V: spherical vesicles, M: membrane-like structure, SA: sheet-like array structure in matrix, MT: sheet-like structures in inner matrix).
Figure 3

(a) the chemical structure of melanin in melanosomes; (b) FTIR spectra and (c) solid state 13C NMR spectra of the isolated melanosomes from human hair in comparison with hair fibres; (e) TGA and (f) DTG curves of the isolated melanosomes from human hair and hair fibres.
Figure 4

UV-vis absorption (a) and UV-vis-transmittance (b) of human hair melanosome suspensions at various concentrations; (c) UV-vis transmittance of PAM and PAM-M films.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- GraphicalAbstract.docx
- schema1.png