Functional Amyloid as a Potential Encoder of Melanin Pigments

Nghia T. K. Le and Kyungtae Kang*

Department of Applied Chemistry, Kyung Hee University, Yongin 17104, South Korea.
**E-mail: kkang@khu.ac.kr*
Received October 16, 2018, Accepted November 28, 2018, Published online January 28, 2019

Keywords: Melanin biosynthesis, Functional amyloid, Peptide assembly

Melanins are insoluble polymeric pigments that have many important biological functions in diverse life forms. In humans, melanin, which appears in skin, hair, and eyes, is synthesized by melanocytes via oxidative polymerization of tyrosine and additives. Depending upon the location and molecular composition, the melanin can be categorized as black eumelanin (mostly composed of *L*-dopa), yellow-to-red pheomelanin (cysteine as the additive), and neuromelanin (synthesized in the brain), each of which exhibits unique physicochemical properties. As evidenced by their prevalence, melanins have many advantageous features, including broad-range optical absorption and thermal relaxation, permanent radical character, and hydration-dependent electric conductance.[1] Use of melanins or their derivatives to other fields, however, is limited except for surface coating,[2] because unlike other polymers, melanins exhibit heterogeneous and oligomeric molecular structures, extreme insolubility in most solvents, and uncontrollable macroscopic properties.

Recently, Lampel *et al*. suggested a new way to tune the macroscopic properties of melanin-like pigments using self-assembly of tyrosine-containing tripeptides (Figure 1(a)).[3] All possible tripeptides of tyrosine (Y; a residue converted into L-dopa upon enzymatic treatment), phenylalanine (F; an aggregation-prone residue), and aspartic acid (D; a charged residue) formed distinct supramolecular structures with different opacities and solubilities; interestingly, enzymatic oxidation of the structures resulted in pigments with different light absorption, morphology, color, and even electrochemical properties (Figure 1(b) and (c)). For example, DXX- and XXD-type peptides (those with paired aromatic residues) formed insoluble hydrogels, while only DXX-type peptides displayed a strong tendency to form dark pigments upon enzymatic oxidation. XDX-type peptides formed neither insoluble supramolecular structures nor strong pigments upon oxidation. The supramolecular orientation of each peptide (before and after oxidation) was investigated with multiple techniques, collectively showing that the differences in pigment properties reflect those in supramolecular order before oxidation.

Although a molecular-level understanding of the relationship between the properties of pigments and the intermolecular orientation of the precursors is yet to be completed,

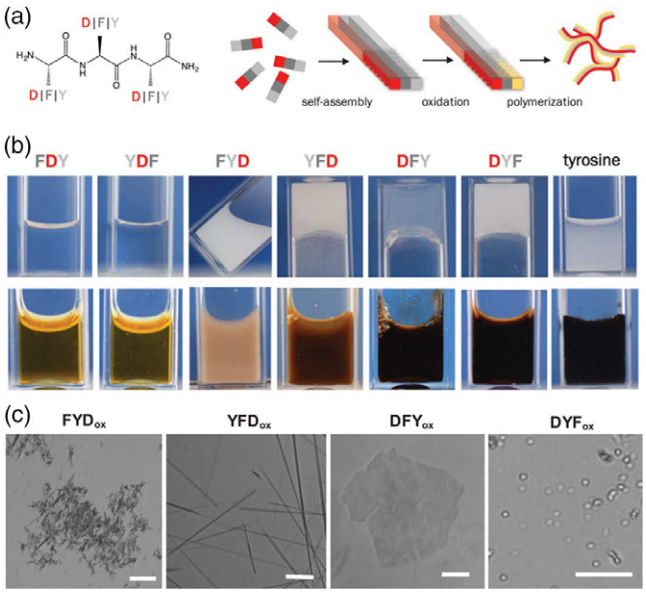

Figure 1. (a) Schematic illustration of the formation of polymeric peptide pigments by enzymatic oxidation of preorganized tripeptides. (b) Images of self-assembled peptides before (top) and after (bottom) 24 h of enzymatic oxidation. (c) Diverse structures of polymeric peptide pigments. Scale bars: 20, 20, 20, 10 μm. Reprinted with permission. Copyright 2017, AAAS.[3]

the results by Lampel *et al*. show promise for controlling the properties of melanin pigments by altering not the identity of the assembled molecules, but the way in which they are assembled. This could be projected to a biosynthetic procedure for melanins, which includes the formation of protein fiber matrix as a catalytic scaffold (Figure 2).[4,5] The intraluminal domain of Pmel17, the major protein component of the matrix, forms amyloid fibrils (non-covalent protein aggregates that are rich in cross-beta sheets) instantaneously under physiological conditions.[6] The currently agreed upon role of Pmel17 matrix is limited to preventing cytotoxic intermediates of melanins to diffuse out of melanosomes (the organelle responsible for melanin synthesis). However, considering that amyloid structures have a highly ordered supramolecular structure, and that the molecular precursors of melanins would likewise be ordered when they penetrate between parallel beta-sheets, Lampel *et al*.'s results imply that the role of amyloid matrix

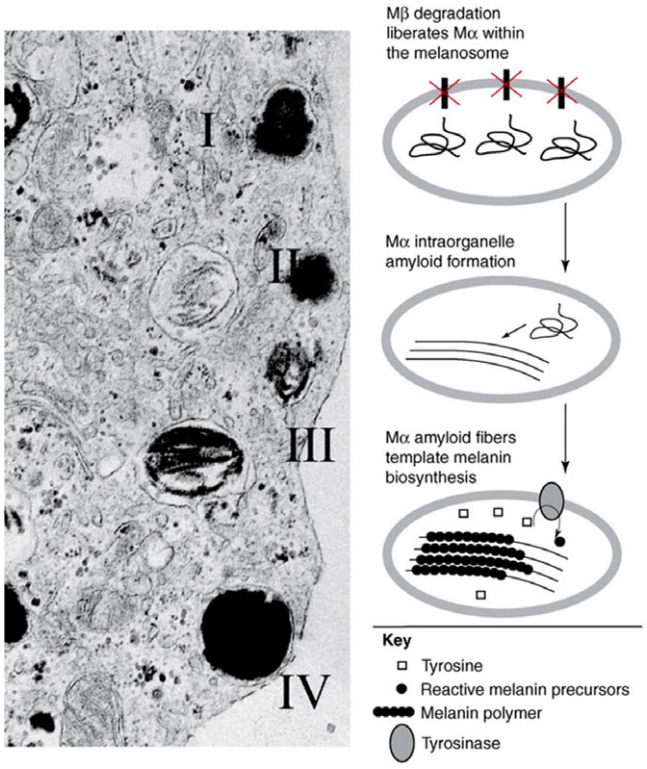

Figure 2. The four-stage (I–IV) process of melanosome maturation and its depiction. Reprinted with permission. Copyright 2007, cell Press.[4]

may be more than acting as a scaffolding for synthesis or sequestering of unwanted intermediates. It has already been shown that alignment of the major chromophore units of melanins (indole derivatives) in the polymeric structure dramatically affects the radical and optical properties of the resulting pigments.[7,8]

Lampel *et al.*'s results and their potential relevance to the role of amyloid matrix in melanosomes may be useful for understanding/designing the functions of other amyloids (either pathological or functional) or in general proteinaceous supramolecular structures. The concept that the supramolecular structure of an amyloid structure enables its function as a catalyst has been validated in numerous recent reports. The Korendovych group demonstrated that amyloid structures can be carefully designed to contain metal binding centers, and when complexed with copper or zinc ions, to catalyze chemical reactions including hydrolysis and oxidation of various substrates (Figure 3).[9,10] The authors also suggested that the catalytic ability of amyloid fibers may be relevant to the cytotoxicity of pathological amyloid oligomers, whose biological mechanisms are not yet well understood.[10]

Collectively, the studies highlighted here support the notion that the three-dimensional orientation of functional groups driven by a myriad of non-covalent interactions can

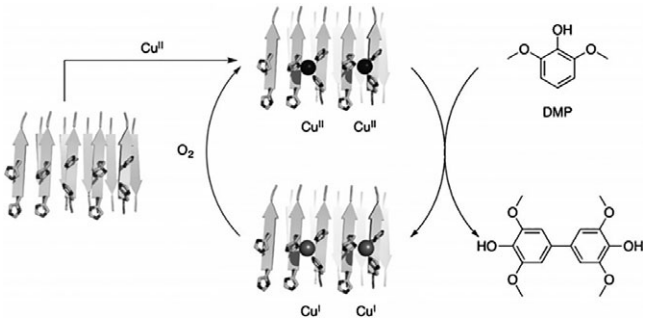

Figure 3. Schematic illustration of copper-mediated oxidation catalyzed by amyloid fibrils. Reprinted with permission. Copyright 2016, Wiley.[9]

be as important for certain purposes as the identity of the functional groups. Nature often utilizes assembled periodic supramolecular structures to control such orientation. Designing functionality based on insoluble, supramolecular structures could open many new possibilities, as organic chemistry tends to focus on approaches that utilize soluble and individual molecular complexes.

Acknowledgments. This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Ministry of Science, ICT, & Future Planning (MSIP) (2016R1C1B2011414).

References

1. M. d'Ischia, A. Napolitano, A. Pezzella, P. Meredith, T. Sarna, *Angew. Chem. Int. Ed.* **2009**, *48*, 3914.
2. H. Lee, S. M. Dellatore, W. M. Miller, P. B. Messersmith, *Science* **2007**, *318*, 426.
3. A. Lampel, S. A. McPhee, H. A. Park, G. G. Scott, S. Humagain, D. R. Hekstra, B. Yoo, P. Frederix, T. D. Li, R. R. Abzalimov, S. G. Greenbaum, T. Tuttle, C. Hu, C. J. Bettinger, R. V. Ulijn, *Science* **2017**, *356*, 1064.
4. D. M. Fowler, A. V. Koulov, W. E. Balch, J. W. Kelly, *Trends Biochem. Sci.* **2007**, *32*, 217.
5. B. Watt, G. van Niel, G. Raposo, M. S. Marks, *Pigm. Cell Melanoma R.* **2013**, *26*, 300.
6. D. M. Fowler, A. V. Koulov, C. Alory-Jost, M. S. Marks, W. E. Balch, J. W. Kelly, *PLoS Biol.* **2006**, *4*, 100.
7. L. Panzella, G. Gentile, G. D'Errico, N. F. Della Vecchia, M. E. Errico, A. Napolitano, C. Carfagna, M. d'Ischia, *Angew. Chem. Int. Ed.* **2013**, *52*, 12684.
8. A. Corani, A. Huijser, T. Gustavsson, D. Markovitsi, P. A. Malmqvist, A. Pezzella, M. d'Ischia, V. Sundstrom, *J. Am. Chem. Soc.* **2014**, *136*, 11626.
9. O. V. Makhlynets, P. M. Gosavi, I. V. Korendovych, *Angew. Chem. Int. Ed.* **2016**, *55*, 9017.
10. C. M. Rufo, Y. S. Moroz, O. V. Moroz, J. Stohr, T. A. Smith, X. Hu, W. F. DeGrado, I. V. Korendovych, *Nat. Chem.* **2014**, *6*, 303.

