Synopsis of Original Research Paper

Novel glycosylation of melanin-related protein and its application

Siro Simizu

Department of Applied Chemistry, Faculty of Science and Technology, Keio University

Premelanosome protein (PMEL) is a functional protein as a scaffold for melanin synthesis and pigmentation in melanosome. PMEL forms non-toxic amyloid fibrils through aggregation of its fragments. In the previous studies, PMEL has several *N*-glycosylation and *O*-glycosylation sites and those are significant for membrane trafficking or fibril formation. *C*-mannosylation is a unique type of protein glycosylation, which the alpha-D-mannopyranose is attached to the first tryptophan residue in the consensus sequence, Trp-Xaa-Xaa-Trp/Cys (Xaa represents any amino acid). PMEL has one predicted *C*-mannosylation consensus sequence, Trp153-Lys-Thr-Trp, in the N-terminal region (NTR) and Trp153 is probably *C*-mannosylated; however, the presence of *C*-mannosylation in PMEL has not been reported. In this study, we first demonstrated by mass spectrometry that PMEL is *C*-mannosylated at predicted Trp153, and also *C*-mannosylated at Trp104 and Trp156, which belong to Trp104-Val-Asn-Asn and Trp156-Gly-Gln-Tyr, respectively. In addition, we identified that *C*-mannosylation at Trp104 can occur near *N*-glycosylation at Asn106. Furthermore, we evaluated the role of *C*-mannosylation in PMEL on its function of fibril formation, using *C*-mannosylation-defective mutant (W104F, W153F/W156F) PMEL transiently overexpressed HeLa cells. Compared with control and W104F, W153F/W156F especially diminished fibrils. Taken together, PMEL is *C*-mannosylated at Trp104, Trp153 and Trp156, and we suggest that the *C*-mannosylation of PMEL regulates amyloid fibril formation and/or pigmentation in melanosome.