Isolation and Identification of Melanosomes from Human Hair
— A New Approach to Morphologic Evaluation —

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Melanosomes were isolated from the human hair by graded centrifugation and identified by transmission and scanning electron microscopic examination. Melanosomes were separated from the keratinous structures by treating with strong NaOH solution for 15 hours. The keratinous structures were removed by centrifugation at 2,500xg and 3,500xg for 10 minutes respectively at 0°C. The isolated melanosomes were collected by centrifugation at 7,800xg at 0°C. Scanning electron microscopic examination made it possible to evaluate the global structure of purified melanosomes. (Ann Dermatol 3:(1) 12—14, 1991)

Key Words: Isolation of melanosomes, Scanning electron microscopy

Within melanocytes, melanin is formed after the tyrosine-tyrosinase reactions on the melanosomal matrix. Fully melanized melanosomes are transferred to the epidermal keratinocytes and hair matrix cells; thus, they are found in the epidermis and hair.

Isolation of purified and nondegraded melanosomes is necessary for morphologic, biochemical and photochemical analysis. Although many isolation methods have been developed\(^1\)\(^-\)\(^7\), they are inadequate due to their inability to breakdown the keratins without the destruction of melanosomal proteins. Borovansky and Hach\(^8\), examined all the recently published methods and suggested several principles which minimize the undesirable effects of the disintegrating agents.

In the present study, melanosomes were separated from the human hair by modification of Borovansky's method\(^1\), and the morphologic evaluation of the isolated melanosomes was performed by transmission and scanning electron microscopy.

MATERIALS AND METHODS

Materials

Black hairs from Korean males in their twenties were used.

Methods

Isolation of melanosomes

Hairs were washed with cold acetone for 10 minutes and diethyl ether for 10 minutes and air dried. These were treated with 1 N NaOH for 15 hours at 37°C in a shaking incubator. The suspension was centrifuged at 2,500xg for 10 minutes. The first supernatant was centrifuged at 3,500xg for 10 minutes. The second supernatant was centrifuged at 7,800xg for 10 minutes. The precipitate was suspended with distilled water, and this suspension was centrifuged at 7,800xg for 10 minutes. The precipitate was melanosomes. All centrifugation procedures were performed at 0°C.

Electron microscopic examinations

Transmission electron microscopy

For transmission electron microscopy, the
diluted pellet of melanosomes was prefixed in a mixed solution of 2.5% glutaraldehyde and 1% paraformaldehyde and postfixed with osmium tetroxide. After dehydration with graded ethanols, the sample was embedded in Epon, cut into several ultrathin sections, and stained with uranyl acetate followed by lead citrate. The sections were examined in a Hitachi H-500 electron microscope.

**Scanning electron microscopy**

For scanning electron microscopy, the diluted pellet of melanosomes was prefixed in a mixed solution of 1% glutaraldehyde and 1% paraformaldehyde. The prefixed melanosomes were filtered through a 0.4 μm nucleopore membrane filter. The filtered melanosomes were arrayed in a single layer and washed with phosphate buffer (pH 7.4), followed by 1% osmium tetroxide. Following osmiation, the sample was dehydrated through graded ethanols, transferred to isoamyl acetate, dried with a critical point drier (Hitachi ICP2), mounted on stubs, coated with gold in a sputter coater, and examined through a Hitachi H-450 scanning electron microscope operated at 20 kV.

**RESULTS**

On the transmission electron microscopic examination, the isolated melanosomes which were irregularly crossected showed a lower degree of degradation and contamination of keratins (Fig. 1).

On the scanning electron microscopic examination, the crossectional features of melanosomes were not observed, but the global morphology of melanosomes which showed varying size and oval to ellipsoidal shape could be observed (Fig. 2).

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**Fig. 1.** Transmission electron microscopic findings of isolated melanosomes. No degradation of melanosomes or contamination of keratins are noted (×12,200).

**Fig. 2.** Scanning electron microscopic findings of isolated melanosomes. The global structures of melanosomes are observed (×14,000).