

Intracellular Distribution of Dopa and 5-S-Cysteinyl-dopa in Pigment Cells with Minimal Pigment Formation

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Abstract. The hypothesis that only melanosomal catecholic amino acids contribute to melanin formation was tested by studying adult bovine eyes in which pigment synthesis is considered to be low or absent. Dopa and 5-S-cysteinyl-dopa were investigated in different cell fractions of the choroid and retinal pigment epithelium of cattle. Most of the dopa and 5-S-cysteinyl-dopa was found in the cytoplasm and very little in the large granule fraction. The presence of cysteinyl-dopa in the adult eye is evidence of tyrosinase activity, but the catechol amino acids in the cytoplasm probably do not give rise to melanin formation. It is assumed that they instead are excreted from the cells.

Key words: Cysteinyl-dopa; Dopa; Intracellular distribution; Pigment, retinal, choroidal

It seems probable that localization of melanin precursors such as dopa and cysteinyl-dopa to melanosomes is a condition for melanin formation. We assume that extramelanosomal tyrosinase produces dopa and cysteinyl-dopa, which do not give melanin, but are excreted. This hypothesis could be tested by studying the intracellular distribution of dopa and cysteinyl-dopa in a tissue containing catecholic amino acids but with absent or minimal melanin formation.

A high rate of melanin formation is present in the fetal eye, but this synthesis is generally considered to end before or soon after birth (3, 4, 6, 7, 9, 10, 11). Tyrosinase activity has been demonstrated in

the eyes of adult rabbits (8), however, and dopa and cysteinyl-dopas have been found in pigmented cells of adult eyes (2, 5). Adult bovine eyes were studied to test our hypothesis.

MATERIAL AND METHODS

Thirty dark-brown eyes from cattle were obtained from the slaughterhouse in Kävlinge, Sweden. They were transported to the laboratory at 4°C, and were dissected within 6 hours. On each occasion 10 eyes were used, and the choroid and retinal pigment epithelium removed together from the underlying sclera were pooled.

Ten pooled choroids and retinas were homogenized in a 0.25 M sucrose solution in a glass homogenizer for 20 min. For each gram of tissue 4 ml of the sucrose solution was used. The homogenate was centrifugated for 10 min at 700 ×g. The sediment consisting of intact cells and nuclei was discarded.

The supernatant was centrifugated for 10 min at 11 000 ×g. The sediment was washed with sucrose solution and after recentrifugation for 10 min at 11 000 ×g precipitated with 0.4 M PCA. This fraction is known to contain large organelles such as mitochondria and melanosomes; we have called it the 'large granule fraction'. The supernatant obtained before the large granule fraction was centrifugated for 60 min at 100 000 ×g. The sediment consisting of microsomes, called the 'small granule fraction', was precipitated with 0.4 M PCA. The supernatant consisting of cytoplasm, not containing organelles, was adjusted to 0.4 M perchloric acid by the addition of 4 N perchloric acid. The fraction was called 'cytoplasm'.

Dopa and 5-S-cysteinyl-dopa was determined by previously described methods (1, 12).

RESULTS AND COMMENTS

The average amount of dopa found in the choroid and retinal pigment epithelium of each eye was 2.3 μg, and the average amount of 5-S-cysteinyl-dopa was 0.7 μg. The distribution of dopa and 5-S-cysteinyl-dopa in the various cell fractions is seen from Table I.

There is no established pathway by which melanin granules can leave the eye, and melanin is

Table I. *Relative amounts of dopa and 5-S-cysteinyl-dopa in the various cell fractions*

Fraction	Dopa (%)			5-S-cysteinyl-dopa (%)		
	Experiment			Experiment		
	I	II	III	I	II	III
Large granule	1.0	0.5	0.9	0.8	2.1	1.1
Small granule	3.1	4.7	3.2	1.6	1.0	1.5
Cytoplasm	95.8	94.6	95.7	97.4	96.8	97.2

broken down with difficulty. It is therefore generally thought that melanin synthesis is low or absent in the adult eye, since pigmentation does not increase with progressive age.

By far the greatest amount of catechols was present in the cytoplasm, and there was very little in the large granule fraction. Since this fraction contains most melanin organelles it would hardly seem likely that the presence of dopa and 5-S-cysteinyl-dopa in the choroid or retina of the adult eye could reflect melanin formation. Our finding that all or almost all dopa and 5-S-cysteinyl-dopa is present in the cytoplasm favours the hypothesis that these pigment precursors, when located outside melanosomes, are excreted from the cell instead of forming pigment.

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Periodic Structures in the Langerhans' Cell: An Optical Diffraction Study

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Abstract. The crystalline-like structure of horizontally sectioned Langerhans cell granules has been studied by optical diffraction. Two different fixation techniques resulted in two different sets of spacings.

Key words: Langerhans cell granules; Optical diffraction

During recent years there has been an increasing interest in the Langerhans cell and its possible functions. This cell is identified in the electron microscope by a specific intracellular organelle, the so-called Birbeck granule or the Langerhans cell granule, first described by Birbeck et al. in 1961 (1). Since then several authors have studied this granule in detail (2, 8). It is described as a disc-shaped structure generally sectioned more or less perpendicularly and therefore appearing like a rod with a central linear periodicity. Occasionally the granule is sectioned horizontally and then gives the impression of a crystalline-like lattice. By using two different preparation methods we have studied the periodicities of such lattice structures by means of optical diffraction.

METHODS

Biopsies were obtained from each of two healthy, adult volunteers. One biopsy was fixed in osmium-zinc-iodide (5) and the other was fixed in glutaraldehyde and postfixed