ELEMENTAL DISTRIBUTION OF Ca AND S IN THE PROCESS OF OTOCONIAL BIOMINERALIZATION

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INTRODUCTION

The vestibular maculae in the inner ear are made up a sensory epithelium formed of two different types of cells: sensory dells and cells supporting and overlying structure, the otolithic mass. This mass consists of two layers: a superficial layer of otoconia, made of $CaCO_3$ crystals in the form of calcite, which contains a protein matrix (1), and an underlying otoconial gelatinous membrane (GM). The GM comprises two layers: a dense peripheral upper layer that supports the otoconia (UGM), and a more loosely arranged lower network called the columnar filament layer (LGM), which connects the surface of epithelium.

Electron probe X-ray microanalysis (EPMA) is a powerful tool able to identify chemical elements in a sample while it is being examined in the electron microscope. EPMA studies of the inner ear have focused mainly on the elemental composition of otoconia (2). However, the GM has seldom been studied by EPMA because of methodological difficulties (3). Quantitative electron probe X-ray microanalysis has rarely been used for inner ear structures.

The aim of the present EPMA study was to determine the concentration and the elemental distribution of Ca an S in the otoconia and in the two layers of the GM in the vestibular maculae, in order to shed light on their role in the dynamic system of otoconial remodeling.

MATERIAL AND METHODS

Five otolithic membranes were obtained from OF1 adult mice, cryofixed in liquid N_2-cooled freon 22, and freeze-dried at -80°C for 24h in a freeze-drying apparatus. The samples were then embedded in EPON-812 resin and polymerized at 70°C. Dried thin sections 500 nm in thickness were cut with an ultramicrotome and transferred to a double aperture holder for the electron microscope. The two holes were previously covered with thin pioloform foil and coated with carbon. These sections were studied in a Philips CM 20 (STEM).

Microanalysis was done in STEM using an EDAX Si (Li) detector system with a multichannel analyzer. Specimen were analyzed under the following conditions for data acquisition: accelerating voltage = 80 kV, point-measurements with a beam diameter of 65nm, magnification = 20000x, tilt angle = 30°, microscan = 0.25 μm^2, take-off angle = 30°, count accumulation time = 100 s live.

Both the otoconia and the GM were microanalyzed. A central zone of individual otoliths measuring 2x5 µm was studied. In the GM, two different regions were analyzed: one no more than 2 µm from the otolith (UGM) and another approximately 5 µm from otolith (LGM). In each specimen 30 analyses were done: 10 for each otolith, and 20 for two regions of the GM. In all, 150 analyses in the otolithic membrane were done to study Ca Kα and S Kα. The background signal was measured at an intensity range of 6.8 to 7.6 kV.

Calcium in GM was quantified using different concentrations of Ca Cl_2 $2H_2O$ standard (0.1%, 0.2%, 0.4% and 0.8%). In the otoliths, calcium was quantified with Ca_5 $(PO_4)_3$ 3OH. To quantify S in otoliths and GM we used different concentrations of $C_4H_{11}O_2PS_2$ standard (0.1%, 0.4% and 2%). These standards were freeze-dried for 24 h in a Edwards-Pearse tissue-drier, embedded in EPON, cut into 50 nm-thick sections on an ultramicrotome, and transferred to double aperture carbon-coated stubs previously coated with pioloform. The standards were observed in STEM and were microanalyzed under the conditions given above for EPMA. A total of 50 microanalytical measurements taken for Ca and S. The mean values obtained for each standard, expressed as Ie/Iw, were related to the concentrations to obtain a regression plot that allowed us to quantify the amount of calcium and sulphur in each specimen. The concentration of Ca and S in the otoconia and GM were calculated with Hall's equation (4).

Table 1

Ca	%wet	SD
Otoconia	42.124	3.908
UGM	1.244	0.484
LGM	0.304	0.263

Table 2

S	%wet	SD
Otoconia	0.199	0.080
UGM	0.544	0.239
LGM	0.489	0.263

RESULTS AND DISCUSSION

Our quantitative microanalytical results show that there is a calcium concentration gradient from the LGM toward the otolith (Figs. 1, 2, and 3). Quantitative microanalyses in the vestibular maculae are shown in Table 1. The distribution of sulfur was comparatively homogeneous in the gelatinous membrane, as shown in Table 2.

The Ca/S ratio in the gelatinous membrane increases from deeper regions of the gelatinous membrane toward the otolith in the vestibular maculae of the inner ear. This gradient may form part of the microenvironment wich, together with other factors (eg, pH, calcium-binding protein, etc.), influences the formation of otoconia (5). The distribution of S in the gelatinous membrane was relatively homogeneous. This may be related with the presence of glycoproteins and sulfatd GAG in the membrane. The precise role of glicoprotein and GAG in the mineralization has been intensely debated. The sulphate groups in the matrix protein have been described as regulators of the biomineralization process of dentine (6), and acidic macromolecules have also been inplicated in calcite nucleation in mollusc shells (7). The topographical distribution of sulphate group in GM may be involved in otoconial biomineralization processes, and may therefore influence the mineral density of the otoconial layer.

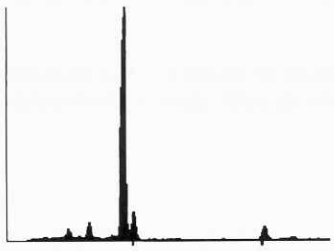

Fig. 1

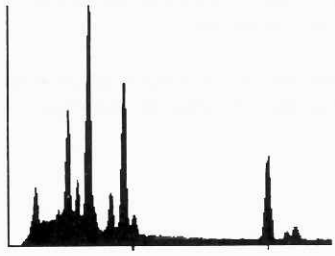

Fig. 2

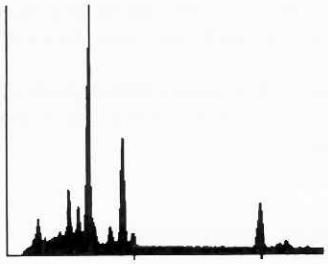

Fig. 3

Figures 1-3: X-ray emission spectrum obtained from the otoconia (Fig. 1), the upper gelatinous membrane (Fig. 2), and the lower gelatinous membrane (Fig. 3).

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