Effect of Deoxycholate on Smooth-surface Membranes

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SEIJI, M. and ITAKURA, H. *Effect of Deoxycholate on Smooth-surface Membranes*. Tohoku J. exp. Med., 1972, 107 (2), 149-157 — The effect of deoxycholate (DOC) on smooth-surface membranes was studied by means of electron microscopy. The smooth-surface membranes were isolated from Harding-Passey mouse melanomas and treated with 0.05% DOC. In the sectioned specimens, the untreated smooth-surface membranes appear as unit membrane-bound, closed vesicles. The unit membranes measure 50 Å across. The treated membranes showed significant vesiculation and swelling. In some parts many small vesicles are grouped in a cluster and enclosed by a membrane. A few to several unit membranes are stuck together to form myelin-like structures. In the negatively stained specimens, varying degrees of smooth-surface membrane vesiculation are apparent. Most large vesicles are found to exhibit myelin-like lamellar structures. From these observations, DOC appears to cause a vesiculation and swelling of the smooth-surface membranes but does not appear to destroy the unit membrane structure. There were a few myelin-like lamellar structures which were thought to have been produced by uniting several unit membranes. ——— deoxycholate; smooth-surface membranes; negatively stained electron microscopy

Although the exact mechanisms of surface active agents on the cytoplasmic membranes are not known, various detergents such as deoxycholate and cholate have been extensively used for the microdissection of mitochondria (Arion and Racker 1970) and for the preparation of the cytoplasmic organelles (Siekevitz and Palade 1966).

The status of tyrosinase has been studied by treating the smooth-surface membranes isolated from Harding-Passey mouse melanoma with deoxycholate (DOC) (Seiji and Iwashita 1964). It was revealed that about 95% of the total tyrosinase activity were found in the soluble phase with the presence of 0.4% DOC. When DOC was removed by gel filtration, however, a considerable amount of membranous material was obtained after centrifugation, and most of the tyrosinase activity, total protein, and phospholipids as well as other enzyme activities were thus recovered in the membranous sediment obtained. From these experimental results it was clear that the "solubilization" of tyrosinase and other constituents of the membranes by the DOC treatment was only apparent and could be reversed by the removal of DOC.

The experiment described in the present paper was performed in an effort to

clarify the effect of DOC on the smooth-surface membranes isolated from Harding-Passey mouse melanoma. Smooth-surface membranes were treated with 0.05% DOC and subjected to electron microscopic observation. The sectioned specimens as well as negatively stained specimens were studied.

MATERIALS AND METHODS

Preparation of the smooth-surface membranes

Harding-Passey mouse melanomas, which had been serially transplanted in Swiss strain mice, were excised when they were about 1.0 to 1.5 cm in diameter and were promptly homogenized in nine volumes of 0.25 M sucrose at 0°C with a Potter glass Teflon homogenizer to give a 10% homogenate (wt/vol). All subsequent procedures were carried out at temperatures below 4°C. The homogenate was centrifuged at $11,000 \times g$ for 10 min and the resulting supernatant was centrifuged at $105,000 \times g$ for 60 min. The sediment was resuspended in 1.32 M sucrose and the smooth-surface membrane fraction was isolated by the Rothschild method (Rothschild 1961) with a slight modification: the small granule suspension instead of the "mitochondrial supernatant" was placed at the bottom of the centrifuge tube.

DOC treatment

DOC in 0.25 M sucrose and 0.05 M Tris buffer, pH 7.8, was mixed with the smooth-surface membrane suspension in 0.1 M phosphate buffer, pH 7.0, for a final concentration of 0.05%. The protein content was determined according to Lowry *et al.* (1951) using bovine serum albumin as a standard.

Morphological methods

For electron microscopy, suitable aliquots of the suspension of the smooth-surface membranes were centrifuged at $105,000 \times g$ for 1 hour. The sediment thus obtained was fixed at 0°C with 2.5% glutaraldehyde in phosphate buffer at pH 7.2 for 2 hrs and then in 1% osmium tetroxide solution for 2 hrs. The sediment was then dehydrated in a graded series of ethyl alcohol and embedded in Epon 812. The specimens were sectioned with a Porter-Blum Ultrathin microtome and stained doubly with uranyl acetate and lead citrate. The suspension of the smooth-surface membranes was also stained negatively with phosphotungstate using the method described by Oda and Nishi (1963).

RESULTS AND DISCUSSION

Under the electron microscope, smooth-surface membranes appear as membrane-bound closed vesicles, as shown in Fig. 1. The membrane appears sectioned normally at A, obliquely at B, and grazing at C. These sections show the characteristic unit membrane appearance. It is noted that most smooth-surface membranes are organized in closed vesicles and that membrane fragments with free margins occur only in small numbers. Some of the vesicles seem to contain part of their content. A unit membrane measured 50 Å across. The nature of the highly electron-dense small particles, some of which are attached to the inside surface of the vesicles, is not known at the present time yet, but they do not seem to be artifacts. As shown in the insert in Fig. 1, it is possible to see a figure in which two unit membranes are united side by side. Figure 2 shows an electron micrograph of smooth-surface membranes treated with 0.05% DOC.

There are numerous small vesicles, 300 to 500 Å in diameter, and some of them are grouped in a cluster and enclosed by a membrane, as shown in Fig. 3. In some parts of the section there are large vesicles that appear to be swelling. Figure 4 shows that the unit membrane structure of 50 Å in diameter appears to be retained, and a few to several unit membranes unite side by side to form a myelin-like lamellar structures.

Figs. 5 and 6 show electron micrographs of negatively stained smooth-surface membranes. The findings revealed in these micrographs are quite similar to those obtained in the sectioned specimens. Varying degrees of vesiculation of the smooth-surface membranes are apparent. Multivesicular body-like structures are also clearly seen. The repeating small particles, which were repeatedly seen on the untreated smooth-surface membranes in the negatively stained specimens, are not clearly visible (Seiji 1967). Most of the large vesicles are found to exhibit myelin-like lamellar structures. Since the negatively stained preparation only shows surface images of the objects, the lamellar structure seen in both specimens does not necessarily indicate the same membrane architecture. It does not seem unreasonable to assume that only certain tangentially sectioned vesicles show such figures in sectioned specimens and that they are multilayered external-compound membranes which are formed by uniting the unit membranes (Robertson 1964). On the other hand, the lamellar structures seen in the negatively stained specimens are actually images of the regular lamellae formed on the surface of the vesicles. How such lamellar structures are formed on the surface of the vesicles, and why the multilayered external-compound membranes are formed by the DOC treatment, are not known. The lamellar structures seen in the negatively stained specimens do not seem to be the same as those formed in the smooth-surface membranes after trypsin digestion (Seiji *et al.* 1971) but they closely resemble structures seen in electron micrographs of artificial lipid spherulites which are formed with ovoidicithin, dicetyl phosphate and cholesterol (70:20:10 by weight, see Sessa and Weissman 1968).

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Fig. 1. Section through a pellet of the untreated smooth-surface membranes isolated from Harding-Passey mouse melanoma. The pellet consists mainly of swollen smooth-surface membranes which have retained membranes and part of their contents. The membrane appears sectioned normally at A, obliquely at B, and grazingly at C. A unit membrane measures 50 Å across. Magnification: 80,000 $\times$, insert 200,000 $\times$

Fig. 2. Section through a pellet of the DOC-treated smooth-surface membranes. There are numerous small vesicles, 300 to 500 Å in diameter, and some of them are grouped in a cluster and enclosed by a membrane. 80,000 $\times$.

Fig. 3. Section through a pellet of the DOC-treated smooth-surface membrane. In some parts there are large swollen vesicles and a few multivesicular body-like structures. The unit membrane structure, 50 Å in diameter, appears to be retained and a few to several unit membranes unite side by side to form myelin-like lamellar structures. 80,000 $\times$.

Fig. 4. High magnification of part of a section through a pellet of the DOC-treated smooth-surface membrane. There are many small vesicles and a myelin-like lamellar structure. 200,000 $\times$.

Fig. 5. Electron micrograph of the DOC-treated smooth-surface membranes isolated from Harding-Passey mouse melanoma and stained negatively with phosphotungstate. Many small vesicles of various sizes and multivesicular body-like structures are also visible. The large vesicles are found to exhibit myelin-like lamellar structures. $200,000\times$.

Fig. 6. A part of the DOC-treated smooth-surface membranes stained negatively. There are lamellar structures on the outer and inner sides of the large vesicle. They seem to be regular lamellae formed on the surface of the vesicles. $200,000\times$.