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ISOLATION AND SOME PROPERTIES OF PLASMA MEMBRANES FROM BOVINE OLFACTORY EPITHELIUM

NORIYUKI KOYAMA, KAZUKO SAWADA AND KENZO KURIHRA
Biological Laboratory, Tokyo Institute of Technology, Meguro-ku, Tokyo (Japan)
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SUMMARY

Plasma membranes were isolated from bovine olfactory epithelium by differential and sucrose density gradient centrifugation. Assays of mitochondrial and microsomal enzymes indicated that very little contamination of these structures was present in the isolated plasma membrane fraction. Electron microscopic examination of the fraction showed that the fraction is composed of membrane profiles essentially uncontaminated by other cellular components. The activity of $(Na^+ + K^+)$-ATPase of the isolated membrane was much lower than that of the nerve membrane.

The chemical composition of the isolated membrane was determined. It was found that the membrane is characterized by a high content of lipid, especially phospholipid. The major components of phospholipids were phosphatidylcholine, phosphatidylethanolamine, and sphingomyelin.

INTRODUCTION

Initial interaction of odorous molecules with the olfactory organ occurs on the surface of the epithelium of the organ. Since olfactory receptor cells of vertebrates are ciliated[1], the initial interaction is most likely to occur on the cilia. However, it was insisted that cilia may not function in olfactory reception at all, on the basis of the observation that neural responses to odorous molecules recovered even after olfactory cilia had been removed by detergents[2].

DAVIES[3] proposed a theory that a temporary "puncturing" of the olfactory receptor cell membrane by adsorption of odorous molecules induces olfactory stimulation. Regardless of whether one admits their puncturing theory or not, it seems to be reasonable to think that the receptor cell membrane is the most probable site at which the initial process of olfactory reception occurs.

While taste receptor cells of vertebrates are distributed sporadically on the surface of the tongue, the olfactory receptor cells which are the terminal swellings of the olfactory nerves are densely localized together with the supporting cells in olfactory epithelium[1]. In the present study, plasma membranes were isolated from bovine olfactory epithelium and some of the chemical properties of the membrane were examined. Recent successful studies[4–7] on the isolation of plasma membranes of animal cells encouraged us to start the present study.

EXPERIMENTAL

Isolation of plasma membrane

The olfactory tissues were obtained from cows as soon as possible after they were killed and brought to the laboratory under cooling at 0°. The olfactory epithelium, which is a tender surface layer resting on the rather hard basement membrane, was shaved off gently with a spatula from the pigmented area of the olfactory tissues[8]. About 11 g (wet wt.) of the epithelium including mucus were collected from twenty cows and used for an experiment.

The collected epithelium was washed twice with 0.3 M sucrose solution containing 1 mM $CaCl_2$ by centrifugation at 6000 × *g* for 15 min and homogenized with a Potter homogenizer with four strokes of a loose Teflon pestle. The homogenate was diluted 100 times the wet wt. of the epithelium with homogenizing medium according to the method of RAY[9]. The diluted homogenate was centrifuged at 700 × *g* for 12 min. The pellet was homogenized in the same medium using two gentle strokes. The suspension was diluted to half the previous volume and centrifuged at 700 × *g* for 12 min. The combined supernatant from the first and second spins at 700 × *g* were centrifuged again at 700 × *g* for 12 min and the scanty pellet was discarded. The supernatant was centrifuged at 7000 × *g* for 15 min. The resulting pellet was washed twice, and resuspended in a small volume of the homogenizing medium by gentle homogenization.

The obtained suspension was carefully layered on a sucrose gradient with steps 37, 35 and 23 % (w/w), and it was centrifuged at 25000 rev./min for 2 h in a RSP 25 A rotor in a Hitachi 55P-2 ultracentrifuge. The brownish-white material at the interface between 23 and 35 % sucrose solution was collected by centrifugation. The pellet was resuspended in a small volume of 1 mM $CaCl_2$ solution and then mixed with 65 % sucrose solution so that the final concentration of sucrose was 43 %. This suspension was pipetted into a centrifuge tube. Over this suspension the following five different concentrations of sucrose solutions were carefully layered in order: 37, 35, 33, 30 and 28 %. This was centrifuged at 25000 rev./min for 2 h in a RSP 25 A rotor in Hitachi 55P-2 ultracentrifuge. All the above operations were carried out at 2°.

Isolation of mitochondrial and microsomal fractions

The olfactory epithelium was homogenized in 0.3 M sucrose solution with a Teflon homogenizer using six strokes, and centrifuged at 900 × *g* for 10 min. The supernatant was centrifuged at 8000 × *g* for 20 min. The suspension of the pellet in a small volume of 0.3 M sucrose solution was fractionated by discontinuous sucrose gradient centrifugation (from 0.8 to 1.6 M), and the fraction at the interface between the 1.2 and 1.4 M sucrose solution was used as the mitochondrial fraction because it had the highest cytochrome *c* oxidase activity.

The supernatant from the above centrifugation (8000 × *g* for 20 min) was centrifuged at 30000 × *g* for 30 min. The pellet was discarded and the microsomal fraction was obtained as the pellet by centrifugation of this supernatant at 150000 × *g* for 60 min.

Enzyme assays

The activity of $(Na^+ + K^+)$-ATPase was measured as a marker of plasma

membrane according to the method of NAKAO *et al.*[10] and the activity of Mg^{2+}-ATPase was also measured[10]. Contamination by microsome and mitochondria was monitored by the activities of the following enzymes as markers: microsomes by NADH–cytochrome *c* reductase[11], mitochondria by cytochrome *c* oxidase[12] and monoamine oxidase[13].

Chemical determinations

Protein was measured according to the method of LOWRY *et al.*[14] with bovine serum albumin as standard.

Lipids of the plasma membrane, immediately after isolation, were extracted with chloroform–methanol (2:1, v/v) and the extracts were washed according to FOLCH *et al.*[15]. The analysis of lipids was carried out by thin-layer chromatography. The plates were prepared with silica gel G (Merck-Darmstad) and developed with chloroform–methanol–water (70:30:5, by vol.). Individual lipids were identified by simultaneous chromatography with standard lipids purchased from Tokyo Kasei Co. The reagent of DITTMER AND LESTER[16] for phospholipids and the anthrone reagent[7] for glycolipids were used as aids in this identification.

For the quantitative analyses of the individual lipid, the spots of lipids on the silica gel plates were visualized by exposure to I_2 vapour. Cholesterol was eluted with chloroform–methanol (2:1, v/v) from the plates and phospholipids were eluted with the same solvents as were used for common phospholipids[17]. The amount of cholesterol was measured by the modified method of GLICK *et al.*[18]. Phosphorus of phospholipid was estimated by the method of BARTLETT[19] and the amount of phospholipid was calculated by multiplying the P value by 25.

Electron microscopy

A preparation of the isolated plasma membrane was fixed with 1 % OsO_4 in 0.14 M veronal–acetate buffer at pH 7.4 and embedded in methacrylate. The ultrathin sections were stained with uranyl acetate followed by lead citrate and examined in a Hitachi HS-7 electron microscope.

RESULTS AND DISCUSSION

When the bovine olfactory epithelium was homogenized gently in a Teflon homogenizer with a loose pestle, most of the activity of $(Na^+ + K^+)$-ATPase, which is a marker enzyme of the plasma membrane, was found in the mitochondrial fraction which sedimented at 7000 × *g* for 15 min. This fraction was fractionated further into four subfractions (A–D) by discontinuous sucrose gradient centrifugation as described under EXPERIMENTAL. As seen from Table I, the highest activity of $(Na^+ + K^+)$-ATPase was found in Subfraction B, while the highest activity of cytochrome *c* oxidase, which is a marker enzyme of mitochondria, was found in Subfraction C.

Subfraction B was fractionated further into five fractions (B-I–B-V) by discontinuous sucrose gradient centrifugation. The activities of the marker enzymes in these fractions are presented in Table I. The highest activity of $(Na^+ + K^+)$-ATPase was found in Subfraction B-II which exhibited low activity of cytochrome *c* oxidase. This result suggested that the subfraction B-II is a plasma membrane fraction contaminated very little by mitochondria. Although relatively high activity of (Na^+

$+ K^+$)-ATPase was also found in Subfraction B-III, this fraction was not as pure as a plasma membrane fraction because contamination by mitochondria was much greater. The relatively high activity of Mg^{2+}-ATPase in Subfractions B-IV and B-V seems to be derived from the higher content of mitochondria in these fractions.

In order to assess the degree of contamination of mitochondria and microsomes in Subfraction B-II, the activities of their marker enzymes in the fraction were compared with those of the mitochondria and microsomes isolated from the bovine olfactory epithelium. As seen from Table II, it is obvious that the degree of contamination of mitochondria and microsomes in Subfraction B-II is very small. The activity of $(Na^+ + K^+)$-ATPase in Subfraction B-II showed an approx. 50-fold increase, when compared with that in the whole homogenate of the olfactory epithelium. While the nerve plasma membrane possesses very high $(Na^+ + K^+)$-ATPase activity, the $(Na^+ + K^+)$-ATPase specific activity of the bovine olfactory plasma membrane

TABLE I

ENZYME ACTIVITIES IN THE FRACTIONS SEPARATED FROM THE HOMOGENATE OF BOVINE OLFACTORY EPITHELIUM BY DISCONTINUOUS SUCROSE GRADIENT CENTRIFUGATION

Subfractions	*Sucrose concn.* (%)	*μmoles P_i released/mg protein per h*		*Cytochrome c oxidase (ΔA units per mg protein per min)**
		$(Na^+ + K^+)$-ATPase	*Mg^{2+}-ATPase*	
Whole homogenate		0.16	1.17	0.483
A	0–23	0.71	2.59	0.492
B	23–35	2.14	4.46	0.527
C	35–37	1.17	6.23	2.270
D	Sediment	0.55	2.41	0.371
B-I	28–30	1.81	3.64	0.128
B-II	30–33	7.48	4.27	0.141
B-III	33–35	5.39	4.84	0.426
B-IV	35–37	3.38	5.99	0.527
B-V	37–43	1.57	6.06	0.633

* 1 *A* unit is defined as the amount of enzyme catalyzing an increase or decrease of 1.00 per min in the absorbance of the substrate[13,20].

TABLE II

COMPARISON OF THE RESPECTIVE MARKER ENZYME ACTIVITIES OF MITOCHONDRIA AND MICROSOME IN THE PLASMA MEMBRANE FRACTION (B-II) WITH THOSE IN MITOCHONDRIAL AND MICROSOMAL FRACTION FROM THE OLFACTORY EPITHELIUM

Fraction	*ΔA units per mg protein per min**		
	Cytochrome c oxidase	*Monoamine oxidase*	*NADH-cytochrome c dehydrogenase*
Plasma membrane fraction (B-II)	0.141	0.004	0.63
Mitochondrial fraction	10.4	0.218	—
Microsomal fraction	—	—	8.46

* Units defined as in Table I.

(B-II) was roughly one order of magnitude lower than that of the squid nerve plasma membrane[21].

Fig. 1 is an electron micrograph of Subfraction B-II which shows that the fraction is composed essentially of membrane profiles, although sometimes a small amount of electron-dense materials which are difficult to identify is seen.

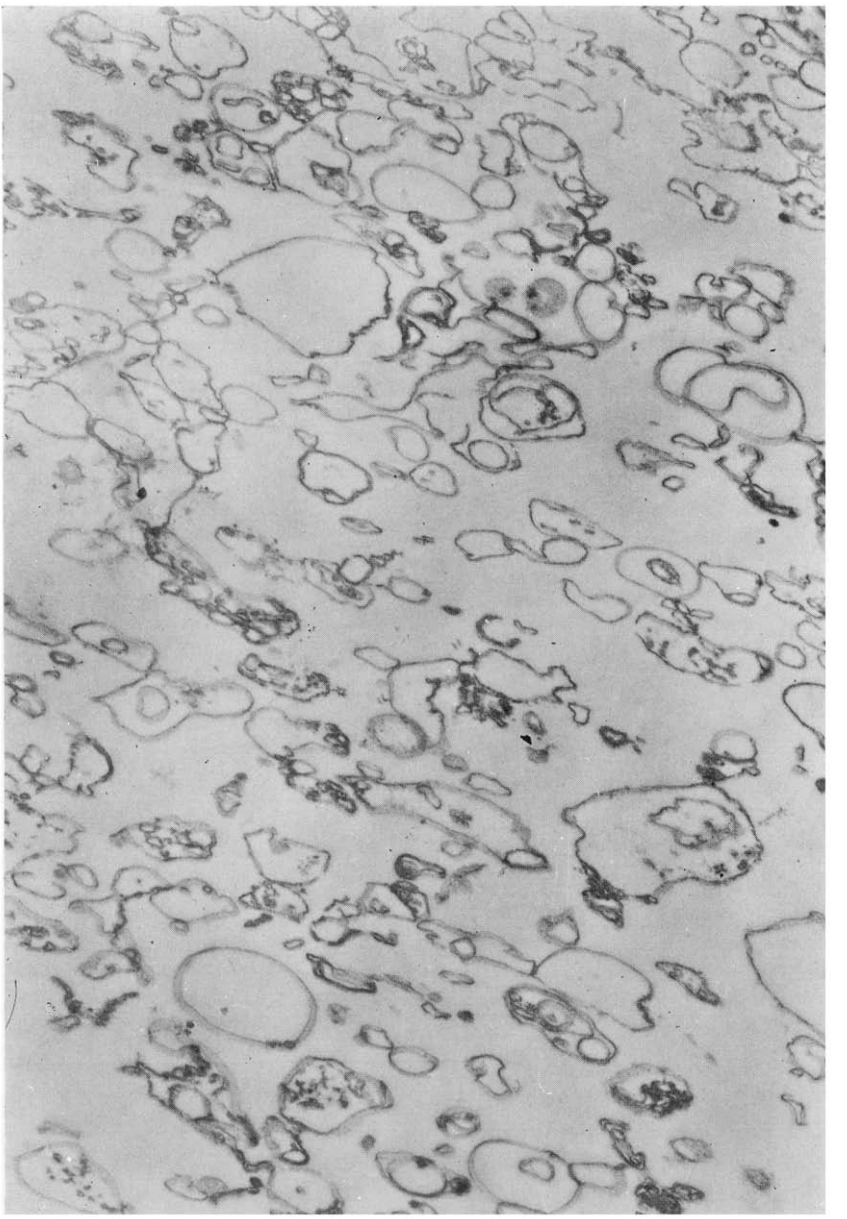

Fig. 1. Electron micrograph of membrane fraction (B-II), which on account of the enzymic data may contain mostly plasma membranes. Magnification × 25000.

TABLE III

CHEMICAL COMPOSITION OF THE PLASMA MEMBRANE FROM THE OLFACTORY EPITHELIUM

Data are expressed in percentages on a weight basis.

	Plasma membrane from the olfactory epithelium	*Whole homogenate from the olfactory epithelium*	*Plasma membrane (Fraction 1)* from the first stellar nerves of the squid (Dosidicus gigas)*[7]	*Plasma membrane from rat liver*[20]
Non-lipid material	40.4	77.8	29.5	60.2
Total lipids	59.6	22.2	70.5**	39.8
Wt. % of total lipids	100	100	100	100
Cholesterol	10.4	6.0	28.1	19.0
Glycolipids	Traces	Traces		5.6
Phospholipids	76.8	72.1	46.8	55.4
Phosphatidyl-ethanolamine	18.7	20.1	16.1	10.2
Phosphatidylcholine	34.8	30.8	21.5	19.3
Sphingomyelin	11.7	11.1	4.7	9.8

* Fraction 1 is considered to be the axolemma fraction.

** According to FISCHER *et al.*[21], total lipids account for 45.4 % of the plasma membrane from the retinal axon of the squid.

Table III shows the chemical composition of the isolated plasma membrane (B-II) and the whole homogenate of the olfactory epithelium. For comparison, the chemical composition of the plasma membrane from the first stellar nerves of the squid[7] and that of rat-liver plasma membrane[20] is presented in the table. The content of total lipids in the isolated plasma membrane is 59.6 % and this percentage of total lipids is fairly high, compared with the lipid content in the rat liver plasma membrane (39.8 %) or that in red cell membrane[22] (22–31 %), although the lipid content in the isolated plasma membrane is a little lower than that in the squid nerve plasma membrane[7] which is one of the membranes having the higest lipid content among plasma membranes isolated from animal sources. Of the total lipids in the isolated plasma membrane, phospholipids account for an extremely high percentage, that is, the content of phospholipids exceeds 76.8 % of the total lipids, while the cholesterol content is only 10.4 % of the total lipids. The phospholipids consist mainly of phosphatidylcholine, phosphatidylethanolamine and sphingomyelin, and phosphatidylcholine accounts for 34.8 % of total lipids. The lipid composition of the isolated plasma membrane resembles that of the whole homogenate of the olfactory epithelium in a general profile, although the plasma membrane of the olfactory epithelium has a much higher lipid content. While glycolipids in rather high amounts were found in the brain white matter or myelin[23], only traces of glycolipids were found in the whole homogenate of olfactory epithelium as well as in the plasma membrane of the epithelium.

An attempt was made by KOCH[24] to isolate the concentrated plasma membrane fraction from the whole homogenate of the septum and turbinate tissues in rabbit olfactory area. In the present study, only part of the olfactory epithelium which consists mainly of the receptor cells and supporting cells[8] were used for isolation of the membrane fraction containing plasma membrane of the receptor cells in the highest

possible content. An attempt to separate different types of plasma membranes from the olfactory epithelium did not succeed in the present study, probably because the density of the receptor cell membrane is not so different from that of the supporting cell membrane.

It is said that odorous molecules are lipoid soluble[25]. The finding that the phospholipid content was very high in the plasma membrane of the olfactory epithelium may suggest that the interaction of odorous molecules with phospholipid in the membrane plays an important role in the primary process of olfactory reception.

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