Biochimica et Biophysica Acta, 597 (1980) 564—576

BBA 78663

THE BINDING OF CYTOCHROME b_5 TO PLASMA MEMBRANES OF RAT LIVER

ITS IMPLICATION FOR MEMBRANE SPECIFICITY AND BIOGENESIS

JOSÉ REMACLE

Department of Biology, University of California at San Diego, La Jolla, CA 92037 (U.S.A.) and Laboratoire de Biochimie Cellulaire, Facultés Universitaires, B-5000 Namur (Belgium)

(Received March 26th. 1979)
(Revised manuscript received September 5th, 1979)

Key words: Membrane specificity; Membrane synthesis; Cytochrome b_5 binding; (Hepatocyte plasma membrane)

Summary

The in vitro incorporation of cytochrome b_5 into purified plasma membranes was investigated by biochemical and immunological methods. Plasma membrane preparations incorporated three times less cytochrome b_5 than did microsomal preparations; 60% of this cytochrome b_5 could not be reduced by the NADH-cytochrome b_5 reductase and was considered as being bound to the plasma membrane. The morphological observations made after the immunochemical labeling of cytochrome b_5 clearly showed a good but asymmetrical distribution of the ferritin labeling: only the inner face of the plasma membrane incorporated cytochrome b_5. These results are discussed with respect to theories which concern the subcellular membrane relationships in the cell.

Introduction

Cytochrome b_5 is an integral protein which has been purified from liver microsomes (D-cytochrome b_5) after solubilization with detergents [1,2]. It is an amphypathic protein consisting of a hydrophobic segment embedded in the lipid bilayer and a hydrophilic moiety which carries the prosthetic group [1,2]. The latter part of the protein can be purified (T-cytochrome b_5) from the microsomes after being solubilized by trypsin [3,4].

D-Cytochrome b_5 can be incorporated in vitro into the membranes of hepatic cells where it is normally located, i.e. in the endoplasmic reticulum outer mitochondrial and peroxisomal membranes [5—8]. Golgi apparatus and inner mitochondrial membranes which do not contain this protein can

incorporate it easily; on the other hand its incorporation in the lysosomal membranes seems to be impossible [8]. Membranes isolated from other cell types can also incorporate cytochrome b_5 [9] and numerous works have been devoted to its binding to artificial membranes [10—15].

In this report, we investigate the in vitro binding of D-cytochrome b_5 in the isolated plasma membranes of rat liver. In this respect, we complete the pattern of the relationships between the natural location of an integral protein and its in vitro binding to the different subcellular membranes of the same cell. Results show that cytochrome b_5, which is not present in the plasma membrane, binds in vitro to the inner face of this membrane. This asymmetric binding is discussed in relation with the asymmetric organization of the plasma membrane.

On the other hand, this positive result reinforces the idea that the non-specific binding of any integral protein to any membrane is possible unless the presence of lipids in a semi-crystalline state, or the presence of some steric factors which hinder the contact between the protein and the lipid phase of the membrane [16].

A brief report of these results has already been given [17].

Materials and Methods

Membrane preparation

Using Neville's method [18], plasma membranes from rat liver were purified but with the difference that the pellet obtained after the low speed centrifugation of the homogenate was not washed. The microsomes were prepared as described in detail by Amar et al. [19]. Both preparations were washed at 4°C with 0.9% NaCl in 10 mM Tris-HCl, pH 7.4, in order to remove any adsorbed hemoproteins which interefere with the cytochrome b_5 assay.

Biochemical and immunological assays

Cytochrome b_5 measurements were realized on a Cary 17 (Cary Instruments, Monrovia, CA) using the difference spectra of the oxidized and reduced hemoprotein and assuming a $\Delta\epsilon_{424-409nm}$ of 160 $cm^{-1} \cdot mM^{-1}$ and a molecular weight of 12900 [20]. The reduction of the hemoprotein was achieved either by cytochrome b_5 reductase in the presence of 63 mM NADH or by the addition of a few grains of $Na_2S_2O_4$. Enzymatic and other chemical components were assayed by the usual methods as described by Beaufay et al. [21].

The activity of the anti-cytochrome b_5 antibodies was estimated by their capacity to bind T-cytochrome b_5 [22]. One unit of anti-cytochrome b_5 activity was expressed as the amount of antibody needed to bind 1 μg of T-cytochrome b_5.

Enzyme and antibody preparations

T-cytochrome b_5 was purified from rat liver microsomes after tryptic digestion, as described by Omura et al. [4] and D-cytochrome b_5 after solubilization with detergents, as proposed by Spatz and Strittmater [1]. T-Cytochrome b_5 was used to produce rabbit anti-cytochrome b_5 antibodies. These antibodies were purified by affinity chromatography on a Sepharose 4B-cytochrome b_5

column. Details on the experimental procedure of the antigen and antibody preparations were given earlier [22]. The two preparations were found pure according to the criteria of the classical immunochemical methods [22]. F(ab) fragments were obtained after papaïn digestion [23]. The anti-F(ab)/ferritin conjugates were prepared by reacting ferritin and affinity purified IgG goat anti-rabbit F(ab) with glutaraldehyde [24]. After the reaction, free IgG, ferritin aggregates and most of the free ferritin molecules were separated from the IgG/ferritin conjugates by chromatography on a 6% agarose gel [25]. The conjugate was adsorbed by rat liver microsomes just before use [26]. The analysis of the reactivity of the anti-cytochrome b_5 F(ab) fragments and of the IgG/ferritin conjugates were reported in a preceding paper [8]

Cytochrome b_5 binding and immunological labeling of the plasma membrane

The plasma membrane preparation (1 mg protein) was incubated for 20 min at 37°C with 30 nmol of D-cytochrome b_5 in 1 ml of 10 mM Tris-HCl buffer, pH 7.4. T-Cytochrome b_5 was used in a control experiment. The membranes were spun down by centrifugation at 30 000 × g for 15 min in a Sorval centrifuge and washed twice with 10 ml buffer. Cytochrome b_5 was assayed on half the preparation. The other half was incubated for 1 h at 4°C, in 1 ml of the same buffer containing 20 mg of bovine serum albumin, with anti-cytochrome b_5 F(ab). The amount of F(ab) fragments was calculated to have a binding activity twice as high as the amount of membrane-bound cytochrome b_5 present in the test. A similar fraction was incubated with normal F(ab) as a control. The membranes were washed twice as described above. They were then incubated for 15 min at 4°C in the presence of 40 mg of bovine serum albumin with the anti-F(ab) ferritin conjugate. The amount of conjugate introduced corresponded to two ferritin molecules per cytochrome b_5 present in the preparation. Finally, the membranes were washed three times as before and resuspended in 0.9% NaCl solution. One sample of the final preparation was incubated with a low amount of digitonin (0.3 mg digitonin per mg protein). Another sample was incubated for 10 min at 4°C with purified vesicular stomatitis virus (Indiana *c* strain) kindly provided by J. Holland. The membranes were again washed twice as mentioned above. All the preparations were fixed with 1.5% glutaraldehyde, collected on millipore filters and embedded in epon [27].

The saturation curves were performed in the same conditions except that the washings were performed at 100 000 × g for 30 min in order to sediment the microsomal membranes.

Results

Membrane preparations

Table I summarizes the biochemical properties of the membrane preparations. The purification of the plasma membrane compared to the homogenate is 24-fold considering the two enzyme markers: alkaline phosphodiesterase 1 and 5′-nucleotidase. The protein content and the enzyme activity of the microsomal preparation are lower than the results reported by Amar et al. [19] but the relative specific activities of the enzymes are quite similar.

TABLE I

BIOCHEMICAL PROPERTIES OF THE MEMBRANE PREPARATIONS

Constituent *	Preparation			
	Plasma membrane I		Microsomes	
	% of homogenate	RSA **	% of homogenate	RSA **
Protein	0.32	1	14.4	1
Alkaline phosphodiesterase I	7.1	22.2	42.3	2.94
5'-Nucleotidase	8.3	25.9	47.4	3.29
Glucose-6-phosphatase	0.12	0.37	63.0	4.37
NADH-dependent cytochrome *c* reductase	0.18	0.57	47.6	3.30
Cytochrome oxidase	0.18	0.55	5.7	0.39
Cytochrome b_5 content (nmol/mg protein)	0.07		0.46	

* Recoveries of fractions compared to the homogenate ranged between 85 and 105%.

** Relative specific activity, calculated as the ratio of the percentage of enzyme activity to the percentage of protein compared to the homogenate.

In Table II, we converted the relative specific activity of the enzymes given in Table I into percentages of the protein of each preparation attributable to the corresponding membranes. The data show that the plasma membrane preparations are not pure and are contaminated by endoplasmic reticulum and mitochondrial membranes. In other preparations, assays for galactosyltransferase were performed and we calculated that Golgi elements represented about 3% of the protein of the plasma membrane preparation and 4% of the microsomes (not shown).

TABLE II

ESTIMATION OF THE AMOUNT OF THE PROTEIN RELATED TO SUBCELLULAR COMPONENTS IN EACH PREPARATIONS

Calculations were performed according to Leighton et al. [28]. Values are expressed in percentages of the protein of the fraction. The relative specific activities were taken on an average of 30 for 5'-nucleotidase and alkaline phosphodiesterase I in the plasma membranes; 4 for NADH-cytochrome *c* reductase, and 5 for glucose-6-phosphatase in the endoplasmatic reticulum, and 10 for cytochrome oxidase in the broken mitochondria. To estimate the amount of endoplasmatic reticulum (ER) membranes in the preparations, we used a corrected value for the NADH-dependent cytochrome *c* reductase activity assuming that the enzyme associated with the mitochondria represents 20% of the total liver activity [19].

Components	Preparations	
	Plasma membrane preparation (%)	Microsomes (%)
Plasma membrane	80.0	10.4
ER membrane	10.0	84.0
Mitochondria	5.5	7.8
Total	95.5	102.2

The binding of cytochrome b_5 to the membranes

The saturation curve of cytochrome b_5 binding to the microsomes is presented in Fig. 1. As previously shown [5], the microsomal membranes bind large amounts of the hemoprotein. The larger part of the bound cytochrome (about 90%) is reduced by the NADH-cytochrome b_5 reductase. The same experiment was performed with the plasma membrane preparation (Fig. 2). In this case, much less cytochrome can be bound and only a small part of the total bound hemoprotein is reduced by the NADH cytochrome b_5 reductase. From Figs. 1 and 2, one can calculate the ratio between the amount of cytochrome b_5 non-reduced to reduced in the presence of NADH. This ratio is constant for all the concentrations of cytochrome b_5 tested and is found to be around 1.6 and 0.1, respectively, for the plasma membrane and the microsomal preparations. When an equal amount, calculated on a protein basis, of both preparations was incubated with cytochrome b_5 and the amount of bound cytochrome measured, we observed a constant ratio of 0.3. All these observations show that this ratio is hardly affected by the amount of cytochrome b_5 incubated with the membrane but mostly depends on the composition of the preparation; the more the amount of plasma membrane there is in the preparations, the higher the ratio.

In the labeling experiments, after cytochrome b_5 incubation with the plasma membrane preparation, the amount of bound hemoprotein reduced by NADH was 0.131 and 1.4 nmol/mg protein for the control (T-cytochrome b_5) and for the test (D-cytochrome b_5), respectively; the total amount reduced by $Na_2S_2O_4$ was 0.22 and 3.5 nmol/mg protein for the control and for the test, respectively.

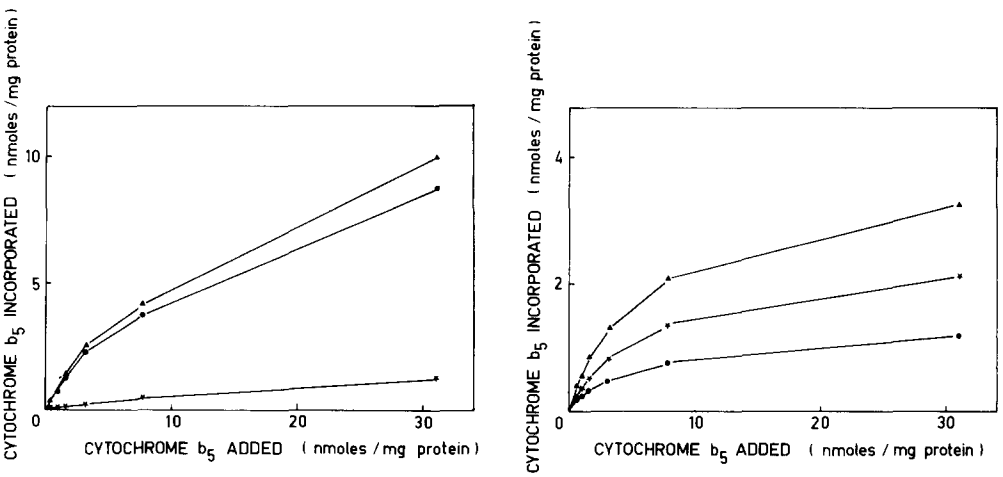

Fig. 1. Binding of cytochrome b_5 to microsomes. The binding was carried out for 20 min at 37°C, as described in Materials and Methods. The values obtained with D-cytochrome b_5 were corrected for the binding of T-cytochrome b_5. ▲———▲, cytochrome b_5 reduced by $Na_2S_2O_4$; ●———●, cytochrome b_5 reduced in the presence of NADH; *———*, cytochrome b_5 non-reduced by NADH.

Fig. 2. Binding of cytochrome b_5 to plasma membrane preparation. The binding was carried out for 20 min at 37°C, as described in Materials and Methods. The amounts of bound D-cytochrome b_5 were corrected for the binding of T-cytochrome b_5. ▲———▲, cytochrome b_5 reduced by $Na_2S_2O_4$; ●———●, cytochrome b_5 reduced in the presence of NADH; *———*, cytochrome b_5 non-reduced by NADH.

Morphological observations

The morphological aspect of the control experiment obtained after the incubation of the plasma membrane with D-cytochrome b_5, normal F(ab) fragments and anti-F(ab)/ferritin conjugate is presented in Fig. 3. The preparation contains mostly large and small vesicles without precise characteristics. Some particular structures can tentatively be associated with broken mitochondria. The non-specific binding of the ferritin is very low. Further control obtained after the incubation of the membranes with T-cytochrome b_5, normal F(ab) and the conjugate was very similar and is not presented.

Fig. 4. presents the labeling of endogeneous cytochrome b_5 in the plasma membrane preparation; only a few small vesicles and some membranes which originate from the mitochondria are labeled. The large membrane fragments are not labeled; they originate from the plasma membrane since they show the typical fenestrated appearance after treatment with digitonin (Fig. 4b). None of the small labeled vesicles have the same behavior and are considered as contaminant membranes probably derived from the endoplasmic reticulum membrane.

After the incorporation of D-cytochrome b_5, the small and most of the large membrane fragments are labeled (Fig. 5). However the larger profiles carry ferritin on one face only which appears in the picture to be the inner face of the vesicle. In Fig. 6, plasma membranes are easily recognizable by their fenestrated aspect; some of them are positively labeled, others not at all. All the con-

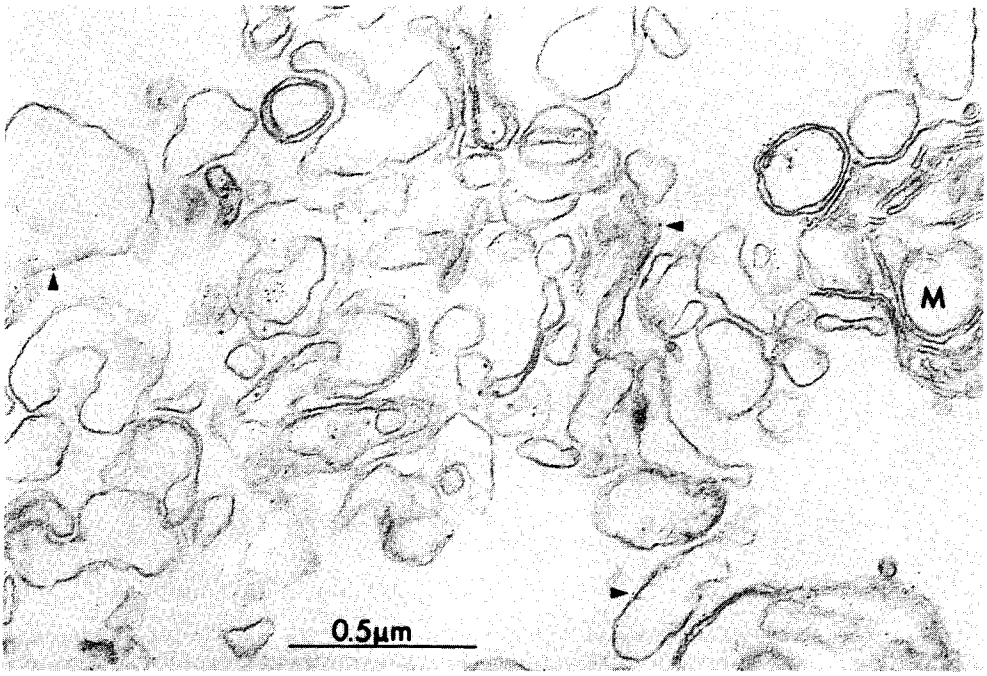

Fig. 3. Plasma membranes incubated with D-cytochrome b_5 and normal F(ab) before labeling with anti-F(ab)/ferritin conjugate. Occasional ferritin molecules on the surface of the membranes (arrow). Probably structures derived from mitochondria (M). ×50 000.

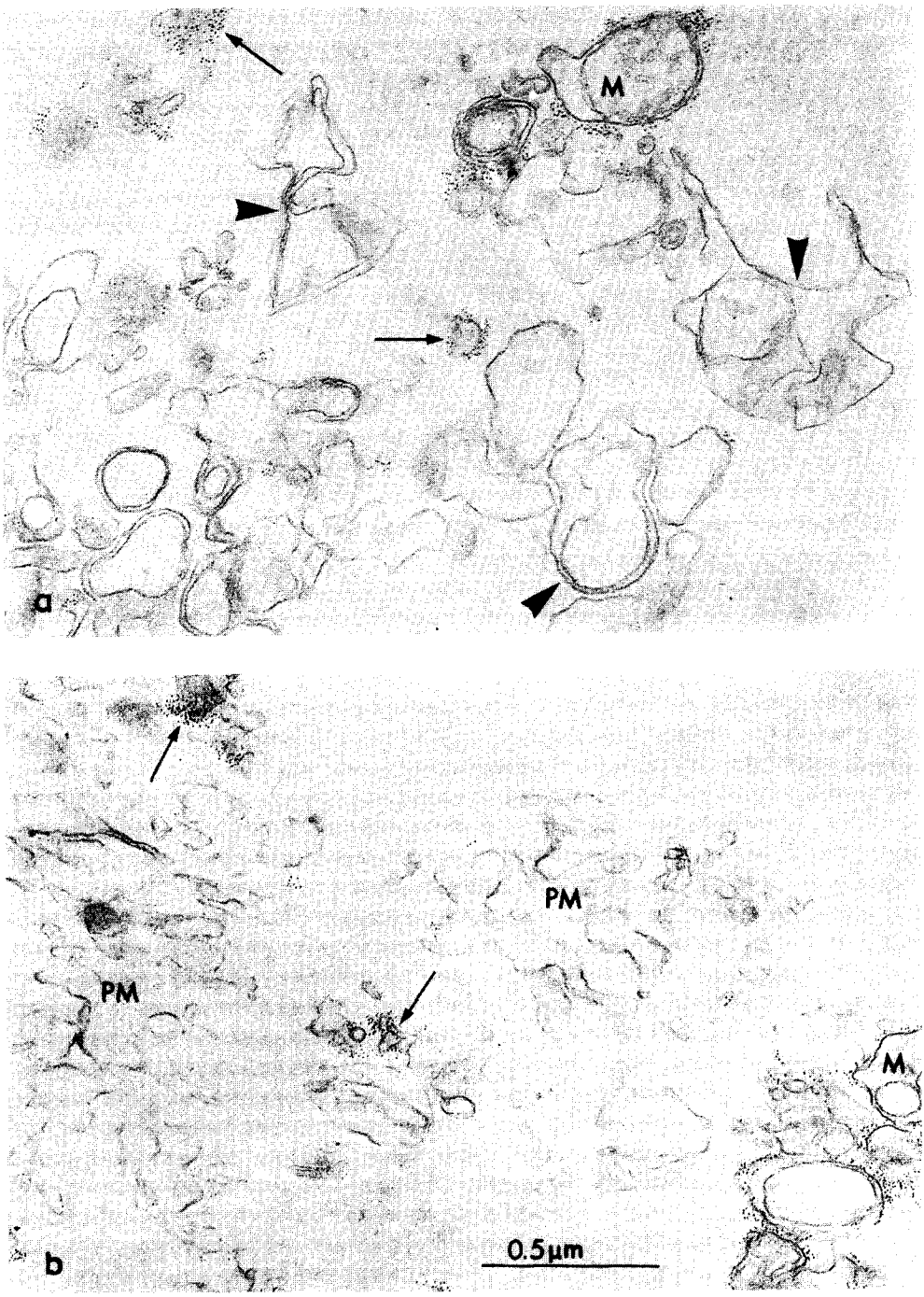

Fig. 4. (a) Plasma membranes incubated with T-cytochrome b_5 and F(ab) anti-cytochrome b_5 before labeling with anti-F(ab)/ferritin conjugate. Large membrane fragments devoid of ferritin (large arrows); small profiles heavily labeled (small arrows). Mitochondria (M) showing a positive reaction on the outside of the outer membrane. ×50 000. (b) Same preparation but incubated with digitonin before fixation with glutaraldehyde. Plasma membranes (PM) recognized by their typical fenestrated appearance are not labeled. Small profiles (small arrows) and mitochondria (M) non altered by digitonin and carrying ferritin molecules. ×50 000.

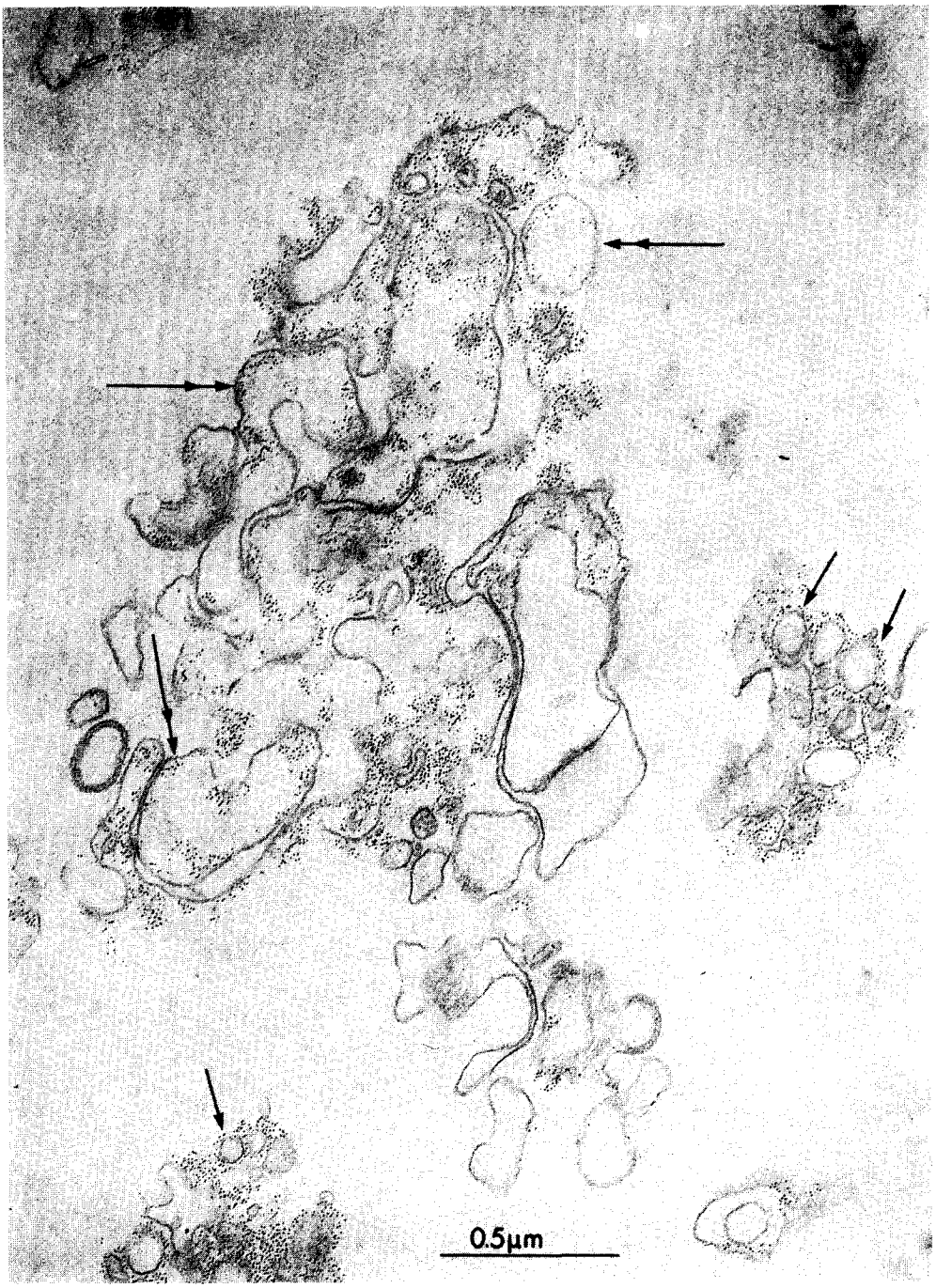

Fig. 5. Plasma membranes labeled with anti-F(ab)/ferritin conjugate after incubation with D-cytochrome b_5 and F(ab) anti-cytochrome b_5. Large membrane fragments significantly labeled mostly on the inner face of the vesicles (double arrows). Small profiles heavily labeled on the outer face (small arrows). ×50 000.

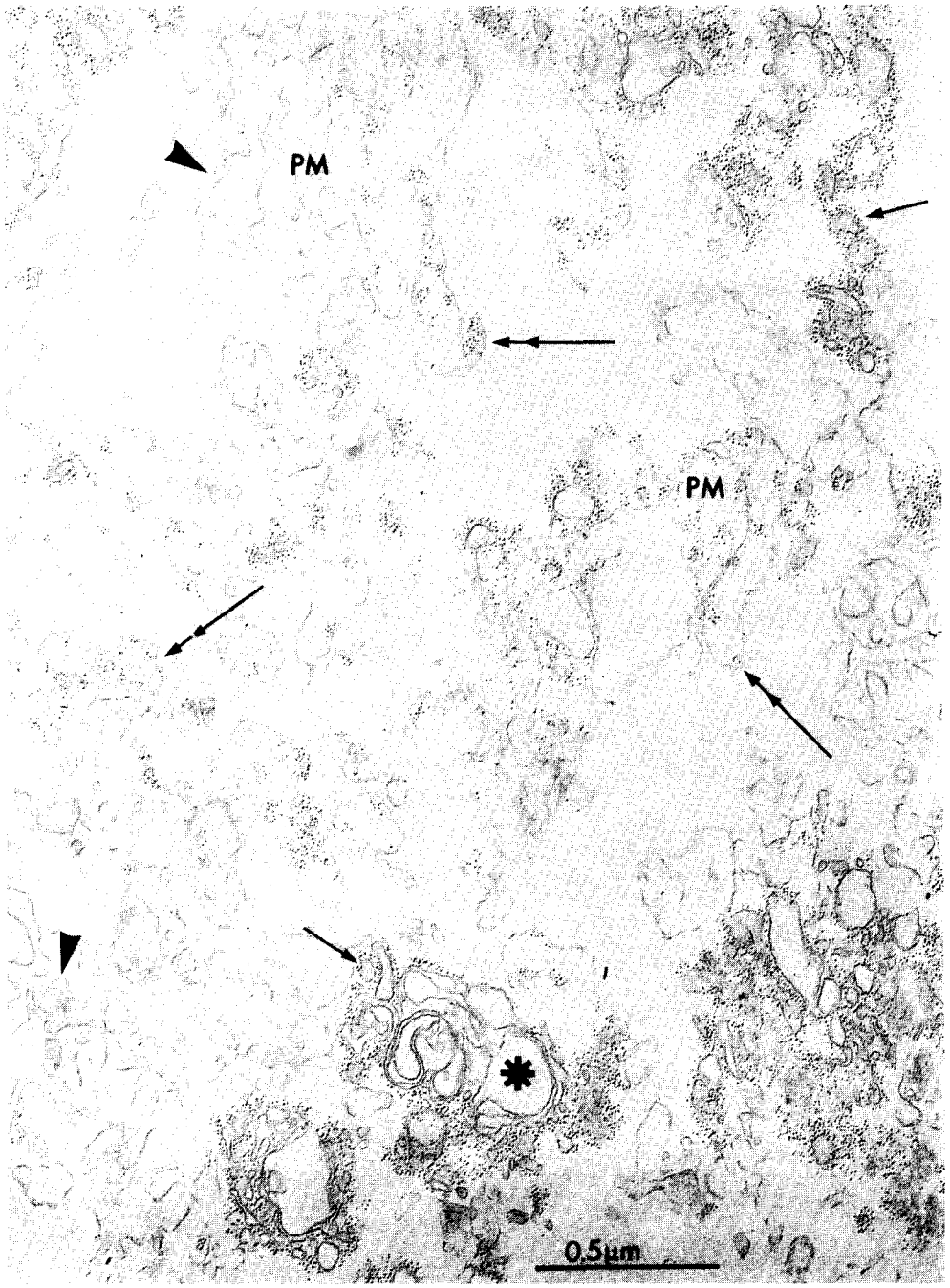

Fig. 6. Plasma membranes prepared as in Fig. 5 but treated with digitonin before fixation with glutaraldehyde. Plasma membranes (PM) identified by their fenestrated appearance are either labeled (double arrows) or completely devoid of ferritin (large arrows). Small vesicles (small arrows) and large membrane complexes (asterisk) probably derived from mitochondria are heavily labeled on the outside of the profiles. ×50 000.

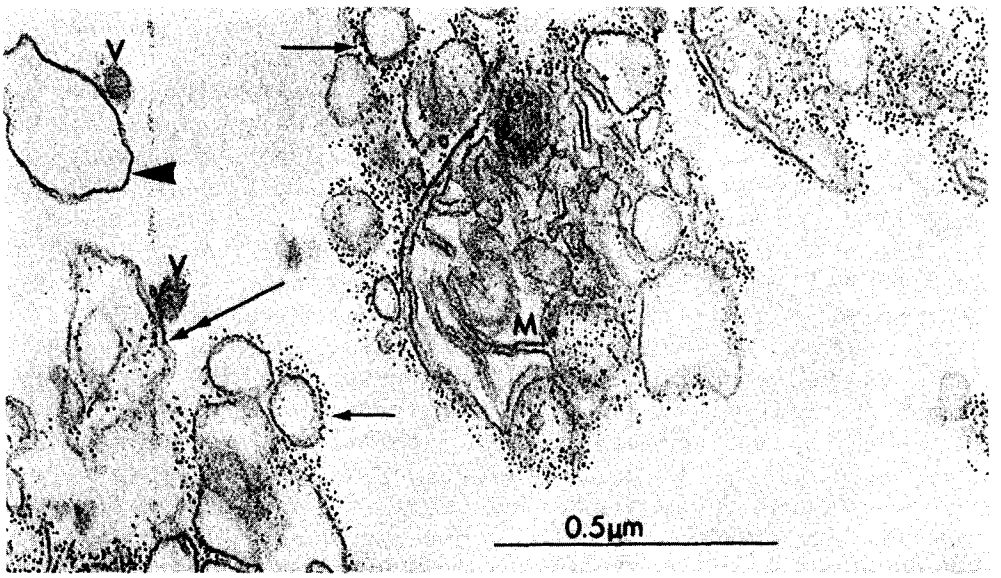

Fig. 7. Plasma membranes prepared as in Fig. 5 but incubated with the vesicular stomatitis virus before being embedded. Virus (V) attached to the membranes. Plasma membranes showing ferritin on the inner face of the profile (double arrows). Plasma membrane non-labeled with ferritin (large arrow). Small profiles without virus particles but heavily labeled on the outer face (small arrow). Mitochondria (M) labeled on the outer face of the outer membrane. ×50 000.

taminants appearing as non-fenestrated membranes are heavily labeled.

In Fig. 7, the membranes were incubated with the vesicular stomatitis virus, washed and then embedded. In each case it is clear that the viruses bind to the opposite face of the ferritin labeling. We know that viruses fix themselves to the plasma membrane of isolated cells. The fact that the outer face of the plasma membrane is the only side on which the virus will fix was demonstrated for the influenza virus [29]. In these pictures we conclude that the plasma membranes appear as right-side fragments with virus bound on the outer and ferritin on the inner face.

Discussion

Compared to the microsomes, the amount of cytochrome b_5 measured in the plasma membrane preparation (Table I) is low. Taking into account the limits of the immunochemical methods, it is clear from Fig. 4 that the cytochrome b_5 detected in the plasma membrane preparation is carried exclusively by contaminant membranes. Fowler et al. [22] reached the same conclusion using an other immunochemical reagent. When the plasma membrane preparation is incubated with D-cytochrome b_5, the amount of bound hemoprotein is 35 times the amount initially present in the preparation (Fig. 2). Most of it cannot be reduced in the presence of NADH. The evidence that the protein is really bound to the plasma membrane is given in Figs. 5 and 6. Since the main contaminant membranes which incorporate D-cytochrome b_5 are endoplasmic reticulum-derived vesicles and outer mitochondrial membranes (Tables I and II)

and since both these membranes contain a NADH cytochrome b_5 reductase, we can consider, with a good approximation, that the hemoprotein non-reduced by NADH, is the cytochrome b_5 bound to the plasma membrane. Assuming that this is correct, we can calculate from Table II and Fig. 1 that the maximum amount of cytochrome b_5 bound solely to pure endoplasmic reticulum-derived vesicles is around 10.5 nmol/mg protein. Similarly when calculated from Table II and Fig. 2 the maximum amount of cytochrome b_5 bound to pure plasma membrane fragments would be 2.56 nmol/mg protein. We conclude that on a protein content, plasma membranes incorporated four times less cytochrome b_5 than the endoplasmic reticulum membranes.

Still assuming that this statement is correct, we consider the ratio of cytochrome b_5 (non-reduced to reduced) in the presence of NADH, as a measurement of the hemoprotein bound to the plasma membrane compared to the two other subcellular membranes (endoplasmic reticulum and outer mitochondrial membranes). This ratio is effectively very high in the plasma membrane preparation (containing mainly plasma membranes), very low in a microsomal preparation (containing mainly endoplasmic reticulum-derived vesicles) and intermediate when the preparations are mixed. More surprising was the fact that these ratios were only slightly modified by the amount of cytochrome b_5 added to the preparation, indicating that the affinity of cytochrome b_5 is identical for the binding sites present in both membranes or that the speed of the cytochrome b_5 incorporation expressed per binding site is identical in both membranes.

All the distribution of the ferritin on the plasma membranes observed after immunochemical labeling of the cytochrome b_5 (Figs. 5—7) are explained if we accept that cytochrome b_5 binds only to the inner face of the plasma membrane. Indeed, ferritin is only found on one face which appears in the picture as the inner face (Figs. 5 and 6) and which is opposite to the virus fixation (Fig. 7). When right-side-out membranes are resealed, no binding of the cytochrome b_5 can be observed, explaining why some vesicular membranes appear devoid of ferritin. Binding of cytochrome b_5 on the inner face of the plasma membranes isolated from human erythrocytes has already been reported [9]. The interpreation given for this asymmetric binding was that cholesterol was mostly present on the outer layer of the membrane and prevented the incorporation of the cytochrome in the lipid bilayer. However, Roseman et al. [30] have shown that cholesterol does not prevent the incorporation of D-cytochrome b_5 in artificial vesicles. It is also possible to incorporate integral proteins isolated from red cell membranes into lipid vesicles containing cholesterol [31]. The debate on the subject is open [32] but it seems to us more plausible to postulate the presence at the periphery of the cell of a barrier which could prevent the incorporation of any foreign hydrophobic molecule into the lipidic phase. This barrier would include glycoproteins and glycolipids which extend to the outside of the plasma membrane [33] and which represent only one example of the asymmetry found in the biological membranes [34].

The binding of D-cytochrome b_5 on isolated rat liver plasma membranes completes the work already realized on other subcellular membranes [1,8] and the studies on the association of cytochrome b_5 with hydrophobic substances like phospholipids and detergents [10—15]. It reinforces the idea that the fixa-

tion of an integral protein on subcellular membranes is a non-specific phenomenon and that the only reason for an integral protein to be inserted into a membrane, is its hydrophobic association with the hydrophobic part of the lipids. We must emphasize that important differences exist in the isolation procedures and fixation conditions of D-cytochrome b_5 on the membranes, so that quantitative comparisons between these results are possible, only if the treatments do not alter the binding. A new report [35] indicates that some integral proteins found in a soluble form in the cytosol are also able to bind in vitro into isolated plasma membranes.

The absence of endogeneous cytochrome b_5 from the plasma membrane is probably linked to its biosynthetic site. Some authors have proposed that the integral proteins are generally synthetized on free ribosomes and then released in the cytoplasm where they would bind to their respective membranes [36—38]. Recently, Harano and Omura [39] showed that cytochrome b_5 is synthetized on endoplasmic reticulum-bound ribosomes. Our results also favor this latest explanation since no endogeneous cytochrome b_5 is ever found on the plasma membranes, whereas it is possible for the protein to bind in vitro. This observation also raised the problem of interrelationship existing between the subcellular membranes. More precisely, we question about the mechanism of secretory protein transit from the endoplasmic reticulum to the plasma membranes through the way of the Golgi apparatus [40—45]. We know from biochemical analysis that membrane enzymes located in the endoplasmic reticulum, like cytochrome b_5, are not found in the Golgi nor in the plasma membrane. The fact that cytochrome b_5 can bind to these two membranes indicates that there is no incompatibility between an integral protein of the endoplasmic reticulum and the plasma membrane, nor for the Golgi membrane [8]; it is our opinion that in the cell, cytochrome b_5 never comes into contact with these membranes; a selection mechanism must exist which prevents the movement of the integral protein from one subcellular compartment to the other although soluble proteins transit through these organelles.

In this context it is very interesting to compare the mechanism of this specific transport protein with the budding of the specific integral proteins of the viral capside [46].

Acknowledgements

This work was supported by a U.S.P.H.S. grant GM-15971 of S.J. Singer. The author wishes to thank Professor S.J. Singer for his helpful criticism and idea on the subject and the members of the laboratory for their very warm welcome. The help of Mrs. De Connick in the preparation of this manuscript is gratefully acknowledged. J.R. was an Aspirant of the Fonds National de la Recherche Scientifique and a fellow of the Belgian American Educational Foundation.

References

1 Spatz, L. and Strittmatter, P. (1971) Proc. Natl. Acad. Sci. U.S.A. 68, 1042—1046
2 Ito, A. and Sato, R. (1968) J. Biol. Chem. 243, 4922—4923

3 Strittmatter, P. and Velick, S.F. (1956) J. Biol. Chem. 221, 253—264
4 Omura, T., Siekevitz, P. and Palade, G.E. (1967) J. Biol. Chem. 353, 2389—2396
5 Strittmatter, P., Roger, M.J. and Spatz, L. (1972) J. Biol. Chem. 247, 7188—7194
6 Enomoto, K. and Sato, R. (1973) Biochem. Biophys. Res. Commun. 51, 1—7
7 Rogers, M.J. and Stritmatter, P. (1973) J. Biol. Chem. 48, 800—806
8 Remacle, J. (1978) J. Cell Biol. 79, 291—313
9 Enomoto, K.I. and Sato, R. (1977) Biochim. Biophys. Acta 466, 136—147
10 Dufourcq, J., Bernon, R. and Lussan, C. (1976) Biochim. Biophys. Acta 433, 252—263
11 Holloway, P.M. and Katz, J.T. (1975) J. Biol. Chem. 250, 9002—9007
12 Poensgen, J. and Ulrich, V. (1974) Hoppe Seyler's Z. Physiol. Chem. 355, 1238
13 Rogers, M.J. and Stritmatter, P. (1975) J. Biol. Chem. 250, 5713—5718
14 Sullivan, M.R. and Holloway (1973) Biochem. Biophys. Res. Commun. 54, 808—815
15 Robinson, N.C. and Tanford, C. (1975) Biochemistry 14, 369—378
16 Singer, S.J. (1974) Annu. Rev. Biochem. 43, 805—833
17 Remacle, J. and Singer, S.J. (1975) Arch. Int. Physiol. Biochim. 83, 201—202
18 Neville, D.M., Jr. (1960) J. Biophys. Biochem. Cytol. 8, 413
19 Amar-Costesec, A., Beaufay, H., Wibo, M., Thines-Sempoux, D., Feytmans, E., Robbi, M. and Berthet, J. (1974) J. Cell Biol. 61, 201—212
20 Klingenberg, M. (1958) Arch. Biochem. Biophys. 75, 376—386
21 Beaufay, H., Amar-Costesec, A., Feytmans, E., Thines-Sempoux, D., Wibo, M., Robbi, M. and Berthet, J. (1974) J. Cell Biol. 61, 188—200
22 Fowler, S., Remacle, J., Trouet, A., Beaufay, H., Berthet, J., Wibo, M. and Hauser, P. (1976) J. Cell Biol. 71, 535—550
23 Porter, R.R.(1956) Biochem. J. 73, 119—126
24 Avrameas, S. (1969) Immunochemistry 6, 43—52
25 Nicolson, G.L., Marchesi, V.T. and Singer, S.J. (1971) J. Cell Biol. 51, 265—272
26 Remacle, J., Fowler, S., Beaufay, H., Amar-Costesec, A. and Berthet, J. (1976) J. Cell Biol. 71, 551—564
27 Wibo, M., Amar-Costesec, A., Berthet, J. and Beaufay, H. (1971) J. Cell Biol. 51, 52—71
28 Leighton, F., Poole, B., Beaufay, H., Baudhuin, P., Coffey, J.W., Fowler, S. and de Duve, C. (1969) J. Cell Biol. 37, 482—513
29 Nicolson, G.L. and Singer, S.J. (1974) J. Cell Biol. 60, 236—248
30 Roseman, M.A., Holloway, P.W. and Calabro, M.A. (1978) Biochim. Biophys. Acta 507, 552—556
31 Barzilay, M., Vries, A.D. and Condrea, E. (1977) Experientia 33, 1102—1105
32 Tajima, S. and Sato, R. (1979) Biochim. Biophys. Acta 550, 357—361
33 Revel, J.P. and Ito, S. (1967) in Specificity of Cell Surfaces (Davis, B.D. and Warren, E., eds.), Prentice-Hall, Englewood Cliffs, NY
34 Rothman, J.E. and Senord, J. (1971) Science 195, 743—753
35 Thompson, J.E., Chambers, J.A. and Semple, N.L. (1978) Exp. Cell Res. 113, 127—137
36 Bretscher, M.S. (1973) Science 181, 622
37 Lodish, H. (1973) Proc. Natl. Acad. Sci. U.S.A. 70, 1526—1530
38 Lowe, D. and Halliman, T. (1973) Biochem. J. 136, 825—828
39 Harano, T. and Omura, T. (1977) J. Biochem. 82, 1541—1550
40 Claude, A. (1970) J. Cell Biol. 47, 745—766
41 Morre, D.J. (1977) in The Synthesis, Assembly and Turnover of Cell Surface Components (Poste, G. and Nicolson, G.L., eds.), pp. 1—83, North-Holland, Amsterdam
42 Jamieson, J.D. and Palade, G.E. (1968) J. Cell Biol. 34, 577—596
43 Bergeron, J.J.M., Ehrenreich, J.H., Siekevitz, P. and Palade, G.E. (1973) J. Cell Biol. 59, 73—88
44 Novikoff, A.B. and Shin, N.Y. (1964) J. Microsc. 3, 187—206
45 Palade, G. (1975) Science 189, 347—358
46 Choppin, P.W., Compans, R.W., Scheid, A., McScharry, J.J., Lazarowitz, S.G. (1972) in Membrane Research (Fox, G.F., ed.), pp. 163—186, Academic Press, New York, NY