

Isolation and characterization of syncytiotrophoblast plasma membrane from human placenta

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Human full-term syncytiotrophoblast plasma membranes isolated by mechanical procedures (sieving and ultrasonic disintegration), purified by phase centrifugation, form a single band of 1.052 ± 0.002 g/ml density in percoll gradient. The purity of the preparation was assessed by electron microscopy, enzyme analysis and β_2 -microglobulin determination.

Plasma membrane isolation Syncytiotrophoblast Human placenta

1. INTRODUCTION

The continuous layer of syncytiotrophoblast surrounding chorion constitutes an elective area for intimate contact between maternal and fetus blood [1]. It could be that the syncytiotrophoblast membrane (STM) plays a role to maintain alive successfully the fetal semi-allograft, inasmuch as cell plasma membranes in general are involved in the cellular processes of contact, recognition and exchange. Techniques have been described using salt extraction [2,3] or high-ground homogenates [4,5], followed by filtration and phase centrifugation for purification. These techniques are time consuming and result in syncytiotrophoblast membranes contaminated with membranes from other placenta or blood cells. We attempted therefore to develop a technique to obtain reasonably pure STM in view of further immunochemical studies.

2. MATERIALS AND METHODS

2.1. Isolation of syncytiotrophoblast membranes

Placentas from uncomplicated full-term pregnancy were collected in crushed ice containers

within 20–30 min post-delivery and immediately transferred to the laboratory. All further steps were carried out at 4°C in phosphate-buffered saline (PBS), pH 7.4, supplemented with 1 mM phenylmethylsulfonyl fluoride (Sigma), 10 mM ethylene diamine tetraacetic acid tetra sodium salt (Merck), 10000 IU/l Iniprol (Laboratoire Choay S.A.), 50 IU/ml penicillin and 50 µg/ml streptomycin (Flow Laboratories). Eighty to 100 g of cotyledons were cut in 2 cm thick cubes on the maternal side of the placenta, then sliced with scissors to approximately 1–5 mm³ pieces. The minced tissue was repeatedly washed with ice-cold calcium chloride 0.1 M, until a perfectly clear supernatant was obtained. The washed villous tissue was then passed under slight pressure through a 100 mesh sieve (Saulas, France). Placental villi were recovered on the sieve, and blood or other cells passing the sieve were discarded. Placental villi were then suspended in ice-cold supplemented PBS medium, as above. Sonication was performed at the amplitude of 18 µ with a Soniprep MSE disintegrator, 150 W, by 12 beams for 30 s each. Filtration on a 63 mesh sieve (Saulas) was used to separate the syncytiotrophoblast layer from placental villi. Cellular organelles were discarded from STM by a three-step phase cen-

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trifugation. Firstly, the filtrate was spun at $1000 \times g$ for 10 min (International Centrifuge) to remove large tissue fragments, if any. The pellet was discarded and the supernatant centrifuged at $10000 \times g$ for 10 min (J2-21 Beckman Centrifuge) to eliminate basement membranes, mitochondria and other debris. The resulting supernatant was then ultracentrifuged at $100000 \times g$ for 1 h ($L_8 70$ Beckman ultracentrifuge) to obtain STM as a sediment. Further purification and density determination of STM were carried out by phase centrifugation on a self-generated gradient of percoll. The syncytiotrophoblast membrane pellet was homogenised in 0.05 M Tris-HCl buffer (pH 7.4) containing 0.25 M sucrose, and 200 μ l of this homogenate were mixed with 10 ml iso-osmotic percoll. Nine parts of commercial percoll (Pharmacia Fine Chemicals) were mixed with 1 part of 2.5 M sucrose, then diluted with 0.05 M Tris-HCl buffer (pH 7.4) containing 0.25 M sucrose to obtain a starting density of 1.315 ± 0.005 g/ml. The percoll gradient was generated by centrifugation at $120000 \times g$ for 15 min ($L_8 70$ Beckman ultracentrifuge 23° angle head rotor). Density marker beads (Pharmacia Fine Chemicals) were used as an external calibration standard.

2.2. Electron microscopy assay

Small pieces of intact placental villi, sonicated villi, and pellets obtained during each step of STM preparation were fixed in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) for 90 min at room temperature and post-fixed with 1% osmium tetroxide in the same buffer. The samples were then dehydrated and embedded in araldite. Ultrathin sections were stained with uranyl acetate and lead citrate for 10 min as described by Reynolds [6], and examined with a JEOL SEM 100 B electron microscope.

2.3. Determination of enzyme activities

The samples obtained during each step of STM preparation were washed in 20 mM Tris-HCl buffer (pH 7.2) supplemented with 5 mM magnesium chloride (Merck), 1 mM calcium chloride (Merck) and 0.15 M sodium chloride, and homogenised in a potter grinder in the presence of the supplemented Tris-HCl buffer, then kept at -70°C until use. Alkaline phosphatase [7], acid phosphatase [8], glutamate dehydrogenase [9] and

6-phosphatase [10], used, respectively, as a marker of plasma membrane [11], lysosome [12], mitochondria [13] and endoplasmic reticulum [14], were determined by previously established procedures.

2.4. Dosage of total proteins and of β_2 -microglobulin

Total protein content was determined by the method of Lowry et al. [15]. β_2 -Microglobulin was determined in the final fraction, according to the technique described by Evrin et al. [16] using the Phadebas β_2 microtest (Pharmacia diagnostic).

3. RESULTS

3.1. Electron microscopy aspects

The various fractions obtained during the process of isolation of MST were checked by electron microscopy. Fig.1 shows the aspect of a placental villus undamaged (A) or after sonication (B). Fig.2 presents the fraction sedimented by centrifugation at $100000 \times g$ for 1 h. At low magnification the material appeared as smooth membrane structures, either round or tubular (fig.2A). The bilamellar structure, characterizing a plasma membrane, was clearly shown at high magnification (fig.2B,C).

3.2. Biochemical characteristics

Table 1 summarizes the enzyme activities of each fraction obtained during the isolation procedure. The marked enrichment in plasma membrane in the final $100000 \times g$ pellet was indicated by an increase in the relative specific activity for phosphatase alkaline to 16.3 with respect to that of the initial filtrate. The mitochondrial and lysosomal markers were concomitantly decreased through the isolation procedure as indicated by the 0.8-fold ratio for relative specific activity both for glutamate dehydrogenase and for acid phosphatase. This is indicative of a negligible contamination by mitochondria and lysosome. Glucose-6-phosphatase, an endoplasmic reticulum marker, has a relative specific activity of 1.6 indicating a weak contamination by endoplasmic reticulum.

As shown in fig.3, STM forms a single band on a percoll gradient. Its buoyant density is $1.052 \pm$

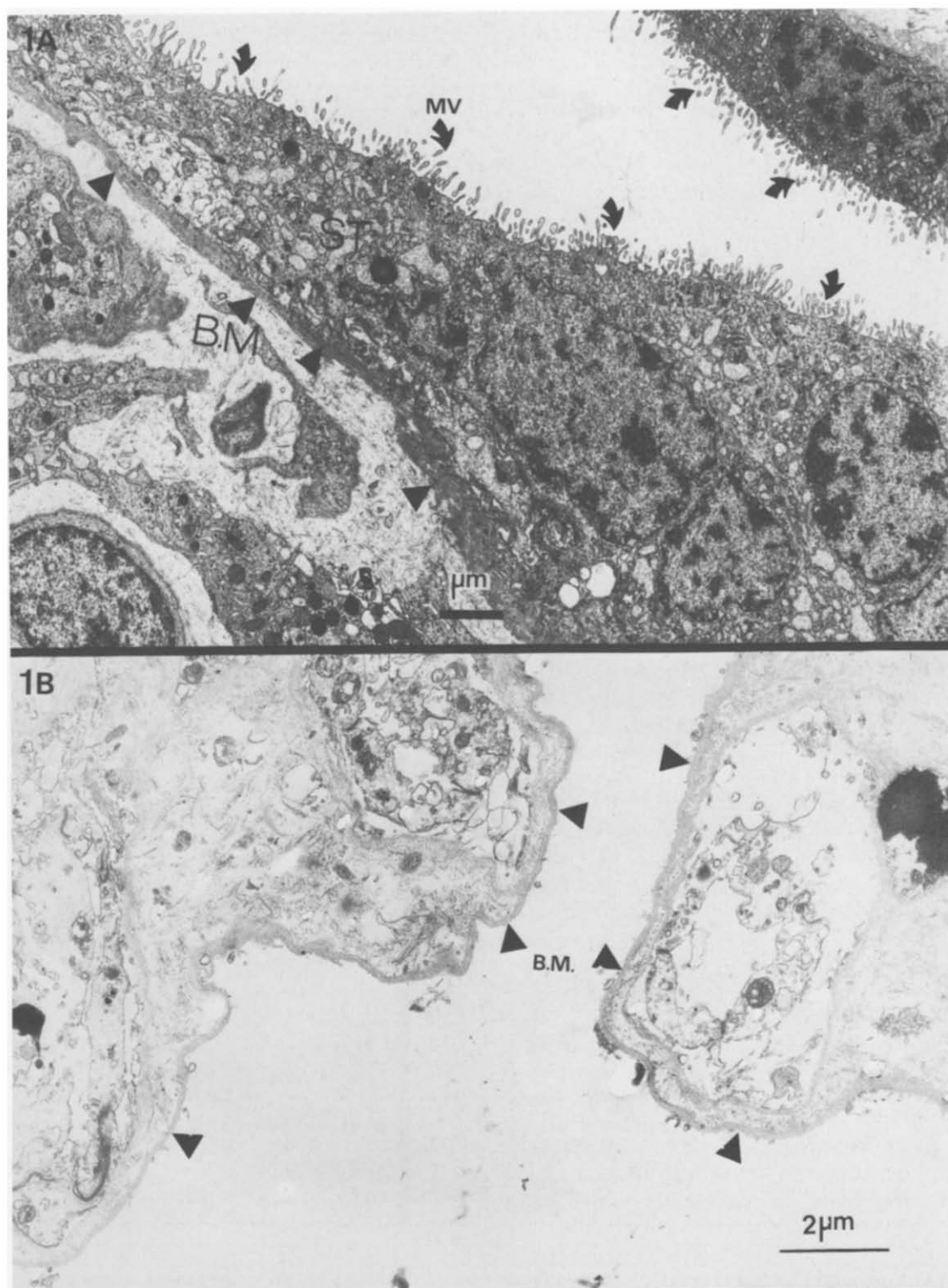


Fig.1. (A) Electron micrograph of an intact placental villus showing microvilli (MV), syncytiotrophoblast (ST) and basement membrane (B.M.). $\times 8400$. (B) Micrograph of villus after sonication. Basement membrane remains intact and adherent to placental villus. $\times 8000$.

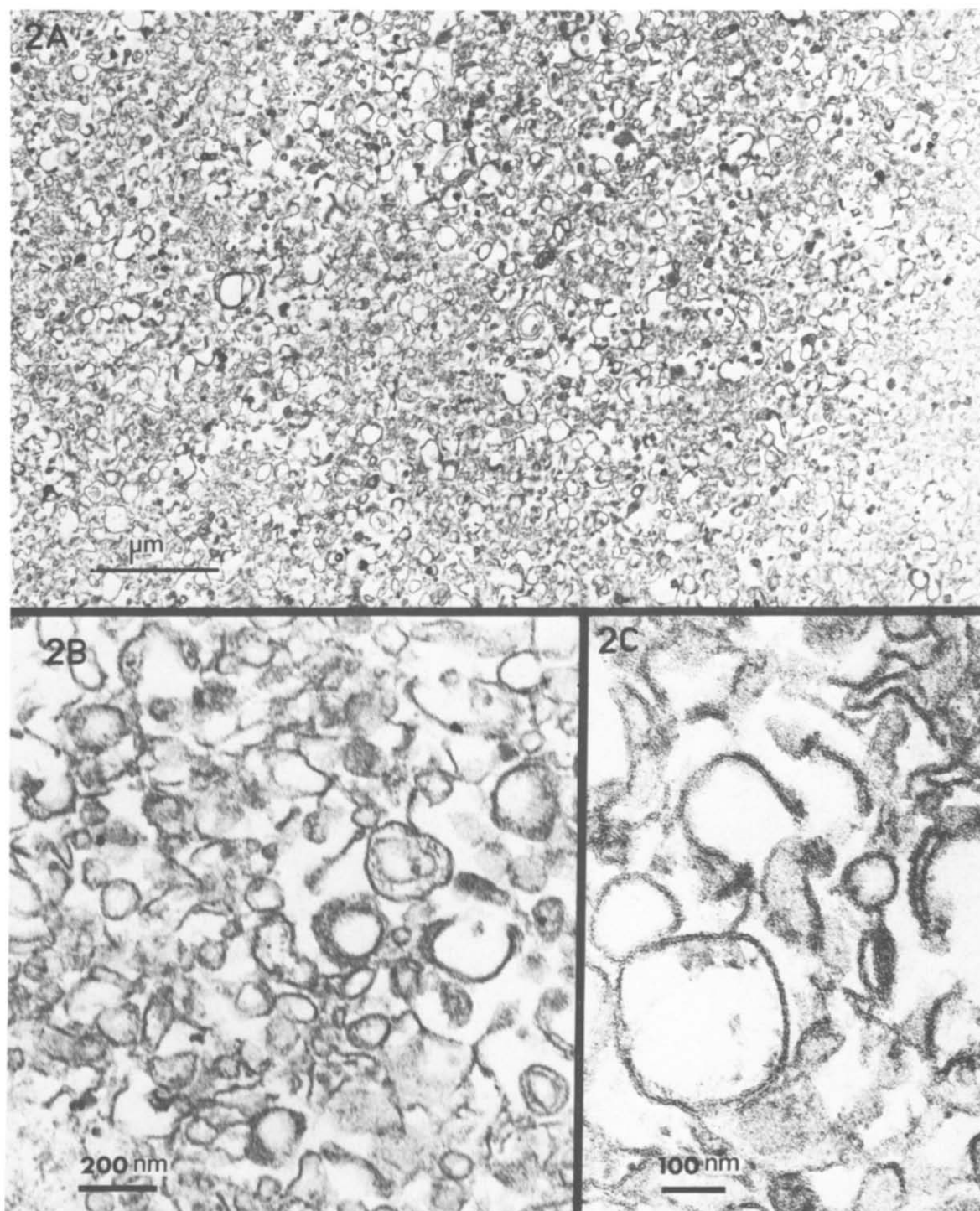


Fig.2. (A) Low magnification micrograph of membrane vesicle fraction. Note the homogeneity of the preparation. $\times 20000$. (B,C) High magnification micrograph of membrane vesicle preparation ($\times 60000$ and 100000 , respectively). Note the bilamellar structure and electron dense material.

Table 1

Specific activity of organelle specific enzymes in fractions obtained during the isolation procedure of MST

	Specific activity ($\mu\text{mol}/\text{min}$ per mg protein)				Relative specific activity enrichment (P_3/F)
	Filtrate	P_1	P_2	P_3	
Alkaline phosphatase ^a	0.36	0.32	1.04	5.9	16.3
Acid phosphatase ^a ($\times 10^{-2}$)	4.7	1.6	1.3	4.2	0.8
Glutamate dehydrogenase ^b ($\times 10^{-2}$)	5.8	2.5	9.5	4.7	0.8
Glucose-6-phosphatase ^a ($\times 10^{-2}$)	1.4	1.1	2.4	2.08	1.6

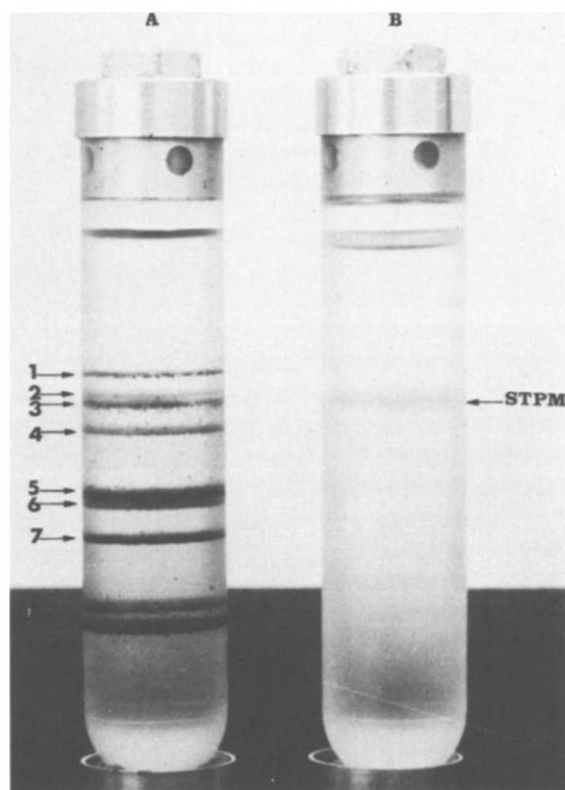
^a Mean of 4 experiments^b Mean of 3 experiments P_1 , P_2 , P_3 pellet obtained by centrifugation at $1000 \times g$, $10000 \times g$ and $100000 \times g$, respectively

Fig.3. Banding of density marker beads and syncytiotrophoblast plasma membrane in gradient of percoll. (A) Density marker beads (g/ml) ± 0.002 : (1) 1.036, (2) 1.049, (3) 1.054, (4) 1.073, (5) 1.090, (6) 1.098, (7) 1.110, (8) 1.133, (9) 1.145. (B) Syncytiotrophoblast plasma membrane (STPM).

0.002 g/ml in comparison with the density marker beads. β_2 -Microglobulin was found at the concentration of 11 ng/mg total proteins in the final STM suspension.

4. DISCUSSION

The technique described here allowed us to obtain a fraction characterized by a high alkaline phosphatase activity (a known marker of syncytiotrophoblast membrane according to [4]) and concomitant low amounts of other enzymatic markers of intracellular structures. Electron microscopy and percoll gradient density advocate also for a high degree of purity. Finally, the syncytiotrophoblast origin of the membrane fraction is suggested by its low content in β_2 -microglobulin [17]. Our data compare favorably with previous reports [2-5,18,19]. The advantages of a preparative technique by sieving, sonication and phase centrifugation can be summarized as follows. It does not require drastic chemical reagents, yet gives a good yield of STM: approx. 100 mg proteins from 80 to 100 g placental tissue. This technique also has the advantage that it can be used for simultaneous isolation of other constituents of placental villi, as in [20]. Finally, the high degree of purity of the preparation indicates that it should be suitable for biochemical and immunological studies and to specify the role of STM in the maternal tolerance of the fetal allograft.

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