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Membrane Vesicles of Cellular Dimensions Fit in Two Geometric Series

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Summary. Preparations of basal-lateral plasma membranes from rat intestinal epithelial cells were analyzed with the analytical centrifuge. In these preparations a number of well-defined membrane fractions were observed. The particle weights of these fractions appear to fit in two geometric series. Until now only relatively small vesicles up to a diameter of about 1 μm were observed. In our preparation we have observed vesicles up to a diameter of 7.5 μm. Therefore, even vesicles with the same size as the plasma membranes of intact cells fit in the two geometric series.

Key Words membranes · lipids · vesicles

Introduction

All biomembranes have a phospholipid bilayer [7] and form vesicles, i.e., closed spherical bodies [1]. The dimensions of the vesicles obtained are dependent upon the isolation procedure: with subtle isolation procedures large vesicles are obtained, whereas the use of rough isolation procedures results in the formation of relatively small vesicles [3]. It has been described that the membrane vesicles are characterized by a relatively small number of well-defined, discrete sedimentation coefficients [5, 6]. The diameters of the vesicles, starting with the smallest, appear to fit in two geometric series [2]. Not less important is the observation that all vesicles found in living cells have diameters that fit in the same two series [2]. However, all the reports about the discreteness of vesicles have been published by only one group of investigators [2–6]. The discreteness of membrane vesicles has not been confirmed by an independent group. This is surprising since the subject seems rather important. Moreover, only relatively small vesicles, up to a diameter of about 1 μm, were observed in preparations of biomembranes. Although it has been *calculated* that even "vesicles" as large as lymphocytic cells fit in the geometric series mentioned above [3], vesicles of such a large size could not be detected in preparations of biomembranes. In this paper we will demonstrate that vesicles of discrete size can be obtained in preparations of basal-lateral plasma membranes from rat intestinal epithelial cells. The diameters of these vesicles fit in the two geometric series. Moreover, provided the detection system is adapted to very large sedimentation coefficients, vesicles as large as cells are observed in these preparations.

Materials and Methods

Isolation of basal-lateral plasma membranes from rat intestinal cells was performed according to Mirchef et al. [11]. The membranes were finally suspended in a buffer containing (in mM) 100, NaCl; 14, EDTA; 40, Tris-HCl; pH = 7.5.

Analytical ultracentrifugation was performed in a Spinco Model E analytical ultracentrifuge, equipped with UV absorption optics and an automatic scanning system, essentially as described earlier [14]. The electronics of the scanning device has been adapted to low centrifugation speeds in order to be able to determine very large sedimentation coefficients. Moreover, the system is adapted in such a way that scanning can be made every 45 sec. Runs were performed at speeds of 3,600, 4,800, 6,000, 6,800, 8,000, 10,000 and 15,000 rpm at 20°C. These speeds were chosen in order to be able to measure sedimentation coefficients ranging from about 150 S up to about 40,000 S.

Sedimentation coefficients were determined, as usual (*see* for instance [8]), from a plot of ln r *vs.* t, where r = radial distance of the sedimenting boundary at time t. Since the sedimentation velocities of very large vesicles are high, it is evident that the displacement of the sedimenting boundary during scanning cannot be neglected. Therefore the real time t' was calculated with the equation

$$t' = t + l/v$$

where t = time at which the scanning starts, l = distance travelled by the scanning device from the starting position to the sedimenting boundary, and v = speed of the scanning device.

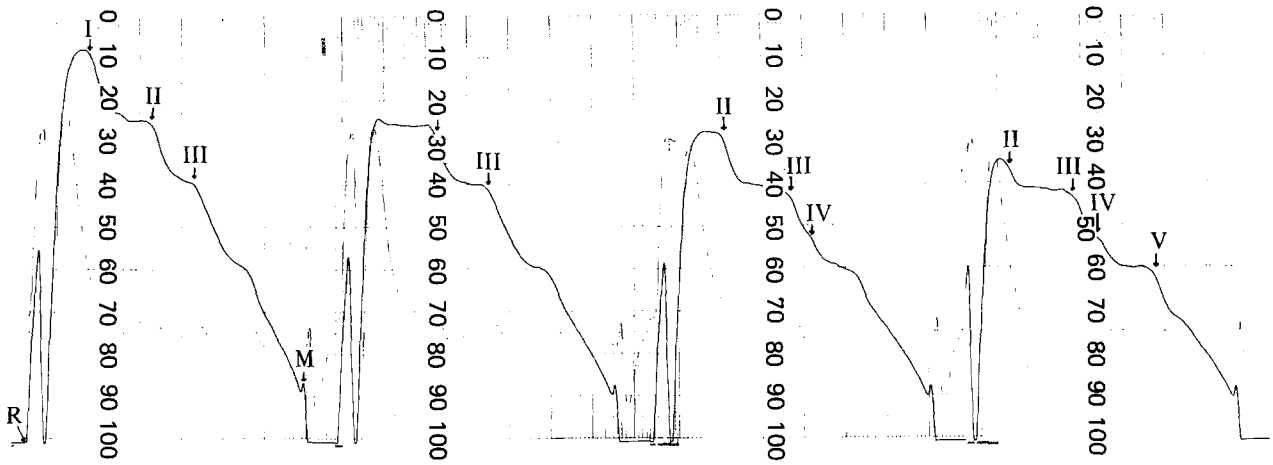

Fig. Sedimentation profiles of membranes. The scans were traced at 180, 225, 270, and 315 sec, respectively, after reaching maximum speed (= 6800 rev min^{-1}). Temperature = 20°C. *R* = reference point at a radial distance of 6.70 cm. *M* = meniscus. The sedimentation coefficients are 10915S, 7190S, 5690S, 4340S and 2970S for numbers *I*, *II*, *III*, *IV* and *V*, respectively

Results

The sedimentation velocity of basal-lateral plasma membranes from rat intestinal epithelial cells was studied in a Spinco E ultracentrifuge. A typical sedimentation profile is given in the Figure. The step-like sedimentation pattern clearly demonstrates that the membrane contains well-defined fractions. By performing the sedimentation velocity studies at different rotor speeds (*see* Materials and Methods) we were able to detect 22 fractions within the same preparation. From the sedimentation coefficients the radius of the corresponding vesicles can be calculated according to [5]:

$$S = 3{,}4(R - 50)^2/3R \qquad (1)$$

where S = sedimentation coefficient and R = radius (in Angstrom). The experimental sedimentation coefficients and the radii of the corresponding vesicles are summarized in the Table. The other data of the Table are discussed below.

It has been shown by Israelachvili et al. [10] that the smallest phospholipid vesicles have a discrete size. These smallest vesicles have also been reported to have a minimum size, the radius being about 200 Å [9]. The thickness of these phospholipids is about 45 Å [1]. Since the thickness of biomembranes is about 100 Å [5], it follows immediately that the radius of the smallest biomembrane vesicles should be approximately 227 Å. This is in excellent agreement with the value of 226 Å for the smallest vesicles as given in the Table. These smallest vesicles are referred to as unit vesicles. It has been reported that larger membrane vesicles are multiples of the unit vesicle and that the mass of their membrane material forms two geometric series [2]. The diameter $D(n)$ of a vesicle (in Angstrom) is related to the number n of unit vesicles according to [2]:

$$(D(n) - 100/D(1) - 100)^2 = n \qquad (2)$$

where $D(1)$ = diameter of unit vesicles and 100 Å is the thickness of membranes. The number of unit vesicles in larger vesicles are also given in the Table. The theoretical values predicted according to the two geometric series are included in the Table. If we compare the experimental data with the theoretical values, we observe that the deviations are small. Note that some vesicles, which can exist according to the two series, are not observed in the preparation.

Discussion

The present study was undertaken in the first place to reproduce the surprising results reported by others on the discreteness of membrane vesicles. We have clearly demonstrated that membrane vesicles are indeed characterized by a relatively small number of well-defined vesicles. The mass of their membrane material forms two geometric series, and therefore the diameters of membrane vesicles also form two geometric series. It has been reported that all vesicles found in living cells, e.g. granular vesicles for neurotransmitters or hormones, the mem-

brane envelopes of viruses and chlamydiae, have diameters that fit in the very same two series [2]. In this study we have found vesicles with diameters as large as 7.5 μm. Therefore, the assumption that even the plasma membranes of intact cells can be considered as vesicles that fit in the two geometric series [3] seems to be justified. The assumption is even proven true by the following quantitative data. The number of unit vesicles in the plasma membranes of the four types of lymphocytic cells in bone marrow, reported to be 3.2^{13}, 3.2^{14}, 3.2^{15} and 2^{16}, respectively [3] has to be compared with 3.2^{13}, 3.2^{14} and 2^{15} observed in vesicles in our membrane preparations.

Not all vesicles that can exist according to the two geometric series are present in all preparations. The vesicles which are absent are not the same in all preparations. Only the vesicles containing two unit vesicles could never be found, in agreement with earlier observations [2].

It is interesting to note that in some preparations we could not detect vesicles of discrete size. In some cases, however, in these preparations vesicles of discrete size could be observed after prolonged storage of the sample. This indicates that the formation of vesicles can take some time. The formation of vesicles of discrete size in membrane preparations is surprising. Even more surprising is the finding that vesicles found in living cells and cells themselves have the same diameters as vesicles formed in membrane preparations. The factors which affect the formation of vesicles of discrete size are still largely unknown and must be studied in detail. A clear understanding of the phenomena described in this paper might be of considerable interest for our insight in many processes of cell biology.

A remarkable finding known as geometric phenotypic variability deals with the fact that production of cellular products such as proteins, often follows a geometric series with a ratio of $\sqrt{2}$ [12, 13]. If we combine the two geometric series for membrane vesicles to one single series (1, 2, 3, 4, 6, 8, 12, ...) we see that (apart from the unit vesicle) the alternate ratios in this series are 1.5 and 1.33, resulting in an average ratio of $1.41 = \sqrt{2}$. A choice between the two alternatives (one series with a ratio of $\sqrt{2}$ or a combination of two series with an average of $\sqrt{2}$) depends on the accuracy of the measurements. The accuracy of the S values is about 3% and therefore the error in the mass will be about 6% and in the mass ratio of two consecutive vesicles about 12%. However, the theoretical ratios of 1.33 and 1.5 for the two combined series deviates about 6% from the average ratio of $\sqrt{2} = 1.41$. Therefore it is impossible within the accuracy of our experiments to discriminate between the two possibilities. Our preference for using two series in the analysis is mainly based on the observation that in preparations of nuclear membranes [5] only one of the two series was present. It seems unlikely that for one series with a ratio of $\sqrt{2}$ all alternate forms are missing.

Table.

Theoretical value of n[a]		$S_{20,w}$	R[b]	n_{exp}[c]	$\Delta n(\%)$[d]
Series I	Series II				
1 (0)		155	226	1	—
2 (1)					
	3 (0)	280	340	2.7	10.0
4 (2)					
	6 (1)				
8 (3)					
	12 (2)				
16 (4)		705	719	15	6.3
	24 (3)	920	909	24	0.0
32 (5)					
	48 (4)				
64 (6)		1510	1431	62	3.1
	96 (5)	1910	1784	97	1.0
128 (7)					
	192 (6)	2750	2525	198	3.1
256 (8)		2970	2720	230	10.2
	384 (7)	3625	3298	340	11.5
512 (9)		4340	3929	485	5.3
	768 (8)	5690	5120	829	7.9
1024 (10)					
	1536 (9)	7190	6443	1318	[e]
2048 (11)		9210	8226	2156	5.3
	3072 (10)	10915	9731	3023	1.6
4096 (12)		12870	11456	4196	2.4
	6144 (11)	15310	13609	5930	3.5
8192 (13)		18320	16265	8481	3.5
	12288 (12)	22910	20315	13247	7.8
16384 (14)		25710	22785	16674	1.8
	24576 (13)	30600	27100	23603	4.0
32768 (15)		36330	32155	33250	1.5
	49152 (14)	42470	37573	45420	7.6

[a] The number of unit vesicles forms two geometric series. The first series is given by $n = 2^m$ ($m = 0, 1, 2, \ldots\ldots$), the second series by $n = 3.2^m$ ($m = 0, 1, 2, \ldots\ldots$). The values in parenthesis are the values of m.

[b] Radius of the vesicles, determined from the S-value, according to Eq. (1).

[c] Number of unit vesicles in a vesicle, as found from Eq. (2).

[d] Relative difference between the theoretical and experimental values of n:

$$\Delta n = |n_{\text{theoretical}} - n_{\text{exp}}|/n_{\text{theoretical}} \times 100\%$$

[e] For this vesicle it is not clear whether it belongs to series I or to series II.

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