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PHYSICS LETTERS A
ELSEVIER
Physics Letters A 310 (2003) 493–497
www.elsevier.com/locate/pla

Possible role of phospholipid nanotubes in directed transport of membrane vesicles

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Received 5 December 2002; received in revised form 27 February 2003; accepted 4 March 2003

Communicated by C.R. Doering

Abstract

We indicate that membrane nanotubes may have an important role in directed transport of membrane vesicles between different membrane-enclosed compartments in cells. We present experimental evidence that small blebs of phospholipid nanotube may travel along the nanotube and act as vehicles for transporting the enclosed solution. We have also observed similar small membrane blebs of a long membrane tube in red blood cells. In both cases the small vesicles seem to be a distended integral part of the membrane tube and not independent vesicles entrapped within the tube.

Keywords: Phospholipid nanotubes; Membrane vesicle; Directed transport; Deviatoric elasticity

Tubular structures seem to be rather common in organic [1,2] and inorganic [3–5] systems. Phospholipid micro and nanotubes [1] may be formed as a result of mechanical excitation in various micromanipulative methods [6,7]. Phospholipid nanotubes have also been found [8,9] in a suspension of giant bilayer liposomes grown in an alternating electric field [10]. In this case the phospholipid nanotubes were attached to giant bilayer liposomes and were stable for many hours without any external stabilizing forces, presumably due to in-plane orientational ordering of the phospholipid molecules [9]. However, tubular structures composed of membraneous material were also found in red blood cells. Membrane nanotubes have been detected in red blood cells connecting the daughter vesicles to the parent cell [11]. Also, it was observed that adding anisotropic dimeric amphiphiles [12] or amphiphiles with a strongly anisotropic hydrophilic head (dodecyl maltoside) [13] to the erythrocyte suspension resulted in a release of long and thin (diameter $\approx$ 30–60 nm) tubular micro and nanoexovesicles from the erythrocyte membrane. It was shown that deviatoric elasticity [4,14,15] may provide an explanation for the stability of the observed micro and nanotubular exovesicles [12].

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0375-9601/03/$ – see front matter
doi:10.1016/S0375-9601(03)00449-3

In this work giant 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) bilayer liposomes were prepared by a modified method of electroformation [9,10] at room temperature. In the procedure [9], the phospholipid was dissolved in a chloroform/methanol mixture and spread over a pair of platinum electrodes. The solvent was allowed to evaporate in low vacuum for 2 hours. The electrodes were then placed 4 mm apart in an electroformation chamber that was filled with sucrose solution. An alternating electric field of magnitude 1 V/mm and a frequency of 10 Hz was applied for 2 hours. Then the magnitude and frequency of the alternating electric field was gradually reduced, first to 0.75 V/mm and 5 Hz (applied for 15 minutes), then to 0.5 V/mm and 2 Hz (applied for 15 minutes) and finally to 0.25 V/mm and 1 Hz (applied for 30 minutes). The contents of the electroformation chamber were poured out into a plastic beaker and diluted with glucose solution. The solution containing the liposomes was then placed into an observation chamber made from a pair of cover glasses and sealed by grease. The prepared liposomes were observed by an inverted microscope with phase contrast optics (Zeiss IM 35).

Fig. 1a shows a bleb (black arrow) of the thin phospholipid tube. It can be seen that one end of the tube is attached to the bilayer membrane of the giant POPC liposome (Fig. 1b). The other end of the nanotube is attached to the glass surface of the observation chamber (Fig. 1c). The length of the tube is several diameters of the liposome.

Fig. 2 shows transport of a small prolate bleb (black arrows) along the thin phospholipid tube. In the beginning the bleb was far away from the surface of the giant liposome (Fig. 1a). The bleb moved continuously toward the surface of the giant liposome (Fig. 2a–c). In the vicinity of the surface of the giant liposome the bleb started to oscillate slightly back and forth along the tube. Also, the shape and size of the bleb changed, i.e., the bleb became elongated and appeared to lose volume. In the final stage the contents of the bleb fused with the giant liposome (Fig. 2d).

We cannot say how the observed bleb of the phospholipid membrane was produced, however we speculate a following time course: immediately after the process of electroformation, spherical liposomes are connected by a network of nanotubes [8]. When we rinsed the liposomes into the observation chamber,

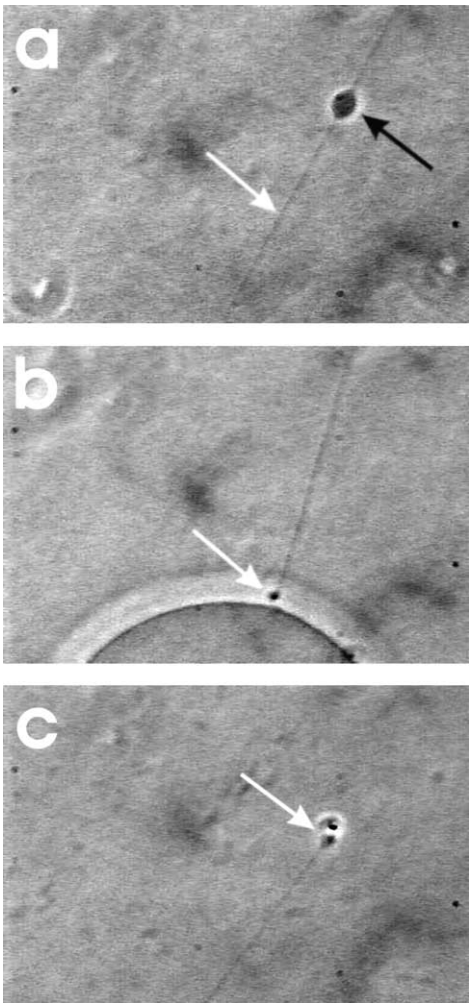

Fig. 1. A bleb (black arrow) of phospholipid tube (white arrow) (a) that acts as a transport vesicle for the enclosed material. One end the phospholipid tube is attached to the bilayer membrane (b), while the other end of the tube is attached to the glass surface of the observation chamber (c).

a configuration where two liposomes were connected by a thin tether was produced. Such configuration is occasionally obtained and was previously observed [8]. One of the liposomes was large, the other was small. The small liposome may have attached to some defect (Fig. 1c) in the glass. There is always some slight convective current in the observation chamber. This current caused the excess pressure on both liposomes. The movement of the larger liposome caused the nanotube to straighten while the excess

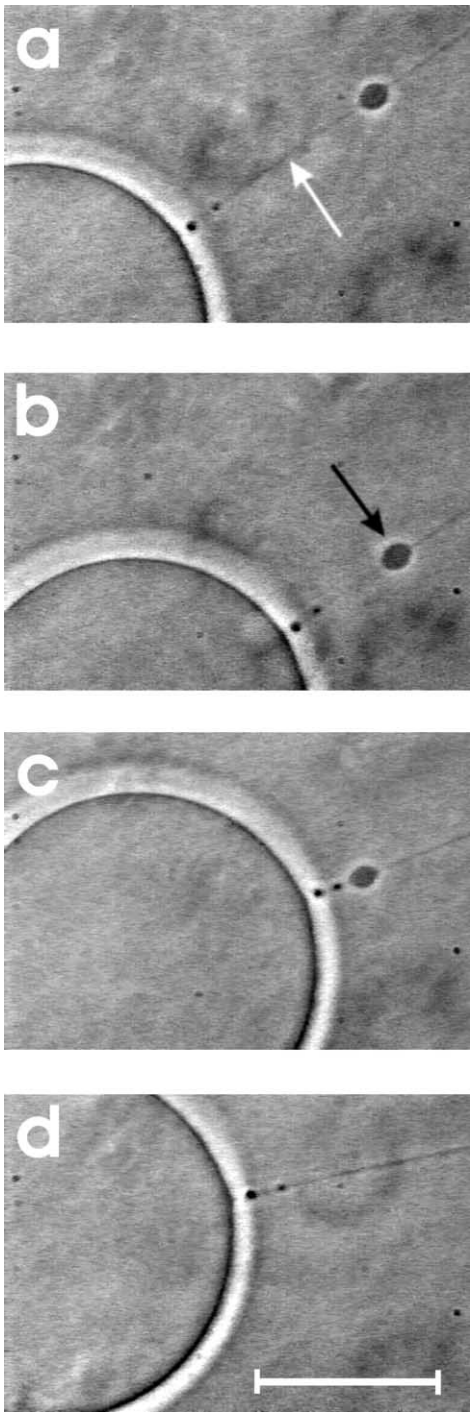

Fig. 2. Transport of a small phospholipid prolate bleb (black arrow) along a thin phospholipid tube (white arrow). Note that the bleb (transport vesicle) is an integral part of the tube membrane. Scale bar, 10 μm.

pressure on the small liposome pushed it along the nanotube. The small liposome started moving and became a bleb (Fig. 1a). We have followed the bleb (transport vesicle) from the moment that we spotted it to the integration with the larger liposome (Fig. 2d) for about half an hour. This observation started about an hour after the process of electroformation was completed and the liposomes were gently rinsed out from the chamber where electroformation took place. When spotted, the bleb was about halfway between the origin of the tube on the glass and the insertion of the tube on the larger liposome. It was moving slowly toward the larger liposome retaining its shape and size throughout. When the bleb reached the larger liposome (Fig. 2c), the current pressure eventually pushed its contents through the neck connecting the bleb and the larger liposome.

There are related phenomena where beads corresponding to transient excited states were produced by a sudden tension in the membrane tubes that is induced either by laser tweezers [16] or by mechanical manipulation [17] (see also [18]). No such procedure was involved in our experiments. Our vesicles produced by electroformation pertain to quasi-equilibrium states and not to transient excited states. We regard the blebs (transport vesicles) that we report of as stable, which was of special interest to us. On the other hand, excited states of the membrane [16] are relaxed after a certain time (depending on the shape of the blebs; slight undulations are relaxed in seconds while sphere-like blebs connected by thin tubular parts are relaxed in minutes [16]).

Small blebs (vesicles) of thin membrane tubes were also observed in red blood cells. Blood was drawn from the authors and red blood cells were washed three times. A few microlitres of the red blood cell stock suspension was pipetted into an Eppendorf tube containing a high pH buffer. Incubation of the red blood cells at high pH resulted first in an echinocytic shape transformation and microvesiculation. As incubation continued, large spherical daughter vesicles were shed from the cell surface. The release of the rather large vesicles was accelerated by addition of dibucaine. The exovesicles were in some cases connected by thin tubular structures to the parent cell [11]. Fig. 3 shows a small prolate bleb that was the integral part of the thin membrane tube connecting two membrane-enclosed parts of the red blood cell into which the cell disin-

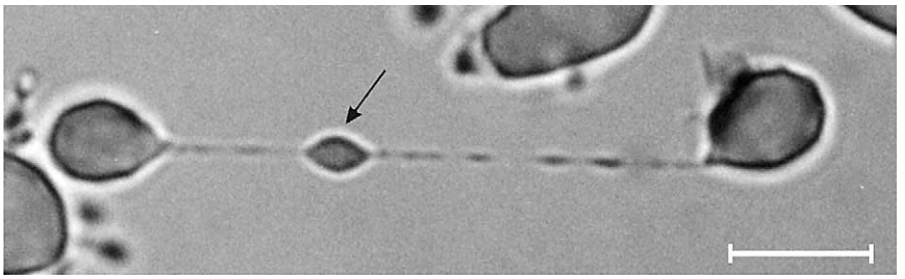

Fig. 3. A micrograph showing a small prolate vesicle (black arrow) of a red blood cell membrane tube connecting the two membrane-enclosed parts of a disintegrated red blood cell. The red blood cells were observed in isotonic physiological solution with added dibucaine at pH $\approx$ 8.5. Scale bar, 3 μm.

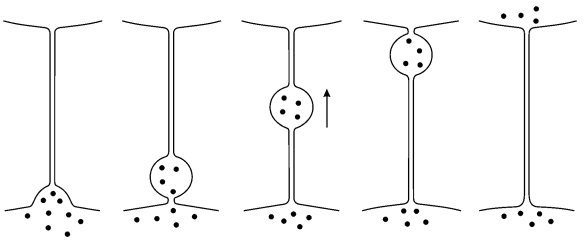

Fig. 4. Schematic presentation of a possible mechanism of micro and nanotube directed traffic of transport vesicles between two membrane-enclosed compartments.

tegrated under the given external conditions. The bleb of erythrocyte membrane tube (Fig. 3) was observed for about half an hour during which it more or less retained its size and shape.

Transport vesicles play a central role in the traffic of molecules between various membrane-enclosed compartments of cellular secretory pathways and in the transport of materials taken up at the cell surface [19]. In this work we observed that the transport vesicles (blebs) may travel along the nanotube attached to the giant liposome. The existence of small blebs of membrane micro and nanotubes was also observed in red blood cells. Based on these results it can be anticipated that micro and nanotube directed transport of vesicles (Fig. 4) could have an important role in the selectivity of specific pathways of transport vesicles in cellular systems where the transport vesicles must move specifically from one membrane enclosed compartment to another compartment. As the nanotubes may be very thin (diameter below 50 nm) [8,9] the connected vesicles may seem as diffusing freely in the solution. We have recently observed in erythrocytes that the daughter vesicles may be connected by nanotubes to the parent cell [11]. The nanotubes could not be directly observed, however the vesicles moved synchronously with the mother cell indicating their connection with the parent cell by nanotubes [11]. The transport to the target point would be much more efficient if the nanotubes directed the vesicles. In the present work, we would like to point to this possibility.

The observed blebs (Figs. 1–3) are large regarding intracellular mechanisms, however, we think that the same mechanism of the transport by blebs should be relevant also for smaller blebs, provided that they are made stable. In the intracellular transport the shape of transport vesicles may be additionally stabilized by mechanisms that are not present in the simple system of one-component liposomes, as for example by clathrin coating [20] or other protein and lipid domain formation mechanism [21,22]. In the transport, the contents of the nanotube and of the bleb are expected to mix, however, if the nanotube is very thin, it could not contain much volume.

Since the nanotubes are difficult to visualize and are also very fragile, the proposed mechanism of directed vesicle transport may have been overlooked in biological systems, though it could have an important role in the function of the Golgi system and other cellular transport systems involving vesicles.

References

[1] J.M. Schnur, Science 262 (1993) 1669.
[2] A. Iglič, V. Kralj-Iglič, in: H. Ti Tien, A. Ottova-Leitmannova (Eds.), Planar Lipid Bilayers (BLMs) and Their Applications, Elsevier, Amsterdam, 2003, pp. 143–172.
[3] N.G. Chopra, R.J. Luyken, K. Cherrey, V.H. Crespi, M.L. Cohen, S.G. Louie, A. Zettl, Science 269 (1995) 966.

[4] V. Kralj-Iglič, M. Remškar, G. Vidmar, M. Fošnarič, A. Iglič, Phys. Lett. A 296 (2002) 151.
[5] M. Remškar, A. Mrzel, Z. Škraba, A. Jesih, M. Čeh, J. Demšar, P. Stadelmann, F. Levy, D. Mihailovič, Science 292 (2001) 479.
[6] A. Karlsson, R. Karlsson, M. Karlsson, A.S. Cans, A. Strömberg, F. Ryttsén, O. Orwar, Nature 409 (2001) 150.
[7] E. Evans, H. Bowman, A. Leung, D. Needham, D. Tirell, Science 273 (1996) 933.
[8] L. Mathievet, S. Cribier, P.F. Devaux, Biophys. J. 70 (1996) 1112.
[9] V. Kralj-Iglič, A. Iglič, G. Gomišček, F. Sevšek, V. Arrigler, H. Hägerstrand, J. Phys. A: Math. Gen. 35 (2002) 1533.
[10] M.I. Angelova, S. Soleau, P. Meleard, J.F. Faucon, P. Bothorel, Prog. Colloid Polym. Sci. 89 (1992) 127.
[11] V. Kralj-Iglič, A. Iglič, M. Bobrowska-Hägerstrand, H. Hägerstrand, Colloids Surf. A 179 (2001) 57.
[12] V. Kralj-Iglič, A. Iglič, H. Hägerstrand, P. Peterlin, Phys. Rev. E 61 (2000) 4230.
[13] H. Hägerstrand, B. Isomaa, Biochim. Biophys. Acta 982 (1989) 179.
[14] V. Kralj-Iglič, V. Heinrich, S. Svetina, B. Žekš, Eur. Phys. J. B 10 (1999) 5.
[15] T.M. Fischer, J. Phys. II (France) 2 (1991) 337.
[16] R. Bar-Ziv, E. Moses, Phys. Rev. Lett. 73 (1994) 1392.
[17] E. Evans, W. Rawicz, Phys. Rev. Lett. 64 (1990) 2094.
[18] R.E. Goldstein, P. Nelson, T. Powers, U. Seifert, J. Phys. II (France) 6 (1996) 767.
[19] G.M. Cooper, The Cell, a Molecular Approach, ASM Press, Sunderland, 2000.
[20] W. Stoorvogel, V. Oorschot, H.J. Geuze, J. Cell. Biol. 132 (1996) 21.
[21] C. Thiele, M.J. Hannah, F. Fahrenholz, B. Huttner, Nature Cell. Biol. 2 (2000) 42.
[22] J.M. Holopainen, M.I. Angelova, K.J. Kinnunen, Biophys. J. 78 (2000) 830.