Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 9590–9592, October 1995
Biophysics

Drug delivery: Piercing vesicles by their adsorption onto a porous medium

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Communicated by Pierre-Gilles de Gennes, Collège de France, Paris, France, June 26, 1995

ABSTRACT **Experimental evidence is presented that supports the possibility of building a "molecular drill." By the adsorption of a vesicle onto a porous substrate (specifically, a lycopode grain), it was possible to increase the permeability of the vesicle by locally stretching its membrane. Molecules contained within the vesicle, which could not cross the membrane, were delivered to the porous substrate upon adsorption. This general process could provide another method for drug delivery and targeting.**

Much effort has been devoted to developing additional ways to deliver and target drugs. The general application of any method is, however, especially difficult because drugs differ widely in their sizes and functions. All of the following constricting conditions must be met: (*i*) penetration of the drug inside the body; (*ii*) preservation of the drug and its activity; (*iii*) transport of the drug to a specific site; and (*iv*) delivery of the drug at this site.

Three types of drug transport are currently available: (*i*) via solubilization in a fluid (digestive apparatus or blood), although this method requires a large quantity of active material because of the loss by degradation; (*ii*) by diffusion (skin stamps or hypodermic capsules); and (*iii*) in compartmentalized media like retroviruses and liposomes (1).

We are presently studying a fourth type of drug delivery, which particularly addresses constraint *iv* in the first list. This method, which was suggested recently by de Gennes and coworkers (2), is based on a previous observation reported by Taupin *et al.* (3) that a membrane bilayer stretched by osmotic pressure becomes more permeable. The basic principle of the "molecular drill" is that tension on the membrane could also be provided by strong adsorption of the vesicle on a porous substrate, as depicted in Fig. 1. The delivery would then occur as follows: the active drug is originally contained in the porous substrate; upon adsorption on the porous target, the membrane of the cell to be treated is locally stressed, creating permeation defects in the bilayer membrane, which allow the drug to invade the cytoplasm (Fig. 2).

In this paper we demonstrate the possibility of such a delivery process by experiments with a simple model. We used giant monolamellar vesicles as cell models and microspores of *Lycopodium clavatum* as porous substrates. The strong adsorption of the vesicles onto the porous surface resulted from attractive electrostatic interactions between the positively derivatized grains and the negatively charged vesicles. The entire process was observed through a phase-contrast microscope.

MATERIALS AND METHODS

Vesicles. Unilamellar, spherical liposomes were obtained by using the technique described by Angelova and Dimitrov (4). A 10-μl solution in $CHCl_3/CH_3OH$, 9:1, of a lipid mixture

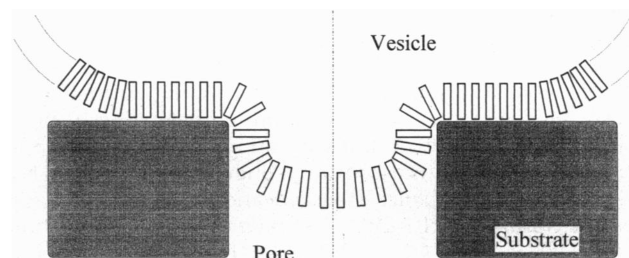

FIG. 1. Basic principle of the molecular drill. The bilayer of the vesicle strongly adsorbs on the porous substrate, and, as a consequence, the central part of the membrane inside the pore is placed under tension and becomes permeable.

(0.126 μM L-α-phosphatidylcholine/0.036 μM dicetyl phosphate/0.018 μM cholesterol; all chemicals were from Sigma) was deposited on each of two glass plates coated with an indium tin oxide conducting film, and the slides were dried in open air to obtain lipid layers. The two slides were glued face-to-face by using a Tygon tube of 760-μm diameter as a spacer to form a liquid cell. We then applied an electric alternating-current voltage (0.5 V, 10 Hz) throughout this cell and filled it with an aqueous solution [1 M 4-hydroxy-2,2,6,6-tetramethylpiperidinooxyl, free radical (4-hydroxy-TEMPO; from Sigma) or 5 mM glucose]; the alternating-current voltage was then increased to 1 V for 5–10 hr. This method produces giant unilamellar vesicles (average diameter is ≈10 μm). The spin label was used for a quantitative and local study of the molecular piercing (L. Dei, M.-A.G.-B., and J.-M.d.M., unpublished work).

To increase the optical contrast between interior and exterior of the vesicles, in some experiments we added 10 μl of a concentrated solution of high-molecular-weight polymer (0.1 g of 75- or 2000-kDa dextran per ml of 5 mM glucose solution) that dissolves everywhere in the vesicular solution but does not penetrate the vesicles under normal conditions to 100 μl of the vesicular suspension (in this case, the "drug" is outside the vesicles).

Substrates. Microspores of *L. clavatum* were purchased from Coopérative Pharmaceutique Française (Paris). Their average diameter was ≈30 μm, and the mean pore size was 2 μm (Fig. 3). These microspores were functionalized by grafting 1,2-diaminoethane (5, 6) to obtain a positively charged surface in aqueous solution at neutral pH that could strongly attract the negatively charged vesicles. Nonporous glass beads (aver-

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Abbreviation: 4-hydroxy-TEMPO, 4-hydroxy-2,2,6,6-tetramethylpiperidinooxyl.

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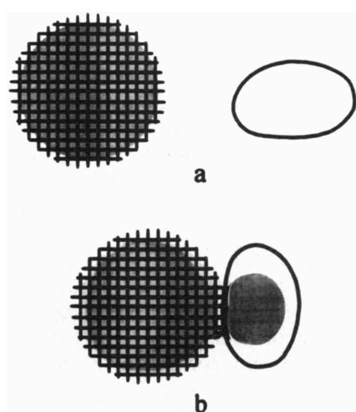

FIG. 2. Schematic illustration of the elementary process for drug delivery using the molecular drill. (*a*) Drug-filled porous vector (at left) with the cell to be treated. (*b*) Cell is adsorbed on the porous substrate; the drug diffuses into the cell through the channels opened by the molecular drill.

age diameter, 40 μm) were functionalized with 3-(2-aminoethylamino)propyltrimethoxysilane, according to ref. 7.

Cell Preparation and Microscope Observation. These observations were made at room temperature directly on the stage of a reverse-phase microscope (Nikon Diaphot-TMD, objective, ×60) with a cell closed just after addition of 2 mg of lycopode to 100 μl of a vesicular suspension without shaking.

RESULTS

Observation under the phase-contrast microscope showed that adsorption of the negatively charged vesicles onto the positively charged substrates occurred without vesicle breakage (Fig. 4) and that no adsorption occurred on corresponding neutral substrates. The microphotographs of Fig. 5 represent the changes with time of a 4-hydroxy-TEMPO-filled vesicle attached to a positively charged lycopode. The 4-hydroxy-TEMPO-filled vesicle is initially bright, presumably due to dilution of the external vesicular medium during overnight storage of the vesicular suspension in an atmosphere saturated with pure water; in this case, the vesicles may not continue to swell with water, despite dilution of the external medium, because they may have reached the limit at which an increase in osmotic pressure cannot further stretch the membrane. [We checked by electron paramagnetic resonance that the rate at which TEMPO crosses the membrane is too small ($t_{1/2}$ > several days) to change in concentration during our experiment.] As a consequence, the refraction indices of the vesicles and the external solution become different, thus creating the contrast. Furthermore, for this experiment it can be reasonably

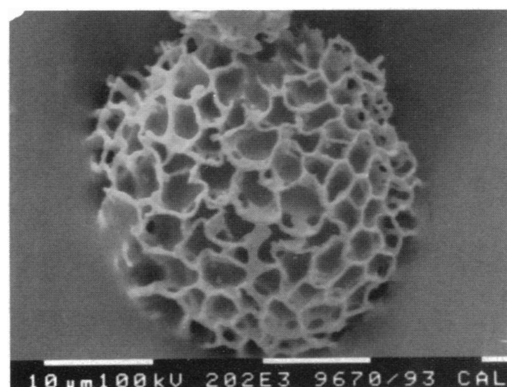

FIG. 3. Scanning electron microscope photograph of a positively charged microspore of *L. clavatum.*

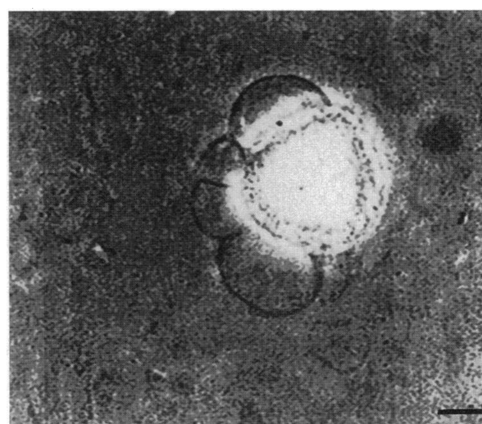

FIG. 4. Negatively charged vesicles adsorbed on a positively derivatized lycopode. (Bar = 10 μm.)

assumed that the membrane is stretched before adsorption onto the lycopode grain. Fig. 5 shows that the vesicle gradually disappears into the lycopode grain, suggesting that the porous substrate has pierced the bilayer and that the membrane of the vesicle has been completely absorbed into the lycopode.

An alternative way of increasing the optical contrast between vesicle and solution is to add a long-chain polymer that dissolves in the external medium without entering the vesicles. In the case of dextran addition just after vesicle preparation, the vesicles appeared white due to the difference of refraction indices. Fig. 6 shows that in contact with the lycopode grains, the vesicles recovered their transparency. The polymer there-

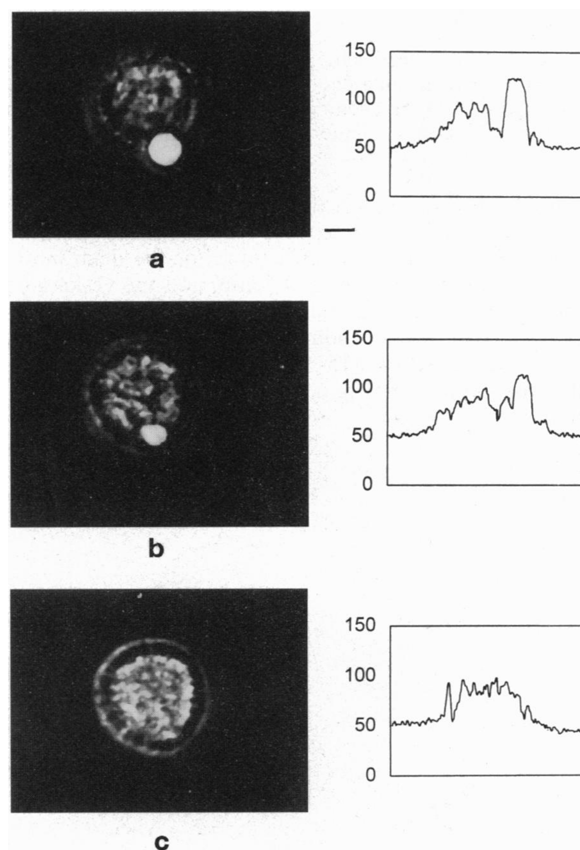

FIG. 5. 4-Hydroxy-TEMPO-embedding vesicle pierced by a lycopode. Graphs at right represent optical-contrast profiles along a line that crosses lycopode and vesicle. Durations of contact were 0, 4, and 9 min for *a*, *b*, and *c*, respectively. Note the decrease in brightness of the vesicle (peak on graph) with time. (Bar = 10 μm.)

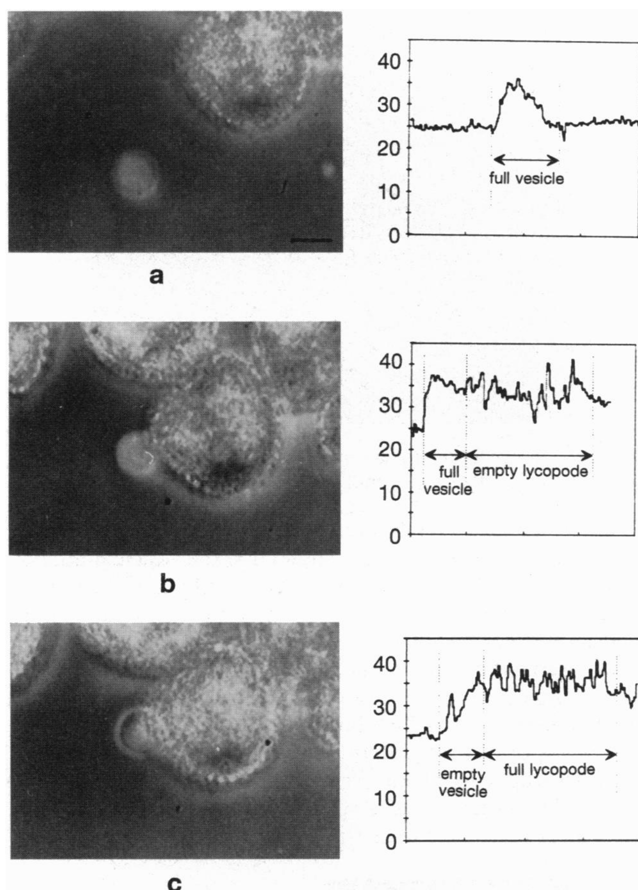

FIG. 6. Polymer entry into adsorbed vesicles. Vesicle became transparent as polymer entered its core through channels created by the molecular drill. Optical contrast profiles are at right. Durations of contact were 0, 2, and 2.8 min for *a*, *b*, and *c*, respectively. (Bar = 10 μm.)

fore entered the vesicle via the defects created in the membrane by the adhesion on the porous substrate and neutralized the difference in indices of refraction. For the dextran of 75 kDa equilibration occurred in <3 min, and the vesicles conserved their integrity. With the dextran polymer of higher mass (2000 kDa), the process of equilibration of an adsorbed vesicle was more drastic. After a 15- to 20-min period, the adsorbed vesicle broke before having completely equilibrated with the solution.

In contrast to the permeation behavior seen upon adsorption of a vesicle onto the positively charged porous lycopodes, we found that no permeation occurred when negatively charged vesicles were adsorbed on positively charged smooth surfaces, either slides functionalized with poly(L-lysine) (poly-Prep, Sigma) or nonporous glass beads (average diameter, 40 μm) functionalized with 3-(2-aminoethylamino)propyltrimethoxysilane. These results show that, although the vesicle was adsorbed to the positively charged surfaces, the membrane curvature due to its contact angle with the surface—even on a surface whose radius of curvature was of the same order of magnitude as that of the vesicle—was insufficient to allow transport across the membrane and that the porosity of the lycopode was, indeed, the cause of molecular piercing.

Our study emphasizes the significance of functional and roughness of the support surface for inducing permeation without vesicle breakage. The corresponding observations are relevant for setting up optimal conditions (*i*) for vesicle formation and/or sizing by using extrusion of vesicle suspensions through membranes containing microscopic or submicroscopic holes; and (*ii*) for patch-clamping or determination of curvature energy by using micropipettes.

In conclusion, these preliminary results support the practicality of the molecular drill concept: a local bilayer stress induced by strong adsorption on highly curved surfaces promotes solute permeation. Extension to new porous substrates and spin-labeling experiments are logical investigational extensions of this work (using 4-hydroxy-TEMPO and spin-labeled dextran). The latter experiments would allow quantitation of the leak induced by the molecular drill, as well as measurement of the local changes occurring in the membranes.

This study was initiated by Prof. P.-G. de Gennes and Dr. M. Dvolaitzky. We thank Dr. M. Strick, Prof. L. Léger, Dr. R. Ober, and Dr. L. Dei for helpful advice, Mr. D. Detry for technical assistance, and Mr. F. Montembault of Elf-Atochem (Paris) for the scanning electron microscope photographs.

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