Communication

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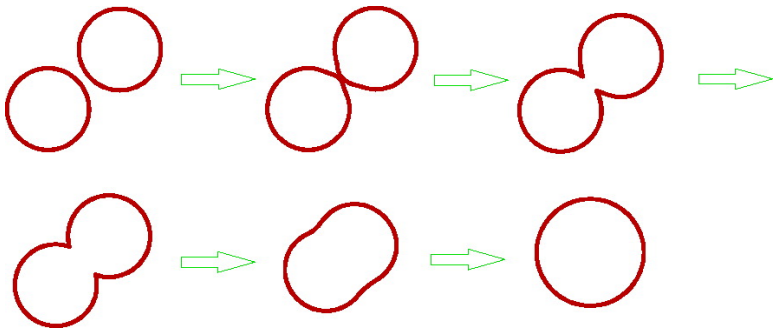

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Real-Time Membrane Fusion of Giant Polymer Vesicles

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Membrane fusion plays an important role in the formation of many complex organs, such as muscles, bones, and placentae in metazoans, and it is even believed to be one of the key events in the origin of life.[1] Despite the great significance of membrane fusion in biology, it is very difficult to directly observe the biomembrane fusion due to the complexity of biomembranes and the high speed of the process. To explore the cellular processes, model systems, such as vesicles for the plasma membrane and intracellular compartments in living cells, have become specific topics of interest in recent years.[2] Menger et al. have coined a novel terminology, "cytomimetic" chemistry, to describe the real-time shape transformations of vesicles in mimicking cellular morphology change.[3] Hotani et al. have already reported the real-time cytomimetic fusion process of lipid vesicles (liposomes).[4] However, the model membranes used in cytomimetic chemistry have been limited to giant liposomes (5−200 μm), and no real-time fusion photos of polymer vesicles have ever been reported. This work demonstrates for the first time the real-time fusion of individual giant polymer vesicles and provides some information for understanding the fusion process.

Polymer vesicles with excellent stability have become attractive and promising research objects since the first observation of block copolymer vesicles by Eisenberg and the developments by Discher and others.[2c,5,6] However, compared with the large amount of articles on the membrane fusion of liposomes, papers on polymer vesicle fusion have seldom been reported. Eisenberg and co-workers have investigated the fusion dynamics of the important polymer vesicles, termed "crew-cut" micelles, and presented fusion sequences by using transmission electron microscopy (TEM) to record the fusing vesicle intermediates.[2c,5c,d] To our knowledge, it has been the only work concerning the fusion of polymer vesicles.

Recently, we developed a new type of polymer vesicles coined as "branched-polymersomes", which were generated from the molecular self-assembly of an amphiphilic multiarm copolymer with a hyperbranched poly(3-ethyl-3-oxetanemethanol) core and many poly(ethylene oxide) arms (HBPO-star-PEO) in water.[7] Giant polymer vesicles with a bilayer structure and an average diameter of 112.8 μm (HB1) were obtained.[7b] The molecular structure and the preparation of HB1 vesicles are shown in the Supporting Information (Figure S1). In this article, we studied the real-time morphology changes of HB1 polymer vesicles after ultrasound treatment. The aqueous solution of HB1 vesicles in beaker was subjected to ultrasound for several minutes (150 W, 25 kHz), and then the sample was transferred immediately into a culture dish for the direct observation by optical microscopy (Leica Dmlp, TMS94) at 20 °C.

At first, HB1 polymer vesicles were suspended freely in water. Then, some HB1 vesicles aggregated (Figure S2) probably due to gravity, Brownian motion, and the formation of intervesicular hydrogen bonds. The aggregated vesicles could be stabilized for at least several hours without further fusion if no disturbance was exerted. When the vesicle suspension was subjected to ultrasound,

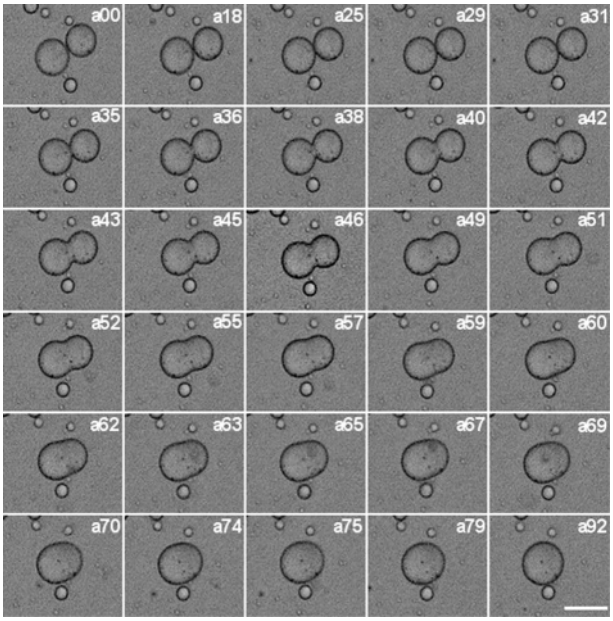

Figure 1. Time sequence of fusion images of two giant polymer vesicles. The number in the symbol labeled on each image denotes the elapsed time (in seconds), and the time of first image is set as zero. The scale bar represents 50 μm.

the average vesicle size decreased greatly and vesicle−vesicle fusion occurred throughout the whole suspension system. A sequential fusion process of two HB1 vesicles is displayed in Figure 1. Undergoing the intermediates of "8" shape, peanut (pear) shape, and oblate sphere, the two separate vesicles gradually fused into one vesicle. The whole fusion process includes four successive steps: membrane contact, formation of center wall, symmetric expanding of the fusion pore, and complete fusion. The fusion sequences presented in Figure 1 are somewhat different from those of the crew-cut micelles[2c,5c] and liposomes[4b] due to the special molecular structure of HB1 vesicles.

The whole fusion process of the polymer vesicles studied here lasted about 1.5 min, which is much longer than the fusion time of liposomes[4b] (in the time scale of seconds) and biomembranes[8a,b,9c] (in the time scale of milliseconds). Compared with the total volume of the two original HB1 vesicles, the volume of the fusing vesicle decreased continuously, and the finally fused vesicle decreased about 16% in volume (Figure S3), which indicated that 84% water encapsulated in the original vesicles mixed after fusion and the rest escaped into the aqueous environment. The content mixing inside vesicles during fusion was also observed directly from the fusion images of rhodamine-encapsulated vesicles by fluorescence microscopy (Figures S4 and S5).

Figure 1 also provides some experimental evidence for the theoretical work on membrane fusion. First, we found that the two

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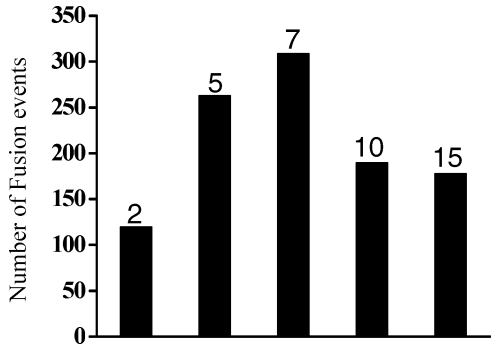

Figure 2. Statistics of the fusion frequency of HB1 vesicles after pretreatment by ultrasound. The number above the bar chart is the sonicated time in minutes.

adjacent membranes formed a softly protruding rim at the contact site in the initial stage of the fusion (0−31 s), which was proven in theory to be energetically favorable for promoting membrane fusion.[10a] Second, we found that the vesicle swelled during fusion, and the fusing vesicle only deformed in the neck domain around the fusion pore in the lateral direction (Figures S6 and S7). The neck stretched laterally, which was followed with the flattening of the vesicle. In other words, the fusing vesicle seemed to be compressed in the vertical direction and expanded in the lateral (Figure S6). In addition, by deleting the necks of the fusing vesicles with different elapsed time and overlapping the residual parts together, we found that the fusing vesicles without necks almost coincided completely (Figure S7), which indicates that the deformation of the fusion vesicle is mainly limited to the neck. The lateral deformation is certainly induced by the lateral tension. The mechanism for the formation and expansion of the fusion pore is controversial. Several theoretical works of Zimmerberg, Lipowsky, Kozlov, and MacDonald show that lateral tension is very important for the fusion pore.[8,10] Our observations support them at the vesicle deformation level, that is, the lateral tension, mainly concentrated on the rim of the neck, leads to the swelling of the fusing vesicle and the enlargement of the fusion pore.

The fusion of sonicated HB1 vesicles, as illustrated in Figure 1, is very common. Several examples of fusing vesicles are shown in Figure S8. The fusion process was observed between two large HB1 vesicles or between a large vesicle and a small one, and the cofusion process of three polymer vesicles was also observed. Evidently, vesicle size is not a severe limitation to membrane fusion. The statistics of the fusion frequency are shown in Figure 2. Hundreds of fusion events were observed in the HB1 vesicle suspension within 5 min after ultrasonic processing. The fusion frequency increased with the sonication time at first and then decreased. However, we noted that fewer vesicles could be detected by optical microscopy at a longer sonication time (exceeds 10 min) due to the strong decrease of vesicle size. As another evidence for vesicle fusion, the average size of the HB1 vesicles increased, and the size distribution became broader with respect to time after sonication (Figure S9). The fused vesicles were able to fuse again; however, the fusion could not proceed indefinitely. We found that the fusion process stopped at a certain stage after sonication, such as 30 min, which is probably attributed to the precipitate of the fused vesicle (Figure S10) and the restabilization of the sonicated vesicles.

Now, one question naturally occurs. What induces the fusion of the HB1 polymer vesicles? There are two different mechanisms for lipid membrane fusion. The proximity model postulates that very close apposition of membranes and small perturbations suffice to induce fusion.[9] Another model emphasizes that the fusion is regulated by membrane proteins through forming proteinaceous pores between apposed membranes.[1b] The fusion process of HB1 polymer vesicles has three characteristics. First, no protein is involved. Second, the close apposition of HB1 vesicles is common (Figure S2). Third, the ultrasound treatment, a perturbation generating defects on the vesicle membrane and destabilizing the vesicles,[9c] is necessary to mediate the fusion process. So the vesicle fusion reported here supports the proximity model. We have proposed a possible defect model on the sonicated vesicle membrane in the Supporting Information (Figure S11). Sonication can partly break the hydrogen bonds and give rise to the molecular packing defects on the membrane, which triggers the membrane fusion. Although the proximity model is based on liposomes, it can be expanded to the fusion of some synthetic and protein-free polymer vesicles, as reported in this paper.

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Supporting Information Available: Molecular structure and preparation of the HB1 vesicles (Figure S1). Aggregated vesicles (Figure S2). Volume changes, content mixing, deformation, and size changes of the fusing vesicle (Figure S3−S7, S9). Fusing vesicles (Figure S8). Precipitated vesicle (Figure S10). Defect model (Figure S11). This material is available free of charge via the Internet at http://pubs.acs.org.

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