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Recognition of Biotin-functionalized Liposomes

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Abstract: Functionalized liposomes were prepared by mixing the biotin in the lipid vesicle suspensions. The experiments through immersing streptavidin deposited mica into the biotin modified liposome solution testify the specifically biological binding interaction and extend the function of liposomes as a biosensor or drug carrier.

Keywords: Recognition, liposome, TEM, AFM.

Molecular recognition has been defined as the strong and specific interaction between two molecules without covalently binding[1]. Molecular recognition occurred at surfaces is eventually more important in controlling of the exchange of signals, compounds and energy[2]. Binding of biotin, vitamin H to the bacterial protein, streptavidin is a typical pair of molecular recognition[3]. Their binding constant, $k_a = 10^{15}$ L mol^{-1} is extremely high and very specific. Thus this pair is widely applied in many systems. In the present work we have chosen biotin as a recognized unit to modify the lipid liposomes and attempted to use such a functional liposome to construct a biosensor in the future[2, 3,4].

Liposome possesses a broad perspective in encapsulating of drugs, minerals, dyes and proteins in a nanoscale[5]. For delivery systems how liposomes can find the target and release the drug molecules in precise way is a practical problem. This work presents an easy route to modify the liposomes with a recognized unit and makes use of it to search for the specific biological matrix.

Liposomes were prepared by ultrasonically stirring a dispersion solution of 1.5 mmol/L l-α-dimyristoylphosphatidylcholine, DMPC at 30°C. Biotin with different ratio to lipids, was added to the DMPC vesicle suspension based on the approach in fabricating the electronic DNA-sensors by using functionalized liposomes[6]. Biotin has a shorter alkyl chain that can be readily adsorbed inside the vesicle bilayers. As a consequence the DMPC liposomes were functionalized by biotins. The rest biotin was removed by using a high-speed centrifugation. The streptavidin with four opposite side binding sites was readily adsorbed onto the surface of mica by deposition. Pure water was used to rinse up the substrate in order to remove the nonadhesive materials. Then

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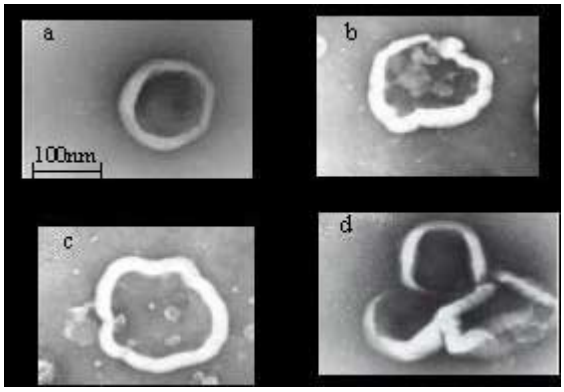
the mica covered by streptavidin was immersed into the solution of biotin-functionalized liposomes for half an hours.

Transition electron microscopy (TEM) images in **Figure 1a** show the direct observation of liposomes formed by DMPC without adding the biotin above the phase transition temperature 30°C[7]. The average liposome diameter is about 150 nm.

Figure 1 TEM images of a) pure DMPC liposome; b) and c) mixed biotin and DMPC liposomes with a ratio of 1: 0.5 and 2:1 d) biotin-functionalized liposomes linked by streptavidin

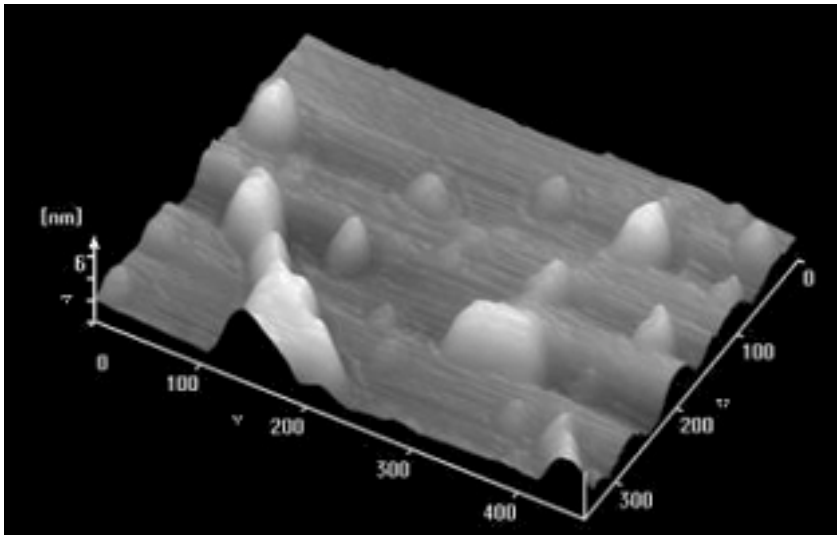


As the biotin powder was added into the vesicle suspension solution stirring with ultrasonic agitation was needed for about one hour. In order to remove excess amount biotin in the solution centrifugation washing was necessary for several times[8]. Then the pure biotin-functionalized liposomes were obtained. The TEM images in **Figures 1b** and **1c** displays the liposomes modified by adding different ratio of lipids to biotins, 1: 0.5 and 1: 2 respectively. To compare the image of pure DMPC liposome in **Figure 1a** we have observed the difference of the vesicle shape and size. The biotin-functionalized liposomes have a flexible and rough surface. However the closed round shape of liposome could not be destroyed. This demonstrates that the hydrophobic chain of the biotin molecules has inserted the bilayers of the lipid molecules. Thus the unilamellar lipid molecules have a rearrangement process. From the TEM images of **Figures 1b and 1c** one can see that the increase of the ratio of biotins to lipids did not successively result in the change of the liposome shape. This means that penetration ability of biotin molecules into the lipid bilayers is limited in a certain range. Since streptavidin has four junction sites, two or more separate biotin modified liposomes can be also linked *via* the biological binding. The aggregation displayed in **Figure 1d** is more likely the combination of three liposomes through streptavidin.

A piece of mica was immersed in a streptavidin solution for adsorption. In 30 minutes the mica covered by the streptavidin molecules was rinsed up with millipore water for several times to remove any weakly adsorbed or nonadsrobed molecules[9]. Then we left the streptavidin deposited mica into the biotin-functionalized liposome solution for around half an hour. Take out the mica from the liposome solution we washed out it

with pure water for the measurements of atomic force microscopy (AFM). The AFM image in **Figure 2** shows that the DMPC vesicles with the recognized units have been bond with the streptavidin on the mica surface.

Figure 2 AFM images of biotin modified DMPC liposome bond with streptavidin deposited in the substrate of mica



Comparing to the pure streptavidin surface, which has a flat surface we can learn that the peaks of **Figure 2** are corresponding to the combination of the two specific binding pair. Each peak height measured by AFM is approximately 6 nm, which is the order of thickness for lipid bilayers. This exhibits that the dried vesicle has been bond with streptavidin *via* biotin recognition unit since the mica was heavily washed out with pure water after the mica covered by streptavidin and was picked out from the biotin-functionalized liposome solution.

In conclusion, we have provided direct experimental evidences by TEM for the liposome modified with a recognized unit through the surface change. The AFM micrographies proved the binding of biotin-modified liposomes with streptavidin through a specifically biological interaction. This may help to understand the application of liposomes as a targeted drug delivery carrier.

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