Molecular Affinity and Permeability of Different Molecular Weight Chitosan Membranes

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Membranes were prepared from chitosan with different molecular weights by a casting method. The molecular affinity and permeability of the membranes for sodium chloride, glucose, tyrosine, and bovine serum protein were measured at 4 °C and pH 7. The molecular permeability of the chitosan membranes was inversely related to molecular weight. All prepared membranes showed a molecular affinity to bovine serum protein. The higher the molecular weight of chitosan membrane, the higher the affinity and the lower permeability of the membrane to bovine serum protein.

KEYWORDS: Chitosan; membrane; molecular permeability; molecular affinity; bovine serum protein

INTRODUCTION

Biopolymer-based membranes have been gaining more attention in the past few years due to their great potential in the application of chemical separations, controlled drug releases, and coating materials. Chitosan, (1−4)-2-amino-2-deoxy-β-D-glucan, is a biopolymer derived from chitin. Due to the presence of an amino group at carbon-2 of its cellulose-like backbone, chitosan is a cationic polyelectrolyte and soluble in acidic media. As a new functional material, chitosan offers a unique set of characteristics: hydrophilicity, biocompatibility, biodegradability, antibacterial properties, remarkable affinity to proteins, and film formation (*1*, *2*).

The membrane made from chitosan has a good mechanical strength with a selective molecular permeability. Therefore, it has been used for molecular separation, food packaging film, artificial skin, drug delivery system, and other applications (*3*−*7*). The molecular permeability of chitosan membrane is affected by various factors such as pore size, thickness of the film, molecular weight of chitosan, degree of deacetylation (DD) of chitosan, and other conditions of the membrane preparation (*8*−*10*). In the same case, the molecular permeability of the membrane depends on the properties of the permeating reagent. The small molecules are easily transported through the membranes, whereas the large molecules move more slowly or cannot be transported on the basis of the theory of the capillary pore model (*11*−*13*).

It was reported that the cross-linking and chemical modification of chitosan membrane influenced the permeability. Cross-linking agents such as glutaraldehyde, ethylene glycol diglycidyl ether, and epichlorohydrin can change the membrane permeability (*14*, *15*). Chemical modification of chitosan membrane with EDTA, Cibacron Blue, hexamethylene diisocyanate, and aldehydes is able to change the membrane ionic charge to influence the permeability (*16*−*19*).

The permeability test conditions also have a strong influence on the results. It was reported that the glucose permeability of chitosan membrane conducted at pH 5.0 showed a higher membrane permeability coefficient than that conducted at pH 7.4 (*20*). Chitosan membrane also has shown a specific molecular affinity toward protein, DNA, and other biomolecules (*17*, *21*, *22*). Therefore, the molecular affinity of the chitosan membrane has a direct influence on membrane permeability.

The objective of this research was to prepare chitosan membranes of different molecular weights (MW) and to study the membrane affinity in relation to permeability using small molecular weight compounds, such as sodium chloride, glucose, tyrosine, and bovine serum protein. Each permeate agent represents a group of compounds common in organisms in order to show a comprehensive test result for the chitosan membranes.

MATERIALS AND METHODS

Materials. Chitosan (MW 480000 Da, DD 90%) made from crab shell was obtained from Biochemical Medicine Plant of Qingdao (Qingdao, China). Sodium chloride, glucose, tyrosine, bovine serum albumin (BSA), and TRIS buffer were Sigma products (Sigma Chemical Co., St. Louis, MO).

Different MW Chitosan Preparations. Chitosan (5 g, 100 mesh powder) was dissolved in 95 mL of 5% aqueous acetic acid, incubated at 50 °C for 0, 5, 10, and 15 h, respectively, and then centrifuged (5000*g*) for 20 min. The supernate was added to 4 N aqueous NaOH to pH 7−9. The sediment was filtered and sequentially rinsed in water and ethanol and dried at 50 °C. The MWs of chitosan samples obtained

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Table 1. Various Molecular Weight Chitosan Samples Prepared and Their Membrane Characteristic

chitosan		membrane	
MW[a] (Da)	DD[b] (%)	MT[c] (mm)	DS[d] (%)
480000	91.4	0.108	10.63
250000	90.2	0.106	13.27
120000	90.0	0.103	20.51
30000	89.2		

[a] Molecular weight. [b] Degree of deacetylation. [c] Membrane thickness. [d] Degree of swelling in water of membrane.

from the above process ranged from 30000 to 480000 Da, the DD ranged from 89.2 to 91.4% (**Table 1**). The MW of the chitosan sample were determined by using a GPC method with a Waters-1525 binary HPLC system, a Waters-2410 refractive index detector (Waters Corp., Miford, MA), and an SEC column (TSK Gel 3000PW, 7.8 × 600 mm, Tosoh Corp., Japan). The mobile phase was 0.25 M acetic acid buffer (pH 4.7). IR and viscometry were used to determine the DD and MW of chitosan, respectively (*1, 23*).

Membrane Preparation. Three chitosan membranes were prepared by a cast method. Chitosan (3 g) was dissolved in aqueous acetic acid (97 mL, 1% v/v). The bubble-free liquid (2 mL) was spread on a plastic dish (Ø = 40 mm) and dried at 50 °C overnight. The dried membrane was soaked in 10% (w/v) aqueous NaOH for 4 h to neutralize the acid. The membrane was separated from the dish, rinsed in distilled water to neutral, soaked in ethyl alcohol (70% v/v), and then dried at 50 °C. The thickness and swelling degree of the membrane were measured by thickness gauges (SM-1201) before and after the membranes were soaked in distilled water for 24 h.

Permeability Determination. The permeability measurements were conducted using a dialysis cell made up of two detachable glass compartments at 4 °C and pH 7. The sample membrane was placed between two compartments. The two sides were clamped together with springtight steel clip. Distilled water (50 mL) and the sample solution (50 mL) were filled into each compartment of the cell at the same time. The liquid of both compartments was stirred by an electromagnetic bar. Sodium chloride (0.9% w/v), glucose (0.1% w/v), tyrosine (0.1% w/v), and bovine serum (20% v/v) solutions were used in the sample solution compartment. The solution in both compartments was taken out after a given period of time to test the migration.

Glucose was determined by using the Anthrone method (*24*). Tyrosine was determined spectrophotometrically with a UV–vis spectrophotometer (DU-650, Beckman) at 280 nm. NaCl was determined by conductance measurement (*25*); the instrument has a sensitivity of $\pm 1 \times 10^{-2}$ μs·cm^{-1}, and the conductance of the doubly distilled water was ≤ 0.05 μs·cm^{-1}. The protein was determined according to the Lowry method (*26*) and the sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) method (buffer: 0.20 M glycine, 0.20 M TRIS, and 0.55% SDS, pH 8.1) (*27*).

Affinity Experiment. Chitosan membrane (0.1 g) was soaked in distilled water for 5 h to equilibrate. The membrane surface liquid was removed with filter paper and then soaked in 2 mL of bovine serum solution (2.5% v/v) at 4 °C. At the given time, the solution was analyzed for the protein content with the Lowry method using BSA as a standard. The protein adsorptive capacity on the membrane (Pa %) was calculated using the following equation:

$$Pa\,\% = (C_1 - C_2)VW^{-1} \times 100\% \qquad (1)$$

Here C_1 and C_2 were the protein concentration of the solution before and after adsorption, respectively, V was the volume of the solution, and W was the weight of the chitosan membrane.

RESULTS AND DISSCUSION

Chitosan Membrane Preparation. The membrane made from the higher MW chitosans (480000, 250000, and 120000 Da) had enough mechanical strength, transparency, and elasticity
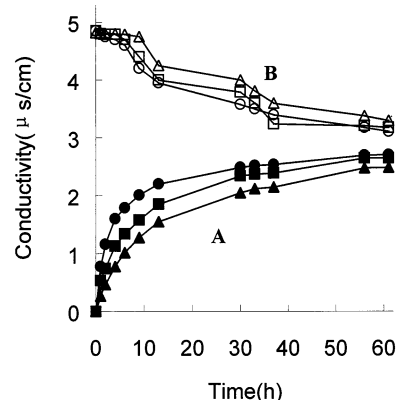

Figure 1. Specific conductance of the sodium chloride solution: (A) distilled water compartment [(▲) 480000, (■) 250000, (●) 120000]; (B) solution compartment [(△) 480000, (□) 250000, (○) 120000].
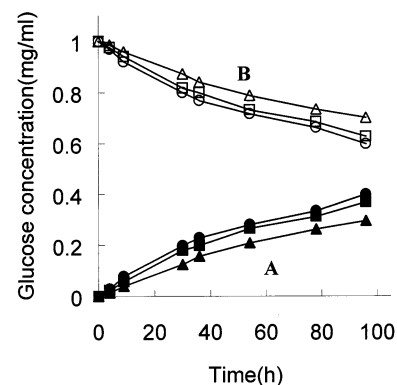

Figure 2. Glucose concentrations in the two sides of chitosan membrane: (A) distilled water compartment [(▲) 480000, (■) 250000; (●) 120000]; (B) solution compartment [(△) 480000, (□) 250000, (○) 120000].

to withstand the test. On the other hand, the low MW chitosan membrane (30000 Da) that contained many pinholes was poor in mechanical strength and easily split, so it was not able to used for the test. The data on swelling efficiency and thickness of the membranes and the molecular weight and deacetylation degree of the chitosan samples are shown in **Table 1**. With the decrease of chitosan MW, the chitosan DD and membrane thickness showed no drastic change, but the membrane swelling degrees, which influence the molecular permeability of the membrane, showed a drastic increase.

Molecular Permeability. Changes in NaCl solution conductivity of each side of the chitosan membrane are shown in **Figure 1**. The conductivity of the solution in the distilled water compartment (**Figure 1A**) increased, whereas the conductivity of the solution in the NaCl compartment (**Figure 1B**) decreased proportionally. The permeability speed (*P*) of NaCl through the membranes has an inverse relationship to the chitosan MW: $P_{120000Da} > P_{250000Da} > P_{480000Da}$.

The permeabilities of glucose and tyrosine are shown in **Figures 2** and **3**, respectively. The permeability curve rose and fell in each side (A, B) of the chitosan membrane as observed with NaCl. The permeability speed (*P*) for both glucose and tyrosine through the membranes also has an inverse relationship to the chitosan molecular weight: $P_{120000Da} > P_{250000Da} > P_{480000Da}$.

Because the rate of the bovine serum protein permeation to the distilled water compartment was very low, the protein concentration could not be detected by the UV–vis method or the Lowry method. Therefore, the total solution in the distilled
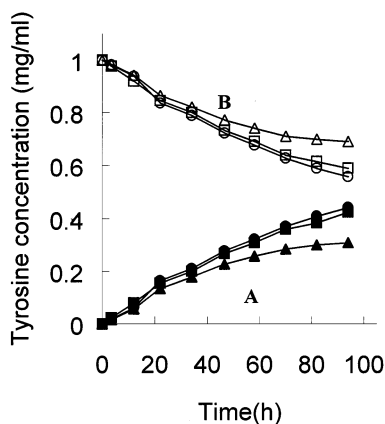
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Figure 3. Concentrations of tyrosine in the two sides of the chitosan membrane: (A) distilled water compartment [(▲) 480000, (■) 250000, (●) 120000]; (B) solution compartment [(△) 480000, (□) 250000, (○) 120000].
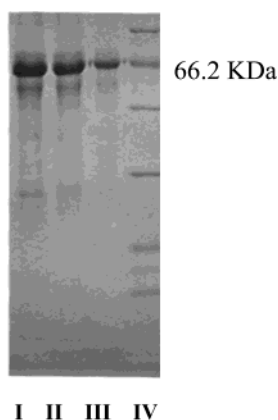

I   II   III   IV

Figure 4. SDS-PAGE of the protein in the distilled water compartment of the chitosan membrane: (lane I) 120000 Da molecular weight chitosan membrane; (lane II) 250000 Da molecular weight chitosan membrane; (lane III) 480000 Da molecular weight chitosan membrane; (lane IV) marker.

water compartment was concentrated to 2 mL, and then the protein was measured by the SDS-PAGE method. As shown in **Figure 4**, the higher MW chitosan membrane showed weak and narrow color bands (**Figure 4**, lane III), whereas the lower MW chitosan membrane showed deep and wide color bands (**Figure 4**, lane I). The highest MW of protein that passed through the chitosan membranes was 66.2 kDa.

Overall, the chitosan MW showed a directly proportional relationship to the membrane molecular permeability, as shown in **Figures 1**−**4**. The molecular permeability was also increased with the increased membrane swelling index, as shown in **Table 1**. It was reported that the swelling index was proportional to the amorphous region of the membrane: the lower the MW of the chitosan membrane, the more amorphous the region, with higher molecular permeability (*10*).

Chitosan is a weak-basic polymer, with a $pK_a = 6.3$−7.0 (*28*). The membrane has a very weak ionic strength at pH 7, and it has no charge influence on small molecule compounds that pass through the membrane. Therefore, sodium chloride, glucose, and tyrosine showed no significant difference in the permeability patterns. The increased amount of the compounds in the distilled water compartment was the same as the decreased amount of the compounds in the solution compartment.

The permeability of bovine serum proteins on the chitosan membranes was very low. It may be due to the fact that the
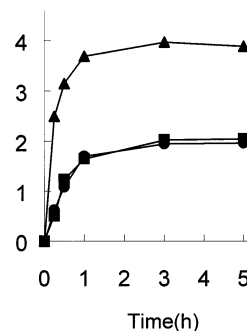

Figure 5. Protein quantity adsorbed on the chitosan membrane: (▲) 480000; (■) 250000; (●) 120000.

protein molecule was too big to pass through the membrane. It was also reported that proteins with MW >70 kDa could not pass through the chitosan membrane (*29*).

Molecular Affinity. Chitosan membrane showed a strong adsorption to the bovine serum protein at pH 7 (2.5% v/v). The main adsorption took place in the first hour. The membrane adsorptive capacity reached 3.69, 1.87, and 1.70 mg/g for the 480000, 250000, and 120000 Da chitosan membranes, respectively. As shown in **Figure 5**, during the extended soaking period, the adsorptive capacity curve stayed flat after 1 h.

Some polymers that have carboxyl groups in the molecule, such as poly(itaconic acid) chitosan, have strong affinity (*30*). Therefore, the reason for the low permeability of the bovine serum protein on the chitosan membrane can be explained by the high molecular affinity of chitosan membrane to the protein. As shown in **Figures 4** and **5**, some proteins were absorbed on the membranes within the initial first hour, and some proteins that have MW <66.2 kDa could pass through the lower MW chitosan membrane (**Figure 4**, lane I) but they could not pass though the higher MW chitosan membrane (**Figure 4**, lane III). Apparently, the molecular affinity of the chitosan membrane played a major role. The unpassed proteins were adsorbed on the membrane by the membrane−protein affinity. The molecular affinity inhibited the permeability of the chitosan membrane to the bovine serum protein. The higher the molecular weight of the chitosan membrane, the higher the affinity and the lower permeability of the membrane to bovine serum protein.

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