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Size-Based Protein Separations in Poly(ethylene glycol)-Derivatized Gold Nanotubule Membranes

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ABSTRACT

An electroless plating method was used to deposit Au nanotubules within the pores of track-etched polycarbonate template membranes. The effect of nanotubule inside diameter on rate and selectivity of protein transport was investigated for three proteins: lysozyme (Lys), bovine serum albumin (BSA), and β-lactoglobulin A. Transport selectivity increased as the inside diameter of the nanotubules within the membrane decreased, and selectivity coefficients in excess of 20 were observed for the separation of Lys from BSA. Protein adsorption, and hence membrane fouling, was eliminated by chemisorbing a poly(ethylene glycol) thiol to the Au nanotubule membranes.

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We, and others, have been exploring the transport properties of gold nanotubule membranes[1−7] prepared via the template method,[8−10] a general approach for making nanomaterials. The gold nanotubules are prepared by electrolessly plating Au within the pores of a microporous track-etched polycarbonate filter. We have shown that by controlling the Au plating time, the inside diameter of the nanotubules can be varied at will down to molecular dimensions (<1 nm).[3] As a result, these membranes can be used in simple membrane-permeation experiments to cleanly separate small molecules on the basis of molecular size.[3] In addition, ion-transport selectivity can be introduced by electrostatically charging the membrane to put either excess positive charge or excess negative charge on the Au nanotubules.[1,2] Ion, and chemical, transport selectivity can also be introduced by chemisorbing thiols to the Au nanotubule walls.[4−7] By appropriate choice of thiol, membranes that preferentially transport cations or anions and hydrophobic or hydrophilic molecules can be prepared. This ability to independently and precisely control both the inside diameter and chemical characteristics of the nanotubules makes these membranes ideal model systems for studying how pore size, chemistry, and charge affect rate and selectivity of permeate transport in membranes.

We have recently been investigating protein transport in the Au nanotubule membranes. In this case, the ability to tailor the chemistry of the nanotubules provides a route for suppressing protein adsorption and thus fouling, a vexing problem for ultrafiltration separations of proteins.[11−16] In particular, there have been a number of investigations of protein transport in the track-etched polycarbonate membranes[17−22] used as the templates to prepare the Au nanotubules, and protein adsorption has proven to be a problem in such studies.[19−22] Protein adsorption to the Au nanotubule membranes was suppressed by chemisorbing a thiol-terminated poly(ethylene glycol). It is well known that PEG-modified surfaces show decreased protein adsorption relative to the unmodified surface.[23−28] The ability to precisely control the inside diameter (i.d.) of the nanotubules (and to produce nanotubules with very monodisperse i.d.s) is also advantageous in that this should allow for size-based separation of proteins of similar molecular weights. This can be a problem for conventional ultrafiltration membranes, which typically have a broad distribution of pore sizes. We describe results of preliminary investigations of protein separations in the Au nanotubule membranes here.

Polycarbonate track-etched membranes (6 μm thickness, either 30-nm or 50-nm diameter pores, 6×10^8 pores cm^{-2}) were obtained from Poretics. Three proteins (Sigma-Aldrich) were investigated: lysozyme (Lys, MW = 14 kDa), β-lactoglobulin A (LGA, MW = 36 kDa), and bovine serum albumin (BSA, MW = 67 kDa). The thiol-terminated poly(ethylene glycol) (PEG-thiol, MW = 5000 Da) was obtained from Shearwater Polymers. Commercial gold-plating solution (Oromerse SO Part B) was obtained from Technic Inc. Purified water was obtained by passing house-distilled water through a Milli Q (Millipore) water purification system.

The electroless plating procedure used to deposit the Au nanotubules within the pores of the polycarbonate template membrane has been described previously.[5] This method yields the Au nanotubules within the pores plus Au surface layers on both faces of the membrane. The PEG-thiol was chemisorbed to the Au by immersion of the nanotubule membrane for 6 days into a degassed 1 mM solution of this

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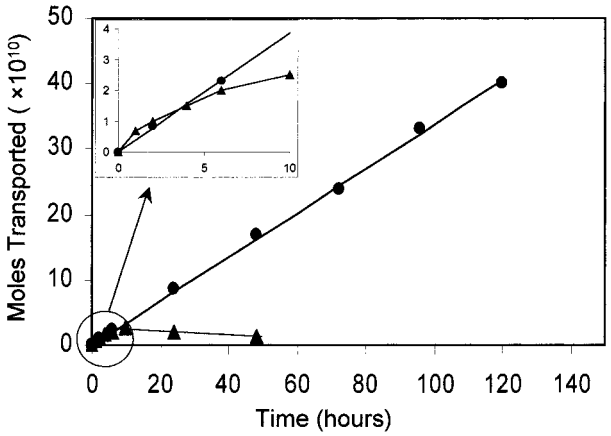

Figure 1. Plots of moles transported vs time for Lys diffusion (no applied pressure) across i.d. = 23-nm Au nanotubule membranes. Upper line: with chemisorbed PEG-thiol. Lower curve: no PEG thiol. The inset shows the initial transport rates of Lys.

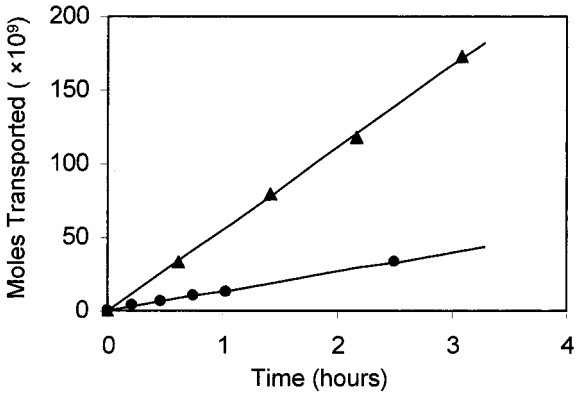

Figure 2. Plots of moles transported vs time for Lys (upper) and BSA (lower) across an i.d. = 40-nm Au nanotubule membrane. Single-protein permeation experiments.

thiol. The i.d. of the Au nanotubules was varied by varying the Au plating time. A gas-flux method, also described previously,[5] was used to obtain nanotubule i.d. values for each membrane. The i.d. values reported here were measured before chemisorption of the PEG-thiol. XPS measurements on a similar 5000 MW PEG-thiol on Au showed that the thiol layer thickness is ~2.4 nm.[23] Membranes with i.d. = 40-nm and 45-nm nanotubules were prepared in the polycarbonate templates with 50-nm diameter pores. Membranes with smaller ID nanotubules were prepared in the templates with 30-nm diameter pores.

The Au nanotubule membranes were mounted between the two halves of a U-tube permeation cell as described previously.[5] Both single-protein and two-protein permeation experiments were done. In the single-protein experiments, the feed half-cell contained 5 mL of a 20 mM sodium phosphate buffer solution (pH = 7.0) that was 0.05 mM in the desired protein. Unless otherwise noted, this solution was forced through the membrane by applying 20 psi pressure to the feed half-cell. The permeate half-cell was initially empty. As solution was forced through the membrane, the permeate half-cell was periodically sampled, and the concentration of the protein was determined via the UV absorbance at 280 nm. The two-protein permeation experiments were done in an analogous fashion except the feed solution was 0.025 mM in each protein. The concentration of each protein in the permeate half-cell was determined by sampling the permeate solution after ~1 mL was transported and doing HPLC analysis. A Shimadzu HPLC system with a YMC−Pack aqueous SEC (diol phase) column and UV/vis detector (280 nm) was used. The mobile phase was 0.1 M phosphate buffer (pH = 6.8) containing 0.2 M NaCl; the flow rate was 1.0 mL/min. In addition to these pressure-driven transport experiments, preliminary single-molecule permeation experiments were done in the absence of applied pressure. In this case, transport occurred by diffusion of the protein across the membrane into 5 mL of the buffer solution.

Figure 1 shows results of single-protein (Lys) permeation experiments in the absence of applied pressure for i.d. = 23-nm Au nanotubule membranes, with and without chemisorbed PEG-thiol. Without the PEG-thiol, transport stops after ~8 h, indicating rapid blockage of the nanotubules by adsorbed protein. (The inset in Figure 1 shows the short time data before the nanotubules are plugged.) In contrast, when the PEG-thiol is present, linear moles-transported vs time data are obtained for up to 5 days. These data clearly show that protein adsorption is strongly suppressed by the chemisorbed PEG. This suppression of protein adsorption is also observed in the pressure-driven transport experiment, where protein flux is ~2 orders of magnitude higher. Indeed, we have conducted such experiments for as long as 6 days with no evidence for protein adsorption. All of the remaining data presented in this paper are from membranes with chemisorbed PEG-thiol and where 20 psi pressure was used to drive the feed solution through the membrane.

Figure 2 shows results of single-protein permeation experiments for Lys (upper line) and BSA (lower line) through a membrane with i.d. = 40-nm nanotubules. The flux of Lys is four times higher than the flux of BSA. The Stokes radii for BSA and Lys are 3.6 and 2 nm, respectively;[29] hence, the Stokes−Einstein equation would predict that in free solution the diffusion coefficient for Lys would be only 1.8 times higher than that for BSA. That the ratio of the fluxes in the nanotubule membrane (Figure 2) is higher than this Stokes ratio is indicative of hindered transport[30,31] of the protein molecules in these nanoscopic tubules. Two-protein permeation experiments were used to explore this issue further.

Figure 3A shows HPLC data for the *feed solution* from a BSA/Lys two-protein permeation experiment. Figure 3B shows HPLC data for an aliquot of the permeate solution after permeation through an i.d. = 45-nm nanotubule membrane. In analogy to the single-protein transport case (Figure 2), the attenuation of the BSA peak, relative to the Lys peak, clearly shows the enhanced discrimination against the larger protein molecule. Figure 3C shows analogous data after transport though an i.d. = 30-nm nanotubule membrane; now the BSA peak is barely discernible. BSA could not be detected in the permeate from an i.d. = 20-nm nanotubule membrane (Figure 3D). This, of course, does not mean that there is no BSA present; it simply means that the concentration is below the detection limit of our analytical method,

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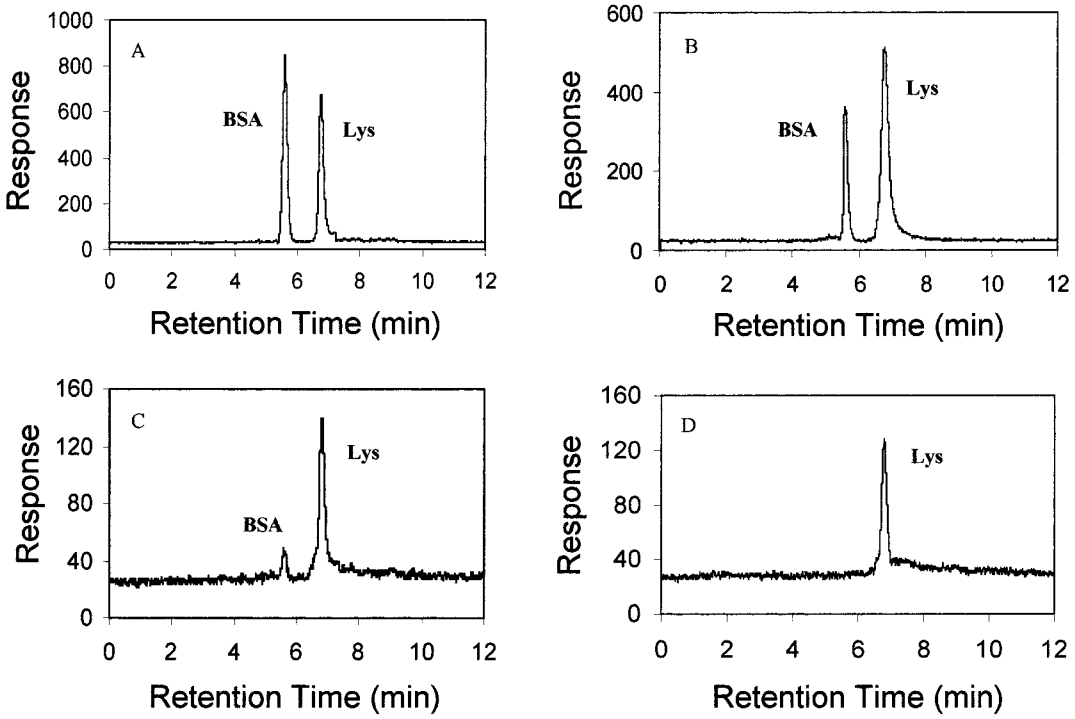

Figure 3. HPLC data for two-protein (Lys/BSA) permeation experiments. (A) Feed solution. Permeate solutions after transport through i.d. = 45-nm (B), 30-nm (C), and 20-nm (D) nanotubule membranes.

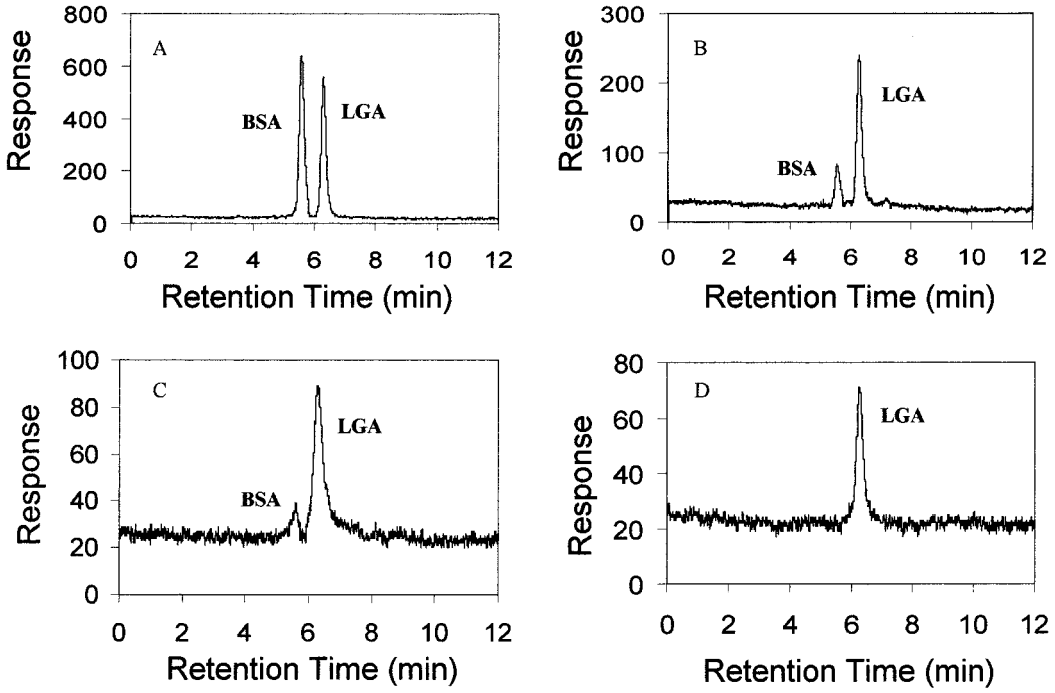

Figure 4. HPLC data for two-protein (LGA/BSA) permeation experiments. (A) Feed solution. Permeate solutions after transport through i.d. = 40-nm (B), 27-nm (C), and 22-nm (D) nanotubule membranes.

which for BSA is ~0.2 μM. In an attempt to obtain a discernible BSA signal in the permeate from the i.d. = 20-nm nanotubule membrane, we increased the permeation volume from 1 to 2 mL, but there was still no detectable BSA in the permeate.

The transport data in Figure 3 can be quantified by defining a Lys vs BSA selective coefficient, $\alpha_{Lys/BSA}$, which is the ratio of the concentration of Lys to the concentration of BSA in the permeate solution. Table 1 shows that $\alpha_{Lys/BSA}$ increases with decreasing nanotubule diameter. Analogous results were obtained for small-molecule permeation in smaller-i.d. Au nanotubule membranes[3,31] and was explained in terms of hindered transport,[30,31] which occurs when the diameter of the molecule approaches the diameter of the pore in which it is being transported. We discuss this issue in some detail in a prior publication.[31] In the case of the i.d. =

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Table 1: Concentrations of Proteins in the Permeate and Transport Selectivity Coefficients for Two-Protein Permeation Experiments

mixture of Lys and BSA				mixture of LGA and BSA			
i.d. (nm)	conc. of Lys (μM)	conc. of BSA (μM)	$\alpha_{Lys/BSA}$	i.d. (nm)	conc. of LGA (μM)	conc. of BSA (μM)	$\alpha_{LGA/BSA}$
20	4.0	0	≥20	22	3.1	0	≥15
30	6.3	0.5	13	27	4.3	0.3	14
45	18.6	8.4	2.2	40	10.5	1.6	6.7

20-nm membrane, where BSA could not be detected, we report a minimal transport selectivity coefficient, defined as before[3] as the detected concentration of the smaller protein (Lys) divided by the detection limit for the larger protein (BSA). As indicated in Table 1, the selectivity coefficient for the i.d. = 20-nm nanotubule membrane is at least 20. That this difference in selectivity is based primarily on the difference in size and not charge of the proteins is reinforced by the fact that at this pH value, BSA is negatively charged and Lys is positively charged.[11,15] Furthermore, the PEG is electrically neutral so there should be no excess charge on the nanotubules.

Table 1 also shows that the increased selectivity for the membranes containing the smaller i.d. nanotubules comes at a price − the concentration of both proteins in the permeate decreases with decreasing nanotubule diameter. Hence, as is typically observed in membrane-based separations processes, membranes that show higher selectivity also show decreased permeate throughput or productivity.[32] Figure 4 shows analogous data for the separation of BSA from LGA, and the corresponding selectivity coefficients are also tabulated in Table 1. Again, we see higher selectivity and lower productivity for the membranes containing the smaller i.d. nanotubules.

These studies have shown that by controlling the i.d.s of the nanotubules, Au nanotubule membranes can show good selectivity for separation of proteins on the basis of molecule size. In addition, by chemisorbing a PEG-thiol, the problem of membrane fouling by protein adsorption can be eliminated. Protein flux in these experiments was enhanced by applying a pressure difference across the membrane. We are currently investigating electrophoresis of proteins across such membranes. This not only provides an alternative method for enhancing flux but also adds the dimension of using protein charge as a way of further discriminating between proteins. Finally, although the Au nanotubule membranes are good model systems for investigating how pore size and chemistry affect protein transport, the porosities of these membranes are too low for practical use in protein separations. We are currently investigating similar membranes with significantly higher porosities. However, the Au nanotubule membranes might prove useful for sensor applications,[33,34] where net flux through the membrane is not as big of a concern.

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