TRANSPORT AND KINETIC FEATURES OF GOLD-FUNCTIONALIZED ARTIFICIAL NANOPORES

ALINA ASANDEI, F. PINTILIE, T. LUCHIAN[#]

Laboratory of Biophysics and Medical Physics, Faculty of Physics, "Alexandru I. Cuza" University, 11,Carol I Blvd., Iaşi, ROMANIA ([#]luchian@uaic.ro)

Abstract. The use of cysteine-functionalized membranes with known porosity may allow a better understanding of separation and transport characteristic through biomembranes. In this work, polycarbonate membrane was gold-coated via electroless deposition onto the pore walls, followed by L-cysteine chemisorption on the modified surface. By measuring the electrical current flow through these membranes and analyzing the current-potential difference diagrams, we showed that such membranes display variable conductivities, depending on the pH value of the contacting solution. Interestingly, the spectral behavior of such functionalized membranes quantified by their electrical impedance shows a strong dependence upon the pH value of the contacting solution, departing visibly at high pH values from the theoretical predictions of a simple parallel-RC model. A possible extension of the present work may be the exploration of the so-called artificial ion channels, which would resemble their biological counterparts from the viewpoint of ion selectivity and gating properties.

Key words: track-etched membranes, cysteine, nanopores, voltage-clamp, impedance.

INTRODUCTION

Biological membranes are molecular structures acting mainly as barriers to the flow of matter between various cellular compartments. With specific ion channels embedded, such membranes can selectively allow for the diffusion of various components of a solution under the influence of pressure, voltage, temperature or concentration gradient.

Recently, some varieties of materials that can be used in the chemical or biological separation came up into the action, including here chemically modified and functionalized polymeric membranes [2]. A good example in this respect are polycarbonate track-etched membranes, which contain nanopores relatively uniformly-distributed, and easily prone to chemical functionalization by depositing noble metals within the pores [8]. The resulting gold (Au) nanopore membranes are

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ideal models for the study of ionic transport through artificial nanopores. For the deposition the metals within the nanopores, the electroless gold plating method [7, 10] is currently being used. It is now known that by controlling the Au deposition time, one can prepare Au nanopores that have effective inside diameters of molecular dimensions (< 1 nm), suggesting that these membranes may be useful as molecular sieves [3]. Such metal nanopore-containing membranes show selective ion transport, analogous to that observed in ion-exchange polymers [1]. Ion permselectivity occurs because excess charge density can be present on the inner walls of the tubules, stemming from the adsorbed chloride ions [5]. These membranes can be either cation- or anion- selective, depending on the potential applied to the membrane; hence, these metal nanotubule membranes can be viewed as universal ion exchange membranes [11]. After metals deposition ends, a further way to chemically modify such nanopores is to attach L-cysteine onto the gold coated surface [9], which brings about the possibility of a pH-switchable ion-transport selectivity property of such functionalized nanopores [4]. At low pH, both the amino and carboxyl groups of the cysteine are protonated, so that the average net electrical charge on the nanopores becomes mostly positive; as a result, such membranes would reject cations and preferentially transport anions. At high pH, when both the amino and carboxyl groups of the bound L-cysteine are deprotonated, and the net charging state of nanopores is negative, which allows them to reject anions and preferentially transport cations. At pH = 7.0, near the isoelectric point of the chemisorbed cysteine, these membranes are expected to show neither cation nor anion transport selectivity.

In this work we explored the transport properties of gold-plated nanopore membranes prepared via the electroless plating method, which contained L-cysteine chemisorbed within the pores of a nanoporous polycarbonate filter. By measuring the electrical current flow through these membranes, and upon analysis of the I-V diagrams, we showed that these membranes display variable conductivities, depending on the pH value of the contacting solution phase. Interestingly, the spectral behavior of such functionalized membranes quantified by their electrical impedance shows a strong dependence upon the pH value of the contacting solution phase, departing visibly from the theoretical predictions of a simple parallel-RC model, especially at high pH values.

EXPERIMENTAL SECTION

MATERIALS

Polycarbonate track-etched membranes (100 μm thickness, 30 nm diameter pores, 6×10^8 pores cm^{-2}) were obtained from Whatman. For the functionalization of the membrane we used $SnCl_2$(1M), $AgNO_3$, trifluoroacetic acid (98%), Na_2SO_3, NH_4OH, NaCl, NaOH, Na_2HPO_4, NaH_2PO_4, Au (24K), KCN, HCl, HNO_3, $[KAu(CN)_2]$, 90% ethanol, L-cysteine, formaldehyde and anhydrous methanol. L-

cysteine hydrogen chloride was obtained from Merck and used as received. The gold-plating solution [KAu(CN)$_2$] was prepared in-house.

MEMBRANE PREPARATION

In order to deposit Au within the pores of the polycarbonate template membrane, we resorted to the electroless plating procedure.

The first step taken in this respect was to prepare the gold-plating solution [KAu(CN)$_2$], containing the following reagents: Au (24K), HCl (36.5%), HNO$_3$ (63%), NH$_4$OH (25%), and KCN.

The procedure used for the electroless deposition was similar to that reported by Yu et al. [11]. That is, the membrane was first immersed in methanol for five minutes, followed by soaking the membrane for 45 minutes in a solution of 0.025 M SnCl$_2$ and 0.07 M trifluoroacetic acid. The membrane was then washed two consecutive times for 2.5 minutes using methanol. Afterwards, the membrane was reimmersed in a solution 0.029 M [Ag(NH$_3$)$_2$]OH (an aqueous ammoniacal solution of AgNO$_3$) for 10 minutes.

As a result, nanoscopic metallic Ag particles will be deposited on the membrane:

$$Sn^{II}_{surf} + 2Ag^{I}_{aq} \rightarrow Sn^{IV}_{surf} + 2Ag^{0}_{surf} \qquad (1)$$

where:

Sn$^{II}_{surf}$ is the oxidation number of tin membrane surface before redox reaction;

Ag$^{I}_{aq}$ is the oxidation number of silver in the aqueous ammoniacal solution of AgNO$_3$;

Sn$^{IV}_{surf}$ is the oxidation number of tin membrane surface after redox reaction;

Ag$^{0}_{surf}$ is the oxidation number of silver of membrane's surface.

The membrane was then rinsed for 5 minutes using methanol, followed by its immersion in a gold-plating bath that contained 0.127 M Na$_2$SO$_3$, 0.0625 M formaldehyde, 0.025 M NaHCO$_3$, and 7.9×10^{-3} M of the gold-plating solution, to yield Au nanoparticles on the surfaces:

$$Au^{I}_{aq} + Ag^{0}_{surf} \rightarrow Au^{0}_{surf} + Ag^{I}_{aq} \qquad (2)$$

where:

Au$^{I}_{aq}$ is the oxidation number of gold in the gold plating solution;

Ag$^{0}_{surf}$ is the oxidation number of silver of membrane surface;

Au$^{0}_{surf}$ is the oxidation number of gold of membrane surface;

Ag^I_{aq} is the oxidation number of silver membrane surface after redox reaction.

The temperature of the bath was maintained at 5 °C throughout. The pH of the gold-plating solution was initially 12.0, but then adjusted to 10.0 by dropwise addition of 0.5 M H_2SO_4, with constant stirring, since it is known that lower pH values of the bath-solution results in gold-nanotubules with more uniform inside diameters [7]. On such gold-functionalized nanopores, L-cysteine chemisorption was accomplished by immersing the membrane into a 2 mM solution of L-cysteine in 80% ethanol for 24 h. The membrane was then rinsed with ethanol and dried in air. L-cysteine becomes chemisorbed to the inside pore walls as well as to the Au surface layers.

To limit the overall current through the nanopores-containing membranes (< 200 nA), we clamped it within a teflon film containing a 0.7 mm diameter hole and the wall of one chamber of experimental set-up (Fig. 1). This hole thus defines the area of membrane exposed to the solution containing 5 mM NaCl and phosphate buffer (10 mM sodium phosphate) buffered at pH values of 2.0, 7.0, or 12.0.
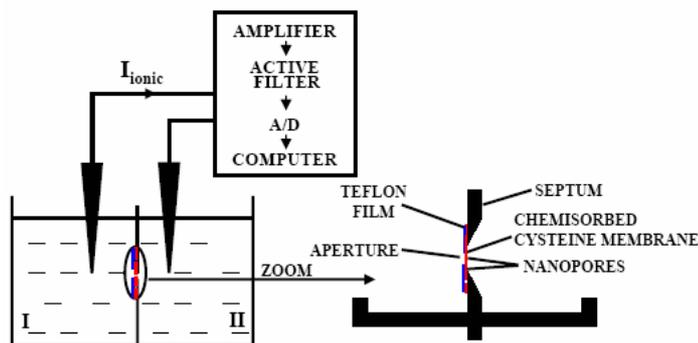

Fig. 1. Experimental set-up for electrical measurements: The two teflon chambers separated by the functionalized membranes contained sodium phosphate buffer (10 mM) with pH 2.0; 7.0; 12.0 and NaCl (5 mM).

The electrical current mediated by nanopores was measured with an integrating headstage Axopatch 200 B amplifier (Molecular Devices, USA) set on the voltage-clamp mode. Data acquisition of the amplified electrical signals was performed with a 16 bits resolution PCI 6014 A/D card (National Instruments) at a sampling frequency of 5 kHz. In order to estimate the square modulus of the membrane impedance we resorted to the use of spectral analysis carried out via the white-noise method. Briefly, a noisy voltage-signal with spectral features resembling those of a 'white noise' was generated at the analog output of a PCI 6014 A/D card (National Instruments) and applied across the nanopores-containing membrane; the resulting current signal was then collected, digitized and used to compute the ratio of the power spectrum of the voltage fluctuations applied at the

input of the membrane and the resulting power spectrum of the current fluctuations generated into it. From elementary electrical circuit analysis, this would correspond to the squared modulus of the electrical impedance of the biomembrane [6].

RESULTS AND DISCUSSION

As a result of L-cysteine chemisorption onto the gold layer deposited with the electroless method on the nanopores surface, the overall electrical charging state of the nanopores will depend upon the pH of the contacting aqueous solution (Fig. 2).
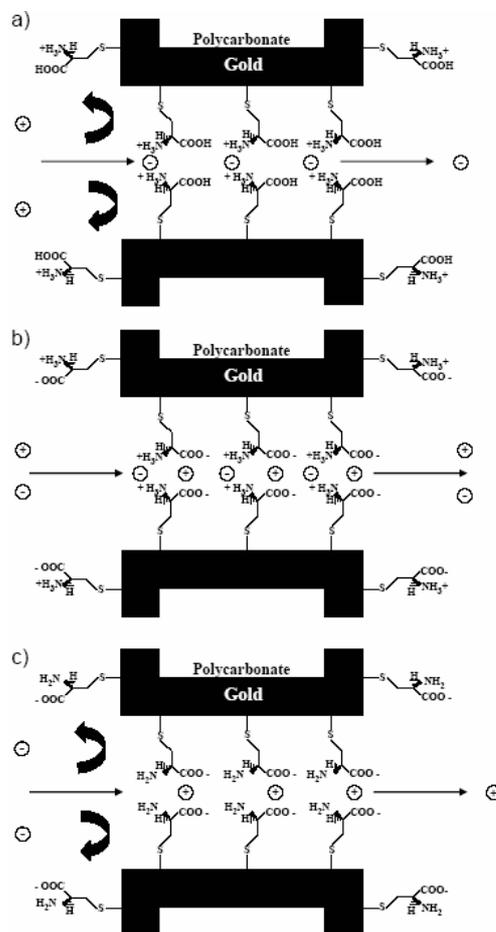
Fig. 2. Schematic representation showing the three states of protonation and the resulting ion-permselectivity of the chemisorbed cysteine. a) low pH, cation-rejecting/anion-transporting state, b) pH = 7.0, non-ion-permselective state, c) high pH, anion-rejecting/cation-transporting state.

We argued that depending on the charging state of the inner surface of the nanopores, the electrical profile experienced by diffusion ions will be greatly affected, and the transport properties of the nanopores will thus be prone to pH modulations.
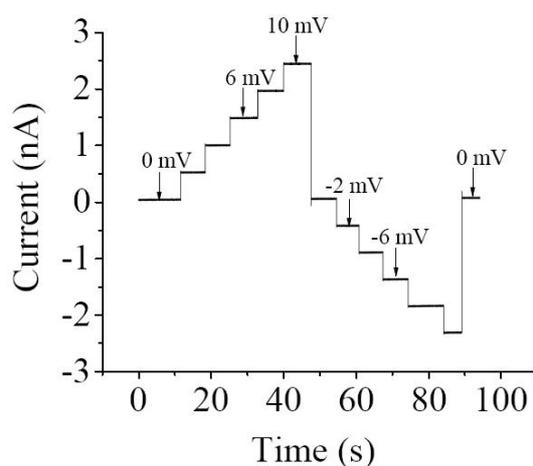

Fig. 3. The electrical current recorded at different potential values applied across the L-cysteine functionalized membrane, at pH 2.0.

In Fig. 3 we show a selected original trace that pinpoints the overall protocol used to infer the I-V behavior of L-cysteine-functionalized nanopores. By evaluating the electrical current mediated by the nanopores in response to various applied holding potentials, we were able to plot I-V diagrams that characterize ion transport through nanopores, as it takes place at various pH values.
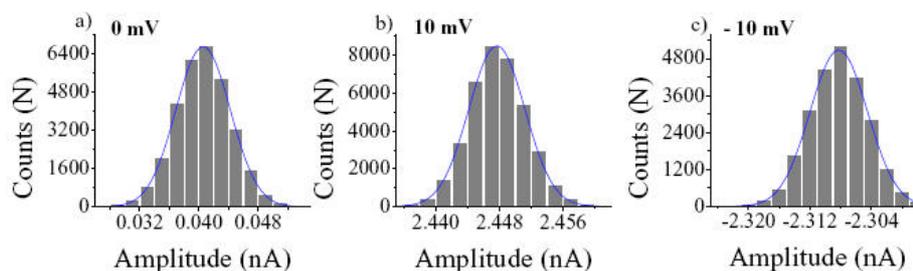

Fig. 4. Amplitude histograms of electrical currents generated across the functionalized membrane at: a) 0 mV, b) 10 mV, and c) –10 mV.

In Fig. 4 we show samples of amplitude histograms of electrical currents flowing through the L-cysteine-functionalized nanopores at 0 (panel a), 10 (panel b) and –10 mV (panel c), when the pH value of the buffer used was set to 2.0.

The result of such evaluations carried out over a wider range of holding potentials is being summarized in the next figure (Fig. 5).
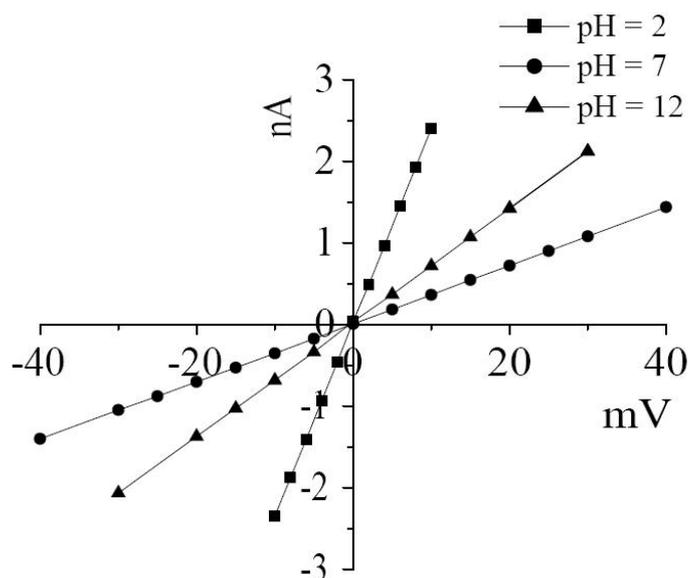

Fig. 5. Electrical current mediated by nanopores containing chemisorbed cysteine at pH-values of 2.0 (■), 7.0 (●) and 12.0 (▲), in response to various holding potentials applied across.

From the linear fit through the I-V pair-points, we inferred values of the electrical conductances of the nanopores functionalized via cysteine chemisorption at pH 2.0, 7.0 and 12.0, as follows: at pH 2.0, $\sigma = 2.39 \times 10^{-7}$ S $\pm 4.6 \times 10^{-10}$; at pH 7.0, $\sigma = 3.55 \times 10^{-8}$ S $\pm 6.4 \times 10^{-11}$; at pH 12.0, $\sigma = 6.99 \times 10^{-8}$ S $\pm 9.5 \times 10^{-11}$.

One plausible explanation for the observed phenomenon of high current values measured at a given holding potential, at pH 2.0, may lie in that due to the protonation of both carboxy- and amino groups of the chemisorbed cysteine, the overall electrical charge of nanopores is mostly positive; by taking into account that, due to a smaller hydrated radius, the permeability of Cl^- ions through such nanopores is greater by comparison to Na^+ ions, the net positive electrical charge of the inner walls of functionalized nanopores, at pH 2.0, does promote the electrodiffusion of Cl^- ions, as compared to less acidic pH values and therefore augment the overall ion current at more acidic pH values.

Interestingly, the spectral analysis carried out via the white-noise method with the purpose of impedance estimation of such functionalized membranes has

revealed that at high pH values (pH = 12.0), the square values of the modulus of the electrical impedance (S. M. I.) of a cysteine-functionalized membrane increases at increasing frequencies (Fig. 6, panel a); obviously, this constitutes a departure from predictions made for a parallel RC circuit, generally used to represent electrically membranes properties.
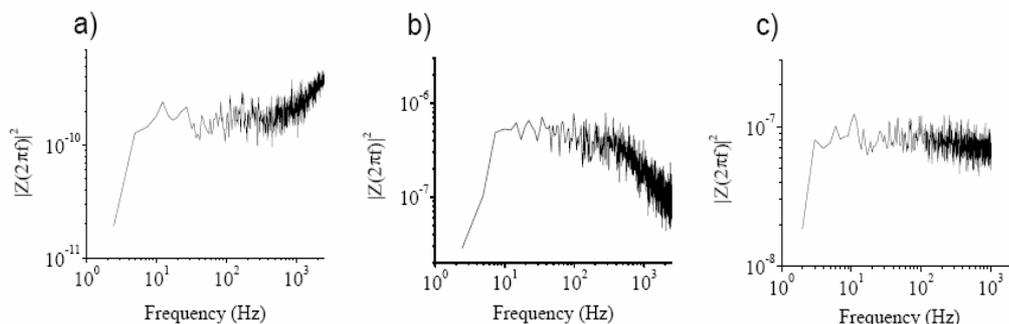

Fig. 6. Square values of the modulus of the electrical impedance of a cysteine-functionalized membrane, estimated under equilibrium conditions via the white-noise method at (a) pH 12.0, (b) pH 7.0, and (c) pH 2.0.

However, at neutral (pH = 7.0) and acidic (pH = 2.0) pH values such prediction are well fulfilled (Fig. 6, panels b) and c)), meaning that under such circumstances the electrical properties of a cysteine-functionalized membrane can be viewed as equivalent to a parallel RC circuit. A plausible explanation for such a discrepancy calls for more in-depth experiments, that are currently underway.

CONCLUSIONS

Herein, we have reported on the transport properties of a pH-switchable membrane that was prepared by chemisorbing cysteine to Au-plated nanopores of a polycarbonate filter. By measuring the electrical current flow through these membranes and analyzing the current – potential difference diagrams, we showed that these membranes display variable conductivities, depending on the pH value of the contacting solution. Interestingly, the spectral behavior of such functionalized membranes quantified by their electrical impedance shows a strong dependence upon the pH value of the contacting solution phase, departing visibly from the theoretical predictions of a simple parallel-RC model at high pH values. Such experimental approaches may become useful for exploring *de-novo* design of artificial nanostructures with kinetic and transport properties resembling those of ion channels. By employing the known affinity of cysteine residue toward a gold substrate, it would prove interesting to ligate artificial Cys-functionalized peptides with known secondary structure and charge to such gold-plated nanopores with the ultimate purpose of endowing them with gating properties similar to those encountered in living cells.

R E F E R E N C E S

1. HOU, Z., N.L. ABBOTT, P. STROEVE, Self-assembled monolayers on electroless gold impart pH-responsive transport of ions in porous membranes, *Langmuir,* 2000, **16**, 2401–2404
2. JIRAGE, K.B., J.C. HULTEEN, C.R. MARTIN, Effect of thiol chemisorption on the transport properties of gold nanotubule membrane, *Anal. Chem.*, 1999,**71**, 4913–4918.
3. LEE, S.B., C.R. MARTIN, Controlling the transport properties of gold nanotubule membranes using chemisorbed thiols, *Chem. Mater.*, 2001, **13**, 3236–3244.
4. LEE, S.B., C.R. MARTIN, pH-switchable, ion-permselective gold nanotubule membrane based on chemisorbed cysteine, *Anal. Chem.*, 2001 **73**, 768 –775.
5. LEE, S.B., C.R. MARTIN, Electromodulated molecular transport in gold-nanotube membranes, *J. Am. Chem. Soc.*, 2002, **124**, 11850-11851.
6. LUCHIAN, T., An automated method for generating analogic signals that embody the Markov kinetics of model ionic channels, *Journal of Neuroscience Methods*, 2005, **147**, 8–14.
7. MARTIN, C.R., M. NISHIZAWA, K.B. JIRAGE, Controlling ion-transport selectivity in gold nanotubule membranes, *Adv. Mat.*, 2001, **13**, 1351–1362.
8. MENON, V.P., C.R. MARTIN, Fabrication and evaluation of nanoelectode ensembles, *Anal. Chem.*, 1995, **67**, 1920–1928.
9. NISHIZAWA, M., V.P. MENON, C.R. MARTIN, Metal nanotubule membranes with electrochemically switchable ion-transport selectivity, *Science, New Series*, 1995, **268**, 700–702.
10. WIRTZ, M., C.R. MARTIN, Template-fabricated gold nanowires and nanotubes, *Adv. Mat.*, 2003, **15**, 455-458.
11. YU, S., S.B. LEE, C.R. MARTIN, Electrophoretic protein transport in gold nanotube membranes, *Anal. Chem.*, 2003, **75**, 1239–1244.