COMMUNICATION

ChemComm
www.rsc.org/chemcomm

Recognition of concanavalin A at the interface between a solvent polymeric membrane and an aqueous sample monitored by electric impedance spectroscopy

Liya Muslinkina and Ernö Pretsch*

Laboratorium für Organische Chemie, ETH Hönggerberg, HCI E 313, CH-8093 Zürich, Switzerland. E-mail: pretsch@org.chem.ethz.ch

Received (in Cambridge, UK) 9th February 2004, Accepted 29th March 2004
First published as an Advance Article on the web 23rd April 2004

In a novel biosensing approach, a stearyl-β-D-glucopyranoside layer is formed by self-organization at the interface between a solvent polymeric membrane and the aqueous sample phase and its interaction with concanavalin A is detected by electric impedance spectroscopy.

There is a growing interest in molecular recognition at the interface of two immiscible liquids.[1] Various methods have been used to detect host–guest interactions at liquid/liquid interfaces including polarography,[1a–d] interfacial tension measurements,[1e] second harmonic generation,[1f] and total internal reflection fluorescence spectroscopy.[1g] Electric impedance (EI) spectroscopy has been widely used for probing biomolecular interfacial recognition on solid surfaces[2] and investigating solvent polymeric membranes[3] but, so far, it has not been applied to monitor specific binding at liquid/liquid interfaces. Recently, it was shown by EI spectroscopy[4,5] and potentiometric measurements[5] that lipophilic nonionic surfactants added to the membrane matrix may form a compact layer by self-organization on the surface of solvent polymeric membranes of ion-selective electrodes in contact with aqueous solutions. Here, we show that the nonionic surfactant, stearyl-β-D-glucopyranoside (*S*-β-D-GP), forms such a layer and the respective EI spectra show distinct changes if the glucose-specific lectin concanavalin A (Con A) is added to the sample solution. The native protein Con A consists of 237 amino acid residues with bound Ca^{2+} and Mn^{2+} ions that provide the necessary activity of the carbohydrate binding sites.[6]

The surfactant *S*-β-D-GP[7] has been selected since it has a similar estimated[8] lipophilicity (log P = 5.9) to sorbitan monostearate, which was shown to form a resistive layer on the surface of ion-selective membranes.[4,5] It was added (1 mmol kg^{-1}) to the cocktail of Ca^{2+}-selective membranes otherwise having the usual composition.[9] In the Nyquist plot representation, the corresponding EI spectrum of the membrane shows a second semicircle due to charge transfer resistance and double-layer capacitance at the membrane/electrolyte interfaces, which does not occur in the absence of the surfactant (Fig. 1, top).[10] The potentiometric response to Ca^{2+} of the ion-selective electrodes[11] with an internal solution of very low Ca^{2+} activities shows a typical apparently super-Nernstian step due to zero-current transmembrane ion fluxes.[12] Upon addition of *S*-β-D-GP to the membrane, the position of this step is shifted by about one logarithmic unit to lower activities due to ion transfer resistance (Fig. 1, bottom).[5]

When membranes doped with *S*-β-D-GP are brought into contact with Con A,[13] their EI spectra show significant changes (Fig. 2, left).[10] The flattening of the low frequency semicircle in the Nyquist plot (top) and the concomitant decrease in the corresponding phase angle, θ, of the Bode phase plot (bottom) indicate an increase in the surface capacitance. No such changes are detected if the membranes do not contain *S*-β-D-GP (Fig. 2, right) showing that they are due to specific interactions and not to unspecific adsorption as was observed with bovine serum albumin.[14] The EI spectroscopy data were fitted to the equivalent circuit shown in the insert of Fig. 1 (top) to obtain the surface resistance and capacitance values (Table 1). No significant changes occurred in the bulk resistance and capacitance (data not shown). In all cases, incubation of the membranes in Con A solutions induced a decrease in the surface capacitance, which was more pronounced with higher concentrations of Con A but did not change if the reaction time was longer than 10 min (see Table 1). The observed decrease in capacitance is in accordance with the attachment of Con A to the membrane surface and the concomitant increase in the thickness of the surface layer.[2] Interestingly, at the same time, the surface resistance also decreases. This might be due to facilitated ion transfer in the

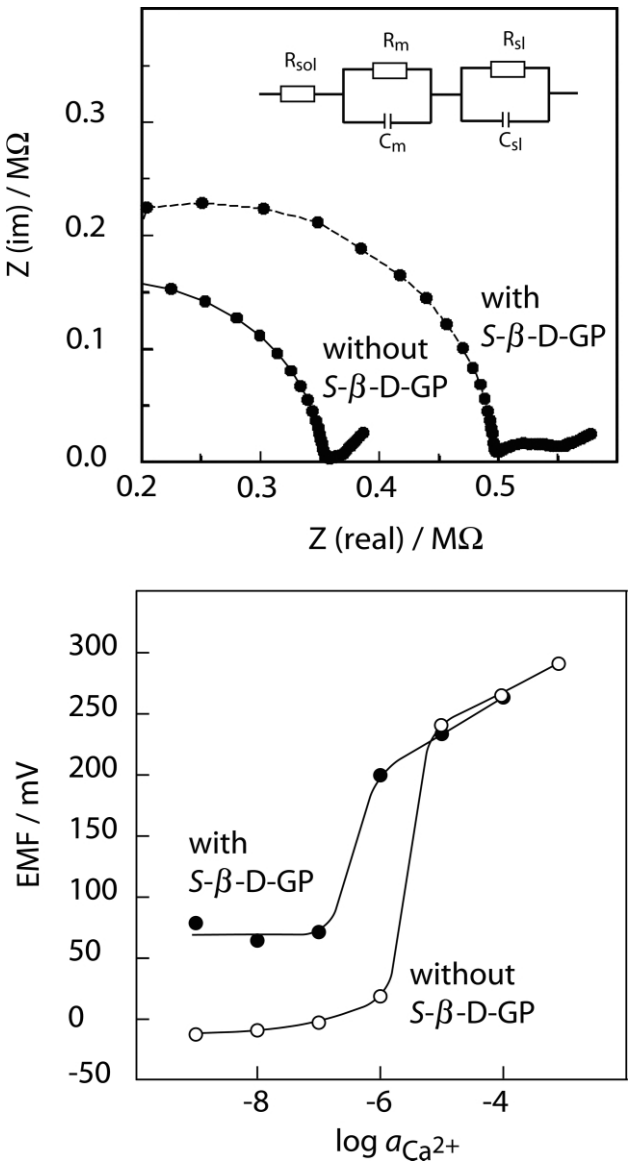

Fig. 1 Top: Low frequency part of the EI spectrum[10] of a Ca^{2+}-membrane without and with *S*-β-D-GP. Insert: Equivalent circuit used for analyzing the data. Bottom: Potentiometric response of Ca^{2+}-ISEs without and with *S*-β-D-GP.[11] The internal solution was designed so as to induce a strong inward flux of Ca^{2+} thus causing an apparently super-Nernstian step.

DOI: 10.1039/b401864h

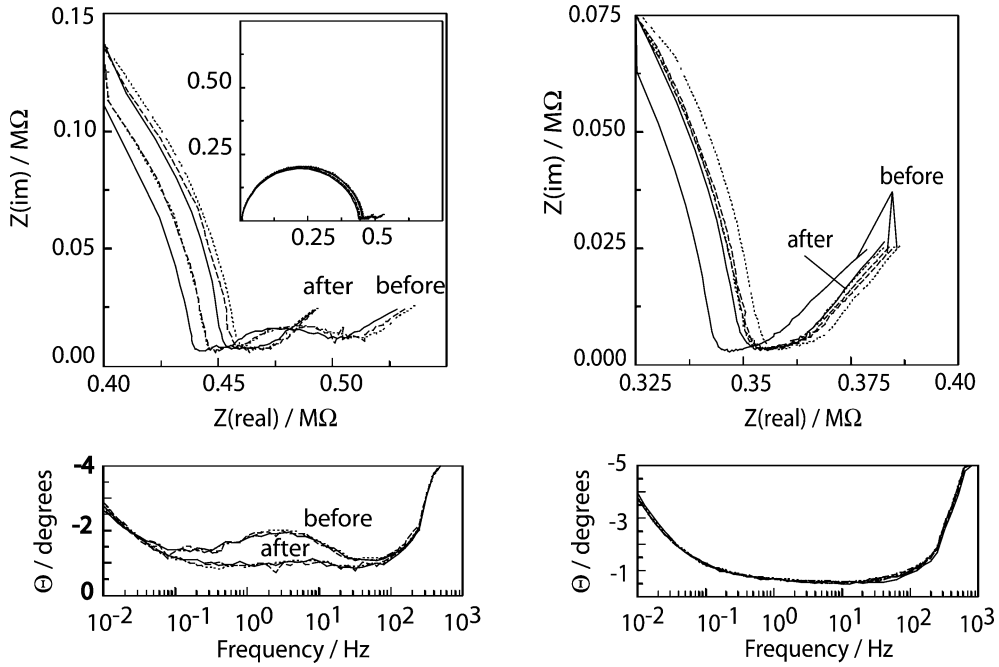

Fig. 2 EI spectra of Ca^{2+}-membranes[10] with (left) and without (right) incorporated *S*-β-D-GP before and after incubation with 1 mM Con A for 1 h.[13] Top: Nyquist plots, bottom: Bode phase plots.

Table 1 Surface layer (sl) capacitance (C) and resistance (R) obtained from the EI spectra for Ca^{2+}-selective membranes[9] doped with *S*-β-D-GP before and after contact with Con A for different concentrations and incubation times[a]

	C_{sl}, μF		R_{sl}, kOhm			
Con A (incubation time)	before	after	before	after	ΔC_{sl}, %	ΔR_{sl}, %
0.05 mM (1 h)	1.4 ± 0.7	1.6 ± 0.9	61.9 ± 18.3	37.1 ± 1.2	10.6	−40.1
0.10 mM (1 h)	1.4 ± 0.8	2.1 ± 2.4	61.2 ± 15.5	34.9 ± 9.0	46.9	−43.0
0.25 mM (1 h)	1.3 ± 0.7	2.4 ± 1.4	36.2 ± 3.0	29.8 ± 16.7	81.2	−17.7
0.10 mM (10 min)	1.4 ± 0.7	1.9 ± 1.1	59.4 ± 7.1	34.0 ± 7.7	33.1	−42.7
0.10 mM (overnight)	1.6 ± 0.3	2.5 ± 0.4	52.8 ± 3.3	32.7 ± 6.8	31.4	−38.1

[a] Error bounds: standard deviations of a total of 9 measurements (3 measurements with each of 3 membranes).

presence of Con A as a consequence of its interference with the ordered surface layer of *S*-β-D-GP or of the presence of cations bound to the protein.[6]

In summary, by EI spectroscopy, we have demonstrated the first example of molecular recognition on the surface of polymeric membranes between Con A and the nonionic surfactant *S*-β-D-GP, which forms a layer on the membrane surface.

This work was financially supported by the Swiss National Science Foundation and the National Institutes of Health (grant 8R01EB002189-04). Dr Alexander Prosvirkin is acknowledged for the synthesis of stearyl-β-D-glucopyranoside. We also thank Dr D. Wegmann for careful reading of the manuscript.

Notes and references

1 (*a*) T. Shioya, S. Nishizawa and N. Teramae, *J. Am. Chem. Soc.*, 1998, **120**, 11534; (*b*) S. Nishizawa, T. Yokobori, T. Shioya and N. Terame, *Chem. Lett.*, 2001, 1058; (*c*) S. Nishizawa, T. Yokobori, R. Kato, T. Shioya and N. Teramae, *Bull. Chem. Soc. Jpn.*, 2001, **74**, 2343; (*d*) S. Nishizawa, T. Yokobori, R. Kato, K. Yoshimoto, T. Kamaishi and N. Teramae, *Analyst*, 2003, **128**, 663; (*e*) K. Shigemori, S. Nishizawa, T. Yokobori, T. Shioya and N. Teramae, *New J. Chem.*, 2002, **26**, 1102; (*f*) K. Nochi, A. Yamaguchi, T. Hayashita, T. Uchida and N. Terame, *J. Phys. Chem. B*, 2002, **106**, 9906; (*g*) S. Ishizaka, S. Kinoshita, Y. Nishijima and N. Kitamura, *Anal. Chem.*, 2003, **75**, 6035.

2 E. Katz and I. Willner, *Electroanalysis*, 2003, **15**, 913.

3 R. P. Buck, *Ion-Sel. Electrode Rev.*, 1982, **4**, 3.

4 Q. Ye, A. Vincze, G. Horvai and F. A. M. Leermakers, *Electrochim. Acta*, 1998, **44**, 125.

5 L. Muslinkina and E. Pretsch, *Electroanalysis*, in press.

6 G. N. Reeke and J. W. Becker, *Curr. Topics Microbiol. Immunol.*, 1998, **139**, 35.

7 V. Vill, H. M. V. Mineden, M. H. J. Koch, U. Seydel and K. Brandenburg, *Chem. Phys. Lipids*, 2000, **104**, 75.

8 TlogP, Upstream Solutions GmbH, Scientific Software Engineering, Imfeldstr. 57, 8037 Zürich, Switzerland.

9 Composition of the ISE membranes: *N*,*N*-dicyclohexyl-*N*′,*N*′-dioctadecyl-3-oxapentanediamide (ETH 5234, 10 mmol kg^{-1}), sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (5 mmol kg^{-1}), bis(2-ethylhexyl) sebacate (66 wt%), high molecular weight poly(vinyl chloride) (33 wt%), *S*-β-D-GP (0 or 1 mmol kg^{-1}). Membranes of *ca.* 150 μm thickness were obtained by casting *ca.* 590 mg of components dissolved in THF into a glass ring of 59 mm diameter fixed on a glass plate and letting the solvent evaporate overnight.

10 EI spectra were recorded with the frequency response analyzer 1255B FRA combined with the electrochemical interface SI 1286 (Solartron Mobrey, Ltd., Berkshire, England) and ZPlot and ZView software packages using an excitation signal of 5 mV amplitude in the frequency range of 1 MHz–0.01 Hz in the following solution on either side of the membrane: 10^{-3} M $CaCl_2$ with 10^{-3} M $MnCl_2$ in 10^{-2} M TRIS·HCl at pH 7.2. Measurements were performed in the 2-electrodes mode in a cell made in the laboratory from Plexiglass.

11 Potentiometric responses were taken in 10^{-2} M TRIS·HCl at pH 7.2 as background.

12 T. Sokalski, A. Ceresa, M. Fibbioli, T. Zwickl, E. Bakker and E. Pretsch, *Anal. Chem.*, 1999, **71**, 1210.

13 Incubation of membranes in aqueous Con A (Type IV, Sigma, Steinheim, Germany) solution (3 mL each) was performed at room temperature (20–22 °C), the Con A powder being dissolved in 10^{-3} M $CaCl_2$ with 10^{-3} M $MnCl_2$ and 10^{-2} M TRIS·HCl, at pH 7.2. After incubation, the membranes were carefully rinsed with 10^{-2} M TRIS·HCl of pH 7.2.

14 Q. Ye, Z. Keresztes and G. Horvai, *Electroanalysis*, 1999, **11**, 729.