Langmuir

Article

Enhanced adsorption of Ca-ATPase containing vesicles on a negatively charged solid supported membrane for the investigation of membrane transporters

Alessio Sacconi, Maria Rosa Moncelli, Giancarlo Margheri, and Francesco Tadini-Buoninsegni

Langmuir, **Just Accepted Manuscript** • Publication Date (Web): 16 Oct 2013

Enhanced adsorption of Ca-ATPase containing vesicles on a negatively charged solid supported membrane for the investigation of membrane transporters

Alessio Sacconi[1], *Maria Rosa Moncelli*[1], *Giancarlo Margheri*[2], *Francesco Tadini-Buoninsegni*[1]*

[1]Department of Chemistry "Ugo Schiff", University of Florence, via della Lastruccia 3, 50019 Sesto Fiorentino, Italy.

[2]Institute for Complex Systems, National Research Council, Via Madonna del Piano 10, 50019 Sesto Fiorentino, Italy.

ABSTRACT

A convenient model system for a biological membrane is a solid supported membrane (SSM), which consists of a gold-supported alkanethiol|phospholipid bilayer. In combination with a concentration jump method, SSMs have been used for the investigation of several membrane transporters. Vesicles incorporating sarcoplasmic reticulum Ca-ATPase (SERCA) were adsorbed on a negatively charged SSM (octadecanethiol|phosphatidylserine bilayer). The current signal generated by the adsorbed vesicles following an ATP concentration jump was compared to that produced by SERCA containing vesicles adsorbed on a conventional SSM (octadecanethiol|phosphatidylcholine bilayer). A significantly higher current amplitude was recorded on the serine-based SSM. The adsorption of SERCA incorporating vesicles on the SSM was then characterized by surface plasmon resonance (SPR). The SPR measurements clearly indicate that in the presence of Ca^{2+} and Mg^{2+} the amount of adsorbed vesicles on the serine-based SSM is about twice that obtained using the conventional SSM, thereby demonstrating that the higher current amplitude recorded on the negatively charged SSM is correlated with a greater quantity of adsorbed vesicles. The enhanced adsorption of membrane vesicles on the PS-based SSM may be useful to study membrane preparations with a low concentration of transport protein generating small current signals, as in the case of various recombinantly expressed proteins.

INTRODUCTION

A convenient model system for a biological membrane is a solid supported membrane (SSM), which has been employed for the functional characterization of several membrane transport proteins (1,2). The SSM consists of a hybrid alkanethiol|phospholipid bilayer supported by a gold electrode. In particular, the SSM is formed by an octadecanethiol (Oct) monolayer covalently bound to the gold surface via the sulphydryl group and a second phosphatidylcholine monolayer on top of the

thiol layer. In combination with a concentration jump method (3), SSMs have successfully been used to study electrogenic transport by ion pumps (3-6), secondary active transporters (7,8) and ion channels (9-12).

In the concentration jump method, membrane preparations (membrane fragments or vesicles) containing the protein of interest are adsorbed on a SSM, which is usually formed by a diphytanoyl phosphatidylcholine (PC) monolayer on top of the gold-supported Oct film (1-3). However, it has not been demonstrated whether this is definitely the SSM sensor that adsorbs the higher amount of membrane vesicles. Aiming to fabricate a SSM with higher affinity to vesicles with incorporated membrane transporters, a possible strategy would be that of using a lipid surface of different composition and charge, i.e. different lipid head groups. It is known that negatively charged lipid headgroups can strongly interact with bivalent cations (13-17), e.g. Ca^{2+} and Mg^{2+}, which were reported to promote the adsorption process of small unilamellar phospholipid vesicles on a gold-supported organophosphate monolayer (18). However, the adsorption of protein containing membrane vesicles on a negatively charged SSM has not yet been investigated. For this purpose, in this work we characterized a new SSM based on diphytanoyl phosphatidylserine (PS), which has the same hydrophobic moiety as that of PC, that is the model molecule to be compared, but a final negatively charged serine headgroup (19). By employing the concentration jump method, we compared the current signal produced by vesicles containing sarcoplasmic reticulum Ca-ATPase (SERCA) adsorbed on our new SSM (Oct|PS bilayer) with that obtained with SERCA incorporating vesicles adsorbed on the conventional SSM (Oct|PC bilayer). A SERCA-generated current transient with much higher amplitude was recorded on the PS-based SSM. To explain this result, the adsorption of SERCA containing vesicles on the SSM was investigated by surface plasmon resonance (SPR) measurements in the presence and absence of Ca^{2+} and Mg^{2+} ions. We found that Ca^{2+} and Mg^{2+} have a fundamental role in the adsorption of SERCA containing vesicles on the SSM

surface. We demonstrate that the higher current peak measured on the PS-based SSM is strictly correlated with a greater amount of adsorbed vesicles.

MATERIALS and METHODS

The lipid solution contained 1,2-glycero diphytanoyl-sn-3-[phospho-L-choline] (PC) or 1,2-glycero diphytanoyl-sn-3-[phospho-L-serine] (PS) (Avanti Polar Lipids) and was prepared at a concentration of 7.5 mg/mL in n-decane or n-octane (Merck).

Native sarcoplasmic reticulum (SR) vesicles containing SERCA were prepared as reported by Eletr and Inesi (20). The total protein concentration, determined by the Lowry procedure (21), was 8.4 mg/ml. SERCA content corresponds to ~50% of the total protein (22).

COS-1 microsomes incorporating recombinant wild type (WT) SERCA were obtained as previously described (23). Total protein concentration was 2.6 mg/ml (78 μg/ml of SERCA corresponding to 3%). The content of expressed SERCA was evaluated by SDS gel electrophoresis and Western blots (23).

Concentration jump method

The SSM was formed as described (3) using a PC or PS solution (7.5mg/mL) in n-decane. In each experiment we added to the SSM the same amount of native SR vesicles or SERCA-incorporating COS-1 microsomes. The vesicles were then adsorbed on the SSM during an incubation time of 90 minutes. Following adsorption, SERCA was activated by an ATP concentration jump. If at least one electrogenic step, i.e. a net charge movement across the vesicular membrane generated by the protein, is involved in the relaxation process following protein activation, a current transient can be recorded due to the capacitive coupling between vesicle membrane and SSM (1,24). In particular, the current signal of the membrane transporter can be detected under potentiostatic conditions. In

this case, movement of a net charge across the activated protein is compensated by a flow of electrons along the external circuit toward the gold electrode surface to keep the applied voltage ΔV constant across the whole metal/solution interphase. The resulting current transient is recorded as a function of time. Normally, experiments are carried out under short circuit-conditions, i.e. at zero applied voltage relative to the reference electrode. The numerically integrated current transient is related to a net charge movement within the protein, which depends upon the particular electrogenic step.

In ATP concentration jumps the buffer solution contained 100 mM choline chloride, 25 mM MOPS, (pH 7.0), 1 mM $MgCl_2$, 0.25 mM EGTA, 0.25 mM $CaCl_2$ (10 μM free Ca^{2+}) and 0.2 mM DTT. Activation was obtained by rapid addition of 100 μM ATP.

The concentration jump experiments were performed by the $SURFE^2R^{One}$ device (Nanion Technologies). The temperature was maintained at 22-23 °C for all the experiments.

To verify the reproducibility of the current transients on the same SSM, each single measurement was repeated 6 times (two minute waiting time after each measurement) and then averaged to improve the signal to noise ratio. Standard deviations did not exceed 5%. Moreover, each set of measurements was usually reproduced using three different SSM sensors.

SPR and electrochemical measurements combined in situ

All electrochemical and SPR measurements were performed using the templated stripped gold (TSG) sensors purchased from Restec (Resonant Sensor Technology). The TSG sensor was incubated in a solution of 0.2 mM Oct in ethanol for 16 hours to allow Oct monolayer formation on the TSG surface by self assembly. The TSG sensor was then rinsed with ethanol to remove thiol molecules not covalently bound to the gold surface. The sensor was finally dried under a stream of N_2 and was oil-matched to the base of a 45° roof LaSFN9 glass prism (Hellma Optik; dielectric constant $n = 1.85$ at $\lambda = 633$ nm). By means of an O-ring tight seal, the thiol-coated TSG sensor was

mounted in a Teflon cell for in situ electrochemical and SPR analysis using the Kretschmann configuration. After cell assembling, a phospholipid layer was formed by spreading 40 μL of a phospholipid (PC or PS) solution (7.5 mg/mL) in n-octane on top of the gold-supported Oct monolayer. Following phospholipid addition, the cell was immediately filled with a buffer solution, whose composition, ionic strength and pH were the same as in the case of current transient measurements. Formation of the gold-supported alkanethiol|phospholipid bilayer (Au|Oct|PC or Au|Oct|PS) usually occurred during an incubation time of 16 hours. After SSM formation, the cell was placed on a rotating stage in a θ-2θ configuration. The monochromatic light beam from a He/Ne laser (JDS Uniphase; λ = 632.8 nm, beam diameter = 480 μm) was focused into the cell through the prism, and the reflected light was collected by a photodiode detector (25). The software Wasplas (A. Scheller, Max Planck Institute for Polymer Research, Mainz, Germany) was used for SPR measurements.

We characterized the SSM by AC Voltammetry and Electrochemical Impedance Spectroscopy (EIS). The electrochemical measurements were performed using a PGSTAT12 Autolab potentiostat/galvanostat (Metrohm), with an in-built frequency response analysis FRA2module. All potentials were referred to a three electrode system, with an Ag|AgCl|0.1 M KCl homemade reference electrode, a Pt electrode as a counter-electrode and the TSG as the working electrode. Capacitance and resistance of the SSM were determined by AC Voltammetry (potential range from 0.0 to + 0.10 V combined with a superimposed wave of 75 Hz frequency and 10 mV amplitude, and in a phase setting of 90° to obtain the capacitive signal) and EIS (bias potential 0.0 V, frequency range from 10^5 to 10^{-2} Hz with 10 mV amplitude). Following formation and characterization of the SSM, the buffer solution in the cell was exchanged with a suspension of native SR vesicles or COS-1 microsomes. In each adsorption experiment the amount of SR vesicles or COS-1 microsomes added to the cell was the same as indicated for the corresponding concentration jump experiment. After an incubation time of 90 minutes, the cell was rinsed three times, every 40 minutes, with 5 mL

of buffer solution to remove scarcely bound vesicles. This volume guarantees the complete exchange of buffer solution in the cell. The fixed angle of incidence (56.00°) was chosen at the lower flank of the decreasing reflectivity before the SPR peak. The percentage of the reflectivity (*%R*) registered at the fixed angle of incidence was reported as a function of time. The plasma angle shift ($\Delta\theta$) was given by the difference between the plasma angle value after the third rinse and the plasma angle value before vesicles injection.

RESULTS and DISCUSSION

Current transient measurements

The SERCA protein has been extensively investigated by the concentration jump method based on current transient measurements (1,4,23,24). This enzyme, which is a prominent and well characterized member of the P-type ATPase family, couples the hydrolysis of one molecule of ATP to the active transport of two Ca^{2+} ions from the cytoplasm to the SR lumen in muscle cells (22,26).

In this study, SR vesicles containing native SERCA (Fig. 1A) were adsorbed on a SSM, i.e. gold-supported Oct|PC or Oct|PS bilayer, and subjected to ATP activation. If we performed a 100 μM ATP jump in the presence of 10 μM free Ca^{2+} and 1 mM Mg^{2+}, we observed a current transient, whose related charge has been attributed to an electrogenic event corresponding to movement of bound Ca^{2+} within the enzyme following utilization of ATP (24). In control experiments on native SR vesicles adsorbed on the PS- and PC-based SSM we confirmed that the ATP-induced current transient measured in the presence of Ca^{2+} was fully suppressed by 100 nM thapsigargin (TG), a highly specific and potent SERCA inhibitor (27,28), as previously reported (24). It is interesting to note that in the case of native SR vesicles adsorbed on a Oct|PS bilayer (Fig. 1A, solid line) the amplitude of the current peak is significantly higher with respect to that measured on a Oct|PC bilayer (Fig. 1A, dotted line). By numerically integrating the current transient recorded on the

Oct|PS bilayer, we found a value for the related charge, which is approximately 2.7 times higher than that obtained on the Oct|PC bilayer (Fig. 1C). It should be pointed out that the current peak value is related to the number of adsorbed ATPase molecules that are activated after the ATP concentration jump, and the charge obtained by numerical integration corresponds to the overall amount of Ca^{2+} ions traslocated by the ion pumps following their activation.

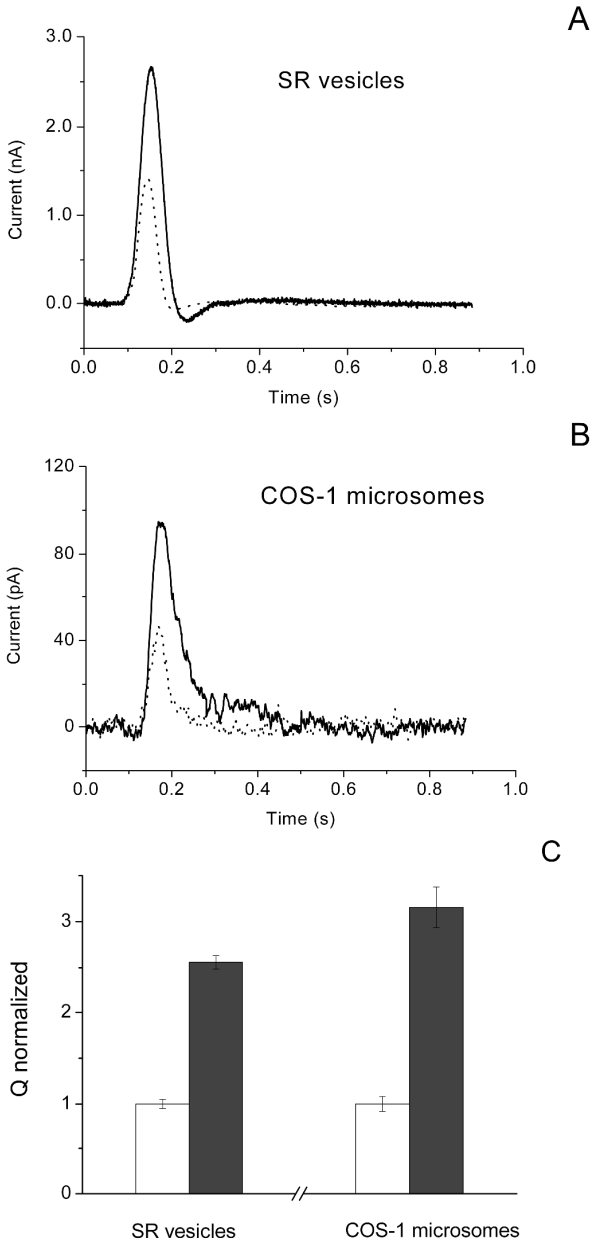


Figure 1. Currents transients induced by 100μM ATP concentration jumps in the presence of 10 μM free Ca^{2+} and 1 mM Mg^{2+} using native SR vesicles containing SERCA (A) or COS-1 microsomes incorporating recombinant WT SERCA (B) adsorbed on a gold-supported Oct|PC (dotted line) or Oct|PS (solid line) bilayer. (C) Normalized charges related to current transients by native SR vesicles containing SERCA or COS-1 microsomes incorporating recombinant WT SERCA adsorbed on a Oct|PC (white columns) or Oct|PS (dark grey columns) bilayer. The charges were normalized to the charge obtained on the Oct|PC bilayer. The error bars represent the s.e.m. of three independent measurements.

The concentration jump method has been employed to investigate not only native SERCA, but also the recombinant WT and mutant enzyme expressed in microsomes of COS-1 cells (23). The study of recombinant mutant SERCA provided useful information for a detailed characterization of the enzyme's transport cycle, especially as concerns Ca^{2+} binding mechanism, Ca^{2+}/H^{+} exchange and competitive Mg^{2+} binding (23). To examine the effects of a negatively charged SSM surface on the current signal generated by recombinant WT SERCA, COS-1 microsomes were adsorbed on a Oct|PS bilayer and activated by an ATP concentration jump. Similarly to native SERCA, we found that the current peak generated by recombinant WT SERCA on a Oct|PS bilayer (Fig. 1B, solid line) is characterized by a much higher amplitude with respect to that measured on the conventional SSM, i.e. Oct|PC bilayer (Fig. 1B, dotted line). Also in the case of recombinant SERCA the ATP-induced current transient recorded on the Oct|PS or Oct|PC bilayer was totally abolished by 100nM TG, thereby confirming again that the measured current transient is related to transport of Ca^{2+} ions by the SERCA enzyme. Moreover, the charge related to the current transient on the PS-based SSM was found to be about three times higher than that observed on the PC-based SSM (Fig. 1C). However, the absolute value of the current peak obtained with COS-1 microsomes is about one-order of magnitude lower as compared to that observed with native SR vesicles, since the protein

concentration in COS-1 microsomes (78 μg/ml) is much lower than that in SR vesicles (~ 4.2 mg/ml).

In conclusion, for both SR vesicles and COS-1 microsomes a much higher current peak was measured when the protein was adsorbed on an Oct|PS bilayer. This increase in the current signal may be explained by a greater quantity of adsorbed vesicles on the PS-based SSM.

In-situ SPR and electrochemical measurements

In order to verify the above statement, vesicles adsorption on the SSM was studied using SPR and electrochemical techniques (EIS and AC voltammetry) combined in situ. We investigated the adsorption of native SR vesicles or COS-1 microsomes on Au|Oct|PC or Au|Oct|PS sensors in the presence or absence of Ca^{2+} and Mg^{2+}. The concentration of Ca^{2+} and Mg^{2+}, as well as the ionic strength and pH of the buffer solution, were the same as in the case of the current transient measurements.

Before vesicles adsorption on the SSM, the Au|Oct, Au|Oct|PC and Au|Oct|PS systems were characterized by AC and EIS techniques to evaluate in situ the correct formation and stability of the alkanethiol monolayer and the alkanethiol|phospholipid bilayer. Both the monolayer and the bilayer can be described by an equivalent circuit which consists of a parallel RC mesh in series with a solution resistance (Fig. S1 in Supporting Information) (29,30). In particular, the capacitance and resistance values (Table 1) of the monolayer and bilayer can be obtained by fitting the equivalent circuit to the EIS data (Fig. S1 in Supporting Information).

	$R_{solution}^{(a)}$ (Ω*cm^2)	$R^{(a)}$ (MΩ*cm^2)	$C^{(a)}$ (μF/cm^2)	$C^{(b)}$ (μF/cm^2)
Au|Oct	50.0 ±1.0	1.50 ± 0.30	1.05 ± 0.10	1.20 ± 0.10
Au|Oct|PC	50.0 ± 1.0	7.00 ± 1.5	0.34 ± 0.05	0.37 ± 0.05
Au|Oct|PS	50.0 ± 2.0	23.5 ± 8.0	0.32 ± 0.04	0.32 ± 0.20

Table 1. Resistance and capacitance values of gold-supported Oct monolayer and Oct|PC or Oct|PS bilayer were obtained by EIS spectroscopy (a) and AC voltammetry (b). The solution resistance was determined by EIS. The EIS error gives the deviation from the fit and the error in the AC voltammetry data represents the s.e.m. of four independent measurements.

The capacitance of the monolayer and bilayer was also evaluated by AC voltammetry (Fig. S2 in Supporting Information), yielding values very similar to those determined by EIS (Table 1). From our AC voltammetry data, that were obtained in a phase setting of 90°, the resistance value cannot be determined. The slightly higher resistance of Au|Oct|PS with respect to that of Au|Oct|PC is probably due to the higher compactness of the PS-based bilayer, which is presumably related to the presence of Ca^{2+} and Mg^{2+} ions interposed between the negatively charged serine headgroups. The capacitance and resistance values in Table 1 are in good agreement with those reported in the literature (31-36), thus confirming the proper formation and integrity of the thiol monolayer and the hybrid thiol|phospholipid bilayer.

After monitoring the correct formation of the SSM on the sensor surface, we performed SPR measurements to characterize the adsorption of SERCA containing vesicles on the alkanethiol|phospholipid bilayer. Figures 2A and 2B show the SPR kinetic curves of the reflectivity signal versus time for native SR vesicles adsorption on Au|Oct|PC or Au|Oct|PS, respectively. For both sensors, we compared the adsorption process in the presence of 10 μM Ca^{2+} and 1 mM Mg^{2+} (solid lines), and in absence of both ions (dotted lines). When the SSM was completely formed and stabilized, the incidence angle was set at 56.00° obtaining the corresponding reflectivity value of ~30%, that represents the baseline of the reflectivity signal before SR vesicles addition.

After SR vesicles injection in the cell, the reflectivity signal progressively increased during the incubation time and finally attained a maximum constant value. Such an increase in reflectivity clearly indicates the occurrence of SR vesicles adsorption on the SSM surface. It is interesting to observe that in the presence of Ca^{2+} and Mg^{2+} ions a greater quantity of SR vesicles was adsorbed on the PS-based SSM (Fig. 2B, solid line) with respect to the PC-based SSM (Fig. 2A, solid line). In the absence of Ca^{2+} and Mg^{2+} ions, very few SR vesicles, if any, were adsorbed on the PC or PS-based SSM (Figs. 2A and 2B, dotted lines).

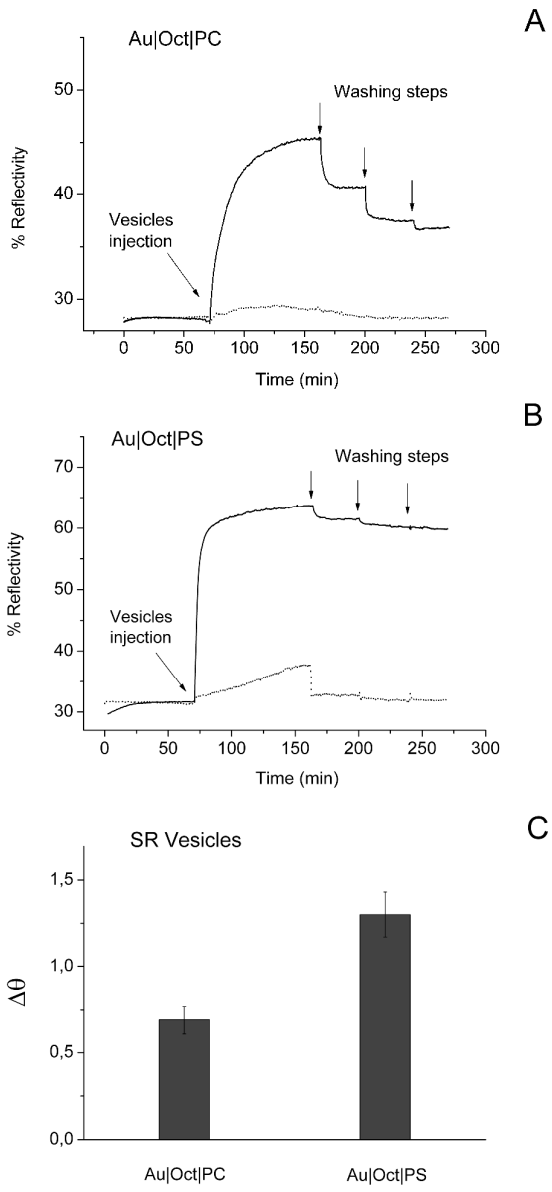


Figure 2. Reflectivity versus time kinetic curves for the adsorption process of native SR vesicles on Au|Oct|PC (A) or Au|Oct|PS (B) sensor in the presence (solid line) and in the absence (dotted line) of Ca^{2+} and Mg^{2+}. (C) The histogram shows the plasma angle shift ($\Delta\theta$) values for SR vesicles adsorption on Au|Oct|PC or Au|Oct|PS sensor in the presence of Ca^{2+} and Mg^{2+}. The vertical bars represent the reproducibility errors of three independent measurements.

Interestingly, we found that the adsorption process in the presence of PC (Fig. 2A, solid line) occurs in longer times than those occurring in the presence of PS (Fig. 2B, solid line). For example, in the presence of both Ca^{2+} and Mg^{2+} the risetime of the reflectivity signal is ~ 890 s using PC on the sensor surface, while it reduces to ~ 260 s in the case of PS, confirming the tendency of SR vesicles to bind preferentially to the PS-based SSM rather than the PC-based SSM and, therefore, indicating a higher affinity of the SR vesicles for the negatively charged SSM, as expected.

As the cell was further rinsed with the buffer solution, we recorded a decrease of the reflectivity signal which can be explained by partial removal of the SR vesicles from the SSM surface. In the presence of Ca^{2+} and Mg^{2+} ions (solid lines), such a decrease in reflectivity is less evident when the SSM is formed with PS (Fig. 2B) rather than PC (Fig. 2A). Moreover, the reflectivity decrease after the rinsing steps is systematically lower for PS than for PC. This is a further indication of the higher affinity of the SR vesicles for the PS-based SSM.

We can compare the amount of adsorbed SR vesicles on the PC and PS-based SSM by determining the values of the plasma angle shift $\Delta\theta$, as this quantity is directly proportional to the adsorbed mass (37). The histogram (Fig 2C) shows that the amount of native SR vesicles adsorbed on the Au|Oct|PS sensor surface is approximately twice that obtained on the Au|Oct|PC sensor, in the presence of Ca^{2+} and Mg^{2+} ions. This result is consistent with the current signal increase observed with SR vesicles adsorbed on the PS-based SSM (Fig. 1A). It is worth noticing that in the absence of both ions the $\Delta\theta$ values are close to zero.

The experimental procedure described above was then repeated using COS-1 microsomes. Figures 3A and 3B show the reflectivity versus time kinetic curves for COS-1 microsomes adsorption on Au|Oct|PC or Au|Oct|PS, respectively. Also in the case of COS-1 microsomes, we observed that in the presence of Ca^{2+} and Mg^{2+} a greater quantity of vesicles can be adsorbed on the PS-based SSM (Fig. 3B) with respect to the PC-based SSM (Fig. 3A). In the absence of bivalent cations, a very low amount of vesicles is adsorbed on the SSM and the adsorbed vesicles can be

completely removed from the SSM surface following buffer rinsing (Figs. 3A and 3B, dotted lines). We note that the risetimes of the reflectivity signals don't exhibit a marked difference as that observed for native SR vesicles (Figs. 2A and 2B). Among the various reasons that reduce this effect, we suggest that the different total protein concentration in the SR vesicles and COS-1 microsomes may play a role, as the association rates are concentration-dependent.

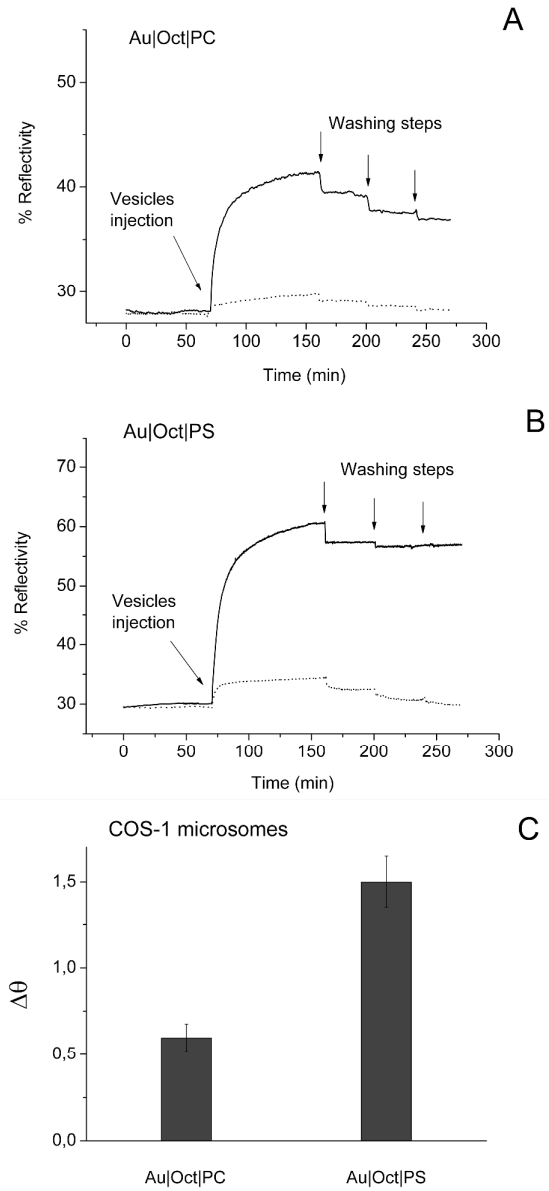


Figure 3. Reflectivity versus time kinetic curves for the adsorption process of COS-1 microsomes on Au|Oct|PC (A) or Au|Oct|PS (B) sensor in the presence (solid line) and in the absence (dotted

line) of Ca^{2+} and Mg^{2+}. (C) The histogram shows the plasma angle shift ($\Delta\theta$) values for COS-1 microsomes adsorption on Au|Oct|PC or Au|Oct|PS sensor in the presence of Ca^{2+} and Mg^{2+}. The vertical bars represent the reproducibility errors of three independent measurements.

In the case of COS-1 microsomes, the $\Delta\theta$ value due to microsomes adsorption by PS is approximately 2.5 times higher that that obtained with PC (Fig. 3C), also in this case consistent with the current signal increase detected on the PS-based SSM (Fig. 1B).

From our SPR measurements it is therefore evident the strong influence of Ca^{2+} and Mg^{2+} in the adsorption process of membrane vesicles on the SSM surface. The higher current peak recorded on the PS-based SSM can be correlated with a greater amount of adsorbed vesicles on the Au|Oct|PS sensor in the presence of Ca^{2+} and Mg^{2+}. On the other hand, without Ca^{2+} and Mg^{2+}, the adsorption of membrane vesicles on the SSM is drastically reduced and all adsorbed vesicles can be easily removed from the SSM surface.

CONCLUSIONS

The current signal generated by SERCA containing vesicles adsorbed on a negatively charged SSM (Oct|PS bilayer) following an ATP concentration jump exhibits a much higher amplitude with respect to that produced by SR vesicles adsorbed on a conventional SSM (Oct|PC bilayer). The SPR measurements clearly indicate that in the presence of Ca^{2+} and Mg^{2+} the amount of adsorbed vesicles on our new PS-based SSM is about twice that obtained using the conventional PC-based SSM, thereby demonstrating that the higher current amplitude recorded on the negatively charged SSM is correlated with a greater quantity of adsorbed vesicles.

It is known that Ca^{2+} and Mg^{2+} ions can strongly interact with negatively charged phospholipid headgroups, as previously reported (13-18). The accumulation of cations at the negatively charged

SSM surface may contribute to a more stable vesicles-SSM interaction. We suggest that Ca^{2+} and Mg^{2+} coordinated to serine headgroups of the PS-based SSM may promote a further interaction with the external surface of the membrane vesicles. It is worth noticing that the external surface of the SR vesicle is the cytoplasmic surface of the SR. Since the cytoplasmic leaflet of membranes contains significant amount of phosphatidylserine, we propose that Ca^{2+} and Mg^{2+} ions may bind to both serine-rich membranes and connect them. The coordinating effect of Ca^{2+} and Mg^{2+} ions may be regarded as a type of "ionic glue", as proposed for Zr^{4+} cations (38). In this connection, it is useful to compare the coordination number (CN) of Zr^{4+} (CN = 7 and 8) with those of Mg^{2+} (CN = 5 and 6) and Ca^{2+} (CN = 6 and 7) (39). However, the studies on the interaction of Zr^{4+} ions with various phospholipid headgroups (40-42) indicate that Zr^{4+}, due to its high ionic charge and specific coordination properties, interacts with lipid headgroups more strongly and effectively than divalent cations, such as Mg^{2+} and Ca^{2+}. Therefore, the term "ionic super glue" (38) appears most appropriate for the coordinating behavior of Zr^{4+} ions.

The enhanced adsorption of membrane vesicles on the PS-based SSM may be particularly useful to study membrane preparations with a low concentration of transport protein generating very small amplitudes in current recordings, as demonstrated here for the recombinantly expressed Ca-ATPase.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Gianluca Bartolommei for very helpful suggestions and critical reading of the manuscript, and Dr. Giuseppe Inesi for providing them with recombinant SERCA. Financial support by Ente Cassa di Risparmio di Firenze (2009.0749) and the Italian Ministry of Education, University and Research (PRIN Project 20083YM37E) is gratefully acknowledged.

Supporting Information Available. Electrochemical characterization (Electrochemical Impedance Spectroscopy and AC Voltammetry) of the octadecanethiol monolayer and octadecanethiol|phosphatidylcholine or octadecanethiol|phosphatidylserine bilayer supported by gold. This information is available free of charge via the Internet at http://pubs.acs.org/.

Corresponding Author

*Francesco Tadini-Buoninsegni, Department of Chemistry "Ugo Schiff", University of Florence, via della Lastruccia 3, 50019 Sesto Fiorentino, Italy,

Phone: +39-055-4573239; Fax: +39-055-4573142; E-mail: francesco.tadini@unifi.it

REFERENCES

(1) Tadini-Buoninsegni, F.; Bartolommei, G.; Moncelli, M. R.; Fendler, K. Charge transfer in P-type ATPases investigated on planar membranes. *Arch. Biochem. Biophys*. **2008**, *476*, 75-86.

(2) Schulz, P.; Garcia-Celma, J. J.; Fendler, K. SSM-based electrophysiology. *Methods* **2008**, *46*, 97-103.

(3) Pintschovius, J.; Fendler, K. Charge translocation by the Na^+/K^+-ATPase investigated on solid supported membranes: rapid solution exchange with a new technique. *Biophys. J.* **1999**, *76*, 814-826.

(4) Lewis, D.; Pilankatta, R.; Inesi, G.; Bartolommei, G.; Moncelli, M. R.; Tadini-Buoninsegni, F. Distinctive features of catalytic and transport mechanisms in mammalian sarco-endoplasmic

reticulum Ca^{2+} ATPase (SERCA) and Cu^{+} (ATP7A/B) ATPases. *J. Biol. Chem.* **2012**, *287*, 32717-32727.

(5) Bartolommei, G.; Moncelli, M. R.; Rispoli, G.; Kelety, B.; Tadini-Buoninsegni, F. Electrogenic ion pumps investigated on a solid supported membrane: comparison of current and voltage measurements. *Langmuir* **2009**, *25*, 10925-10931.

(6) Zebrowska, A.; Krysiński, P. Incorporation of Na^{+},K^{+}-ATPase into the thiolipid biomimetic assemblies via the fusion of proteoliposomes. *Langmuir* **2004**, *20*, 11127-11133.

(7) Ganea, C.; Fendler, K. Bacterial transporters: charge translocation and mechanism. *Biochim. Biophys. Acta* **2009**, *1787*, 706-713.

(8) Khafizov, K.; Perez, C.; Koshy, C.; Quick, M.; Fendler, K.; Ziegler, C.; Forrest, L. R. Investigation of the sodium-binding sites in the sodium-coupled betaine transporter BetP. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, E3035-E3044.

(9) Schulz, P.; Dueck, B.; Mourot, A.; Hatahet, L.; Fendler, K. Measuring ion channels on solid supported membranes. *Biophys. J.* **2009**, *97*, 388-396.

(10) Balannik, V.; Obrdlik, P.; Inayat, S.; Steensen, C.; Wang, J.; Rausch, J. M.; DeGrado, W. F.; Kelety, B.; Pinto, L. H. Solid-supported membrane technology for the investigation of the influenza A virus M2 channel activity. *Pflugers Arch.* **2010**, *459*, 593-605.

(11) Blesneac, I.; Ravaud, S.; Machillot, P.; Zoonens, M.; Masscheylen, S.; Miroux, B.; Vivaudou, M.; Pebay-Peyroula, E. Assaying the proton transport and regulation of UCP1 using solid supported membranes. *Eur. Biophys. J.* **2012**, *41*, 675-679.

(12) Garcia-Celma, J.; Szydelko, A.; Dutzler, R. Functional characterization of a ClC transporter by solid-supported membrane electrophysiology. *J. Gen. Physiol.* **2013**, *141*, 479-491.

(13) Ekeroth, J.; Konradsson, P.; Björefors, F.; Lundström, I.; Liedberg, B. Monitoring the interfacial capacitance at self-assembled phosphate monolayers on gold electrodes upon interaction with calcium and magnesium. *Anal. Chem.* **2002**, *74*, 1979-1985.

(14) Rossetti, F. F.; Bally, M.; Michel, R.; Textor, M.; Reviakine, I. Interactions between titanium dioxide and phosphatidyl serine-containing liposomes: formation and patterning of supported phospholipid bilayers on the surface of a medically relevant material. *Langmuir* **2005**, *21*, 6443-6450.

(15) Sinn, C. G.; Antonietti, M.; Dimora, R. Binding of calcium to phosphatidylcholine–phosphatidylserine membranes. *Colloids Surf. A* **2006**, *282-283*, 410-419.

(16) Garcia-Celma, J. J.; Hatahet, L.; Kunz, W.; Fendler, K. Specific anion and cation binding to lipid membranes investigated on a solid supported membrane. *Langmuir* **2007**, *23*, 10074- 10080.

(17) Monson, C. F.; Cong, X.; Robison, A. D.; Pace, H. P.; Liu, C.; Poyton, M. F.; Cremer, P. S. Phosphatidylserine reversibly binds Cu^{2+} with extremely high affinity. *J. Am. Chem. Soc.* **2012**, *134*, 7773-7779.

(18) Ekeroth, J.; Konradsson, P.; Höök, F. Bivalent-ion-mediated vesicle adsorption and controlled supported phospholipid bilayer formation on molecular phosphate and sulfate layers on gold *Langmuir* **2002**, *18*, 7923-7929.

(19) Coronado, R. Effect of divalent cations on the assembly of neutral and charged phospholipid bilayers in patch-recording pipettes. *Biophys. J.* **1985**, *47*, 851-857.

(20) Eletr, S.; Inesi, G. Phospholipid orientation in sarcoplasmic membranes: spin-label ESR and proton MNR studies. *Biochim. Biophys. Acta* **1972**, *282*, 174-179.

(21) Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265-275.

(22) Inesi, G.; Toyoshima, C. In *Handbook of ATPases: Biochemistry, Cell Biology, Pathophysiology*; Futai, M., Wada, Y., Kaplan, J. H., Eds; Wiley-VCH: Weinheim, 2004; p 63.

(23) Liu, Y.; Pilankatta, R.; Lewis, D.; Inesi, G.; Tadini-Buoninsegni, F.; Bartolommei, G.; Moncelli, M. R. High-yield heterologous expression of wild type and mutant Ca^{2+} ATPase: Characterization of Ca^{2+} binding sites by charge transfer. *J. Mol. Biol.* **2009**, *391*, 858-871.

(24) Tadini-Buoninsegni, F.: Bartolommei, G.; Moncelli, M. R.; Tal, D. M.; Lewis, D.; Inesi, G. Effects of high-affinity inhibitors on partial reactions, charge movements, and conformational states of the Ca^{2+} transport ATPase (sarco-endoplasmic reticulum Ca^{2+} ATPase). *Mol. Pharmacol.* **2008**, *73*, 1134-1140.

(25) Guidotti, C.; Minunni, M.; Moncelli, M. R. Probing DNA hybridization in thiolipid monolayers by means of impedance spectroscopy. *Electrochem. Commun.* **2007**, *9*, 2380-2386.

(26) Møller, J. V.; Olesen, C.; Winther, A. M.; Nissen, P. The sarcoplasmic Ca^{2+}-ATPase: design of a perfect chemi-osmotic pump. *Q. Rev. Biophys.* **2010**, *43*, 501-566.

(27) Sagara, Y.; Inesi, G. Inhibition of the sarcoplasmic reticulum Ca^{2+} transport ATPase by thapsigargin at subnanomolar concentrations. *J. Biol. Chem.* **1991**, *266*, 13503-13506.

(28) Lytton, J.; Westlin, M.; Hanley, M. R. Thapsigargin inhibits the sarcoplasmic or endoplasmic reticulum Ca-ATPase family of calcium pumps. *J. Biol. Chem.* **1991**, *266*, 17067-17071.

(29) Steinem, C.; Janshoff, A.; Ulrich, W. P.; Sieber, M.; Galla, H. J. Impedance analysis of supported lipid bilayer membranes: a scrutiny of different preparation techniques. *Biochim. Biophys. Acta* **1996**, *1279*, 169-180.

(30) Diao, P.; Jiang, D.; Cui, X.; Gu, D.; Tong, R.; Zhong, B. Unmodified supported thiol/lipid bilayers: studies of structural disorder and conducting mechanism by cyclic voltammetry and AC impedance. *Bioelectrochem. Bioenerg.* **1999**, *48*, 469-475.

(31) Plant, A. L. Self-assembled phospholipid/alkanethiol biomimetic bilayers on gold. *Langmuir* **1993**, *9*, 2764-2767.

(32) Bilewicz, R.; Majda, M. Monomolecular Langmuir-Blodgett films at electrodes. Formation of passivating monolayers and incorporation of electroactive reagents. *Langmuir* **1991**, *7*, 2794-2802.

(33) Tadini Buoninsegni, F.; Herrero, R.; Moncelli, M. R. Alkanethiol monolayers and alkanethiol|phosholipid bilayers supported by mercury: an electrochemical characterization. *J. Electroanal. Chem.* **1998**, *452*, 33-42.

(34) Krysinski, P.; Moncelli, M. R.; Tadini-Buoninsegni, F. A voltammetric study of monolayers and bilayers self-assembled on metal electrodes. *Electrochim. Acta* **2000**, *45*, 1885-1892.

(35) Laredo, T.; Leitch, J.; Chen, M.; Burgess, I. J.; Dutcher, J. R.; Lipkowski, J. Measurement of the charge number per adsorbed molecule and packing densities of self-assembled long-chain monolayers of thiols. *Langmuir* **2007**, *23*, 6205-6211.

(36) Knoll, W.; Köper, I.; Naumann, R.; Sinner, E.-K. Tethered bimolecular lipid membranes—A novel model membrane platform. *Electrochim. Acta* **2008**, *53*, 6680–6689.

(37) Homola, J. Surface plasmon resonance sensors for detection of chemical and biological species. *Chem. Rev.* **2008**, *108*, 462-493.

(38) Brzozowska, M.; Oberts, B. P.; Blanchard, G. J.; Majewski, J.; Krysinski, P. Design and characterization of novel tether layer for coupling of a bilayer lipid membrane to the surface of gold. *Langmuir* **2009**, *25*, 9337-9345.

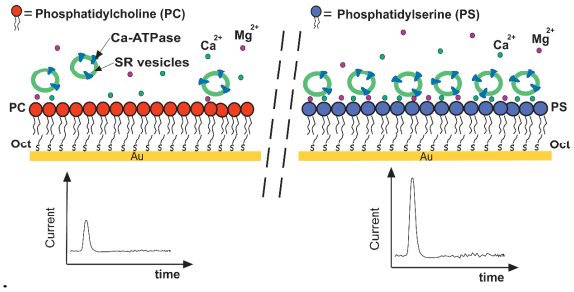
(39) Tunell, I.; Lim, C. Factors governing the metal coordination number in isolated group IA and IIA metal hydrates. *Inorg. Chem.* **2006**, *45*, 4811-4819.

(40) Oberts, B. P.; Blanchard, G. J. Formation of air-stable supported lipid monolayers and bilayers. *Langmuir* **2009**, *25*, 2962–2970.

(41) Oberts, B. P.; Blanchard, G. J. Ionic binding of phospholipids to interfaces: Dependence on metal ion identity. *Langmuir* **2009**, *25*, 13025-13033.

(42) Oberts, B. P.; Blanchard, G. J. Headgroup-dependent lipid self-asse,bly on zirconium phosphate-terminated interfaces. *Langmuir* **2009**, *25*, 13918-13925.

TOC Graphic


= Phosphatidylcholine (PC)
Ca-ATPase
SR vesicles
Ca2+
Mg2+
PC
Oct
Au
Current
time
= Phosphatidylserine (PS)
Ca2+
Mg2+
PS
Oct
Au
Current
time