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Electrokinetic Properties of the Sarcoplasmic Reticulum Membrane Obtained from Reconstitution Studies

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Abstract. Electrophoretic mobility data of SR vesicles reconstituted with uncharged and two mixtures of charged and uncharged lipids (Brethes, D., Dulon, D., Johannin, G., Arrio, B., Gulik-Krzywicki, T., Chevallier, J. 1986. Study of the electrokinetic properties of reconstituted sarcoplasmic reticulum vesicles. *Arch. Biochem. Biophys.* **246:**355–356) were analyzed in terms of four models of the membrane-water interface: (I) a smooth, negatively charged surface; (II) a negatively charged surface of lipid bilayer covered with an electrically neutral surface frictional layer; (III) an electrically neutral lipid bilayer covered with a neutral frictional layer containing a sheet of negative charge at some distance above the surface of the bilayer; (IV) an electrically neutral lipid bilayer covered with a homogeneously charged frictional layer. The electrophoretic mobility was predicted from the numerical integration of Poisson-Boltzmann and Navier-Stokes equations. Experimental results were consistent only with predictions based on Model-III with charged sheet about 4 nm above the bilayer and frictional layer about 10 nm thick. Assuming that the charge of the SR membrane is solely due to that on Ca^{++}-ATPase pumps, the dominant SR protein, the mobility data of SR and reconstituted SR vesicles are consistent with 12 electron charges/*ATPase*. This value compares well to the net charge of the cytoplasmic portion of *ATPase* estimated from the amino acid sequence (-11e). The position of the charged sheet suggests that the charge on the *ATPase* is concentrated in the middle of the cytoplasmic portion. The frictional layer of SR can be also assigned to the cytoplasmic portion of Ca^{++}-ATPase. The layer has been characterized with hydrodynamic shielding length of 1.1 nm. Its thickness is comparable to the height of the cytoplasmic portion of Ca^{++}-ATPase.

Key words: Sarcoplasmic reticulum — Electrophoretic mobility — Membranes — Reconstitution — Frictional layer — Ca^{++}-ATPase

Introduction

Sarcoplasmic reticulum (SR) is an intramuscular membrane system that plays a vital role in excitation-contraction coupling through its ability to regulate intracellular concentration of Ca^{++}. Pioneering studies of electrokinetic properties of SR and of SR reconstituted with phosphatidylcholine (PC) and mixtures of PC with phosphatidylserine (PS) were done by Arrio, Brethes, Johanin and coworkers (Arrio et al., 1984; Brethes et al., 1986). The latter work provided useful insight into the effect of electric charge of the bilayer on the function of Ca^{++} pump and, what is pertinent to this work, the electrophoretic mobility data for the batch of SR vesicles that were used for reconstitution of SR with added PC and two mixtures of PC + PS (75:25 and 50:50). Their analysis of mobility data suggest that the classical treatment of electrophoretic mobility of SR and reconstituted SR vesicles may not be applicable. Specifically, (i) the measured mobility of vesicles reconstituted with mixed lipids (PC + PS) was found to be several times smaller (Brethes et al., 1986) than that predicted from the classical electrophoretic mobility theory that works well with smooth particles such as liposomes (McLaughlin & Harary, 1976; McLaughlin et al., 1981; Smejtek et al., 1990; Smejtek & Wang, 1990; Tatulian, 1993, 1994, 1995). (ii) Assuming that the charge of the reconstituted SR membrane is determined by the charge of the Ca^{++} pump

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and the incorporated PS, the analysis of the measured electrophoretic mobility data using classical model yielded highly variable value for the charge of the Ca^{++} pump depending on the content and type of lipids used, which is not satisfactory. The charge/*ATPase* varied from 1.1e for the native SR to 16.5 e for the SR reconstituted with the mixture of PC + PS (50:50) (Brethes et al., 1986). There is also other evidence suggesting that the electrokinetic properties of SR surface are very different from those of lipid vesicles. We have studied sorption of positively and negatively charged lipophilic ions to SR vesicles and observed effects that cannot be understood in terms of the classical electrophoretic mobility model (*unpublished results*). In view of these failures and greater simplicity of the experimental system (absence of adsorbing ions) used in studies (Brethes et al., 1986), we have reanalyzed the experimental mobility data of reconstituted SR vesicles using the concepts developed for electrokinetic properties of red blood cells (Donath & Pastushenko, 1979; Levine et al., 1983; Sharp & Brooks, 1985; Ohshima & Kondo, 1989; Kawahata et al. 1990; Ohshima, 1994) and gangliosides containing liposomes (McDaniel et al., 1984; McDaniel et al., 1986; Pasquale et al., 1986).

The electrophoretic mobility method is very useful for study of properties of membrane surfaces since it provides information on the membrane surface in its ''natural'' state without removing membrane particles from their aqueous environment. Charged layer models of biomembrane surface proved to be very useful for understanding the electrophoretic mobility data of biomembrane particles. At present, the electrokinetic properties of human red blood cells are best reproduced with a three-layer model of the frictional layer (Nakano et al., 1994). A charged layer model was also used to study electrokinetic properties of other cells, such as rat B and T cells (Morita et al., 1991). Here we report results of analysis of electrophoretic mobility data of SR and reconstituted SR vesicles in terms of four models (*see* Fig. 1) in which the charge is either present at the surface of the bilayer, concentrated in a surface separated from the bilayer or evenly smeared within a layer on the top of the bilayer. Another feature explored is the presence of the frictional layer. Our analysis is based on numerical solution of the nonlinear Poisson-Boltzmann equation and thus avoiding restriction on the magnitude of the electrostatic potential $\psi < 25$ mV associated with the linearization. The electrophoretic mobility of vesicles is calculated from the Navier-Stokes equation which accounts for the spatial distribution of the driving and frictional force. The objective was to explore applicability of simple charged layer models to mobility of SR vesicles reconstituted with neutral and charged lipids, to find out whether it is possible to reproduce the measured mobility of native SR and SR vesicles reconstituted with neutral and charged lipids with set of parameters and to estimate the charge of *ATPase*.

Our results show that electrophoretic mobility of reconstituted SR vesicles are consistent with Model-III in which the charge is concentrated within a layer separated from the bilayer surface and embedded in a frictional layer at the bilayer surface. Using this model it was possible to reproduce the experimental mobility data of SR vesicles reconstituted with neutral and charged lipids with one set of parameters. The agreement was obtained for charge densities corresponding to 12 negative charges per *ATPase* which is close to the net negative charge of the cytoplasmic portion of the *ATPase*. In addition to obtaining a simple model for electrophoretic mobility of reconstituted SR vesicles this analysis also illustrates the usefulness of integrating electrophoretic mobility measurements into reconstitution studies and the importance to perform the reconstitution with both the neutral and charged lipids.

Models of Membrane Surface of SR Vesicles

Lipids represent about 45% of the mass of lyophilized SR and phospholipids about 90% of the lipid content. Phosphatidylcholine is the dominant phospholipid (50–70%), also present are phosphatidylethanolamine, sphingomyelin, phosphatidylserine and phosphatidylinositol. There are five proteins present: Ca^{++}-ATPase is the dominant protein component representing 70–80%, calsequestrin and the high-affinity Ca-binding protein account for approximately 20–30%, there are also small amounts of glycoprotein and proteolipid (de Meis, 1981).

Brethes et al. (1986) have indicated that the classical electrophoretic model of native and reconstituted SR based on charged smooth sphere concept is inconsistent with the mobility data obtained in their reconstitution studies. Since it is not *a priori* known how electric charges are distributed at the surface of SR vesicles and what is the effect of proteins protruding from the surface of the lipid bilayer on the particle hydrodynamics, the surface of SR and SR vesicles is modeled in four ways (*see* Fig. 1). In Model-I the charge resides at the surface of the lipid bilayer, the frictional surface layer is absent, so that the only frictional force acting on the vesicle is that due to the viscosity of the aqueous medium. This model has been successfully used to describe the mobility of charged liposomes. In Models II–IV the additional frictional force acting on the vesicle is due to the presence of a surface frictional layer. Model-II is an extension of Model-I to which an additional electrically neutral frictional layer was added. Model-III differs from Model-II by the insertion of a negatively charged sheet within the electrically neutral frictional layer. This charged sheet is separated from the bilayer surface by a distance *s*. In Model-IV the negative charge of SR mem-

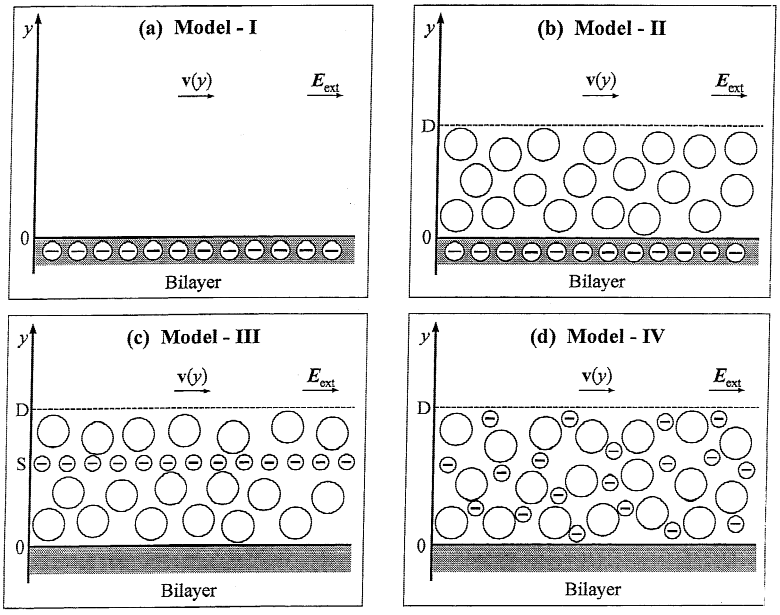


Fig. 1. Schematic diagrams of the models of surface of SR vesicles. (I) A smooth, negatively charged surface. (II) A negatively charged surface of lipid bilayer covered with an electrically neutral frictional layer of thickness D. (III) An electrically neutral lipid bilayer covered with a neutral frictional layer. The negative charge of the SR vesicle surface is concentrated within a thin sheet at a distance s above the surface of the bilayer. (IV) An electrically neutral lipid bilayer covered with a homogeneously charged frictional layer.

brane is assumed to be evenly distributed within the frictional surface layer.

For simplicity we ignore effects associated with the curvature of the vesicles and consider a laminar flow of water above the surface of the bilayer. Ionic strength of suspension used in the reconstitution studies was rather low, 4.7 mM (Brethes et al., 1986), so that discrete charge effects can be neglected. It is further assumed that the fixed charges are evenly spread within the charged surface (Models I, II and III) or within the volume of the frictional layer (Model-IV). The frictional layer is represented by a random array of spheres, a model introduced by Debye and Bueche (1948), and used widely in modeling the frictional layers of red blood cells and liposomes containing gangliosides. The frictional layer is assumed to be permeable to water and ions in the suspending solution whose volume density is determined by the Boltzmann factor $\exp(-z_iF\psi(y)/RT)$ where $\psi(y)$ is the electrostatic potential at distance y above the bilayer surface. A volume element of the suspending solution containing the space charge of mobile ions experiences an electric force originating from an external electric field parallel to the surface E_{ext}. In our models this force is equal to $\rho_{mob}\, E_{ext}$ and sets the suspending solution into motion relative to the surface of the bilayer. Its flow is opposed by two forces: the viscous force, $\eta \cdot (d^2v/dy^2)$, and the frictional force exerted by the frictional layer, γv where η is viscosity and γ the frictional coefficient of the surface layer. In steady state the net force acting on a volume element is equal to zero, which is expressed by the following form of the Navier-Stokes equation

$$\eta \frac{d^2v}{dy^2} - \gamma v + \rho_{mob}E_{ext} = 0 \qquad (1)$$

This equation describes the distribution of velocity of the suspending solution as a function of the distance from the surface of the bilayer. The boundary conditions are $v = 0$ at the surface of the bilayer ($y = 0$) and $dv/dy \to 0$ as $y \to \infty$. Outside the frictional layer, i.e., for $y > D$, we set $\gamma = 0$. By integrating Eq. 1 we obtain v_∞ which is the velocity of the aqueous solution relative to the flat surface of the bilayer. In reverse, the velocity of the flat surface relative to the bulk aqueous solution is $-v_\infty$. The mobility of the vesicle, μ, can be determined according to

$$\mu = -\frac{v_\infty}{E_{ext}} \qquad (2)$$

To obtain the mobility from Eq. 1 it is necessary to know the distribution of the density of space charge due to free ions, ρ_{mob}. This space charge varies with distance from the surface of the bilayer and depends on the distribution of electrostatic potential $\psi(y)$.

In order to avoid the limitation of ψ <25 mV, we obtain the electrostatic potential $\psi(y)$ from the nonlinearized Poisson-Boltzmann equation,

$$\frac{d^2\psi}{dy^2} = -\frac{1}{\varepsilon\varepsilon_0}(\rho_{fixed} + \rho_{mob}) \qquad (3)$$

where ε is the relative dielectric constant of water, ρ_{fixed} is the volume density of the charged frictional layer (Model IV). The density of space charge due to ions in the suspending solution is

$$\rho_{mob}(y) = F\sum_i 1000 z_i c_i \exp(-z_iF\psi(y)/RT) \qquad (4)$$

where c_i is the molar concentration of ions type ''*i*'' with valency z_i and F is the Faraday number. The boundary conditions for solving Eq. 3 are (i) that the electric field at the surface of the bilayer ($y = 0$) is determined by the charge density at the bilayer, σ_m, i.e. $-d\psi/dy = \sigma_m/\varepsilon\varepsilon_0$ and (ii) the electrostatic potential in the bulk aqueous solution, $\psi \to 0$ as $y \to \infty$.

In Model III we assume the presence of charged sheet within the frictional layer. In this case, there is a discontinuity of the electric field in the space charge region at $y = s$ caused by the charged sheet

$$\left[\frac{d\psi}{dy}\right]_{s+\delta} = \left[\frac{d\psi}{dy}\right]_{s-\delta} - \frac{\sigma_s}{\varepsilon\varepsilon_0} \tag{5}$$

where σ_s is the surface charge density of the sheet.

The Frictional Surface Layer

The coefficient γ in Eq. 1 determines the magnitude of the frictional force exerted by the frictional layer. Although the numerical integration method would make it possible to use any function, we assume that γ has a constant, nonzero values within $D \geqslant y \geqslant 0$. Such a layer can be modeled by an random array of spheres of effective radius a and volume density n. In such a case the frictional coefficient $\gamma = 6\pi n a \eta$.

We define the reciprocal hydrodynamic shielding length, λ, according to Ohshima and coworkers and use it as the suitable measure of the hydrodynamic drag effect within the surface frictional layer (Ohshima & Kondo, 1989; Ohshima, 1994; Nakano et al., 1994; Ohshima, 1997). It is equal to

$$\lambda = \sqrt{\frac{\gamma}{\eta}} \tag{6}$$

The dimensionless product λD is further used as a variable parameter in models II-IV. Clear physical meaning of the hydrodynamic shielding length is given by Debye and Bueche (1948).

Reconstituted SR Vesicles

Since Ca^{++}-ATPase is the dominant protein in the SR membrane, SR and reconstituted SR vesicles are characterized by two quantities (Brethes et al., 1986): (a) the molar ratio of lipids and *ATPase*

$$X = \frac{[PL]_{tot}}{[ATPase]} \tag{7}$$

(b) the molar ratio of uncharged (*PC*) and negatively charged (*PS*) lipids

$$R_L = \frac{[PC]}{[PS]} \tag{8}$$

Charge density on the native SR membrane is attributed to the membrane surface (Model I and II), to the charged sheet (Model III) or to the charged frictional layer (Model IV). The charge associated with membrane surface patch containing single *ATPase* is

$$q_{patch} = \sigma_{SR}(A_{ATP} + A_L X_{SR}/2) \tag{9}$$

where σ_{SR} is the surface charge density of the native SR membrane in any of the models, A_{ATP} is the membrane surface area of the stem of the *ATPase*, and A_L is the membrane surface area of phospholipid molecule and X_{SR} is the value of X for the native SR membrane. The factor ½ accounts for the fact that lipids in the membrane are distributed between two monolayers whereas *ATPase* penetrates both lipid monolayers. In model calculations we used $A_{ATP} = 12.6$ nm^2 as the average cross section[1] of the segment of *ATPase* penetrating the bilayer. It was estimated using the model of *ATPase* (Toyoshima, Sasabe & Stokes, 1993). Membrane surface area per lipid was set at $A_L = 0.7$ nm^2 (Small, 1986).

In reconstitution experiments, the membrane charge per *ATPase* originating from the native SR, q_{patch}, is conserved upon the incorporation of additional lipids. The charge density of the membrane modified by the addition of neutral lipids (new lipid/*ATPase* molar ratio X') is

$$\sigma' = \sigma_{SR}\frac{(A_{ATP} + A_L X_{SR}/2)}{(A_{ATP} + A_L X'/2)} \tag{10}$$

On the addition of mixed lipids there is an additional negative charge at the surface of the bilayer due to the incorporation of negatively charged lipids,

$$\sigma_{bil} = -e\frac{X'/2}{(1 + R_L)(A_{ATP} + A_L X'/2)} \tag{11}$$

Upon reconstitution the membrane surface of SR vesicle expands due to the incorporation of additional lipids[2] resulting in the lower volume density of the retarding

[1] It is not possible to determine an accurate effective value of the cross section because the organization of the annulus lipids is not known. Since the size of membrane patch is determined primarily by the value of the area covered by lipids, the error in A_{ATP} is not critical for the results obtained from the models.

[2] We estimate the value of surface expansion factor to be 2.2 for SR reconstituted with PC, 3.9 for PC + PS (75:25) and 6.3 for PC + PS (50;50).

spheres within the surface layer. The number of retarding spheres per *ATPase*, N_{patch}, is equal to

$$N_{patch} = nD(A_{ATP} + A_L X_{SR}/2) \tag{12}$$

This quantity is also conserved with the incorporation of additional lipids. It is assumed that the thickness of the frictional layer, D, does not change. Since the frictional coefficient γ in Eq. 1 is proportional to the volume density of retarding spheres within the surface layer, we use for the frictional coefficient of the surface layer after reconstitution, γ',

$$\gamma' = \gamma_{SR} \frac{A_{ATP} + A_L X_{SR}/2}{A_{ATP} + A_L X'/2} \tag{13}$$

The objective of this study is to explore the properties of the four models by attempting to reproduce the measured mobility from several reconstitution experiments (Brethes et al., 1986). The experimental results are summarized in Table 1. In model calculations the ionic strength of suspending solution was 4.7 mM, the same as in the reconstitution experiments.

Results

The electrophoretic mobility is determined by several model parameters such as charge density, the reciprocal shielding distance, λ, and the thickness of the surface layer, D. To avoid presentation of results in 4-dimensional space, the computational approach was to find the charge density of the SR vesicles for a given λ and D with which the given model reproduces the measured value of the mobility of SR vesicles (1.6 mobility units[3]). This charge density is subsequently used to compute the mobility of vesicles reconstituted with neutral and mixed lipids after taking into account the effect of dilution of charge originating from the native SR, the reduction of the density of the retarding spheres within the surface layer due to incorporation of additional lipids into the bilayer, and introduction of charge at the surface of the bilayer due to the incorporation of negatively charged PS.

MODEL-I

Here we assume that the surface of the vesicle is smooth and the charge resides on the membrane surface. On incorporation of uncharged lipids the surface charge density of the membrane is reduced resulting in the decrease of the mobility. Figure 2*a* illustrates the dependence of the mobility on the molar ratio $[PL]_{\text{tot}}/[ATPase]$ predicted from Model-I. The computed curve shows that theoretical mobility of vesicles reconstituted with PC is substantially smaller than the measured value.

The effect of incorporation of mixed lipids is depicted in Fig. 2*b* for PC:PS = 75:25 and in Fig. 2*c* for PC:PS = 50:50. In contrast to the case of uncharged lipids, the mobility predicted from Model-I is substantially higher than the measured value for the mixed lipids. These disagreements exclude Model I from further consideration.

When performing calculations with Models II, III and IV, the thickness of the surface frictional layer, D, was regarded as the primary independent variable. In each run, the vesicle mobility is computed as a function of the value of the dimensionless product λ.D. Each combination of D and λ.D is used to determine the charge density of the native SR membrane, the surface charge of the bilayer in Model II, the density of charge of the sheet separated distance s from the bilayer in Model III or volume density of charge in Model IV. This charge density and corresponding friction coefficient γ_{SR} are further used for computation of the mobility of reconstituted vesicles. The range of values of the thickness of the frictional layer, D, was obtained using grid search method.

Table 1. Results of reconstitution experiments

Vesicles	Molar ratio $[PL]_{\text{tot}}/[ATPase]$	Mobility, (mob. units)
SR	95 ± 5	1.6 ± 0.1
SR reconstituted with PC	254 ± 45	1.30 ± 0.07
SR reconstituted with PC:PS = 75:25	476 ± 93	1.7 ± 0.1
SR reconstituted with PC:PS = 50:50	788 ± 129	2.4 ± 0.3

MODEL-II

In this model we examine the effect of the presence of a frictional surface layer. For illustration purposes we assume that the surface frictional layer is 10 nm thick. In Fig. 3 we plot the theoretical dependence of mobility on the value of dimensionless parameter, λ.D, which is a measure of the retardation effect of the surface layer. The horizontal lines indicate the average value and standard deviation of the experimental mobility. Introduction of the surface frictional layer makes it possible to reproduce the mobility of the vesicles reconstituted with uncharged lipids (Fig. 3*a*). However, this model is inconsistent with the mobility data for mixed lipids (Fig.

[3] Conventional unit of mobility is $\mu m \cdot sec^{-1} \cdot V^{-1}$ cm.

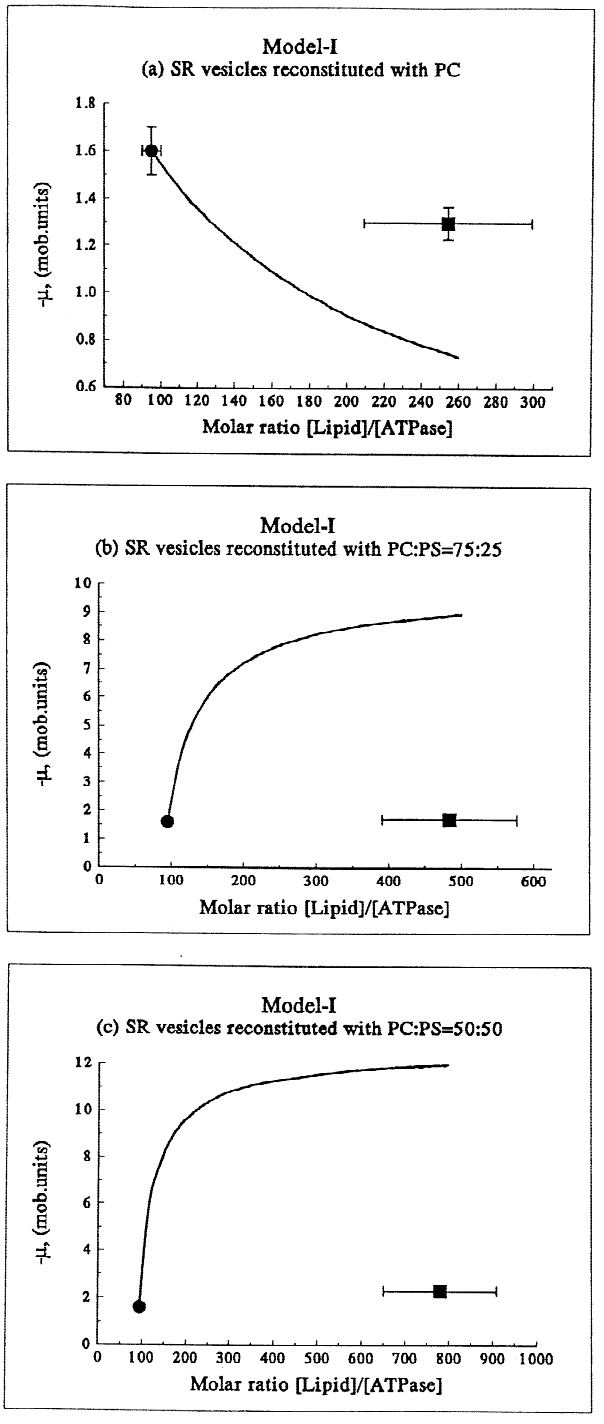


Fig. 2. Dependence of the mobility of reconstituted SR vesicles on the molar ratio $[PL]_{tot}/[ATPase]$ predicted from Model-I. The filled circle data point shows the measured mobility of SR vesicles and the filled square is the mobility of vesicles reconstituted with PC or PC + PS. (*a*) SR vesicles reconstituted with uncharged lipids. (*b* and *c*) SR vesicles reconstituted with mixed lipids.

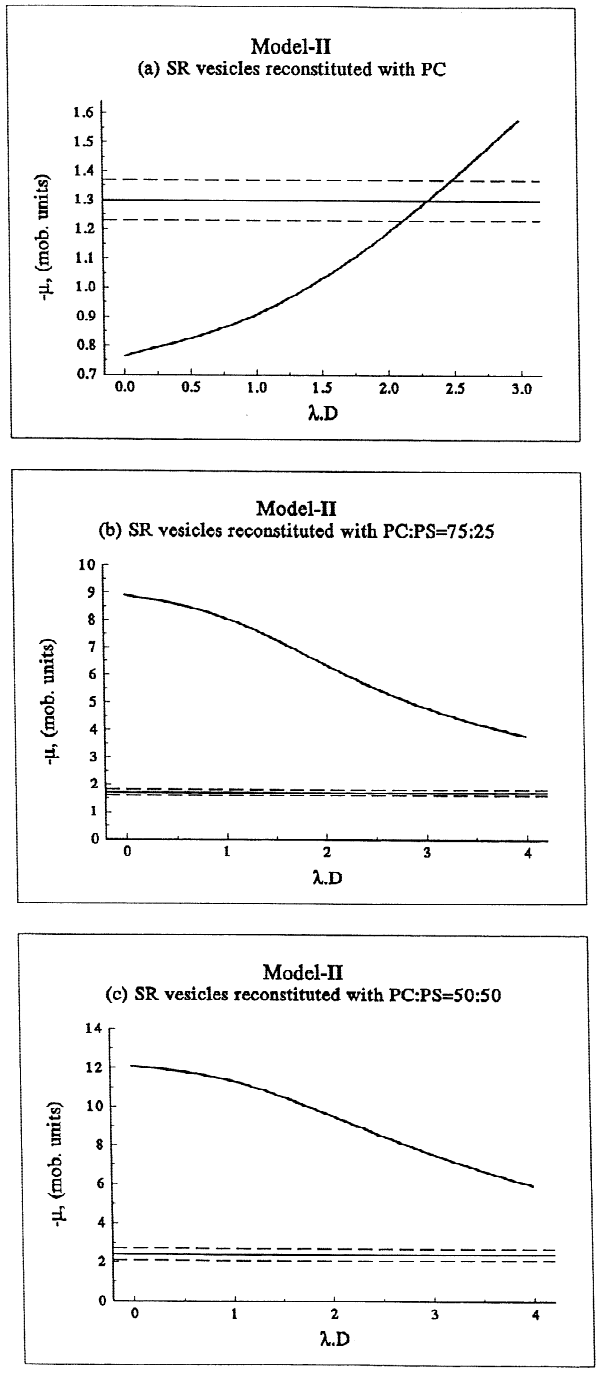
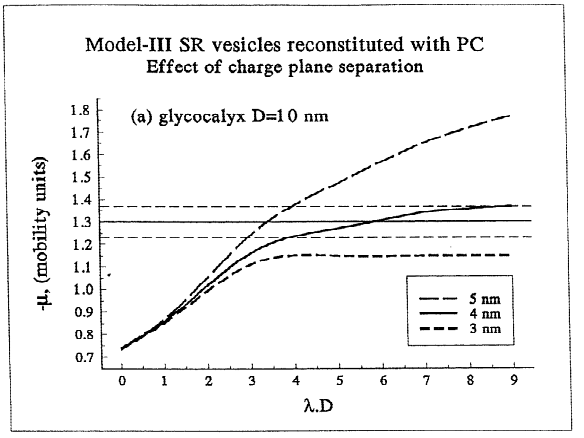


Fig. 3. Dependence of the mobility of SR vesicles on the retardation strength of the surface layer, λ.D, predicted from Model-II. The horizontal lines indicate average and standard deviation of mobility in each case. Model-II has the ability to reproduce the mobility of vesicles reconstituted with uncharged lipids (*a*) but overestimates the mobility of vesicles reconstituted with mixed lipids (*b* and *c*).

3*b* and *c*). This model and the value of λ.D used to fit of the mobility of vesicles reconstituted with PC predicts a mobility several times the measured value for vesicles containing mixed lipids.

The common feature of models I and II is the assumption that the charge on the SR membrane is spread at the surface of the bilayer. Both models fail to reproduce experimental results regardless of the absence or the presence of the frictional layer. In the following



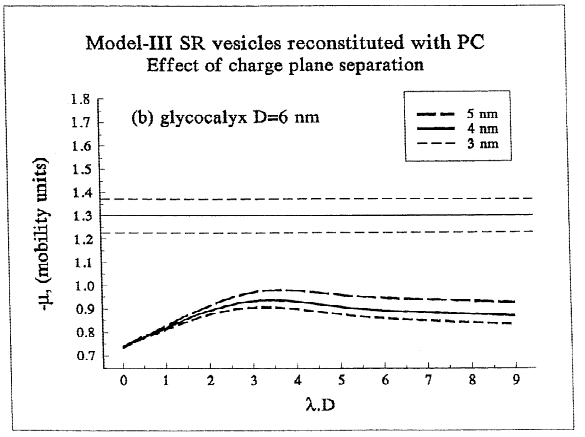
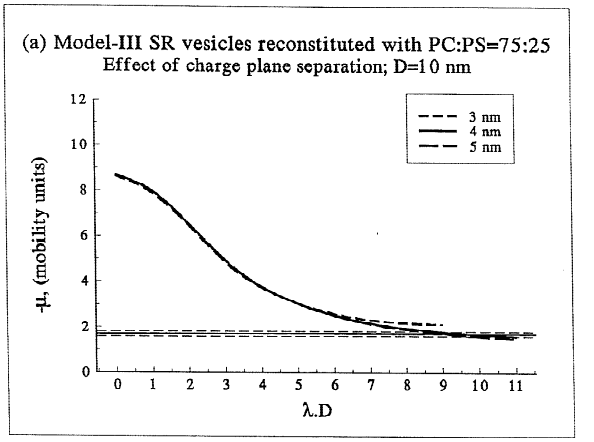


Fig. 4. Model-III. Dependence of the mobility of SR vesicles reconstituted with uncharged lipids on the retardation strength of the surface layer, λ.D for different thickness of the frictional layer: (*a*) D = 10 nm, (*b*) D = 6 nm. Computed results are for molar ratio of 250 lipids per *ATPase*. The horizontal lines indicate the measured value and its standard deviation. The theoretical curves illustrate that in order to reproduce the measured mobility, the frictional layer has to be sufficiently thick and the charged sheet sufficiently far from the bilayer surface.



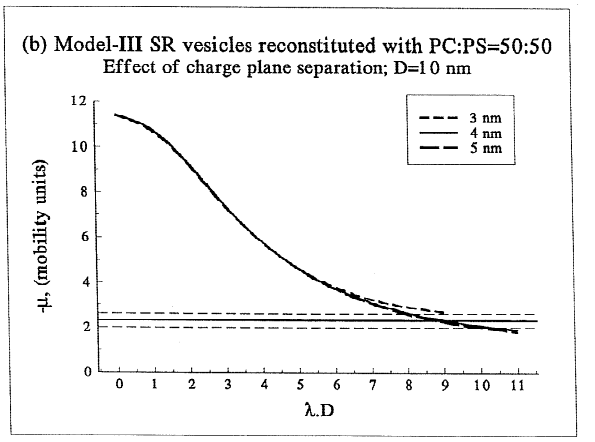


Fig. 5. Model-III. Thickness of the frictional layer D = 10 nm. Dependence of mobility of SR vesicles reconstituted with mixed lipids on the frictional strength of the surface layer, λ.D for different positions of the charged plane and different [Lipid]/[*ATPase*] ratio: (*a*) Molar ratio 480 lipids per *ATPase*. (*b*) Molar ratio 790 lipids per *ATPase*. The horizontal lines indicate the measured value of the mobility ± SD. The mobility of vesicles reconstituted with mixed lipids is weakly dependent on the position of charged sheet.

models we explore the effect of different distribution of charges at the membrane-water interface.

MODEL-III

Model-III differs from Model-II by the assumption that the charges in SR membrane are localized in a layer separated from the surface of the bilayer. The charged layer is modeled by the charged sheet embedded in the frictional layer. With this model we explore the effect of the position of the charged sheet relative to the surface of the bilayer and the effect of the thickness of the frictional layer.

Figure 4*a* illustrates the dependence of vesicle mobility as the function of the product λ.D for three positions of the charged plane (3, 4, and 5 nm) and for a 10 nm thickness of the frictional layer. The computed mobility data indicate that the separation of the charged layer from the bilayer has to be greater than 3 nm in order that the theoretical mobility of SR vesicles reconstituted with PC is equal to the measured value (1.3 ± SD). Computed mobility curves shown in Fig. 4*b* indicate that a frictional layer of substantial thickness must be present. The theoretical mobility of reconstituted SR vesicles does not agree with the measured value for the thickness of the frictional layer of 6 nm (Fig. 4*b*).

Figure 5*a* and *b* shows the mobility of SR vesicles reconstituted with two mixtures of neutral and charged lipids: (*a*) with molar ratio PC:PS = 75:25 and (*b*) with PC:PS = 50:50. The major feature of this model is that it can reproduce the measured mobility for charge plane separation greater than 4 nm. In contrast to the case of reconstitution with neutral lipids (Fig. 4) the mobility of vesicles reconstituted with mixed lipids is not very sensitive to the position of the charged sheet.

Results of similar computations for the thickness of frictional layer D = 6 nm illustrate the mobility properties for a thinner frictional layer. Results are shown in

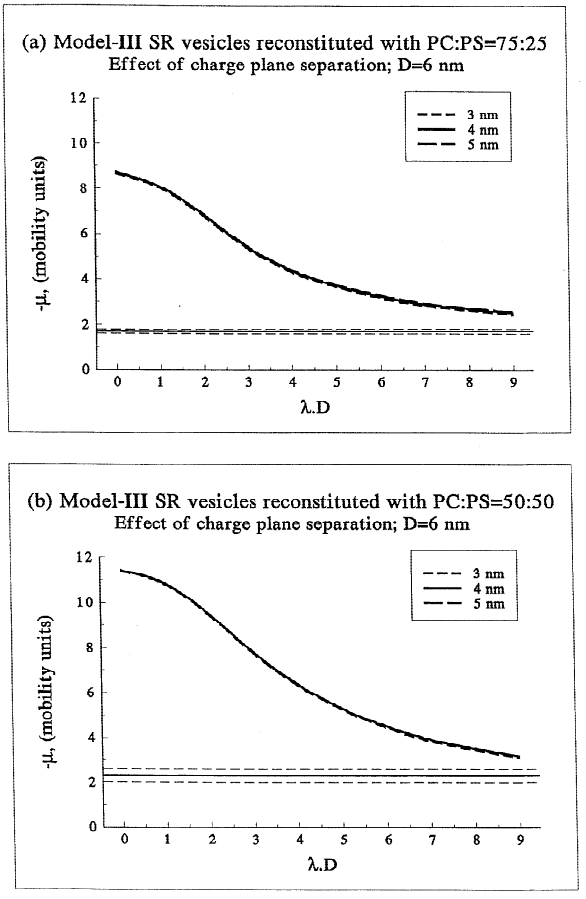


Fig. 6. Model-III. Thickness of the frictional layer D = 6 nm. Dependence of mobility of SR vesicles reconstituted with mixed lipids on the retardation strength of the surface layer, λ.D for different positions of the charged plane and different [Lipid]/[*ATPase*] ratio: (*a*) Molar ratio 480 lipids per *ATPase*. (*b*) Molar ratio 790 lipids per *ATPase*. The horizontal lines indicate the measured value of the mobility ± SD. For smaller thickness of the frictional layer, the model overestimates the mobility of SR vesicles reconstituted with mixed lipids.

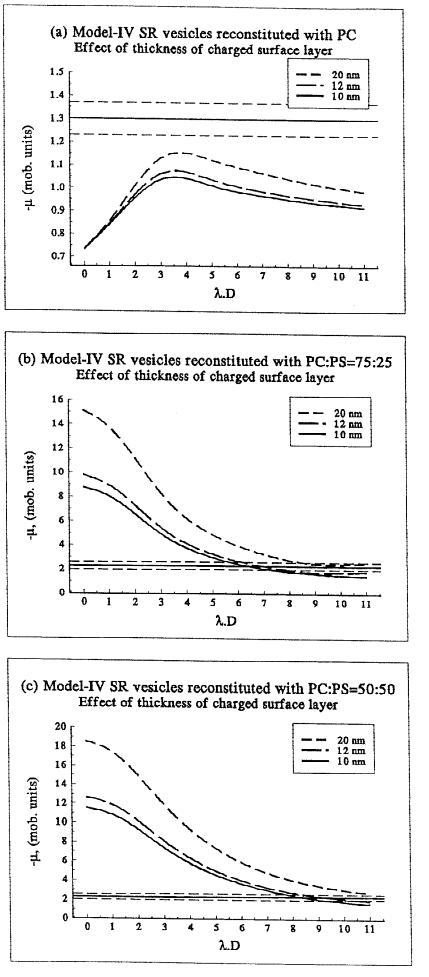


Fig. 7. Model-IV. Dependence of the mobility of SR vesicles on the retardation strength of the surface layer, λ.D, for different thickness of the charged layer. The horizontal lines indicate the average and the standard deviation of mobility in each case. Predictions of the model are inconsistent with experimental results.

Fig. 6*a* and *b*. The notable difference between the D = 6 nm and D = 10 nm results is that the model does not reproduce the measured mobility values of the vesicles reconstituted with mixed lipids for the thinner frictional layer.

MODEL-IV

In Model-IV the charges are assumed to be evenly distributed throughout the frictional layer. In this study we compute the mobility of both types of vesicles as a function of λ.D for different thickness of the frictional layer as a parameter. The computed results for vesicles reconstituted with PC are shown in Fig. 7*a* and for mixed lipids in Fig. 7*b* and *c*. The computed curves show that Model-IV cannot reproduce the measured mobilities of vesicles reconstituted with PC and PC + PS with one set of parameters.

The major result of this study is that Model-III can reproduce the measured mobility values for vesicles reconstituted with both uncharged and charged lipids with one set of model parameters. The computed mobility data[4] are consistent with the experimental results for charged sheet located about 3–4 nm above the surface of the bilayer, the value of λ.D ≅ 9 ± 1 and the thickness of the frictional layer D ≅ 10 ± 1 nm. Under these conditions the density of charge of SR membrane obtained from Model III is −0.27 e/nm^2. The surface area of the membrane patch that can be associated with single

[4] The uncertainties represent the uncertainties associated with the particular model.

Table 2. Electrokinetic parameters

Biomembrane	SR Present work	Erythrocytes (Sharp & Brooks, 1985)	Erythrocytes (Nakano et al., 1994)	Lymphocytes (Morita et al., 1991)
Surface charge density, (e/nm^2)	−0.27	−0.22	Three sublayers	Not available
Reciprocal shielding distance, λ (nm^{-1})	0.9	0.74	0.9	0.3
Frictional layer thickness, D, (nm)	10	7.8	10	Not available

Table 3. Ca^{++}-ATPase charge estimated from mobility of SR vesicles reconstituted with uncharged and mixed lipids

SR vesicles	Q, (e) present work	Q, (e) (Brethes, et al. 1986)
Reconstituted with PC	12	3.4
Reconstituted with PC:PS = 75:25	12	7.8
Reconstituted with PC:PS = 50:50	12	16.5

ATPase is determined from the cross sectional area of the stalk 12.6 nm^2 which was based on the data of Toyoshima and coworkers (Toyoshima, Sasabe & Stokes 1993) and the area of the lipid bilayer. For $X_{SR} = 95$ (*see* Table 1), and using 0.7 nm^2/lipid, the surface area of lipids in the patch is 0.5(95)(0.7 nm^2). These considerations yield the total surface area of the membrane patch associated with single *ATPase* equal to 44 nm^2. Assuming that the charge of the SR membrane originates from *ATPase*s, the charge of the *ATPase* is equal to $Q_{ATPase^+} = (-0.27\ e/nm^2)(44\ nm^2) = -12$ e.

Discussion

The computed mobility data of SR vesicles reconstituted with uncharged and charged lipids clearly indicate that the bilayer surface of SR vesicles has to be covered by a frictional layer. The agreement with experimental results can be achieved if the frictional layer is about 10 nm thick, and if it contains negative charges with highly inhomogeneous distribution represented by a charged sheet positioned about 4 nm above the surface of the bilayer. The next question to be addressed is how the electrokinetic parameters of SR surface obtained in this work compare with similar parameters obtained for other biomembranes.

In Table 2 electrokinetic parameters of SR vesicles: surface charge density, thickness of the frictional layer, and the reciprocal hydrodynamic shielding distance are compared with quantities obtained from analysis of electrophoretic mobility studies using a combination of Poisson's and Navier-Stokes equations. It is interesting that the electrokinetic parameters of SR membrane vesicles are quite similar to those of erythrocytes in terms of surface charge density, the hydrodynamic frictional parameter λ and the thickness of the surface layer. Although the earlier studies of red blood cells were based on single layer models (Model-IV), there are convincing experimental results indicating more complex structure of the charge distribution in the frictional layer of biomembranes (Nakano et al., 1994) (Morita et al., 1991). Our study shows that the SR mobility results are not compatible with the homogeneously charged layer model, but with the model in which charges are concentrated within a narrow layer of the frictional layer, perhaps associated with a certain region of Ca^{++}-ATPase pumps.

Recent studies show that Ca^{++}-ATPase consists of three major segments, references in (Yonekura et al., 1997). In addition to cytoplasmic segment containing 70% of the total mass, there are transmembrane and luminal segments. The structure is approximately 12 nm tall with Ca^{++}-ATPase cytoplasmic head positioned above 2.5 nm stalk and protruding about 7 nm into the cytoplasmic space (Toyoshima, Sasabe & Stokes, 1993). According to Model-III, the layer of negative charges is located about 3–4 nm above the surface of the bilayer. This suggests that negative charges are concentrated in the lower partion of the Ca^{++}-ATPase head. Using data from reconstitution studies (Brethes et al., 1986) and the conditions of fit of Model-III to measured mobilities, and assuming that the membrane charge of native SR originates from the Ca^{++}-ATPase we estimated the amount of charge on Ca^{++}-ATPase pump. In Table 3 we compare the magnitude of charge of Ca^{++}-ATPase obtained in this work with earlier results based on the analysis of electrophoretic mobility data in terms of the classical model (Brethes et al., 1986). How does the electrokinetic parameters of Model III compare with surface properties of SR and molecular structure of Ca^{++}-ATPase?

There are several pivotal studies of amino acid sequence in Ca^{++}-ATPase that can be used to obtain a reference value for the above 12e/*ATPase*. We have

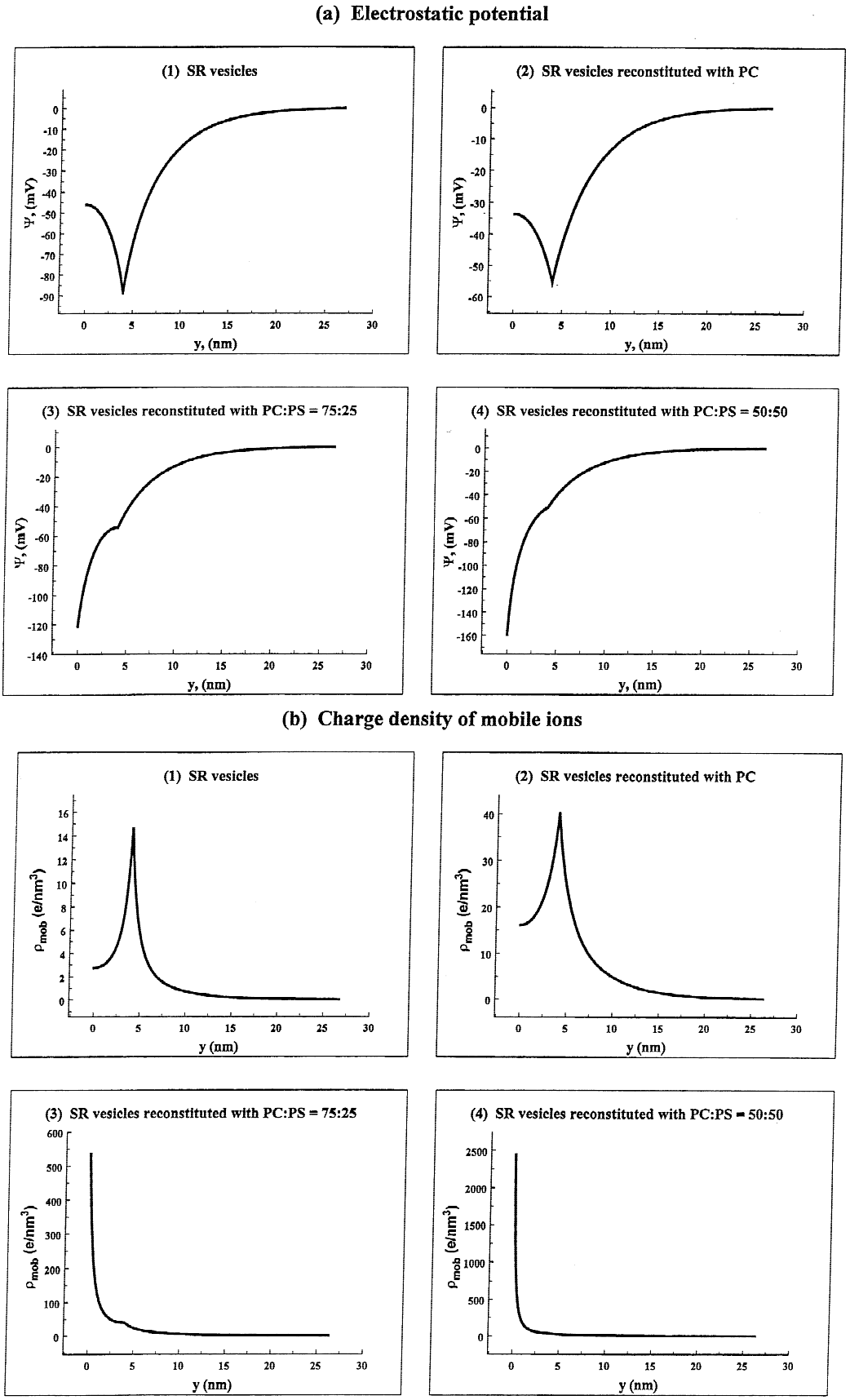


Fig. 8. *Continued on next page.*

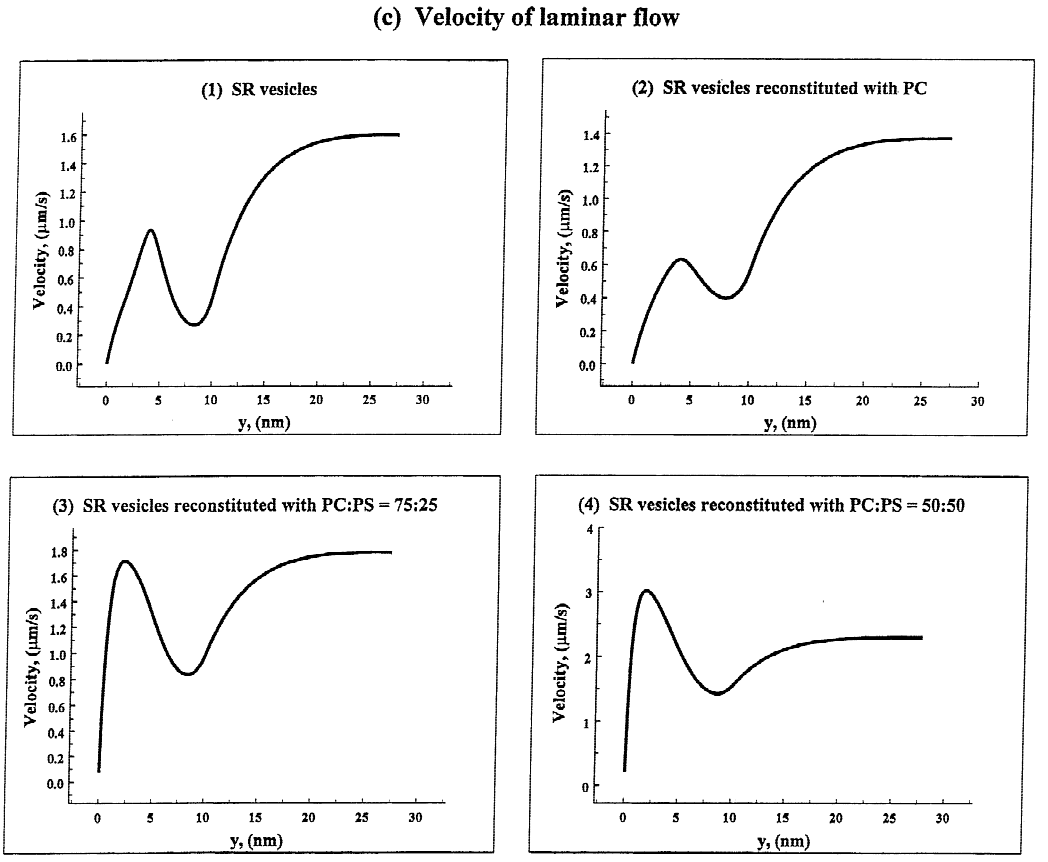


Fig. 8. Panels illustrating the distribution of electrostatic potential $\psi(y)$ (*a*), the charge density of mobile ions $\rho_{mob}(y)$ (*b*), and the velocity of flow $v(y)$ (*c*) for the condition of fit of Model-III to measured mobilities of SR and SR vesicles reconstituted with both neutral and mixed lipids. Computations done for univalent salt concentration 4.7 mM, thickness of the frictional layer D = 10 nm, λ.D = 9, and the position of charged sheet 4 nm above the surface of the bilayer. Vesicle velocity was computed for external applied electric field E_{ext} = 100 V/m.

used the structural model of Ca^{++}-ATPase presented by Andersen (Andersen, 1995) which is very suitable for the determination of net charge on the cytoplasmic portion of Ca^{++}-ATPase. Considering only charges of the cytoplasmic domain including those at the membrane surface we counted 105 negative and 94 positive charges yielding net 11 negative charges, which is surprisingly close to 12 negative charges obtained from the fit of Model III. Our computational results indicate the presence of a frictional layer at the surface of SR since without it one cannot reproduce the measured mobilities of reconstituted SR vesicles. What is the origin of the frictional layer?

The outer membrane of the SR in its native environment faces the cytoplasm. The fact that the proteins and lipids facing the cytoplasm do not get glycosylated rules out the presence a glycocalyx that has been identified as the frictional layer of red blood cells and other biomembranes. These arguments force us to conclude that the frictional layer originates from Ca^{++}-ATPases protruding from the surface of the SR bilayer. The effective thickness of the frictional layer, 10 ± 1 nm, is compatible with the geometry of Ca^{++}-ATPase (Toyoshima, Sasabe & Stokes 1993). It is interesting to observe, that the electrokinetic parameters obtained for the SR membrane correspond well to those of red blood cells despite the different origin of the frictional layer (Table 2). This similarity is accidental.

To illustrate the underlying physical conditions under which Model-III reproduces the measured mobilities we plot in Fig. 8 the spatial distribution of electric potential $\psi(y)$, the charge density of mobile ions $\rho_{mob}(y)$ and the flow velocity distribution $v(y)$.

Panel 8*a* illustrates how the electrostatic potential profile changes when SR vesicles are reconstituted with neutral (*a*2), and mixed lipids (*a*3,*a*4) relative to SR vesicles (*a*1). On incorporation of uncharged lipids (*a*2) the magnitude of the potential decreases whereas the spatial profile remains the same as for SR (*a*1). Note the change of the potential profile due to the incorporation of mixed lipids (compare plot *a*3 with *a*1). In the mixed lipid case, the potential profile results from the superposition of the potential due to the presence of negatively charged PS in the lipid bilayer and the charged sheet of

the SR whose density is reduced by the presence of lipids added to the bilayer. When the concentration of negatively charged lipids is increased, the potential at the bilayer surface becomes more negative and the charged bilayer dominates the distribution of the electrostatic potential (*a*4).

The associated change of the spatial distribution of the charge density due to mobile ions are depicted in Panel 8*b*. This charge density distribution determines the spatial distribution of the driving force, the term $\rho_{mob}E$ in Navier-Stokes equation, and thus it determines the velocity distribution, and ultimately the vesicle mobility. While in SR and SR vesicles reconstituted with uncharged lipids (plots *b*1 and *b*2) the mobile charge localized in the vicinity of the charged sheet determines the distribution of the driving force, the mobile space charge is compacted in front of the lipid bilayer surface when the SR is reconstituted with mixed lipids (compare *b*3 and *b*4 with *b*1 and *b*2). This spatial redistribution of density of mobile ions has a profound effect on the vesicle mobility since fixed charges detached from the surface have a greater effect on vesicle mobility than charges on the bilayer surface (Pasquale et al., 1986).

The velocity profiles obtained from the solution of the Navier-Stokes equation are shown in Panel 8*c*. The presence of velocity maximum and minimum is the consequence of the presence of the frictional layer. Notable among the velocity distributions is the high velocity gradient in the vicinity of the bilayer for vesicles reconstituted with mixed lipids which is due to the high density of space charge in front of the bilayer (*see* plots *b*3 and *b*4).

In closing, it is appropriate to restate the major simplifying assumptions defining the SR membrane used in our models: First, it was assumed that the charge of the SR membrane originates from SR proteins whereas the charge contributed by SR lipids was ignored. This assumption is justifiable in view of the fact that negatively charged lipids represent only a small fraction of the total lipid content of SR (de Meis, 1981). Second, since Ca^{++}-*ATPase* is the dominant membrane protein, the charge density in Model III can be associated with that of Ca^{++}-*ATPase*. The contribution of negatively charged lipids and other proteins present in SR membrane would lower the value of the net charge of the *ATPase*[5] and change slightly the position of charged sheet. When modeling the frictional layer, the retarding force was produced by a layer of randomly distributed spheres. The origin of the friction coefficient γ in Eq. (1) is not essential for the Model III. The structural details determining the hydrodynamic properties of the SR surface are not known, the *ATPase*s tend to oligomerize and there is no consensus on the spatial arrangement of Ca^{++}-ATPases at the SR surface (Maguire & Ohlendieck, 1996). The issues of electrokinetic properties of the SR frictional layer should be addressed in future studies. As suggested by one of the reviewers, it is desirable to measure the electrophoretic mobility of lipid vesicles as a function of the membrane concentration of *ATPase* incorporated into the bilayer, a study similar to that done with gangliosides (McDaniel et al., 1986). In addition to determining electrokinetic parameters of the SR membrane vesicles, this study shows the usefulness of integration of electrophoretic mobility measurements into reconstitution studies of biological membranes. The analysis of electrophoretic mobility data of reconstituted vesicles illustrates the need to use both the uncharged and charged lipids. Addition of uncharged lipids reduces the native charge as well as the retarding force of the surface layer in a controlled way. The addition of known amount of charged lipids changes, also in a well defined way, the charge density at the surface of the bilayer and the reduced drag of the surface layer. From this combination of conditions it is possible to determine the electrokinetic parameters uniquely.

[5] Similarly we expect that the lower dielectric constant of the surface layer would result in the decrease of the net charge/*ATPase*. We think that the assumption of the dielectric constant of the surface layer equal to that of water is applicable in view of the substantial dilution of the surface layer by the additional lipids incorporated into the bilayer.

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References

Andersen, J.P. 1995. Functional consequences of alterations to amino acids at the M5S5 boundary of the Ca^{2+}-ATPase of sarcoplasmic reticulum. *J. Biol. Chem.* **270:**908–14

Arrio, B., Johannin, G., Carrette, A., Chevallier, J., Brethes, D. 1984. Electrokinetic and hydrodynamic properties of sarcoplasmic reticulum vesicles: A study by laser Doppler electrophoresis and quasi-elastic light scattering. *Arch. Biochem. Biophys.* **228:**220–229

Brethes, D., Dulon, D., Johannin, G., Arrio, B., Gulik-Krzywicki, T., Chevallier, J. 1986. Study of the electrokinetic properties of reconstituted sarcoplasmic reticulum vesicles. *Arch. Biochem. Biophys.* **246:**355–365

Debye, P., Bueche, A. 1948. Intrinsic viscosity, diffusion, and sedimentation rate of polymers in solution. *J. Chem. Phys.* **16:**573–579

Donath, E., Pastushenko, V. 1979. Electrophoretical study of cell surface properties. The influence of the surface coat on the electric potential distribution and on general electrokinetic properties of animal cells. *J. Electroanal. Chem.* **104:**543–554

Kawahata, S., Ohshima, H., Muramatsu, M., Kondo, T. 1990. Charge distribution in the surface region of human erythrocytes as estimated from electrophoretic mobility data. *J. Colloid Interface Sci.* **138:**182–186

Levine, S., Levine, M., Sharp, K.A., Brooks, D.E. 1983. Theory of the electrokinetic behavior of human erythrocytes. *Biophys. J.* **42:**127–135

McDaniel, R.V., McLaughlin, A., Winiski, A.P., Eisenberg, M.,

McLaughlin, S. 1984. Bilayer membranes containing the ganglioside GM1: Models for electrostatic potentials adjacent to biological membranes. *Biochemistry* **23:**4618–4624

McDaniel, R.V., Sharp, K., Brooks, D., McLaughlin, A.C., Winiski, A.P., Cafiso, D., McLaughlin, S. 1986. Electrokinetic and electrostatic properties of bilayers containing gangliosides GM1, GDLa or GT1. Comparison with a linear theory. *Biophys. J.* **49:**741–752

McLaughlin, S., Harary, H. 1976. The hydrophobic adsorption of charged molecules to bilayer membranes: A test of applicability of the Stern Equation. *Biochemistry* **15:**1941–1948

McLaughlin, S., Mulrine, N., Gresalfi, T., Vaio, G., McLaughlin, A. 1981. Adsorption of divalent cations to bilayer membranes containing phosphatidylserine. *J. Gen. Physiol.* **77:**445–473

de Meis, L. 1981. The Sarcoplasmic Reticulum. New York: John Wiley & Sons

Maguire, P.B., Ohlendieck, K. 1996. Oligomerization of sarcoplasmic reticulum Ca-ATPase from rabbit skeletal muscle. *FEBS Lett.* **396:**115–18

Morita, K., Muramatsu, N., Ohshima, H., Kondo, T. 1991. Electrophoretic behavior of rat lymphocyte subpopulations. *J. Colloid Interf. Sci.* **147:**457–461

Nakano, Y., Makino, K., Ohshima, H., Kondo, T. 1994. Analysis of electrophoretic mobility data for human erythrocytes according to sublayer models. *Biophys. Chem.* **50:**249–254

Ohshima, H. 1994. Electrophoretic mobility of soft particles. *J. Colloid Interf. Sci.* **163:**474–483

Ohshima, H. 1997. Electrophoretic mobility of a polyelectrolyte-adsorbed particle: effect of segment density distribution. *J. Colloid Interf. Sci.* **185:**269–273

Ohshima, H., Kondo, T. 1989. Approximate analytic expression for the electrophoretic mobility of colloidal particles with surface-charge layers. *J. Colloid Interface Sci.* **130:**281–282

Pasquale, L., Winiski, A., Oliva, C., Vaio, G., McLaughlin, S. 1986. An experimental test of new theoretical models for the electrokinetic properties of biological membranes. The effect of $UO2^{++}$ and tetracaine on electrophoretic mobility of bilayer membranes and human erythrocytes. *J. Gen. Physiol.* **88:**697–718

Sharp, K.A., Brooks, D.E. 1985. Calculation of the electrophoretic mobility of a particle bearing bound polyelectrolyte using the nonlinear Poisson-Boltzmann equation. *Biophys. J.* **47:**563–566

Small, D. 1986. Handbook of Lipid Research. The Physical Chemistry of Lipids. Plenum Press, New York

Smejtek, P., Riker, W.K., Oxyzoglou, A., Wright, C., Bennett, M. 1990. Adsorption of aminopyridines to phosphatidylserine membranes. *Biochim. Biophys. Acta* **1029:**259–266

Smejtek, P., Wang, S. 1990. Adsorption to DPPC membranes in gel and fluid state: Pentachlorophenolate, dipicrylamine and tetraphenylborate. *Biophys. J.* **58:**1285–1294

Tatulian, S. 1993. Ionization and ion binding. *In:* Phospholipids Handbook, G. Cevc, editor. pp. 511–552. Marcell Dekker, New York

Tatulian, S. 1994. Evaluation of ion binding of zwitterionic membranes based on extended Gouy-Chapman-Stern theory. *J. Phys. Chem.* **98:**4963–4965

Tatulian, S.A. 1995. Evaluation of divalent cation binding to phosphatidylserine membranes by an analysis of concentration dependence of surface potential. *J. Colloid Interface Sci.* **175:**131–137

Toyoshima, C., Sasabe, H., Stokes, D.L. 1993. Three-dimensional cryo-electron microscopy of the calcium ion pump in the sarcoplasmic reticulum membrane. *Nature* **362:**469–471

Yonekura, K., Stokes, D.L., Sasabe, H., Toyoshima, C. 1997. The ATP-binding site of Ca^{2+}ATPase revealed by electron image analysis. *Biophys. J.* **72:**997–1005