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Effect of temperature on the formation and inactivation of syringomycin E pores in human red blood cells and bimolecular lipid membranes

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Abstract

The effects of temperature on the formation and inactivation of syringomycin E (SRE) pores were investigated with human red blood cells (RBCs) and lipid bilayer membranes (BLMs). SRE enhanced the RBC membrane permeability of ^{86}Rb and monomeric hemoglobin in a temperature dependent manner. The kinetics of ^{86}Rb and hemoglobin effluxes were measured at different temperatures and pore formation was found to be only slightly affected, while inactivation was strongly influenced by temperature. At 37°C, SRE pore inactivation began 15 min after and at 20°C, 40 min after SRE addition. At 6°C, below the phase transition temperature of the major lipid components of the RBC membrane, no inactivation occurred for as long as 90 min. With BLMs, SRE induced a large current that remained stable at 14°C, but at 23°C it decreased over time while the single channel conductance and dwell time did not change. The results show that the temperature dependent inactivation of SRE pores is due to a decrease in the number of open pores.

Keywords: Syringomycin E; Pore formation; Pore inactivation; Red blood cell membrane; Bilayer lipid membrane; Temperature

1. Introduction

The cyclic lipodepsipeptide syringomycin E (SRE) produced by some strains of the plant bacterium *Pseudomonas syringae* pv. *syringae* [1–4] has antifungal activities against a broad spectrum of pathogenic fungi [5–8] and contributes also to plant diseases [9–13].

The primary site of syringomycin action is the plant and fungal plasma membrane. SRE alters several membrane functions such as ion fluxes, membrane potential [14–16] and activity of K^+-H^+-ATP-ase [17–20]. All of these effects are suggested to be related to SRE pore formation in the host membrane [21,22]. SRE-induced pores with weak anion selectivity were demonstrated in planar bilayer lipid membranes (BLMs) [23–26]. The pore radius was estimated to be about 1 nm [21–24]. SRE was reported to form pores also in horse [21] and human erythrocytes (RBCs) [22,27].

The lipid composition of the membrane was shown to influence SRE pore formation [26,27]. Feigin et al. [26] found that cholesterol increases the energy barrier for SRE pore formation in planar lipid bilayer membrane. Our previous study [27] showed that the

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partial depletion of the membrane cholesterol increased the pore forming activity of SRE in the human RBC membrane. Recent genetic studies performed on yeast *Saccharomyces cerevisiae* suggested that sterols [28–30] and phospholipids, especially sphingolipids [31,32] play important roles in the action of SRE. Results on the effects of sphingolipids (SLs) on channel forming activity of SRE in BLM have shown that the ability of SLs to influence membrane sensitivity to SRE depends on the structural features of the SL molecule [33].

It has been shown in our previous work [27] performed at room temperature (20°C) that SRE addition to human RBCs caused lysis of a minor fraction of the cells within 2 min. The extent of the lysis remained unchanged as long as 100 min. During this time period the membranes of the unlyzed cells had enhanced permeability for ^{86}Rb and monomeric hemoglobin. The ion flux kinetics suggested that the SRE pores had discrete life times and were eventually inactivated. Recently it has been shown with BLMs that the SRE pores are formed from SRE–lipid complexes [23,24,34]. We may suppose that similar SRE pores are formed in RBC membranes. In the kinetics of SRE pore formation and inactivation both the lipid composition and the fluidity of the membrane may have a role. The nature of the influence of the latter is not yet clear. Temperature-dependent channel inactivation was observed with another channel-former, the antibiotic gramicidin A [35] and was concluded to be a result of the interaction between gramicidin A and cholesterol molecules [36].

In this work, transport kinetic studies were performed on RBCs and BLMs at different temperatures. It is shown that the SRE pore inactivation is a strongly temperature dependent process.

2. Materials and methods

Human blood from healthy volunteers was stabilized with citrate buffer and stored at 4°C up to 3 days. Synthetic 1,2-dioleoyl-*sn*-glycero-3-phosphoserine (DOPS) and 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) were purchased from Avanti Polar Lipids, Pelham, AL, USA. SRE was purified to homogeneity as described earlier [37] and stored frozen. All chemicals used were of analytical grade.

2.1. ^{86}Rb efflux from human RBCs

Blood was loaded with ^{86}Rb for 1.5 h at 37°C. After centrifugation RBCs were washed 3 times with buffered salt (BS) solution (in mmol/l): 3.2 KCl, 138 NaCl, 1 $CaCl_2$, 1 $MgCl_2$, 27 sucrose, MOPS, (pH 6.8) and then resuspended in the same solution to obtain a hematocrit value of 0.4–0.5. The RBC suspension was either kept at 37°C or chilled to 20 or 6°C and maintained at this temperature.

SRE solution (0.9 ml of stock SRE solution (2 mg/ml SRE in 1 mmol/l HCl) diluted with 12 ml BS solution) was mixed with 28 ml of the RBC suspension (final syringomycin E concentration was 6×10^6 molecules/cell (150 μg/ml RBCs)). The suspension was incubated at a given temperature (37, 20 or 6°C) in a shaking water bath. Samples of the suspension were taken at designated time intervals. The samples were centrifuged and the radioactivity of aliquots of the supernatant fluids was measured with a γ-scintillation counter (Gamma, Hungary).

^{86}Rb transport was characterized by the amount of tracer found in the supernatant fluids after a time period t and expressed as the percentage of the total activity of the suspension (N_t). The tracer efflux was presented in an N_t versus t curve as well as in a semilogarithmic plot: $-\ln(1-N_t/N_\infty(\mathrm{mod}))$ over time, where $N_\infty(\mathrm{mod})$ was the percentage of radioactivity in the external medium at tracer equilibrium between the extracellular space and the intracellular space of the modified cells. The number of SRE modified cells was calculated as described previously [27].

RBC concentration and hematocrit values were determined using an Automated Hematology Analyzer (COBAS MICROS OT 18). The SRE induced hemolysis was calculated as the difference between the RBC concentrations of the untreated (A_0) and SRE-treated (A) RBC suspension and expressed as a percentage of A_0. The hemoglobin concentration (c_t) was determined in cyanmethemoglobin form by measuring the OD at 540 nm with a Perkin-Elmer Lambda 15 UV/VIS spectrophotometer. The hemoglobin efflux was presented in a c_t versus t curve and in a semilogarithmic plot: $-\ln(1-c_t/c_\infty)$ over time, where c_t was the hemoglobin concentration in the external medium at time t and c_∞ was that of at equilibrium distribution of hemoglobin between the

extracellular space and the intracellular space of the modified cells.

2.2. Electrical conductance measurements on BLMs

Virtually solvent-free membranes were prepared as described by Montal and Muller [38]. Bilayer membranes were formed from an equimolar mixture of DOPS and DOPE in hexane. The BLM was bathed in 100 mmol/l NaCl, 5 mmol/l MOPS (pH 6). Two symmetrical halves of Teflon chamber with solution volumes of 1 cm^3 were divided by a 15-μm thick Teflon partition containing a round aperture of about 100 μm diameter. Hexadecane in *n*-hexane (1:10, v/v) was used for aperture pretreatment. A pair of Ag–AgCl electrodes was used to maintain membrane potential and to detect current fluctuations. 'Virtual ground' was maintained at the *trans* side of the bilayer. Hence, positive voltages mean that the *cis* side compartment is positive with respect to the *trans* side. Positive currents are therefore those of cations flowing from *cis* to *trans* [23,25,39]. The design of chambers heating block permitted the use of 8 mm bore rubber tubing for the circulation of temperature-controlled water. A water bath thermostat (constant temperature circulator) RM 6 (Brinkman Instrument Co., Germany) was used to perform experiments at 14 and 27°C. These temperature values were chosen to have stable bilayers to perform long-term experiments. Bilayer formation was indicated by the subsequent increase in membrane capacitance to its final value of 80–100 pF. The stability of the bilayers was checked by measuring their conductance. For unmodified bilayers a conductance value of about 1 pS was found which remained unchanged under conditions chosen during several hours. Syringomycin E was added to the aqueous phase at one (*cis*) side of the bilayer from water stock solutions (1 mg/ml) and the current records began 10 min later to allow the system to equilibrate.

3. Results and discussion

SRE added to the RBCs at 37°C, at a concentration of 6×10^6 molecules/cell (150 μg/ml RBCs), caused lysis of a minor portion of the cells within 2 min. This lysis remained unchanged for as long as 100 min. During this time period the membranes of the unlyzed cells had enhanced permeability for ^{86}Rb (a tracer analog of K^+ ion). Fig. 1 shows a typical ^{86}Rb efflux time course of SRE treated RBCs. ^{86}Rb efflux rapidly increased and then it ceased at a level far from the equilibrium tracer distribution between the intra- and extracellular spaces.

It should be noted that the kinetics of the ^{86}Rb efflux measured at 37°C was very similar to that obtained with cholesterol depleted RBCs (see Fig. 6 in [27]). The transport kinetics of the latter was explained by the presence of three populations of cells: lyzed, unmodified and SRE modified cells, with the last having pores in their membranes that allowed ^{86}Rb transport to reach an equilibrium. The same explanation applies to the kinetics of SRE induced ^{86}Rb efflux at 37°C (and also at 20 and 6°C). Thus the saturation level of transport was considered as the level of tracer equilibrium between the SRE modified cells and the extracellular space.

In some experiments, however, we did not observe

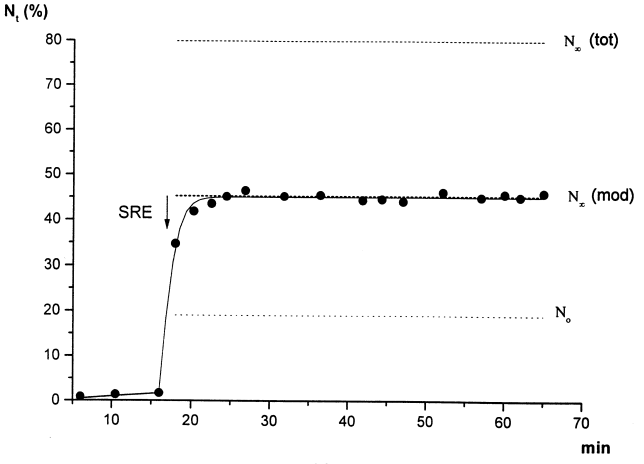

Fig. 1. Effect of SRE on the ^{86}Rb efflux through RBC membranes. RBCs were suspended in BS solution (in mmol/l: 3.2 KCl, 138 NaCl, 1 $CaCl_2$, 1 $MgCl_2$, 27 sucrose, 5 MOPS, (pH 6.8), temperature 37°C). The ordinate gives the amount of radioactive ions effluxed expressed as the percentage of the total activity of the RBC suspension (N_t). N_∞(tot) is the percentage amount of ^{86}Rb in the extracellular solution at tracer equilibrium between the extracellular space and the intracellular space of all lysis survived cells. N_∞(mod) is the percentage amount of ^{86}Rb in the extracellular solution at tracer equilibrium between the intracellular solution of modified cells and the extracellular solution. N_0 is the percentage value of the ^{86}Rb due to lysis and contamination. SRE concentration: 6×10^6 molecules/cell (150 μg/ml RBCs). The arrow indicates addition of SRE.

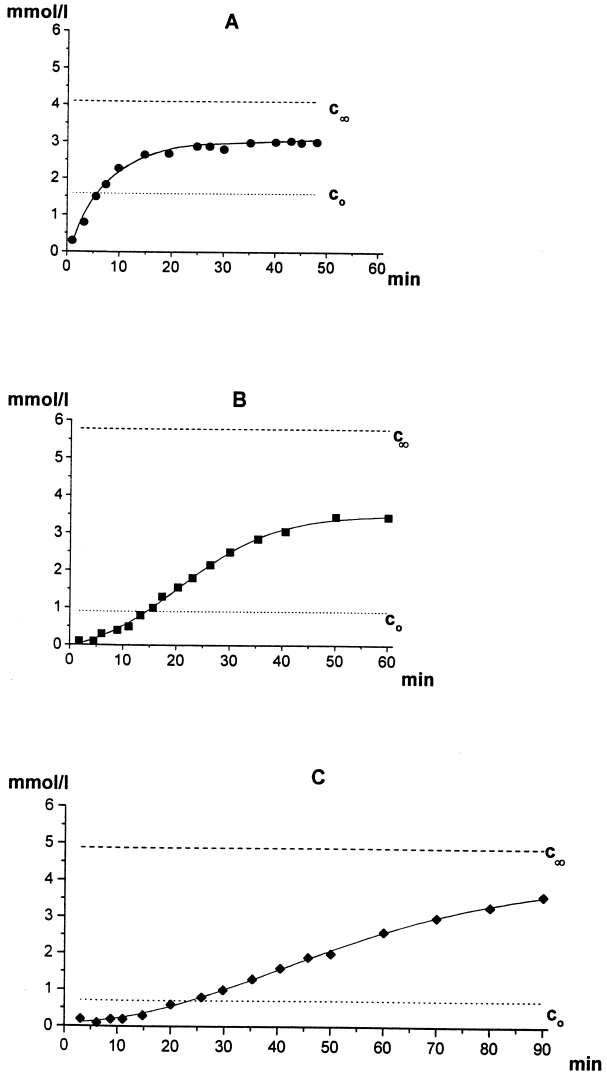

Fig. 2. Time course of SRE-induced hemoglobin efflux through RBC membrane at 37°C (A), 20°C (B) and 6°C (C). RBCs were suspended in BS solution (composition see Fig. 1). The ordinate gives the extracellular hemoglobin concentration (c_t). c_∞ is the hemoglobin concentration at equilibrium when hemoglobin is distributed between the modified cells and the extracellular solution of the RBC suspension. c_0 is the extracellular hemoglobin concentration due to lysis. SRE concentration: 6×10^6 molecules/cell (150 μg/ml RBCs). SRE was added at 0 min.

a real saturation level in the transport curve of ^{86}Rb efflux, especially when it was measured at 20°C. The second part of the transport curve (i.e. see Fig. 3 in [27]) had a slope which was only slightly larger than the slope of the control ^{86}Rb efflux curve. These observations can be explained by the cluster organization of SRE channels. It has been shown [23,24] that SRE when incorporated into BLMs, forms two types of channels (large and small ones) that are different in conductance by a factor of six. It was concluded that the large SRE channels are clusters of small ones exhibiting synchronous opening and closing. We suggest similar channels in the membranes of the SRE-treated RBCs and further suggest that the modified cells have clusters in their membranes, while the unmodified cells have only small pores. Taking into account the conductance of the large pore at about 0.03 nS in 150 mmol/l NaCl solution (at $V\approx0$) [24], the ion flux through these pores is about 10^{-17} mol/s [40]. Therefore, for one pore per one RBC, it requires 2×10^3 s (about 30 min) for complete tracer equilibration. The transport curve reached saturation level in about 10 min after SRE addition. Thus we may suggest an average of three open large pores in each modified cell. On the other hand the second part of the ^{86}Rb efflux curves occasionally observed might be due to the ^{86}Rb transport through small pores that open randomly. Since the conductivity of the small pores is about six times less than the large ones, a longer time is required to achieve tracer equilibrium. Also, pore inactivation will further decrease the transport rate.

It is possible that the Na/K pump could be involved in the ^{86}Rb efflux kinetics measured at 37°C. To check this possibility the same experiments were performed in the presence of 40 μg/ml of oubain in the extracellular solution. Oubain inhibition of the Na/K pump had no effect on the ^{86}Rb transport of SRE treated membrane (data not shown).

While at 37°C the ^{86}Rb efflux from the SRE modified RBCs reached tracer equilibrium and stopped within minutes after SRE addition, an efflux of hemoglobin from these cells did not cease for as long as 50 min (Fig. 2A). As for ^{86}Rb, the time course and kinetics of the hemoglobin efflux were similar to that found with RBCs having an altered sterol composition [27]. A semilogarithmic plot of the hemoglobin efflux (Fig. 3A) revealed that the kinetic data could not be fitted to one exponential curve. The deviation from the first exponent in 15 min after SRE addition indicates a time dependent inactivation of SRE pores in the modified cells. This provides an explanation for the inability of hemoglobin to reach an equilibrium distribution between the modified cells and the extracellular solution.

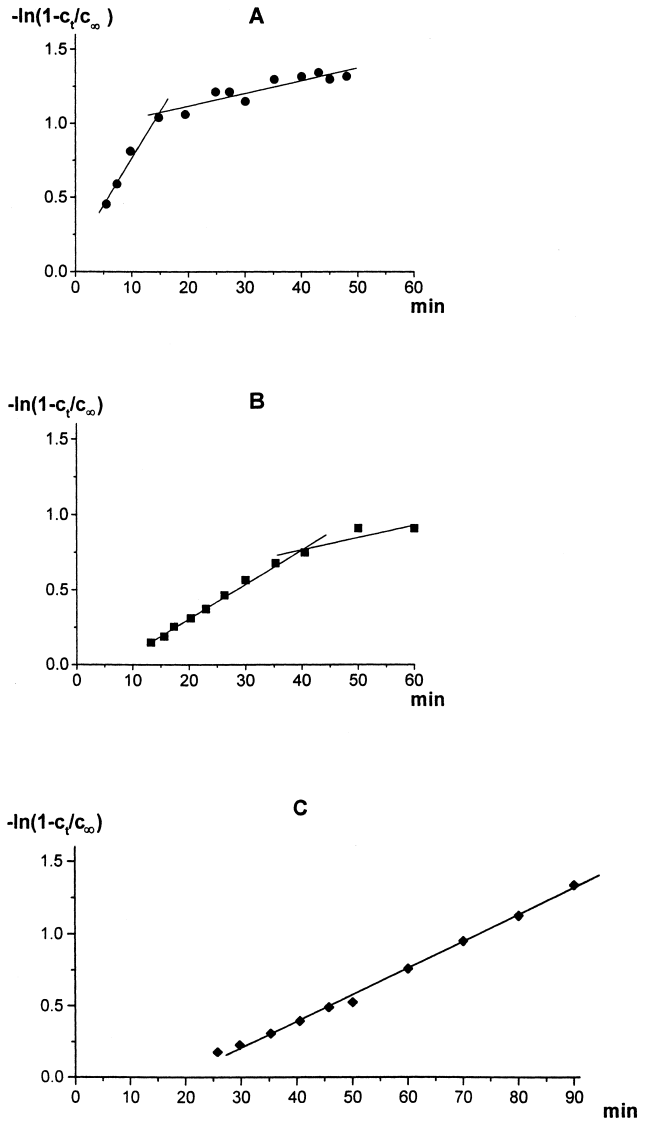

Fig. 3. Semilogarithmic representation of the kinetic data given in Fig. 2. The hemoglobin concentrations below c_0 were omitted (see text for explanation).

A discrepancy was observed in the measured and expected extracellular hemoglobin concentrations. We measured less extracellular hemoglobin in the first several minutes of transport than was calculated from lysis. The initial parts of the hemoglobin efflux curves (see Fig. 2B,C) reflected an adsorption phenomenon that obviously deformed the kinetic of the hemoglobin diffusion through SRE pores. The adsorption of hemoglobin to the vessel's wall was measured and it was shown that less than 20% of the 'lost' hemoglobin was adsorbed. Electrostatic interactions

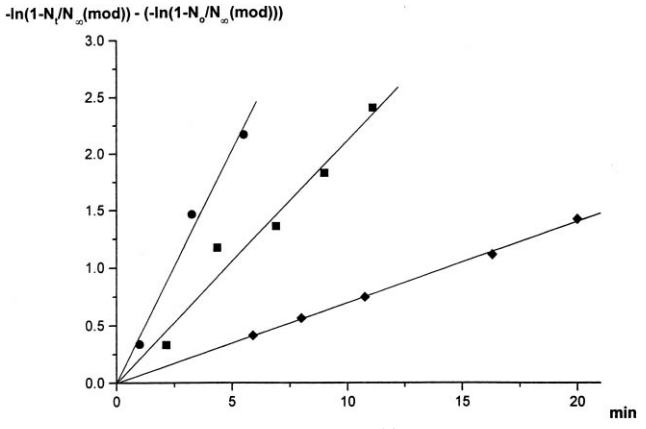

Fig. 4. Temperature dependence of ^{86}Rb efflux through SRE modified RBC membranes in semilogarithmic representation. Temperatures: (●, 37°C; ■, 20°C; ◆, 6°C). SRE was added at 0 min. N_0 is the percentage value of ^{86}Rb due to contamination and lysis. For an explanation of N_∞ and N_t as well as for the experimental conditions see Fig. 1.

were demonstrated between hemoglobin and human RBC membrane at a pH range of 6–7.4 [41–47]. At the pH of the present experiment (pH 6.8), 10% of the hemoglobin released by lysis may be adsorbed to the membrane [44]. Other mechanisms of the hemoglobin adsorption (e.g. to SRE or the membrane) are possible.

The ^{86}Rb (Fig. 4) and hemoglobin (Figs. 2B,C and 3B,C) effluxes were measured also at 20 and 6°C, below the phase transition temperature of the major lipid components of the RBC membranes [48]. In the temperature range from 6 to 37°C there was no significant difference either in the extent of lysis or in the portion of modified cells (Table 1). These results suggest a temperature independent interaction of SRE molecules with the membrane.

On the other hand rates of both ^{86}Rb and hemoglobin effluxes from the SRE modified RBCs showed temperature dependence (Table 2). From these data a mean Q_{10} value of 1.8 was obtained. This large Q_{10}

Table 1
Portion of lyzed and modified cells expressed as a percentage of the total number of RBCs at different temperatures

T (°C)	Lysis (%)	Modified cells (%)	n
6	9.3 ± 1.3	55.0 ± 13.2	2
20	10.2 ± 2.0	59.2 ± 11.8	5
37	16.2 ± 4.4	44.1 ± 9.6	6

SRE concentration was 6×10^6 molecules/cell (150 μg/ml RBCs).

value as compared to $Q_{10} = 1.2–1.3$ for free diffusion of ^{86}Rb and hemoglobin through water filled pores [49] may be a result of increasing numbers of SRE pores in the membranes of modified cells when increasing the temperature.

The inactivation of the SRE pores is a temperature dependent process. From the semilogarithmic representation of the hemoglobin transport kinetic data it was observed at 37°C that the transport curve deviated from the first exponent in about 15 min after the SRE addition (Fig. 3A). At 20°C this occurred 25 min later (Fig. 3B) and no sign of pore inactivation was obtained at 6°C for as long as 90 min (Fig. 3C).

The inactivation may be due to either a reduction in the number of channels or their dwell time of open state or reduction of single channel conductance. To clarify these possibilities the effect of temperature on inactivation of SRE pores was studied with planar lipid bilayers made from a mixture of phosphatidylserine and phosphatidylethanolamine, a negatively charged and a neutral phospholipid of RBC membrane. Fig. 5A,B shows the time course of integral current through the bilayer in the presence of SRE at different temperatures. With a temperature of 14°C (Fig. 5A) at a positive potential applied, the membrane conductance increased and a stable high conductance value was registered for about 10 min. However, at 23°C (Fig. 5B), a decrease in macroscopic conductance over time followed the increase in the SRE-induced conductance.

Experiments performed at the level of single channel conductance at 23°C showed that conductance of single channels and their dwell time did not change over time (Fig. 6A,B). This indicates that the inactivation shown in Fig. 5B is due to a marked decrease in the number of open pores rather then to a reduction of a single pore conductance or dwell time. This process may be called time-dependent inactivation of the channels and it may take place in both bilayers and RBCs.

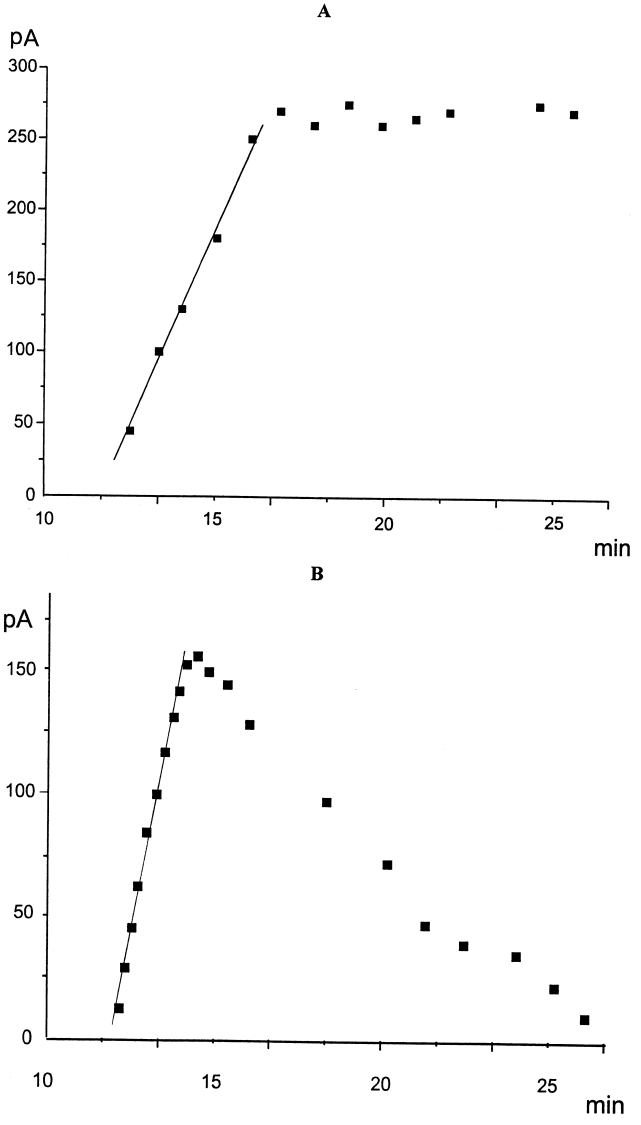

Fig. 5. Time courses of integral current in SRE modified bilayers recorded at 120 mV transmembrane potential difference at 14°C (A) and at 23°C (B). Salt concentration was: 100 mmol/l NaCl (pH 6). The membrane was formed from a lipid mixture of DOPS/DOPE (1:1, M/M). SRE was added to the bathing solution (*cis* side only) at a concentration of 2 μg/ml at $t = 0$ min.

Table 2
Rate constants of SRE-induced ^{86}Rb efflux (k_{Rb}) and Hb efflux (k_{Hb}) at different temperatures

T (°C)	k_{Rb}(1/min)	k_{Hb}(1/min)	n
6	0.06 ± 0.014	0.010 ± 0.006	2
20	0.24 ± 0.147	0.021 ± 0.003	3
37	0.34 ± 0.050	0.055 ± 0.010	6

SRE concentration was 6×10^6 molecules/cell (150 μg/ml RBCs).

Modification of RBC membranes showed no significant temperature dependence (Table 1) suggesting that the membrane easily accepted this foreign molecule. As the cluster formation is an interaction between the SRE and neighboring lipid molecules, the formation of pores may lead to the destruction of the

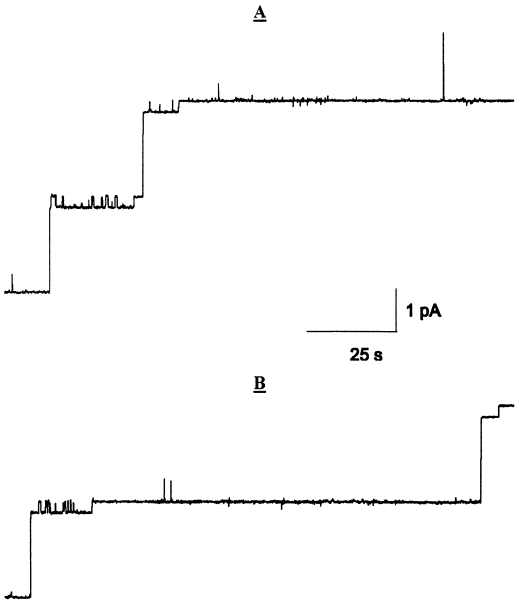

Fig. 6. The records of current fluctuations at −100 mV transmembrane potential difference in SRE modified bilayers in 13 min (A) and in 25 min (B) after SRE addition. Salt concentration was: 100 mmol/l NaCl (pH 6). The membrane was formed from a lipid mixture of DOPS/DOPE (1:1, M/M). SRE was added to the bathing solution (*cis* side only) at a concentration of 2 μg/ml. The temperature was 23°C.

structural integrity of the membrane. It is conceivable that pore inactivation may be a mechanism to repair lipid bilayer of the membrane. The temperature dependence of pore inactivation indicates that membrane fluidity is significant in this repairing process.

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References

[1] D.C. Gross, J.E. De Vay, F. Stadman, J. Appl. Bacteriol. 43 (1977) 453–460.
[2] A. Segre, R.C. Bachmann, A. Ballio, F. Bossa, I. Grgurina, N.S. Iacobellis, G. Marino, P. Pucci, M. Simmaco, J.Y. Takemoto, FEBS Lett. 255 (1989) 27–31.
[3] N. Fukuchi, A. Isogai, J. Nakayama, S. Takayama, S. Yamashita, K. Suyama, J.Y. Takemoto, A. Suzuki, J. Chem. Soc. Perkin Trans. 1 (1992) 1149–1157.
[4] E. Vaillo, A. Ballio, P.-L. Luisi, R.M. Thomas, Biopolymers 32 (1992) 1317–1326.
[5] I. Grgurina, A. Barca, S. Cervigni, M. Gallo, A. Scaloni, P. Pucci, Experimentia 50 (1994) 130–133.
[6] K.N. Sorensen, K.-H. Kim, J.Y. Takemoto, Antimicrob. Agents Chemother. 40 (1996) 2710–2713.
[7] K.N. Sorensen, A.A. Wanstrom, S.D. Allen, J.Y. Takemoto, J. Antibiot. (Tokyo) 51 (1998) 743–749.
[8] A.J. De Lucca, T.J. Jacks, J.Y. Takemoto, B. Vinyard, J. Peter, E. Navarro, T.J. Walsh, Antimicrob. Agents Chemother. 43 (1999) 371–373.
[9] J.E. De Vay, F.L. Lukezic, S.L. Sinden, H. English, D.L. Coplin, Phytopathology 58 (1968) 95–101.
[10] S.L. Sinden, J.E. De Vay, P.A. Backman, Physiol. Plant Pathol. 1 (1971) 199–213.
[11] D.C. Gross, J.E. De Vay, Physiol. Plant Pathol. 11 (1977) 1–11.
[12] J.J. Rich, D.K. Willis, J. Bacteriol. 179 (1997) 2247–2258.
[13] F.C. Adetuyi, A. Isogai, D. DiGiorgio, A. Ballio, J.Y. Takemoto, FEMS Microbiol. Lett. 131 (1995) 63–67.
[14] H.H. Reidl, J.Y. Takemoto, Biochim. Biophys. Acta 898 (1987) 59–69.
[15] L. Zhang, J.Y. Takemoto, Biochim. Biophys. Acta 987 (1989) 171–175.
[16] J.Y. Takemoto, L. Zhang, N. Taguchi, T. Tachikawa, T. Miyakawa, J. Gen. Microbiol. 137 (1991) 653–659.
[17] L. Zhang, J.Y. Takemoto, Biochim. Biophys. Acta 861 (1986) 201–204.
[18] A.P. Bidwai, J.Y. Takemoto, Proc. Natl. Acad. Sci. USA 84 (1986) 6755–6759.
[19] L. Camoni, D. Di Giorgio, M. Marra, P. Aducci, A. Ballio, Biochem. Biophys. Res. Commun. 214 (1995) 118–124.
[20] H. Batoko, A. de Kerchove-d'Exaerde, J.M. Kinet, Biochim. Biophys. Acta 1372 (2) (1998) 216–226.
[21] M.L. Hutchison, M.A. Tester, D.C. Gross, Mol. Plant-Microbe Interact. 8 (1995) 610–620.
[22] M. Dalla Serra, G. Fagiuoli, P. Nordera, I. Bernhart, C. Della Volpe, D. Di Giorgio, A. Ballio, G. Menestrina, Mol. Plant-Microbe Interact. 12 (1999) 391–400.
[23] Y.A. Kaulin, L.V. Schagina, S.M. Bezrukov, V.V. Malev, A.M. Feigin, J.Y. Takemoto, J.H. Teeter, J.G. Brand, Biophys. J. 74 (1998) 2918–2925.
[24] L.V. Schagina, Y.A. Kaulin, A.M. Feigin, J.Y. Takemoto, J.G. Brand, V.V. Malev, Membr. Cell Biol. 12 (1998) 537–555.
[25] A.M. Feigin, J.Y. Takemoto, R. Wangspa, J.H. Teeter, J.G. Brand, J. Membr. Biol. 149 (1996) 41–47.
[26] A.M. Feigin, L.V. Schagina, J.Y. Takemoto, J.H. Teeter, J.G. Brand, Biochim. Biophys. Acta 1324 (1997) 102–110.
[27] K. Blasko, L.V. Schagina, G. Agner, Y.A. Kaulin, J.Y. Takemoto, Biochim. Biophys. Acta 1373 (1998) 163–169.

[28] J.Y. Takemoto, Y. Yu, S.D. Stock, T. Miyakawa, FEMS Microbiol. Lett. 114 (1993) 339–342.
[29] N. Taguchi, Y. Takano, C. Julmanop, Y. Wang, S. Stock, J.Y. Takemoto, T. Miyekawa, Microbiology 140 (1994) 353–359.
[30] R. Wangspa, J.Y. Takemoto, FEMS Microbiol. Lett. 167 (1998) 215–220.
[31] P. Cliften, Y. Wang, D. Mochizuki, T. Miyakawa, R. Wangspa, J. Hughes, J.Y. Takemoto, Microbiology 142 (1996) 477–484.
[32] M.M. Grilley, S.D. Stock, R.C. Dickson, R.L. Lester, J.Y. Takemoto, J. Biol. Chem. 273 (1998) 11062–11068.
[33] Y.A. Kaulin, L.V. Schagina, A.M. Feigin, R. Wangspa, J.Y. Takemoto, J.H. Teeter, J.G. Brand, Biophys. J. 76 (1999) A182.
[34] V. Malev, Y.A. Kaulin, J.Y. Takemoto, J.H. Teeter, J.G. Brand, L.V. Schagina, Biophys. J. 76 (1999) A181.
[35] L.V. Schagina, K. Blasko, A.E. Grinfeldt, Y.E. Korchev, A.A. Lev, Biochim. Biophys. Acta 978 (1989) 145–150.
[36] L.V. Schagina, Y.E. Korchev, A.E. Grinfeldt, A.A. Lev, K. Blasko, Biochim. Biophys. Acta 1109 (1992) 91–96.
[37] A.P. Bidwai, L. Zhang, R.C. Bachmann, J.Y. Takemoto, Plant Physiol. 83 (1987) 39–43.
[38] M. Montall, P. Muller, Proc. Natl. Acad. Sci. USA 69 (1972) 3561–3566.
[39] S.M. Bezrukov, I. Vodyanoy, Biophys. J. 64 (1993) 16–25.
[40] M.T. Tosteson, S.J. Holmes, M. Razin, D.C. Tosteson, J. Membr. Biol. 87 (1985) 35–44.
[41] N. Shaklai, J. Yguerabide, H.M. Ranney, Biochemistry 16 (1977) 5585–5592.
[42] N. Shaklai, J. Yguerabide, H.M. Ranney, Biochemistry 16 (1977) 5593–5597.
[43] P.B. Rauenbuehler, K.A. Cordes, J.M. Salhany, Biochim. Biophys. Acta 692 (1982) 361–370.
[44] L.W.M. Fung, Biochemistry 20 (1981) 7162–7166.
[45] G.L. Lilley, L.W.M. Fung, Life Sci. 41 (1987) 2429–2436.
[46] J. Eisinger, J. Flores, J.M. Salhany, Proc. Natl. Acad. Sci. USA 79 (1982) 408–412.
[47] S. Claster, E. White, V. Woolworth, A. Quintanilha, Arch. Biochem. Biophys. 285 (1991) 147–152.
[48] G. Zimmer, H. Schwimmer, Biochim. Biophys. Acta 345 (1974) 314–317.
[49] B. Hille, Ionic Channels of Excitable Membranes, Sinauer Associates Inc., Sunderland, MA, 1984, p. 426.