ISSP International Workshop on Soft Matter Physics: Biomembranes and Vesicles
on 24 Aug. 2010, at IPMU Lecture Hall

Kinetic Pathway of Antimicrobial Peptide Magainin 2-Induced Pore Formation in Lipid Membranes

1. Antimicrobial peptide magainin2-induced pore formation
2. Toxin protein lysenin-induced pore formation
3. Antimicrobial substance EGCg-induced pore formation

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Antimicrobial peptides:

Defensive weapons produced by animals (amphibians, mammals), insects, and plants to kill bacteria and fungi

More than 500 kinds of AMPs

Structure	Name of AMPs	origin	Number of aa	Positive aa
α-helix	Cecropin A	Silk moth	41 aa	6K+1R
	Magainin 2	frog	23	4K
	Dermaseptin 1	frog	41	4K
	LL-37	human	41	4K+4R
	Buforin II	vertebrate	22	1K+4R
	PGLa	frog	21	4K
1 S-S bond	Bactenesin 1	Cow	12	4R
3 S-S bond	α-defensin	human	30	4R
	β-defensin	Cow	38	5R
β-sheet	Protegrin-1	Porcine	18	6R
	Lactoferricin B	Bovine milk	15	2K+5R
linear, non-α-helix	indolicidin	Cow	13	1K+2R

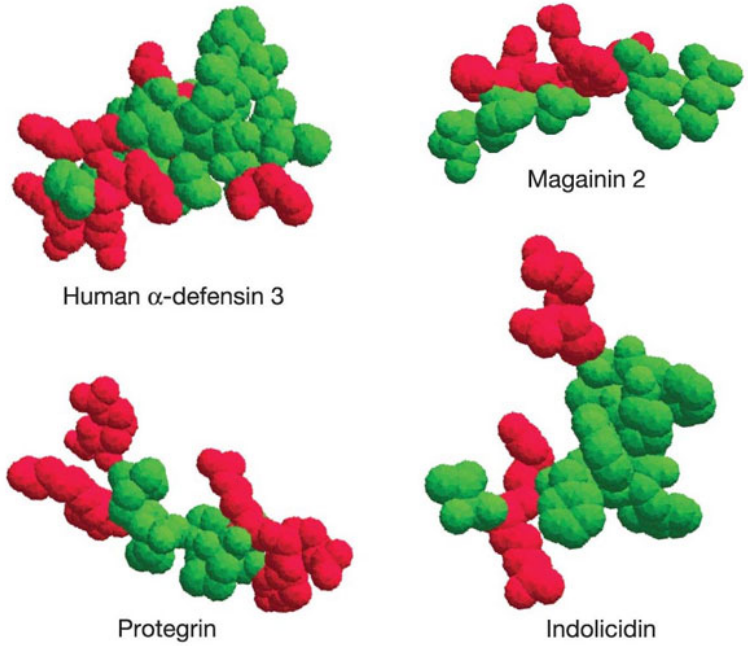
Structure of antimicrobial peptides

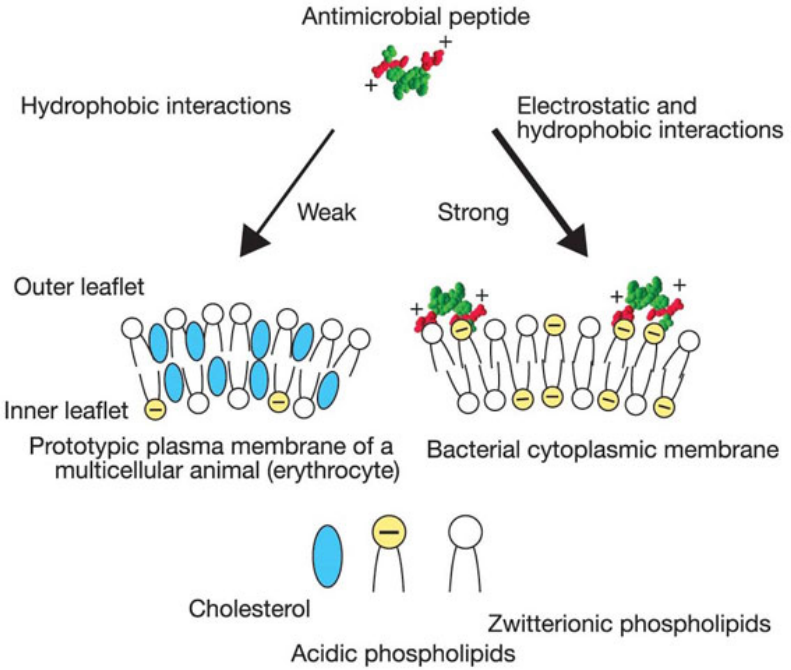
1.Peptides with 10-50 amino acids

2. Containing many cationic amino acids such as Lysine (K) and Arginine (R)

3. Clustering of cationic and hydrophobic amino acids into distinct domains

Binds to negatively charged lipid membranes such as external surface of bacterial membranes





Nature, 415, 389, 2002, M. Zasloff

Antimicrobial peptide magainin 2

(from African clawed frog Xenopus laevis)

the first AMP discovered in vertebrates (1987)

its main target is lipid membrane region in cell membranes

(All D-amino acid magainin 2 had the same antibacterial activity as that of natural, all-L amino acid magainin 2)

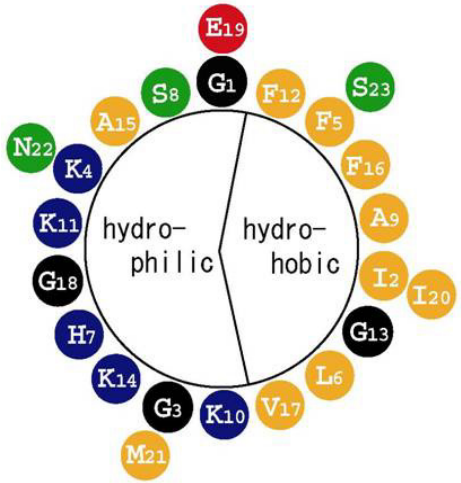
Magainin 2 has 23 amino acids,
and net positive charges due to 4 Lys residues.

⇨

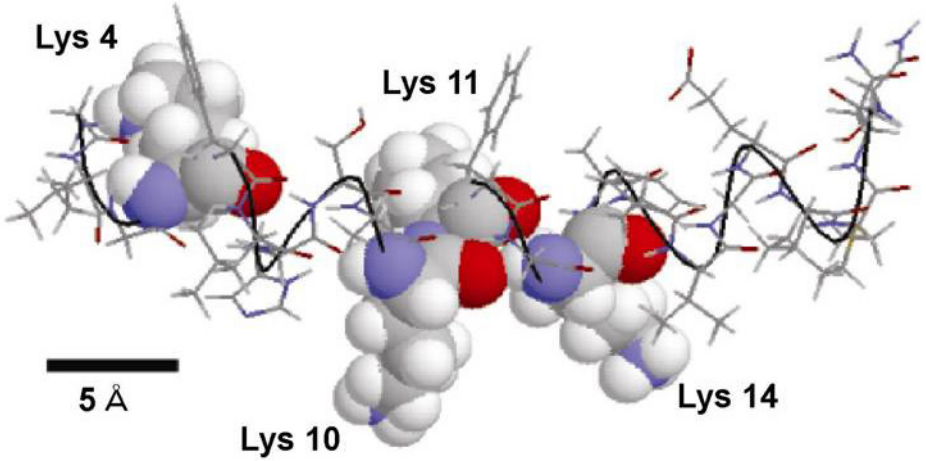
Binds to negatively charged lipid membranes such as external surface of bacterial membranes

Gly-Ile-Gly-Lys-Phe-Leu-His-Ser-Ala-Lys-Lys-Phe
–Gly-Lys-Ala-Phe-Val-Gly-Glu-Ile-Met-Asn-Ser

Magainin 2 forms α -helix structure in membrane interface



Side view

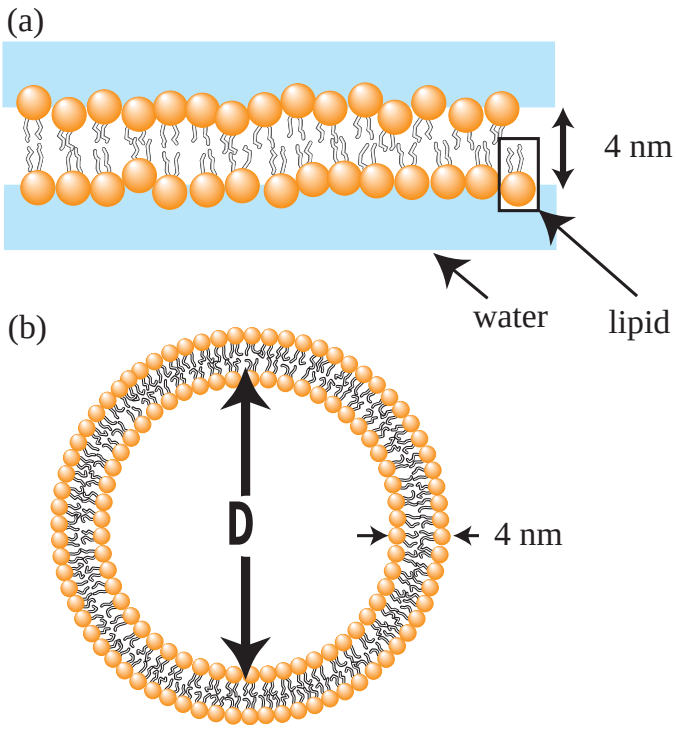


To reveal the mechanism of the bactericidal activity of AMPs

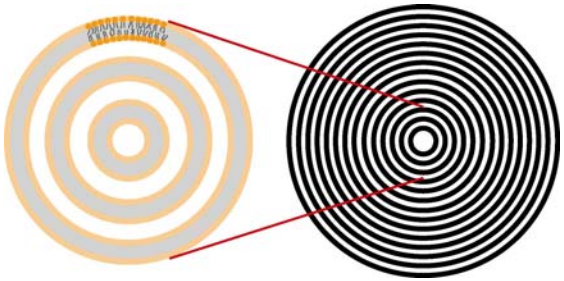
- The interaction of AMPs with lipid membranes
  ➔ using liposomes (or vesicles) of lipid membranes

Unilamellar Vesicle (Liposome)

Closed surfaces composed of lipid membranes with various shapes such as sphere, prolate, discocyte and tube.



Multilamellar vesicle (MLV)



Small Unilamellar Vesicle (SUV)

D: 25 ~ 50 nm

Large Unilamellar Vesicle (LUV)

D: 50 nm ~ 10 μm

Giant Unilamellar Vesicle (GUV)

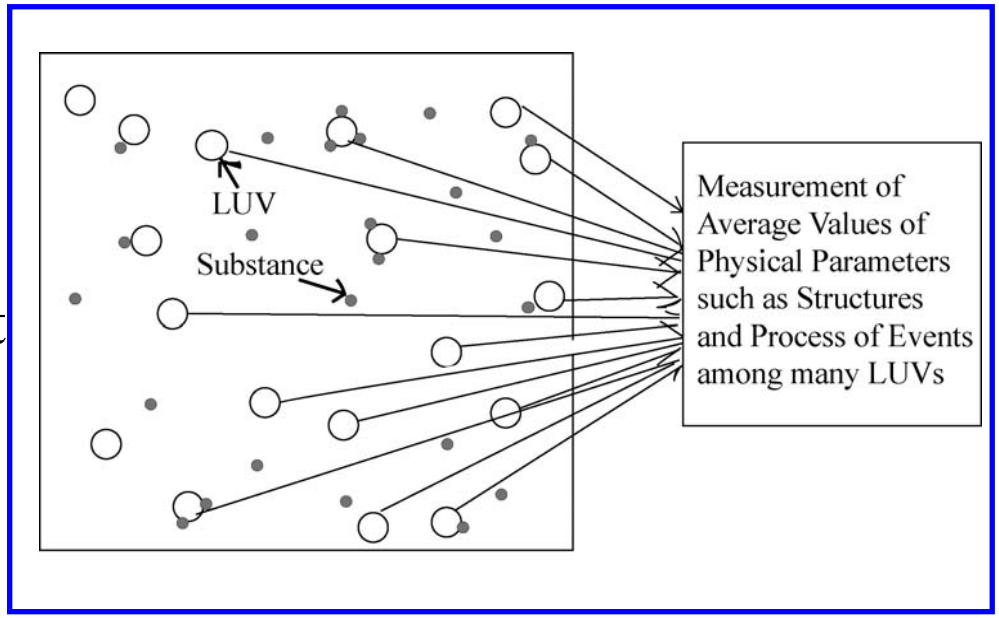
(Giant liposome)

D ≥ 10 μm

cell size (10~50 μm)

LUV suspension method

Most studies of liposomes of biomembrane/lipid membrane



So far, almost all studies of liposomes have been carried out on a suspension of many small liposomes (their diameter 50~500 nm) such as LUV (Large Unilamellar Vesicle) using fluorescence spectroscopy, light scattering, and ESR.

⇒ **(1) The average values of physical parameters of LUVs can be obtained.**
(2) Various events such as membrane fusion and pore formation in each LUV do not occur simultaneously.

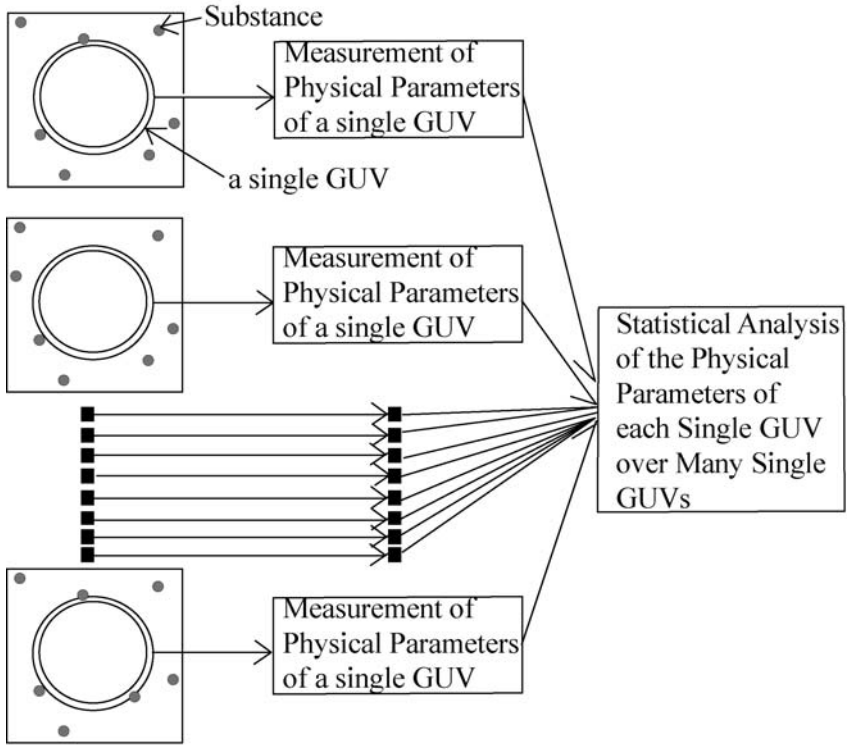
⇒ A lot of various information is lost.
Elementary process of many events cannot be observed.

The Single GUV method

(1) Observe structure and physical properties of a single GUV (Giant Unilamellar Vesicle) and interaction of substances with single GUVs as a function of time and spatial coordinates, using various optical microscopes

(2) Statistical analysis of physical properties of a single GUV over many "single GUVs"

⇒ **Individual events** in single GUVs such as pore formation and membrane fusion can be observed, and so we can investigate the **detailed elementary process** of these events. Statistical analysis of single events in single GUVs over many GUVs can give important information such as **rate constants of elementary process**.



Biophys. J., 92, 3178, 2007, Yamazaki et al.
Adv. Planar Lipid Bilayers & Liposomes, *Elsevier*, *7*, 121-142, 2008, Yamazaki

the Single GUV Method

＜Contents＞

1. Interaction of antimicrobial peptides and antibacterial substances with lipid membranes
2. Membrane fusion and vesicle fission

＜Ref.＞

1.*e-Journal Surface Science and Nanotechnology*, 3, 218-227, 2005

Adv. Planar Lipid Bilayers and Liposomes, *Elsevier*, 7, 121, 2008.

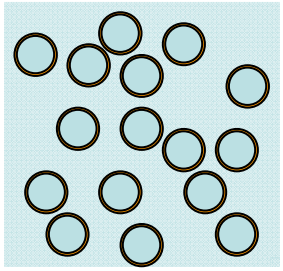
2. *Biochemistry*, 44, 15823, 2005, *J. Phys. Chem. B.*, 113,4846, 2009

4. *Biophys. J.*, 92, 3178, 2007

5. *Langmuir*, 20, 5160, 2004, *ibid*, 20, 9526, 2004,

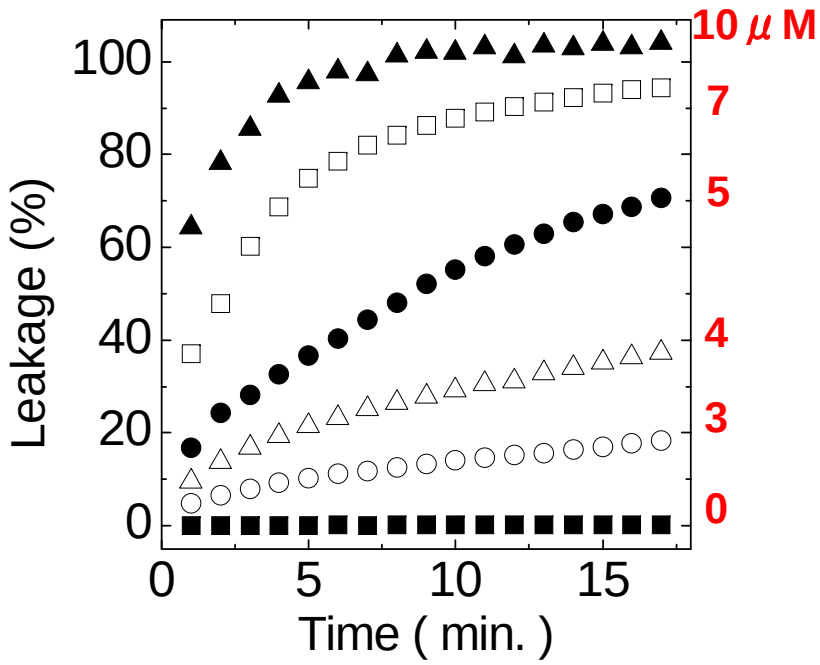
Langmuir, 23, 720, 2007

A typical experiment to detect the interaction of substances (e.g., antimicrobial peptides) with lipid membranes:
⇒ The measurement of leakage of internal contents (such as a fluorescent probe) from small liposomes using LUV suspension (i.e., the LUV suspension method)



The leakage from the LUV suspension increased gradually with time. ⇒

Leakage of calcein from a suspension of 50%DOPG/DOPC-LUV induced by magainin 2

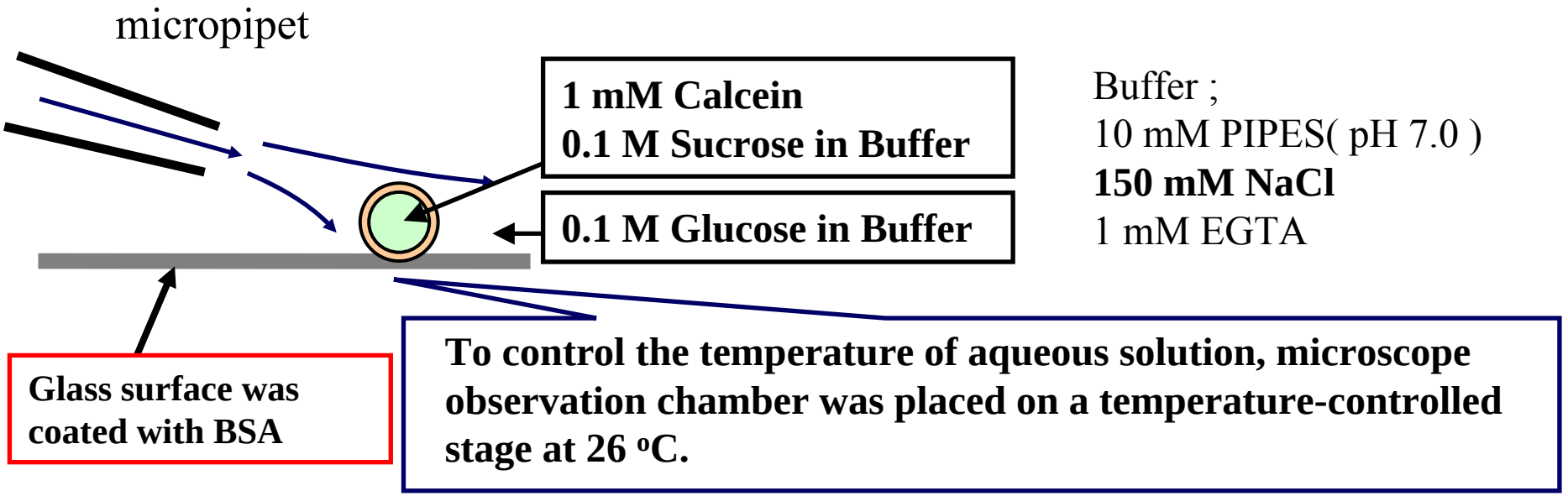


Various causes of leakage

1. Instability of membrane structure at large deformation, membrane fusion
2. Formation of pores and ion channels
3. Rupture of liposomes

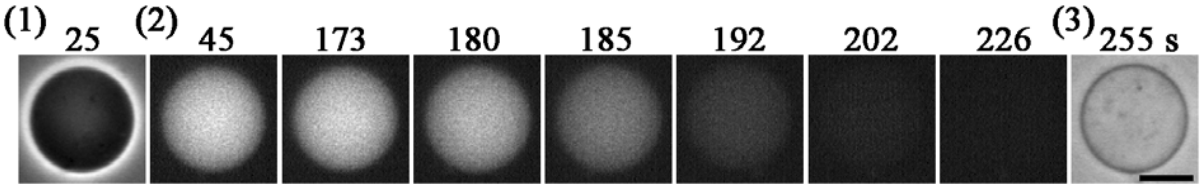
Method

Mixture membranes of negatively charged lipid, DOPG, and electrically neutral lipid, DOPC, were used to change the surface charge density.



Magainin 2 solutions were introduced in the vicinity of a single GUVs through a micropipete. And the structure and the fluorescence intensity of single GUVs were observed using a fluorescence phase-contrast microscope with EM-CCD camera.

Induction of calcein leakage from 60%DOPG/40%DOPC-GUV by 3 μM magainin 2

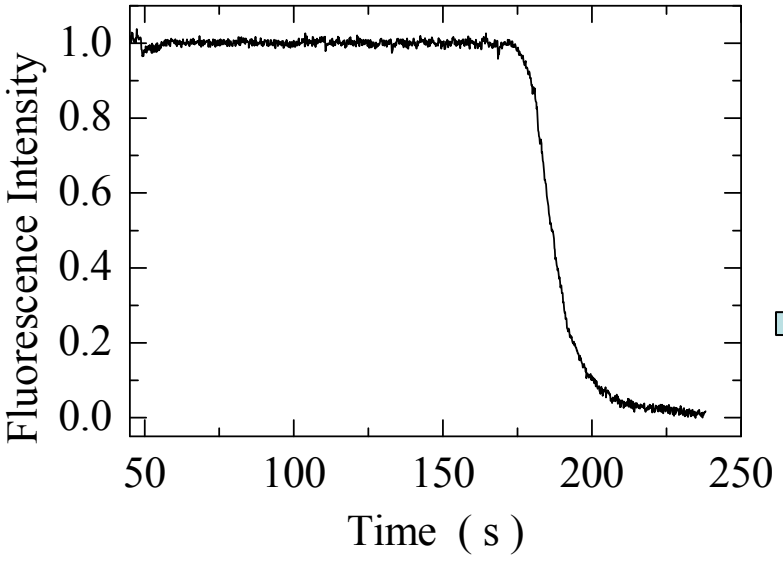


The GUV was not broken and not deformed

(1)(3) Phase contrast image

(2) Fluorescence microscopic image

Scale Bar; 10 μm

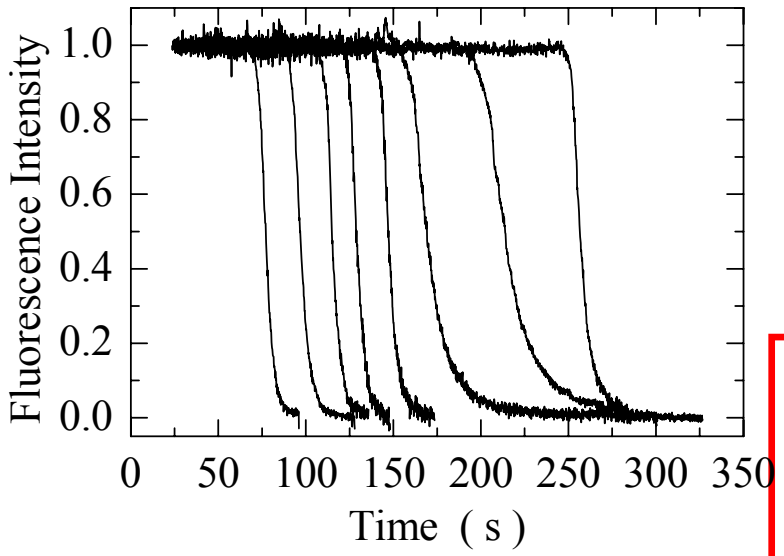


The rapid decrease in the fluorescence intensity occurred due to the leakage of calcein. During the leakage, the GUV was not broken, and no association and no fusion occurred.

Magainin 2 formed pores in the GUV membrane, and calcein and sucrose leaked through the pores.

Induction of calcein leakage from 60%DOPG/40%DOPC-GUV by 3 μM magainin 2

We made the same experiments using many single GUVs.



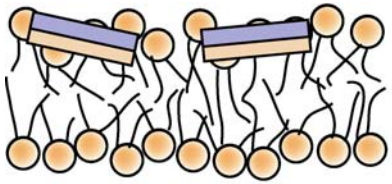
The leakage of calcein from a GUV started stochastically, but once it began, the complete leakage occurred rapidly within 30 s.

To estimate the leakage, the fraction of the leaked GUV at *t*, $P_{LS}(t)$, is important.

$P_{LS}(t)$, the probability that leakage had already started in a GUV, or that leakage had been completed in a GUV, among the population of GUVs examined, at any given time *t* during the interaction between magainin 2 and the GUV.

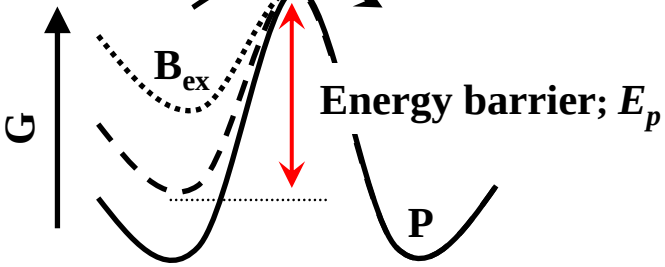
$P_{LS}(t)$ increased with time.

Two-state transition model



k_p → **Pore state (P state)**

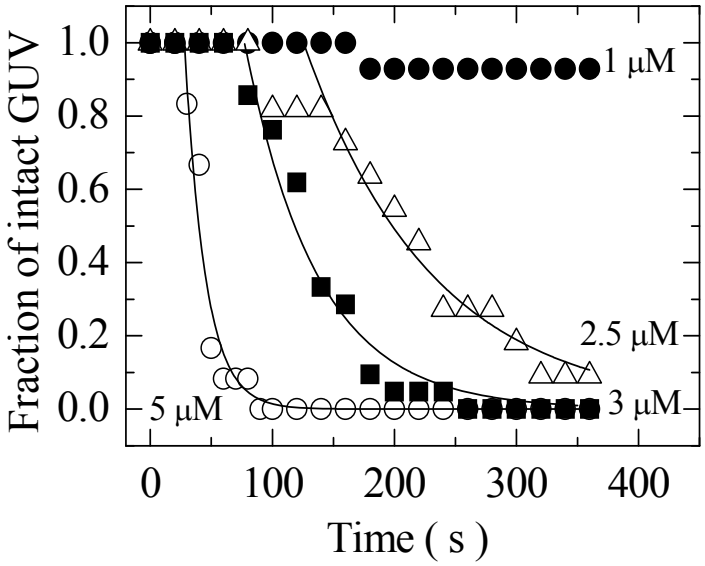
$$k_p = A\exp\left(-E_p / k_B T\right)$$



B_{ex} state

The fraction of the B_{ex} state equals to the fraction of intact GUV from which fluorescent probe did not leak, among all the examined GUVs, $P_{intact}(t)$.

The rate constant of the transition from the B state to the P state, k_p, can be obtained by the analysis of the time course of the fraction of intact GUV.



$$P_{\text{intact}}(t) = \exp\{-k_P (t - t_{eq})\}$$

5 μM magainin 2: $k_p = (5 \pm 1)\times 10^{-2}\ s^{-1}$

2 μM magainin 2: $k_p = (1.7 \pm 0.7)\times 10^{-3}\ s^{-1}$

***Biochemistry*, 44, 15823, 2005, Tamba & Yamazaki**

Effect of Surface Charge Density of Lipid Membranes on the Pore Formation Induced by Antimicrobial Peptide Magainin 2: the Single GUV Method Study

<Purpose>

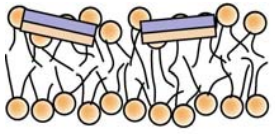
To elucidate the mechanism of the magainin 2-induced pore formation, we investigated the effect of surface charge density of membranes on the pore formation.

<Method>

Surface charge density was modulated by using GUVs composed of a mixture of negatively charged DOPG, and electrically neutral DOPC in which the concentration of DOPG (mol%) in the membrane was controlled.

J. Phys. Chem. B., 113,4846, 2009, Tamba and Yamazaki

Dependence of the rate constant of magainin 2-induced pore formation on surface charge density of membranes

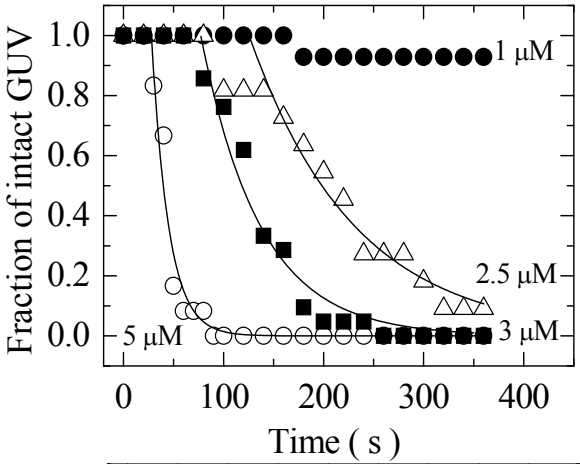


Pore state (P state)

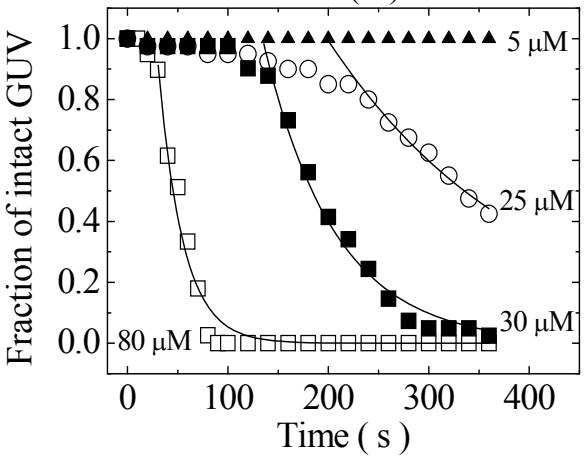
B_{ex} state

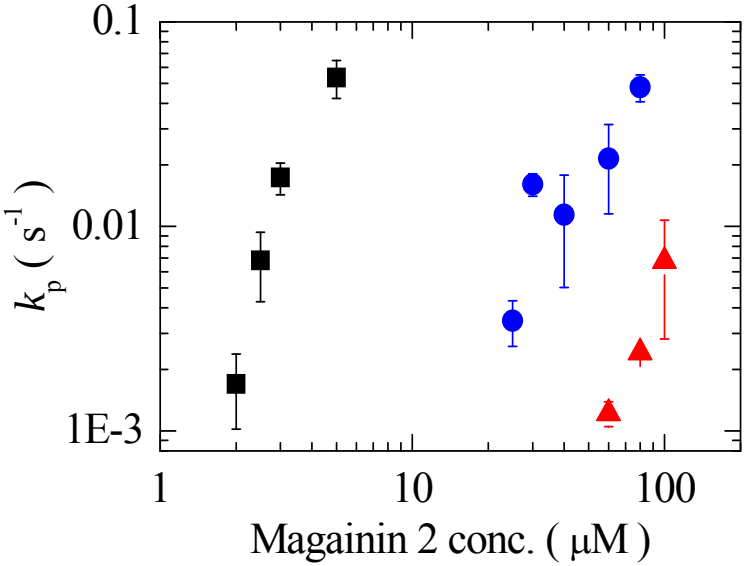
$$P_{intact}(t) = \exp\{-k_P(t - t_{eq})\}$$

60%DOPG/ DOPC-GUV



40%DOPG/ DOPC-GUV





■ ; 60% DOPG / 40%DOPC

● ; 40% DOPG / 60% DOPC

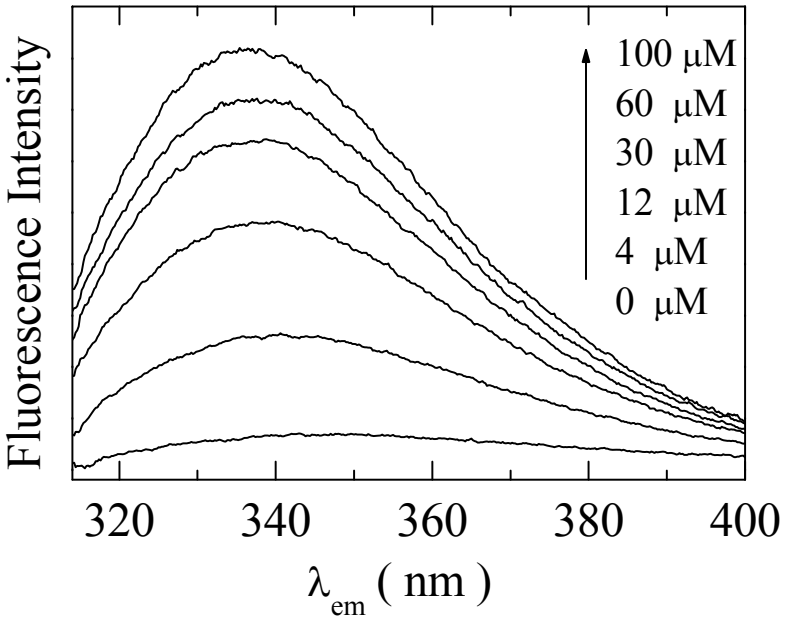
▲; 30% DOPG / 70% DOPC

We can consider that the amount of magainin 2 bound with the membrane interface of GUVs (magainin 2 surface conc.) decreased with a decrease in the surface charge density in the presence of the same magainin 2 concentration in the buffer, due to the decrease in the electrostatic attraction of magainin 2 with the membranes.

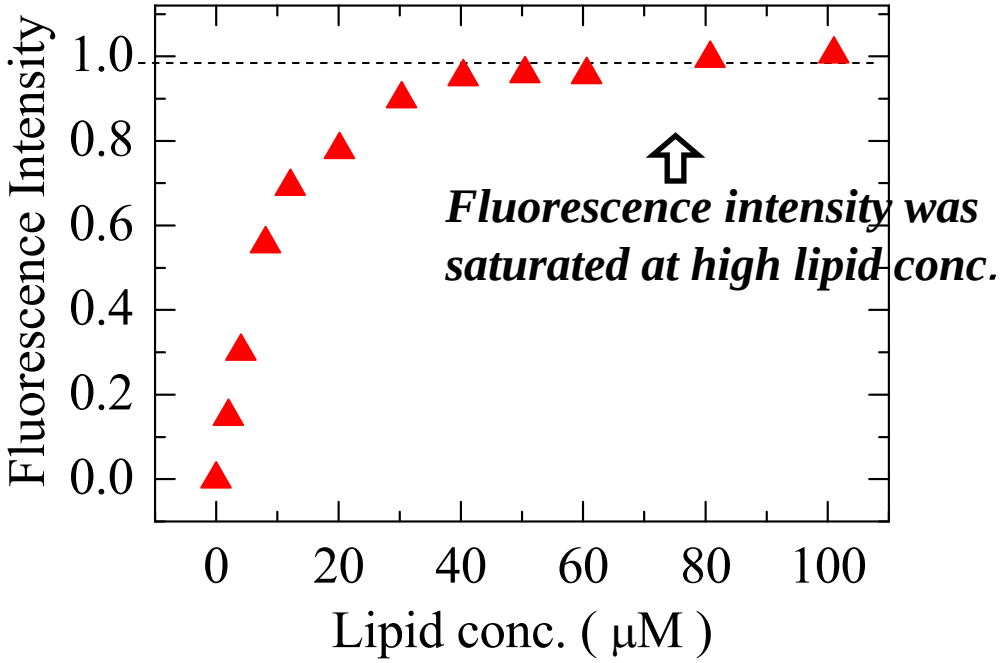
Estimation of the magainin 2 concentration in the membrane interface

F5W-magainin 2 **H-GIGKWLHSAKKFGKAFVGEIMNS-CONH$_2$**

Fluorescence Spectra of 0.1 mM F5W-magainin 2 with 30%DOPG/DOPC-LUVs



Dependence of the fluorescence intensity of F5W-magainin 2 on the lipid concentration



Fluorescence Intensity increased with an increase in lipid concentration. This increasing of fluorescence Intensity indicate that the F5W-magainin2 bound to lipid membrane.

Relationship between the magainin2 concentration in the membrane interface, X_b, and magainin2 concentration in the bulk, C_{eq}

Magainin 2 conc. immediately above the membrane surface, C_M is much larger than C_{eq}.

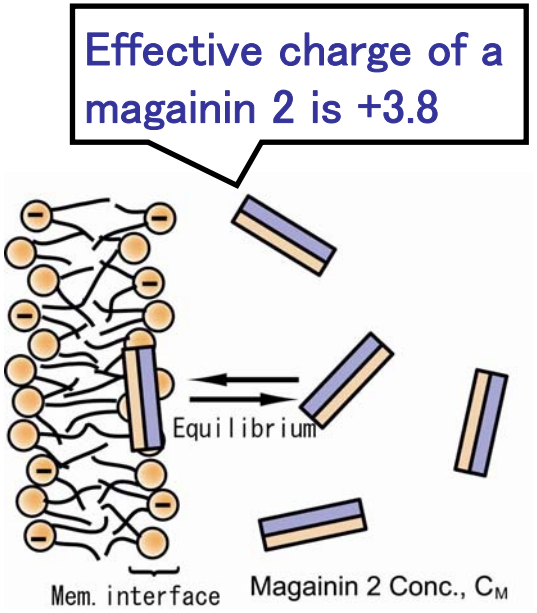
$$C_M = C_{eq} \exp\left(-3.8 e \varphi_o / k_B T\right)$$

where φ_o is the surface potential of the membrane

$$X_b = K_{int} C_M = K_{int} \exp\left(-3.8 e \varphi_o / k_B T\right) \cdot C_{eq}$$

X_b : The molar ratio of the magainin 2 bound with the membrane interface to lipids in the membrane (mol/mol)

K_{int} : The intrinsic binding constant of magainin 2 with lipid membrane



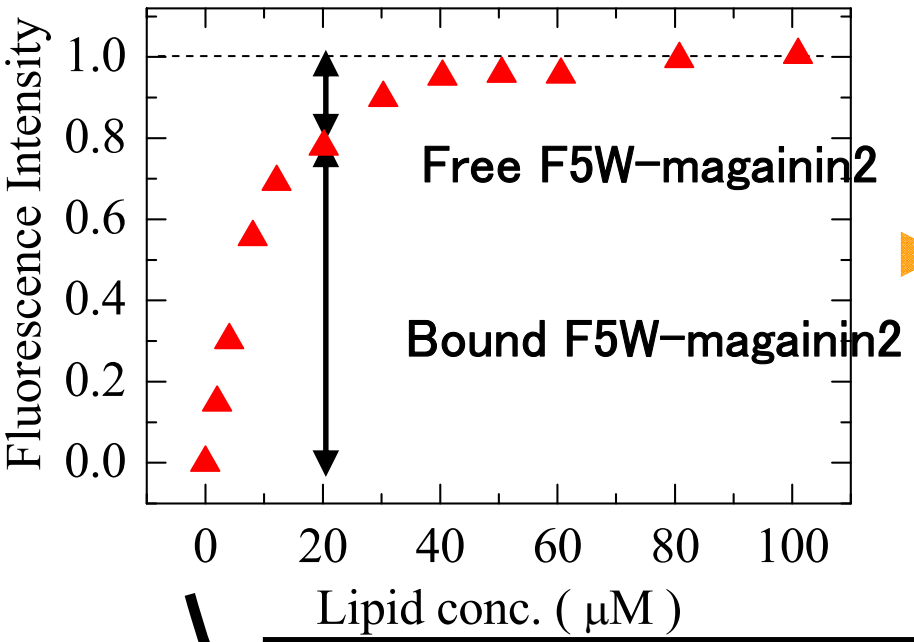
φ_o is determined by the surface charge density σ.

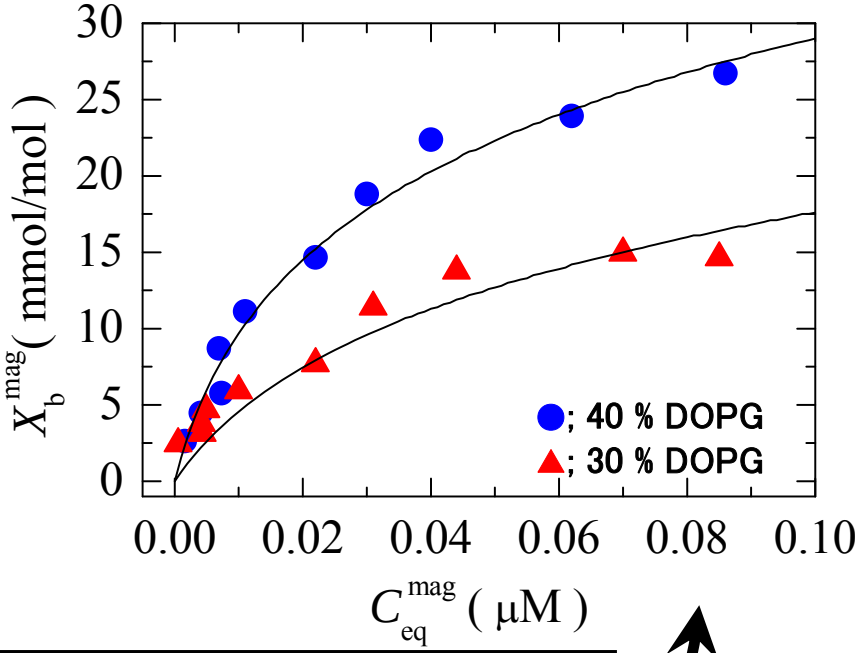
$$\varphi_o = \frac{2k_B T}{e} \sinh^{-1}\left(B \cdot \sigma\right) \quad ; \quad B = \left(8 \times 10^3 \cdot \varepsilon_o \varepsilon_r CRT\right)^{-1/2}$$

$$\sigma = \frac{e}{A}\left(-X_{PG} + X_{Na} + 3.8 \cdot X_b\right)$$

where C is salt concentration, A is the surface area of lipid, X_{PG} and X_{Na} are the molar ratio of the DOPG and bound Na^+ ions# (K_{int}=0.6 M^{-1}) per total Lipids, respectively.

Determination of intrinsic binding constant of magainin2 with lipid membrane, K_{int}





$X_b = 2 \cdot$ (Total magainin2 conc.) × (Normalized F at C_L) / C_L

$C_f =$ (Total magainin2 conc.) × (1 − Normalized F at C_L)

C_L : Lipid concentration

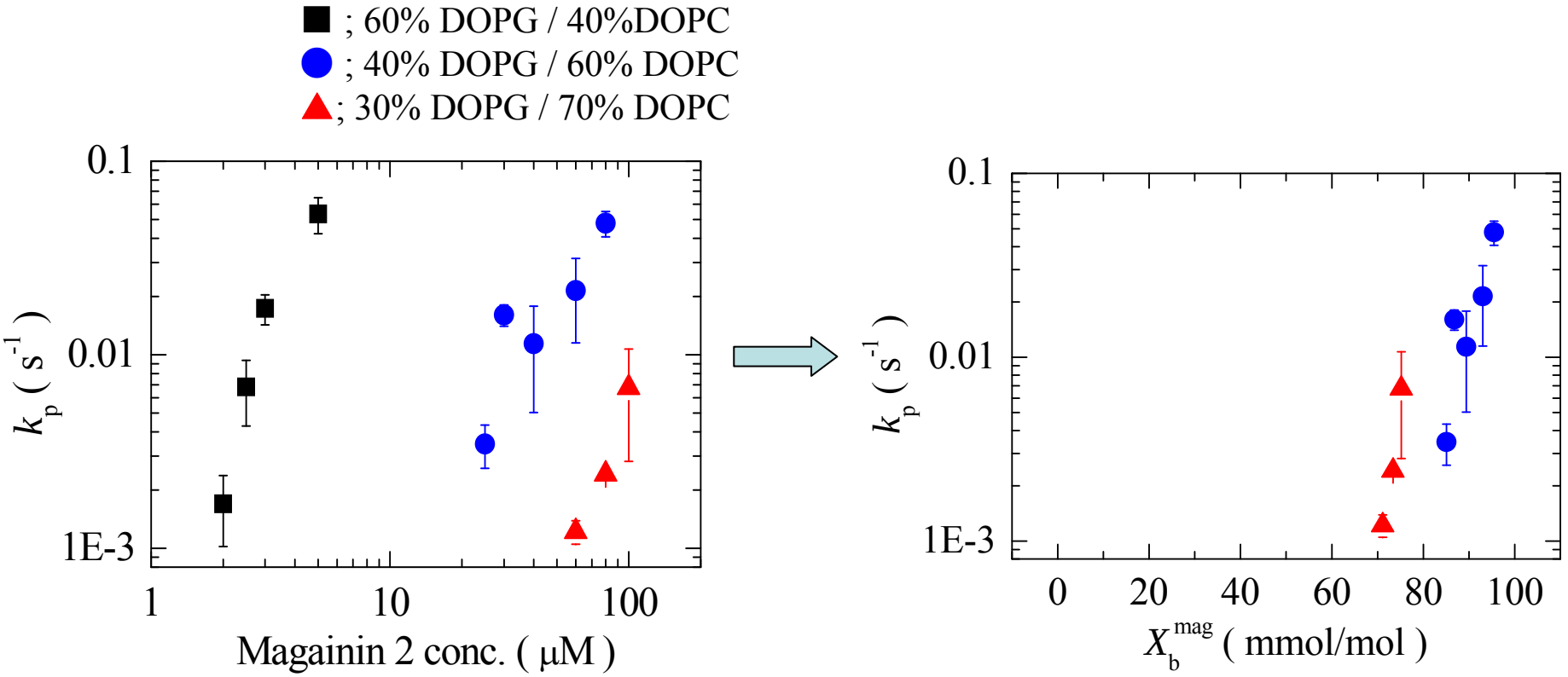
The solid line represent the fit of the data to the equation :

$$X_b = K_{int} \exp\left(-3.8e\varphi_o / k_B T\right) \cdot C_{eq}$$

PG Conc.	K_{in}
30%	1100±100
40%	1200 ±100

The theoretical curves were in good agreement with the experimental ones for 30 and 40%DOPG/DOPC Membranes.

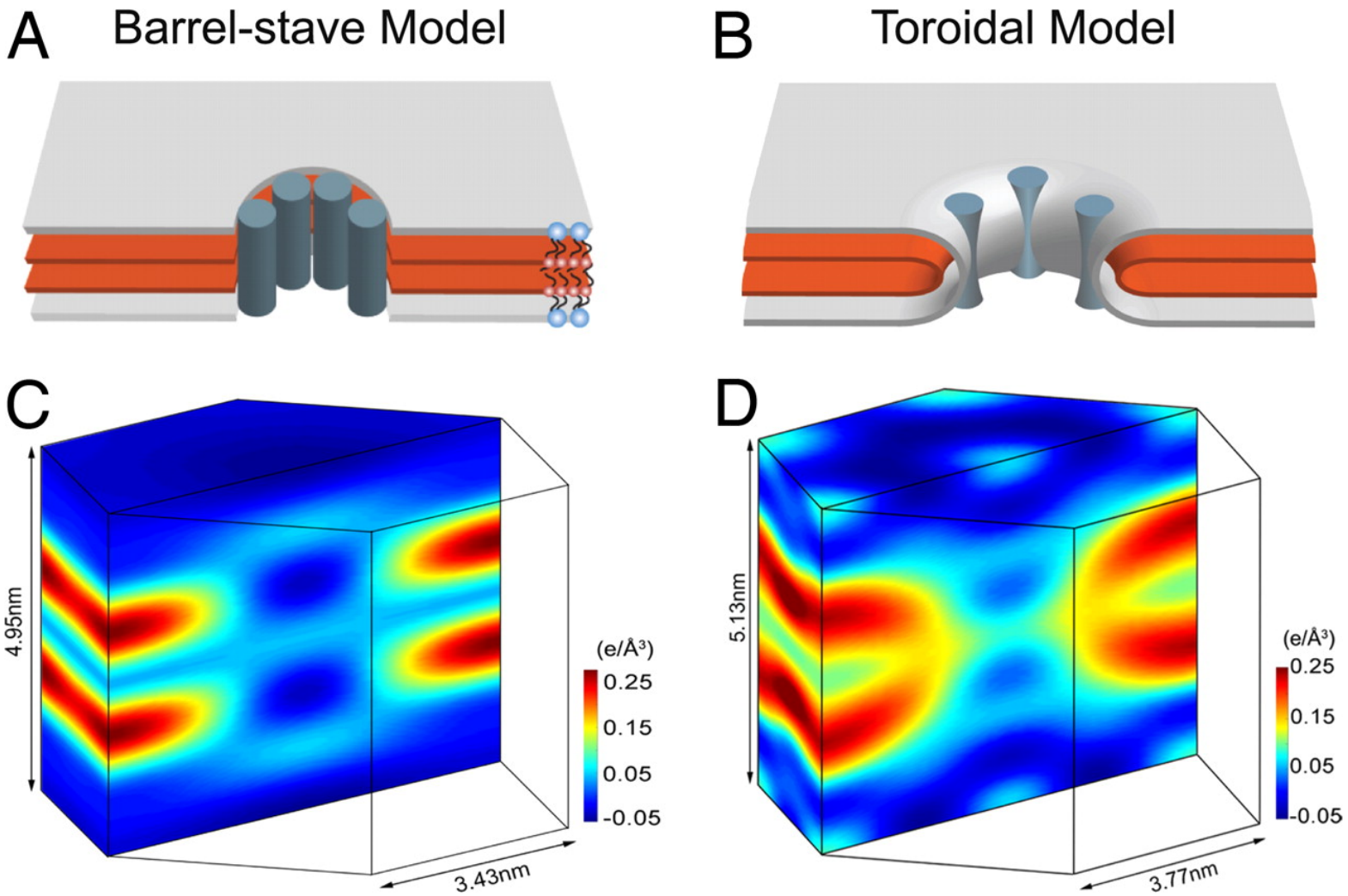
Dependence of the rate constant of pore formation on the magainin 2 concentration in the membrane interface



The rate constant of the magainin 2-induced pore formation is mainly determined by the magainin 2 concentration in lipid membrane interface.

J. Phys. Chem. B., 113,4846, 2009

Two models of pores composed of α-helix in membranes



PNAS 2008;105:17379-17383
Qian S. H.W. Huang et.al.

Kinetic Pathway for the magainin 2-induced pore formation in lipid membranes

<Purpose>

It is important to elucidate how to form the magainin 2-induced pores in lipid membranes, i.e., the kinetic pathway of the pore formation in lipid membranes.

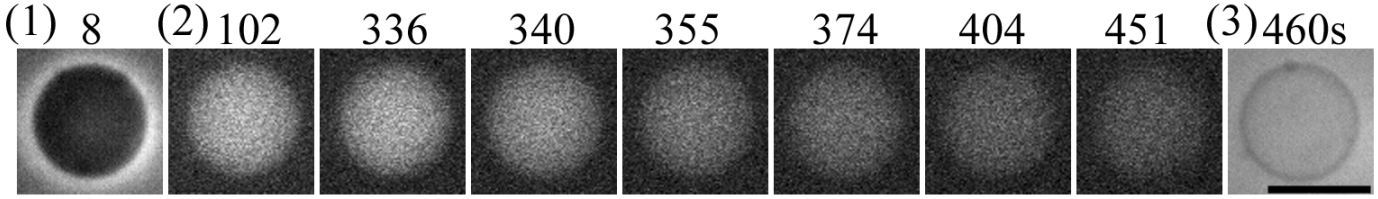
<Method>

To reveal the sizes of the magainin 2-induced pores in lipid membranes and its change during the formation of the stable pores, we investigated the permeability of various sizes of fluorescent probes through the magainin 2-induced pores in single GUVs of 50mol%dioleoylphosphatidylglycerol (DOPG)/50mol%dioleoyl-phosphatidylcholine (DOPC) membranes using the single GUV method. For fluorescent probes, we used Texas-Red Dextran (TRD) of various molecular weight and FITC-albumin.

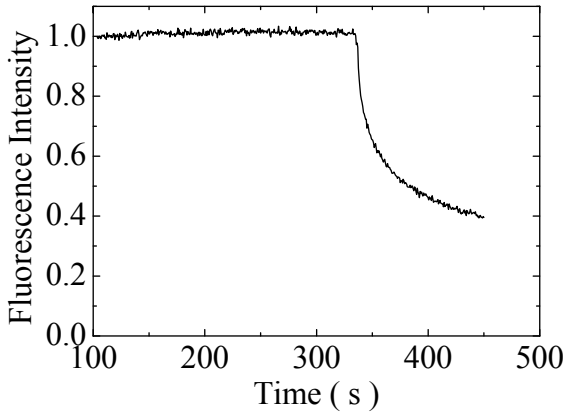
***J. Phys. Chem. B., in press,* 2010, Ariyama, Tamba, Levadny,Yamazaki**

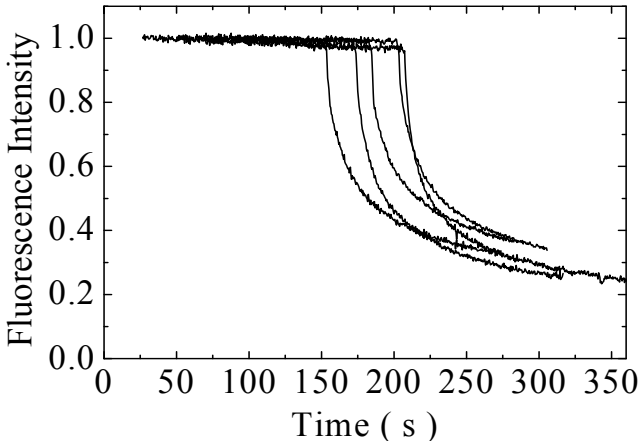
Induction of leakage of Texas Red Dextran 10k from 50%DOPG/DOPC-GUV by 7 μM magainin 2

Texas Red Dextran 10,000 (TRD-10k) (R_{SE}=2.7 nm)



(1)(3) Phase contrast image **(2) Fluorescence microscopic image** **Scale Bar; 10 μm**



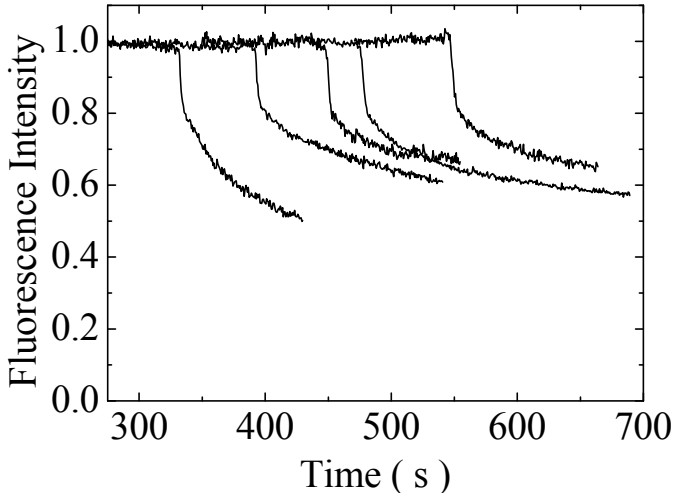


The magainin 2-induced leakage of TRD-10k had two phases:
The transient rapid leakage in the initial stage and the following slow leakage.

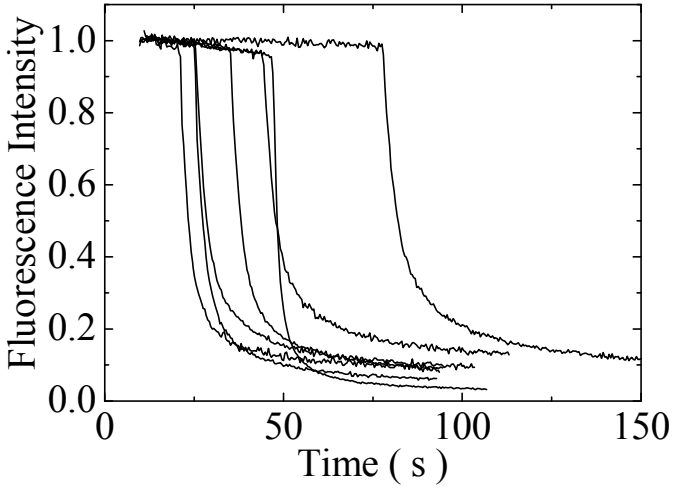
Magainin 2 molecules formed a large pore in the lipid membrane in the initial stage and then they rearrange to form smaller pores.

Induction of leakage of TRD-10k from 50%DOPG/DOPC-GUV

4 μM magainin 2



15 μM magainin 2



The two phases of leakage was more clearly observed.

The transient rapid leakage in the initial stage and the following slow leakage.

The amount of the leakage of TRD-10k in the initial stage increased with an increase in magainin 2 concentration.

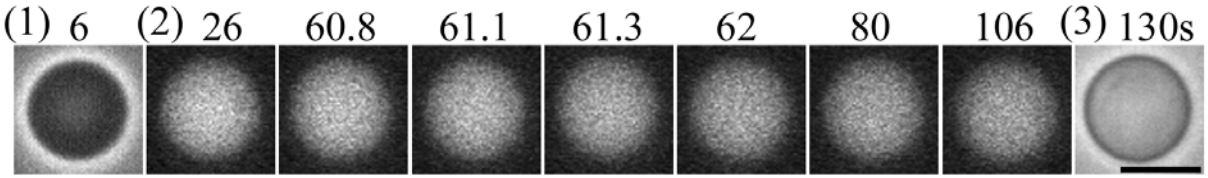
4μM magainin 2: ~20% leakage, 7μM magainin 2: ~40% leakage,

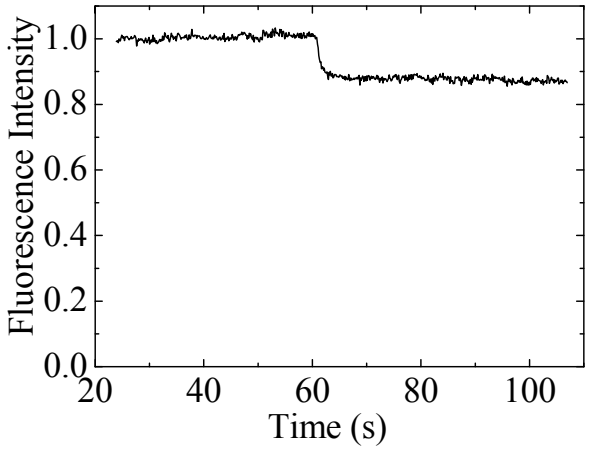
15μM magainin 2: ~70% leakage,

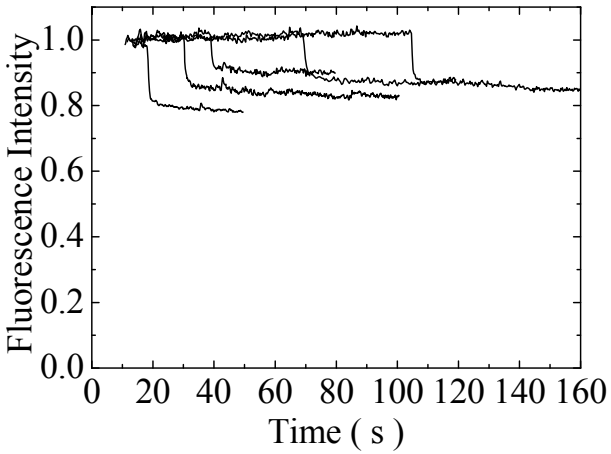
The radius of the large pore in the initial stage increased with an increase in magainin 2 concentration.

Induction of leakage of FITC-BSA from 50%DOPG/DOPC-GUV by 15 μM magainin 2

FITC-BSA (R_{SE} = 3.5 nm)



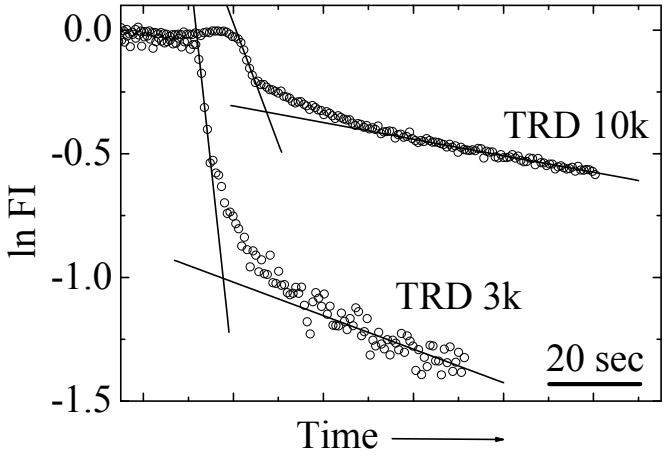




The magainin 2 induced only a transient, small amount of leakage of FITC-BSA occurred for a short time (~5 s).

FITC-BSA leaked through the transient large pore, but did not leak through the steady pore in the final steady stage.

Rate constant of Magainin 2-induced leakage



Determination of the rate constants of the leakage, k_{leak}

$$I(t)/I(t_{tr}) = C^{in}(t)/C_0^{in} = \exp\{-k_{leak}(t-t_{tr})\}$$

The transient, rapid leakage in the initial stage and the slow leakage in the final steady stage.

Magainin 2 molecules formed a large pore in the lipid membrane in the initial stage and then they rearrange to form smaller pores.

Fluorescent probes	R_{SE} nm	Mode of leakage	Initial stage $k_{leak}^{initial}$ (s^{-1})	Final steady stage k_{leak}^{steady} (s^{-1})
TRD-3k	1.4	two phases	**$(1.9 \pm 0.1)\times10^{-1}$**	**$(1.0 \pm 0.1)\times10^{-2}$**
TRD-10k	2.7	two phases	**$(8.2 \pm 0.8)\times10^{-2}$**	**$(3.3 \pm 0.4)\times10^{-3}$**
AF-SBTI	2.8	Initial leakage	**$(1.2 \pm 0.1)\times10^{-1}$**	**No leakage**
TRD-40k	5.0	initial leakage	**$(4.0 \pm 0.3)\times10^{-2}$**	**No leakage**
FITC-BSA	3.6	initial leakage	**$(4.8 \pm 0.3)\times10^{-2}$**	**No leakage**

The radius of the small pore in the final steady stage is smaller than 2.8 nm, but larger than 1.4 nm

(in the presence of 7 μM magainin-2)

Theoretical analysis of the rate constant of the leakage in the initial stage

$$J = -P(C^{\text{in}}(t) - C^{\text{out}}(t)) = -\frac{D}{h}(C^{\text{in}}(t) - C^{\text{out}}(t))$$

$$V\frac{dC^{\text{in}}}{dt} = -\frac{D}{h}S_{\text{p}}C^{\text{in}}$$

$$\therefore \quad \frac{C^{\text{in}}(t)}{C_0^{in}} = \exp(-k_{\text{leak}}t)$$

where $k_{\text{leak}} = \frac{DS_{\text{p}}}{hV}$

P: permeability coefficient of the substance in membrane
D: diffusion coefficient of fluorescent probes
h: the length of the pore (h = 3.5 nm)
S_{p} : the effective cross-sectional area of a pore
V : the volume of each GUV
$C_{\text{in}}(t)$: concentration of the substance inside of a GUV

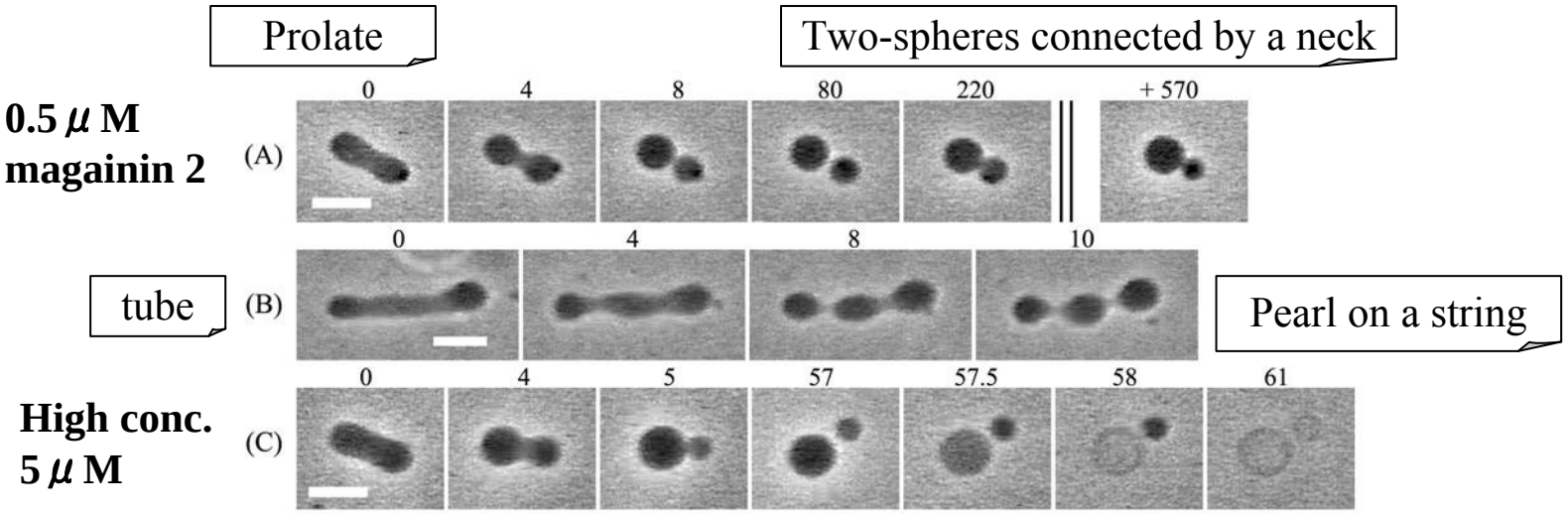
We assume that only one large pore is formed in the initial stage of the leakage (i.e., $n_{\text{p}} = 1$).
⟶ We can estimate the radius of the large pore in the initial stage, r_{lp} (nm).

For GUVs whose radius was 5 ± 1 μm.

***J. Phys. Chem. B., in press,* 2010**

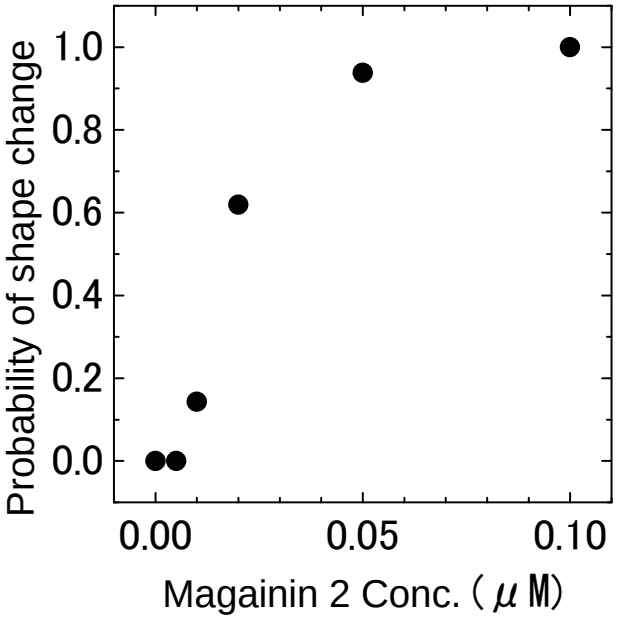
Fluorescent probes	D (m^2s^{-1})	4 μM magainin-2	7 μM magainin-2	15 μM magainin-2
TRD-3k	1.7×10^{-10}	18 ± 1	26 ± 1	46 ± 2
TRD-10k	9.1×10^{-11}	20 ± 1	25 ± 1	40 ± 3
AF-SBTI	8.8×10^{-11}	16 ± 3	26 ± 2	39 ± 2
TRD-40k	4.9×10^{-11}	N.D.	24 ± 2	38 ± 2
FITC-BSA	6.2×10^{-11}	N.D.	20 ± 1	44 ± 4

Shape changes of 50%DOPG/DOPC-GUVs induced by magainin 2



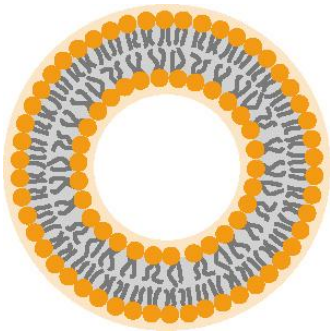
Probability of shape change

Leakage of sucrose occurred.



◎Low concentrations of magainin 2 induced the shape change of single GUVs from prolate to two-spheres connected by a neck.

Area-difference Model (ADE model)



Monolayer membranes can stretch elastically around their equilibrium areas.

$$F_{el} = \frac{\kappa_c}{2}\int (C_1 + C_2)^2 dA + \frac{\kappa_r}{2Ah^2}(\Delta A - \Delta A_o)^2$$

where κ_c : bending modulus of the membrane

κ_r : nonlocal bending modulus of the membrane

C_1 and C_2: two principle curvatures of the monolayer membranes

h: membrane thickness

Shape of GUV is determined by the minimum of elastic energy F_{el} of the membrane for a given difference of the areas of two monolayers under the relaxed conditions:

ΔA_0 $(= A_0^{ex} - A_0^{in})$.

A_0^{ex}: the area of external monolayer

A_0^{in} : the area of internal monolayer under relaxed conditions

ΔA_0 $\Uparrow$

$\Rightarrow$

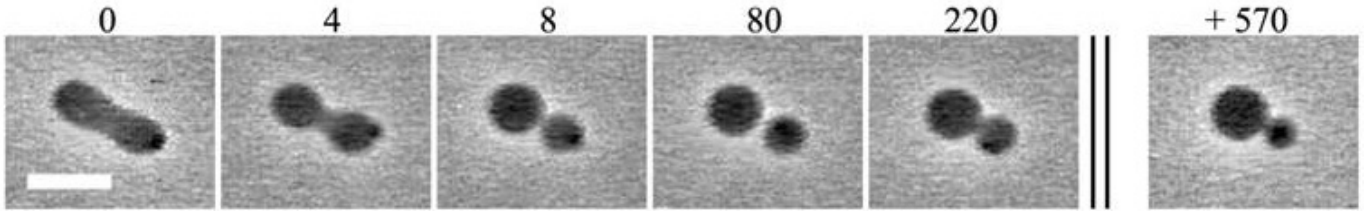
(1) Prolate $\rightarrow$ Pear (or dumbbell)

$\rightarrow$ Two-spheres connected by a neck

(2) Cylinder $\rightarrow$ Pearl on a string

(Phys. Rev. E 48, 3112, 1993;
Phys. Rev. E. 49, 5389, 1994)

Effect of magainin 2 on shape of GUV

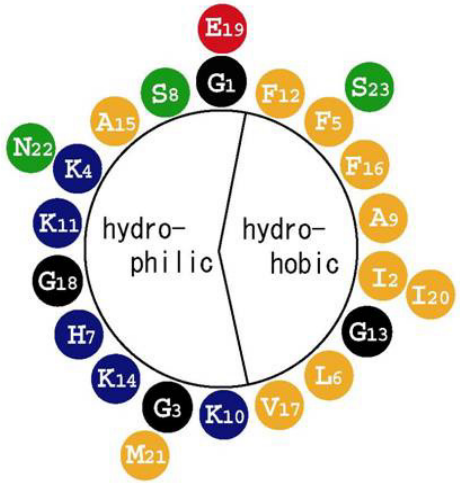


Theoretical analysis based on the ADE model

⇒ Magainin 2 binds to the external monolayer membrane of a GUV and increases its area, inducing the increase in ΔA_0

Magainin 2 has several Phe and Leu residues with high interfacial hydrophobicity and thereby, magainin 2 can be partitioned deeply into the membrane interface of DOPG/DOPC-GUV

⇒ the increase the area of the external monolayer

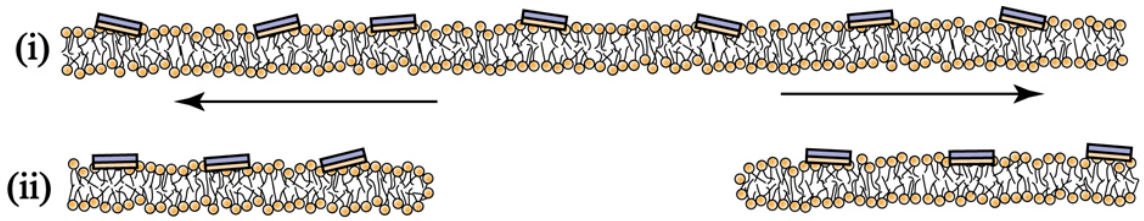


Gly-Ile-Gly-Lys-Phe-Leu-His-Ser-Ala-Lys-Lys-Phe
–Gly-Lys-Ala-Phe-Val-Gly-Glu-Ile-Met-Asn-Ser

The detailed theory of the pore formation was deleted because it is unpublished at present.

A hypothesis on the mechanism for the magainin 2-induced pore formation in lipid membranes

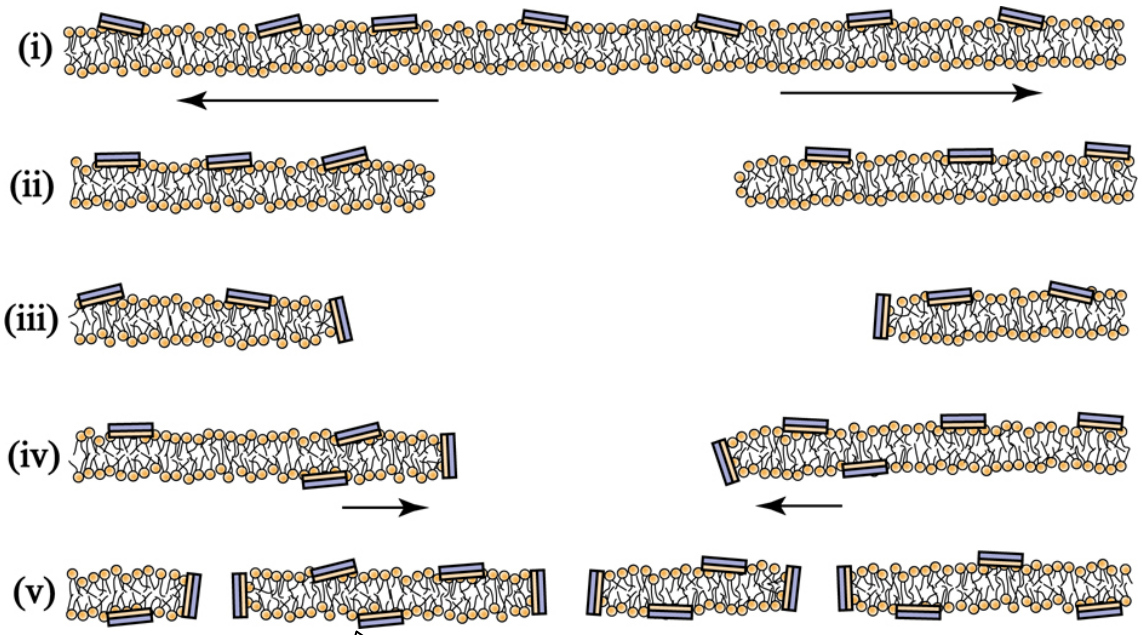
(i) The binding of magainin 2 increases the area of the external monolayer. It increases the tension of the internal monolayer σ_{in} (<0). $|\sigma_{in}|$ increases with X_{ext}.



(ii) The tension induces a pore in lipid membrane stochastically as a result of thermal fluctuation of the lipid membrane. The transmembrane pore appearance decreases the stretch of the internal monolayer (thereby $|\sigma_{in}|$ decreases) and induces the compression of external one (thereby σ_{ex} increases). The pore size changes with time. At the beginning $|\sigma_{in}| >> \sigma_{ex}$ and pore grows rapidly. The pore size is determined by tension balance $|\sigma_{in}| = \sigma_{ex}$

(ii-b) The unbalance of the tension in both the monolayers may induce the transfer of lipid molecules from the external to the internal monolayers through the rim of the pore, which decreases the difference in the absolute value of the tension of these monolayers to zero.

A hypothesis on the mechanism for the magainin 2-induced pore formation in lipid membranes

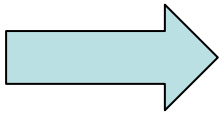


During the large pore formation, magainin 2 molecules in the external monolayer transfer through the rim of the pore (iii), and then into the internal monolayer (iv). It increases the magainin 2 surface concentration in the internal monolayer X_{int}, and thereby its area increases and $|\sigma_{in}|$ decreases. As a result, the diameter of the pore decreases.

(v) During the step of the decrease in the pore size, magainin 2 molecules in the rim of the large pore rearrange to form several stable pores. The stability of these final pores is determined by the interaction free energy between magainin 2 molecules and the total free energy of the lipid membranes containing the pores.

A hypothesis on the mechanism for the magainin 2-induced pore formation in lipid membranes

Working hypothesis.



To construct the mechanism, we need more experimental data and theory (or simulation) in elementary processes of the pore formation.

Comparison with other substances-induced leakage

2. Protein Toxin Lysenin-Induced Pore Formation in Lipid Membranes : the Single GUV Method Study

(Saga, Alam, Kobayashi, and Yamazaki)

⇧

The data on lysenin was deleted because they are unpublished at present.

3. Effect of tea catechin, (-)epigallocatechin gallate (EGCg), with lipid membranes

－the Single GUV method study－

(Tamba, Ohba,Yamazaki et al., *Biophys. J.* 92, 3178, 2007)

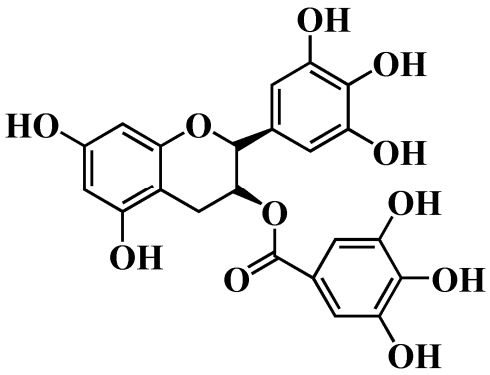
3. Effect of tea catechin, (-)epigallocatechin gallate (EGCg), with lipid membranes

－the Single GUV method study－

(Tamba, Ohba,Yamazaki et al., *Biophys. J.* 92, 3178, 2007)

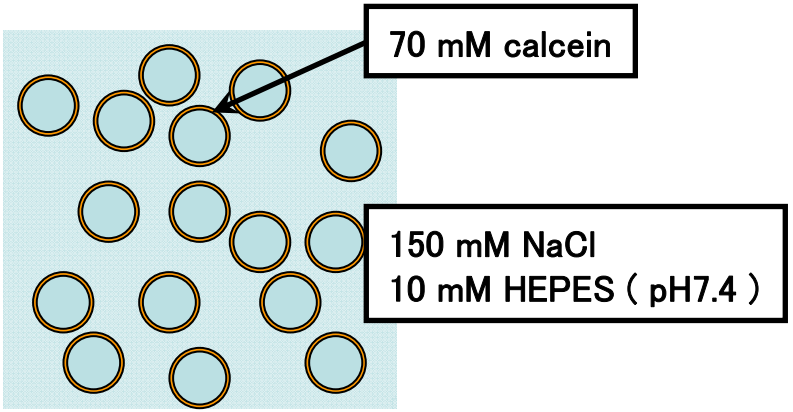
Interaction of tea catechin, epigallocatechin gallate with PC-LUVs

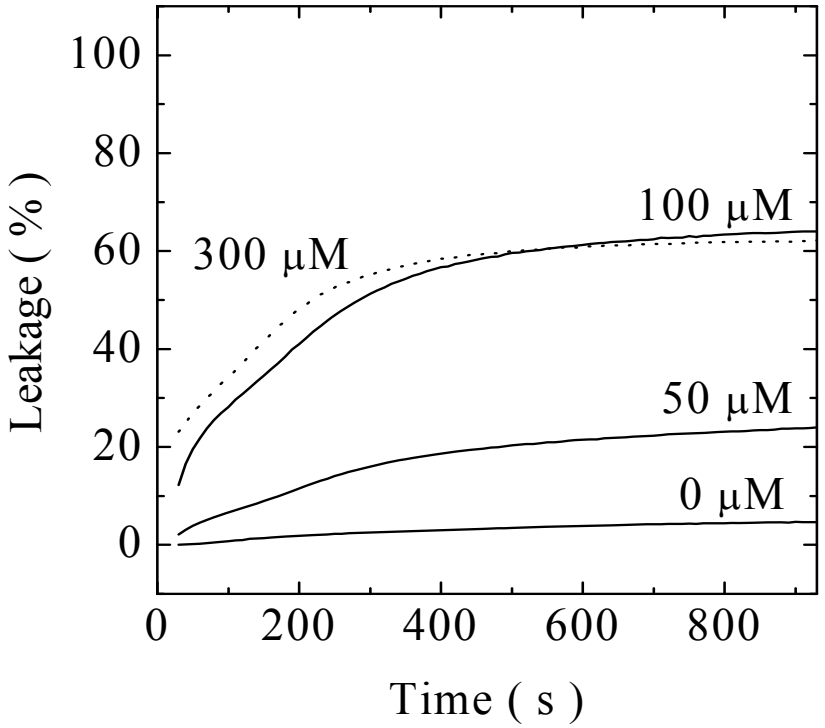
Epigallogatechin gallate (EGCg)



Antibacterial activity
Antioxidant activity

The LUV suspension method

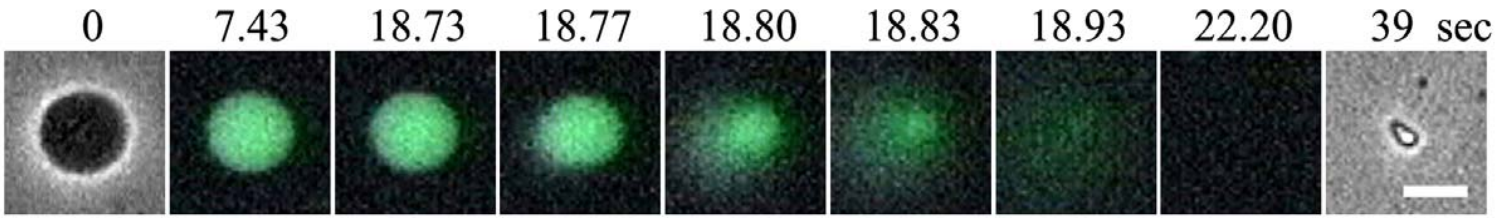




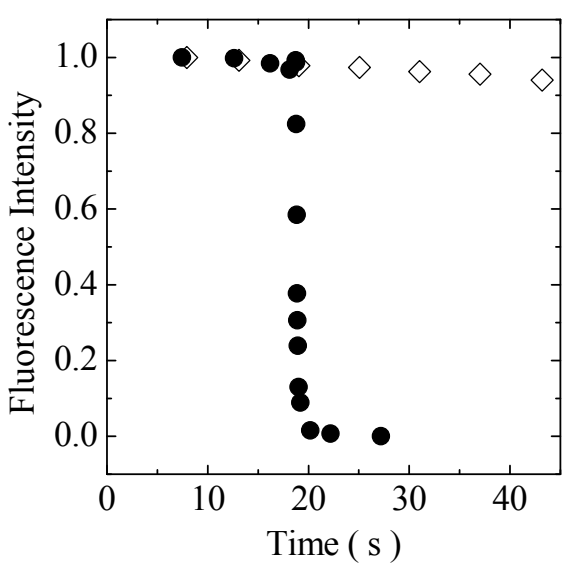
The EGCg-induced leakage of calcein from the LUV suspension increased gradually with time.

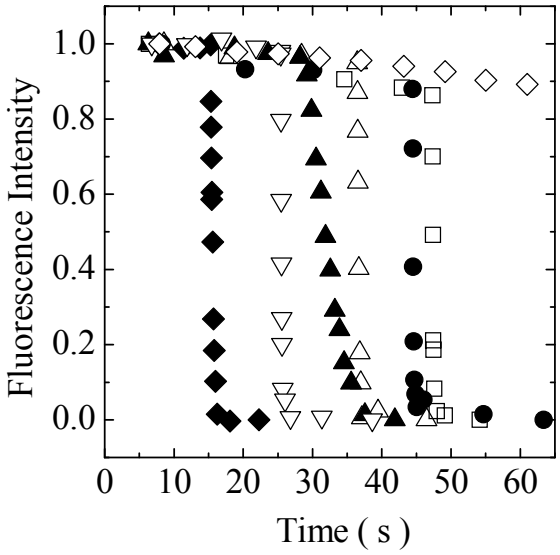
Induction of calcein leakage from egg PC-GUV by 100 μM EGCg

A small lump of lipid membranes



Scale Bar; 10 μm

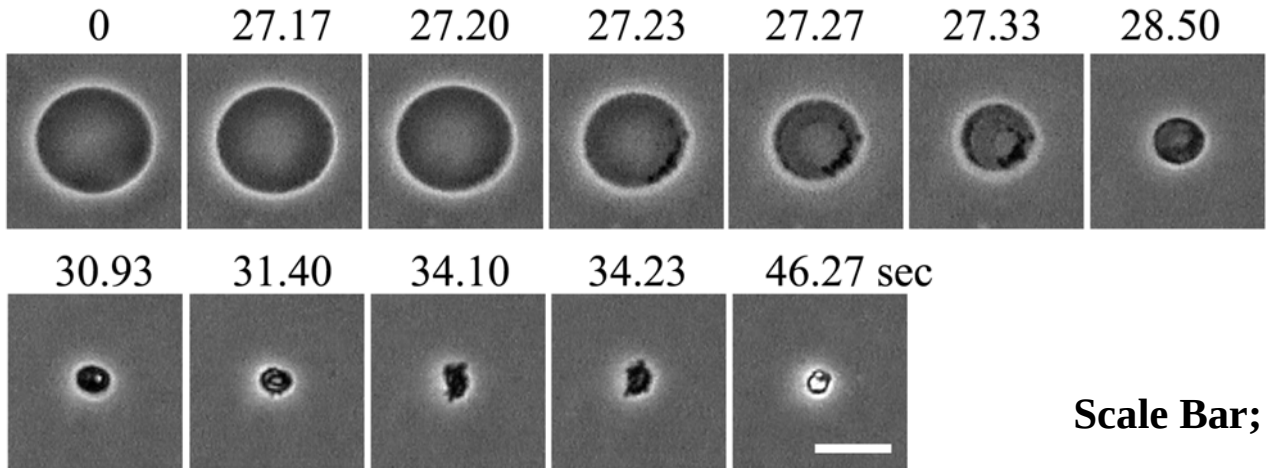




Biophys. J. 92, 3178, 2007
Tamba, Ohba, Yamazaki, et al.

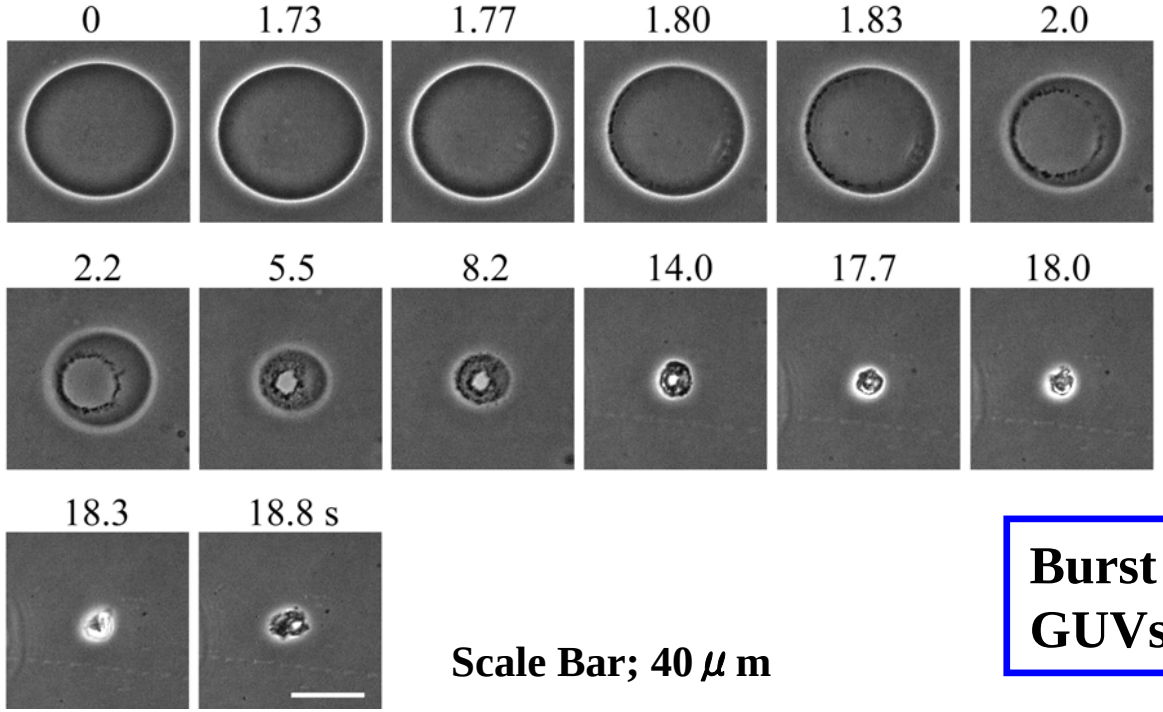
Structural change of single egg PC-GUVs induced by EGCg.

100 μM EGCg



Scale Bar; 20 μm

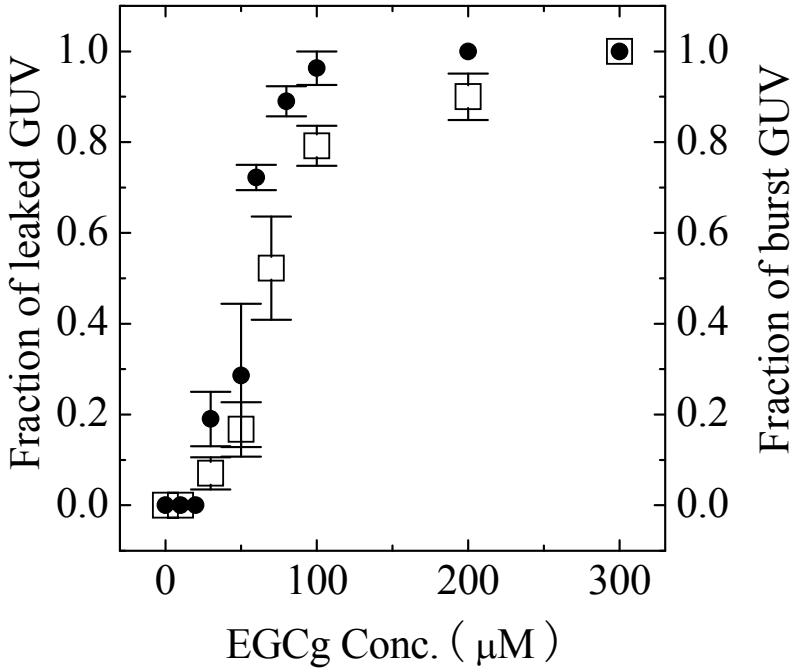
300 μM EGCg



Scale Bar; 40 μm

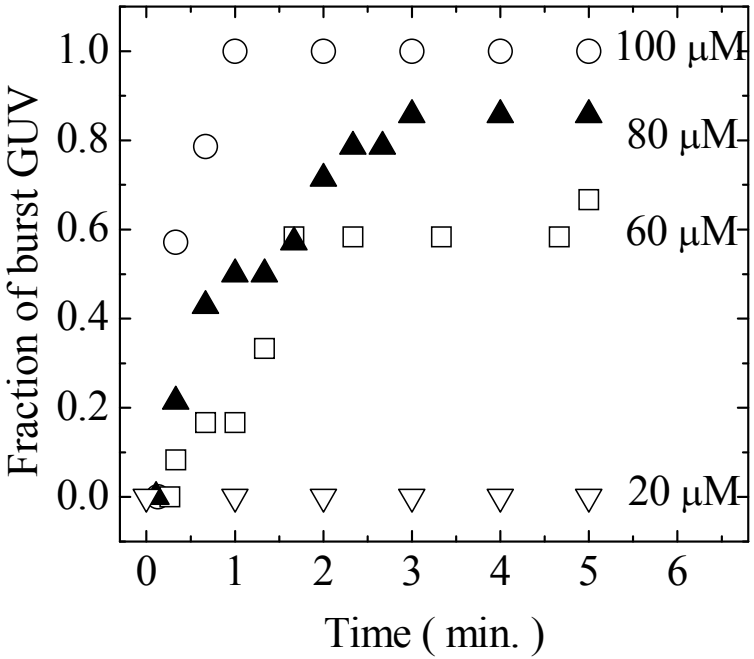
Burst of single GUVs occurred.

Strong correlation between leakage and burst of egg PC-GUV



The leakage of calcein occurred as a result of the burst of single GUVs.

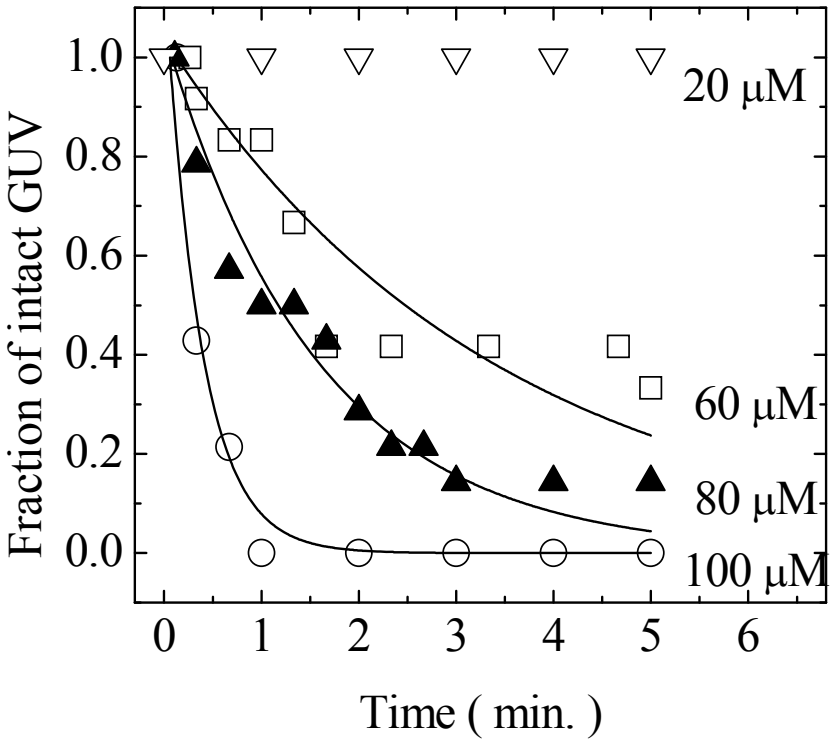
Time course of the fraction of EGCg-induced burst of egg PC-GUV



The leakage from the LUV suspension increased with time.

The number of leaked LUV due to the burst increased with time.

EGCg-induced burst of GUVs



EGCg-induced bursting of the GUV followed the first-order reaction. The rate constant increased with an increase in EGCg concentration.

$$P_{\text{int}\,act}(t) = \exp\{-k_P(t - t_{eq})\}$$

100 μM EGCg: $k_p = 2.5\ \text{min}^{-1}$

80 μM EGCg: $k_p = 1.2\ \text{min}^{-1}$

60 μM EGCg: $k_p = 0.35\ \text{min}^{-1}$

Biophys. J. 92, 3178, 2007
Tamba, Yamazaki et al.

Cholesterol decreased the fraction of burst GUV.

e.g. fraction of burst GUV = 0.3 at 500 μM EGCg

Conclusion

Using the single GUV method, we succeeded in observing the elementary processes of the substances (magainin 2, lysenin, and EGCg)-induced pore formation in lipid membranes. We could separate the step of the substances-induced pore formation in lipid membranes from the step of the leakage of fluorescent probes through the pores. We succeeded in determining two kinds of rate constants of the elementary processes of the substances-induced pore formation.

⇒ **(A) the rate constant of the substances-induced pore formation.**

For magainin 2-induced pore formation, the magainin 2 concentration in lipid membrane interface mainly determines the rate of the pore formation.

⇒ **(B) the rate of the leakage (permeability) of the internal contents**

Time course of the change of the pore size

The dependence of the pore size on substance concentration