Binding Affinity of Basic Amino Acids to the Surface of a Neutral Phospholipid Monolayer

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The binding of basic amino acids to the surface of a zwitterionic phospholipid monolayer at the water–1,2-dichloroethane interface was demonstrated by voltammetry for the ion transfer at the liquid–liquid interface, in which the phospholipids transfer from the aqueous side of the interface to the bulk of 1,2-dichloroethane as the positively charged complex with the basic amino acid.

Most antimicrobial and fusion peptides are known to have the common feature of a net cationic charge due to the presence of arginine and lysine, Arg and Lys, residues.[1–3] These cationic peptides bind to biomembranes and disrupt the membrane structure.[1–5] Their high affinity for membranes has been mainly explained by the electrostatic interaction of the positive charge of the peptide to anionic phospholipids.[1,6–9] These cationic peptides also have an affinity for zwitterionic phospholipid membranes composed of phosphatidylcholine.[6,10] Many works have explained the affinity for the zwitterionic phospholipid membrane by the amphipathic secondary structure of the cationic peptide.[5,6,10–14] In a previous work, we reported that a hydrophilic head group of a phosphatidylcholine forms a complex with mono-, di-, or trialkylammonium cations and not with anions such as carboxylate.[15] These results suggest that the amino group in the basic amino acid residue binds to the hydrophilic head group of a zwitterionic phospholipid membrane even if the cationic peptide has a nonamphipathic secondary structure. However, the complex formation of basic amino acids with zwitterionic phospholipids has been scarcely reported in the previous work.[16] In this paper, we demonstrate the affinity of basic amino acids to the surface of a phosphatidylcholine monolayer adsorbed at an aqueous–1,2-dichloroethane, W–DCE, interface on the basis of the voltammetry for the ion transfer at the liquid–liquid interface.

The basic amino acids employed in the study are Lys and a dipeptide of Lys, Lys–Lys, both of which have positive charges (1+ for Lys, 2+ for Lys–Lys) in the W of neutral pH solutions. L-α-Phosphatidylcholine dioleoyl, PC, was used as a lipid. Since PC is a zwitterion in a W of neutral pH, the PC monolayer formed at a W–DCE interface is not influenced by applying an interfacial potential, E, across the interface. If the basic amino acid in W binds to the hydrophilic head group of the PC monolayer at an aqueous side of the interface, the positively charged PC complex will form at the interface and transfer from the aqueous site of the interface to the bulk phase of DCE by applying E across the interface. Therefore, the current for the transfer of the positively charged PC complex would appear as a positive current related to an adsorbed species in the voltammogram. A W–DCE interface with the phospholipid monolayer was formed in an electrochemical cell (35 mm diameter) with two reference electrodes (a Ag/AgCl electrode in W and a tetraphenylarsonium ion-selective electrode, TPhAsE, in DCE) and two counter electrodes (Pt wire in W and Pt net in DCE), as reported in a previous paper.[15] The measurement of the interfacial tension, γ, was carried out using the same electrochemical cell by the Wilhelmy vertical plate technique.[15,17] The phospholipid monolayer was made by setting W of Lys^+Cl^- or $Lys–Lys^{2+}Cl^-_2$ on DCE containing PC and by holding E at 0.3 V until the adsorption equilibrium of PC was achieved[15] (ca. 20 min with stirring DCE). The applied E was the potential of W referred to that of DCE. The pH of W was adjusted to 7.0 by titration of HCl. Bis(triphenylphosphoranylidene)ammonium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate, $BTPPA^+TFPB^-$, was added to DCE as a supporting electrolyte.

Figure 1 shows voltammograms for the ion transfer at the W–DCE interface obtained by the potential scan from the initial potential of 0.3 V. The positive currents at 0.7 and 0.9 V in the absence of PC was confirmed to be due to the transfers of $Lys–Lys^{2+}$ and Lys^+, respectively, from W to DCE. In the presence of the phospholipid monolayer, a positive current of about 100 mA appeared at a lower potential than the positive limit in the cases of both Lys^+ and $Lys–Lys^{2+}$. The positive current corrected by taking off the background current without PC, ΔI, is indicated in Figure 2. Here, the ΔI values are those obtained by the potential scan from 0.4 V to the positive limits of the potential windows. From the definition of the current, a positive current in the voltammogram means a cation transfer from W to DCE or an anion transfer from DCE to W. Since the potential range for an appearance of the positive current depended on the species of the basic amino acid, we considered that ΔI was caused by the transfer of the positively charged PC complex with the basic amino acid from the aqueous side of the W–DCE interface to the bulk of DCE. The experimental evidences supporting our hypothesis are as follows. (1) ΔI is observed only for an ion having an affinity to PC. In our previous work, the tetramethyl-

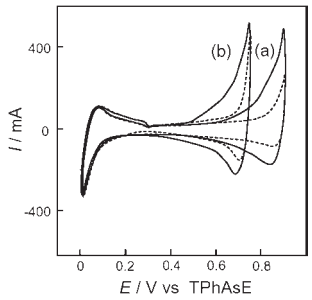

Figure 1. Cyclic voltammograms of (a) 0.01 M Lys^+Cl^- and (b) 0.01 M $Lys–Lys^{2+}Cl^-_2$ in W and 0.01 M $BTPPA^+TFPB^-$ in the absence (····) and presence (—) of 2×10^{-6} M PC in DCE: scan rate, 200 mV s^{-1}.

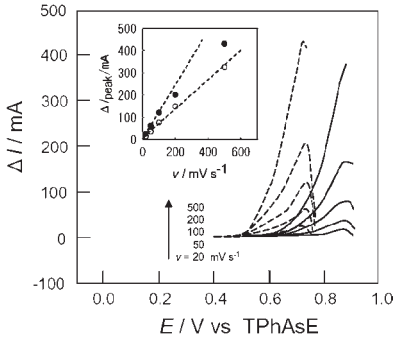

Figure 2. Dependence of ΔI on the scan rate, v, in the presence of 0.01 M Lys^+Cl^- (—, ○) and $Lys–Lys^{2+}Cl^-_2$ (- - -, ●) in W and 0.01 M $BTPPA^+TFPB^-$ and 2×10^{-6} M PC in DCE.

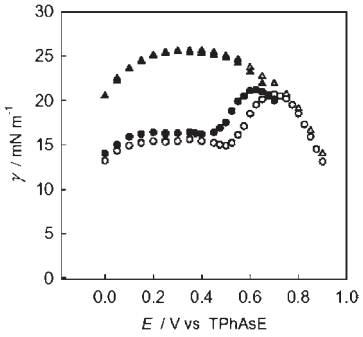

Figure 3. The potential dependences of the interfacial tension of 0.01 M Lys^+Cl^- (△, ○) and $Lys–Lys^{2+}Cl^-_2$ (▲, ●) in W and 0.01 M $BTPPA^+TFPB^-$ in DCE in the presence (○, ●) and absence (△, ▲) of 2×10^{-6} M PC in DCE.

ammonium cation and anions such as carboxylate had little affinity to PC while mono-, di-, and trimethylammonium cations formed PC complexes.[15] Using a tetramethylammonium cation instead of Lys^+ or $Lys–Lys^{2+}$, ΔI did not appear in the voltammogram. (2) ΔI is attributed to the charge-transfer process related to the adsorbed species at the W–DCE interface. The ΔI showed the linear dependence on the scan rate as shown in Figure 2. The peak current of ΔI, ΔI_{peak}, was proportional to the scan rate in the case of both Lys^+ and $Lys–Lys^{2+}$. (3) The PC desorption from the interface occurred in the potential range for the appearance of ΔI in the voltammograms. As seen in Figure 3, the presence of PC reduced γ in the potential range from 0 to 0.4 V ($Lys–Lys^{2+}$) or 0.5 V (Lys^+). In this potential range, PC adsorption occurred. When E was changed to a potential more positive than about 0.4 V, an increase in γ was observed. In this potential range, PC desorption from the W–DCE interface occurred.[15] Compairing Figure 3 with Figure 2, good agreement exists between the potential range for the PC desorption and that for the appearance of ΔI. This result indicates that ΔI is accompanied by the PC desorption. (4) ΔI was dependent on the concentration of the adsorbed PC at the W–DCE interface and independent of the PC concentration in the bulk phase of DCE. The PC adsorption at the W–DCE interface is saturated in the PC concentration range from 10^{-6} to 10^{-5} M, since γ decreased linearly with an increase of the logarithmic concentration of PC, $\log C_{PC}$, in DCE. From the slope analysis of the γ vs $\log C_{PC}$ plot, the surface excess concentration of PC was calculated to be 1.96 μmol m^{-2}, which indicates the PC monolayer formation.[15] When the bulk concentration of PC in DCE was changed within the range from 2×10^{-6} to 10^{-5} M, the ΔI_{peak} showed little dependence on the bulk concentration of PC: 149 mA for 2×10^{-6} M PC, 199 mA for 5×10^{-6} M PC, and 203 mA for 10^{-5} M PC in the case of Lys^+. Therefore, ΔI is caused mainly by the PC adsorbed at the interface rather than by the PC in the bulk DCE. Experimental results of (1)–(4) support the conclusion that Lys^+ and $Lys–Lys^{2+}$ form positively charged complexes with the adsorbed PC at the interface; the transfer of the positively charged PC complex from the aqueous side of the W–DCE interface to the bulk phase of DCE was observed as ΔI in the voltammogram. That is to say, Lys^+ and $Lys–Lys^{2+}$ have an affinity to the hydrophilic head group of the PC monolayer because of the complex formation.

In the present work, the affinity of Lys^+ and $Lys–Lys^{2+}$ to the PC membrane surface was demonstrated despite the fact that these basic amino acids have no amphipathic secondary structure. This result suggests that the basic amino acid residue has an intrinsic binding ability to the PC membrane surface. The complex formation of an amino group with the PC membrane surface has been considered to be negligible because of lower interaction levels than other metal cations.[16] However, the affinity of the amino group to the PC membrane surface would become significant in the case of a cationic polypeptide, which has a net cationic charge. The present result provides an alternative concept, not only for the effect of antimicrobial and fusion peptides on biomembrane structures, but also for membrane protein localization on the membrane surface.

References

1 W. L. Maloy, U. P. Kari, *Biopolymers* **1995**, *37*, 105.
2 R. Maget-Dana, *Biochim. Biophys. Acta* **1999**, *1462*, 109.
3 R. E. W. Hancock, *Lancet* **1997**, *349*, 418.
4 R. B. Gennis, in *Biomembranes: Molecular Structure and Function*, Springer-Verlag, New York, **1989**, Chap. 3.
5 E. T. Kaiser, F. J. Kezdy, *Annu. Rev. Biophys. Biophys. Chem.* **1987**, *16*, 561.
6 Y. Shai, *Biochim. Biophys. Acta* **1999**, *1462*, 55.
7 M. Jelokhani-Niaraki, E. J. Prenner, C. M. Kay, R. N. McElhaney, P. S. Hodges, *J. Pept. Res.* **2002**, *60*, 23.
8 D. Murray, L. Hermida-Matsumoto, C. A. Buser, J. Tsang, C. T. Sigal, N. Ben-Tal, B. Honig, M. D. Resh, S. Mclaughlin, *Biochemistry* **1998**, *37*, 2145.
9 J. Seeling, S. Nebel, P. Ganz, C. Bruns, *Biochemistry* **1993**, *32*, 9714.
10 R. Maget-Dana, D. Lelièvre, *Biopolymers* **2001**, *59*, 1.
11 T. C. Terwilliger, L. Weissman, D. Eisenberg, *Biophys. J.* **1982**, *37*, 353.
12 C. R. Dawson, A. F. Drake, J. Helliwell, R. C. Hinder, *Biochim. Biophys. Acta* **1978**, *510*, 75.
13 A. Kitamura, T. Kiyota, M. Tomohiro, A. Umeda, S. Lee, T. Inoue, G. Sugihara, *Biophys. J.* **1999**, *76*, 1457.
14 K. Kono, S. Kimura, Y. Imanishi, *Biochemistry* **1990**, *29*, 3631.
15 Y. Yoshida, K. Maeda, O. Shirai, *J. Electroanal. Chem.*, **2005**, *578*, 17.
16 S. A. Tatulian, in *Phospholipids Handbook*, ed. by G. Cevc, Marcel Dekker Inc., New York, **1993**, Chap. 14, p. 511.
17 Y. Yoshida, K. Maeda, O. Shirai, *J. Nucl. Radiochem. Sci.* **2005**, *6*, 631.

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