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Voltammetry of the Phase Transfer of Polypeptide Protamines across Polarized Liquid/Liquid Interfaces

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In this Communication, using natural polypeptide protamines as the illustrating example, we report for the first time on voltammetric observations of the phase transfer of biological polyions at polarized interfaces between two immiscible electrolyte solutions (ITIES).

Ion transfer at ITIES serves as a basis or model of many important biomembrane, industrial, and analytical systems.[1] In the last three decades, it has been shown that electrochemical techniques and theories are valuable to study the transfer process at ITIES.[2] These studies, however, have been focused on the transfer of small ions so that there are relatively few studies of biological polyions such as oligonucleotides and proteins at ITIES.[3] Herein, we demonstrate that phase transfer of polypeptide protamines across polarized ITIES gives very well-defined voltammograms.

Micrometer-sized ITIES were formed at the tip of micropipets[4] and characterized by voltammetry of tetraethylammonium (TEA^+) transfer (see Supporting Information for experimental details). As shown in Figure 1A, the sigmoid shape of the forward wave is due to spherical diffusion of TEA^+ in the aqueous phase outside the pipet. The peak-shaped wave during the backward scan is due to linear diffusion of TEA^+ inside the pipet. The diffusion-limited steady-state current, i_{ss}, was used to determine the radius of the disk-shaped interface at the tip, a, using the equation

$$i_{ss} = 4.64zFDca \quad (1)$$

where the electrode-geometry factor of 4.64 rather than 4 was used because of the thin wall of the pipet tip (the ratio of outer and inner tip diameters = 1.5),[4c,d] z is the charge number of the ion, F is the Faraday constant, D is the diffusion coefficient (9.3×10^{-6} cm^2/s for TEA^+),[5] and c is the concentration.

Micropipet voltammetry was carried out to study the phase transfer of protamines, which are simple, highly positively charged proteins.[6] They have similar sequences and molecular weights (4000−4250). Importantly, two-thirds of the approximately 30 total amino acids are arginine, which is the only amino acid with an ionizable side chain that is contained in the proteins. Therefore, protamines have 20 or 21 excess positive charges in a solution at physiological pH or lower. The voltammogram of protamines at water/nitrobenzene interfaces (Figure 1B) is similar in shape to that of TEA^+, indicating that protamines carry the current. The wave was observed at more negative potentials, corresponding to the very hydrophilic nature of protamines. Analysis of i_{ss} by eq 1 (R^2 = 0.998 between 5 and 40 μM) gives $D = 1.1 \times 10^{-6}$ cm^2/s ($1.2-1.3 \times 10^{-6}$ cm^2/s[6a]) with $z = 20$, which supports that each protamine carries 20 positive charges.

With the charge number determined from i_{ss}, the current−potential curve was analyzed to obtain kinetic information. When the current is diffusion-limited, the forward wave is expressed by

$$i = i_{ss}/\{1 + \exp[zF(E - E^{0'})/RT]\} \quad (2)$$

where $E^{0'}$ is the formal transfer potential. Indeed, it is the case of

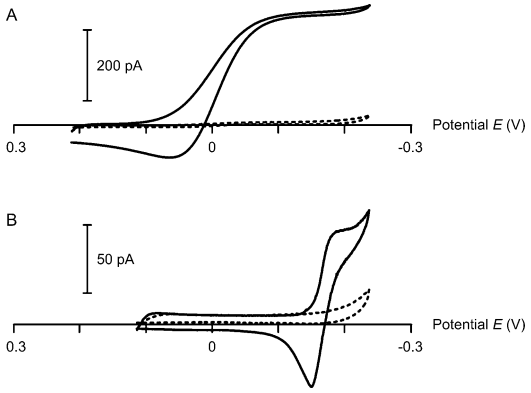

Figure 1. Cyclic voltammograms of (A) 0.15 mM TEA^+ and (B) 12 μM protamines at water/nitrobenzene interfaces as observed with a 4.8 μm-radius pipet. Dotted lines are backgrounds. Scan rate: 10 mV/s.

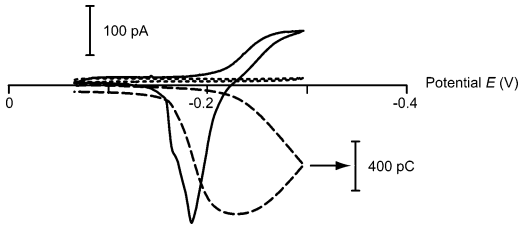

Figure 2. Cyclic voltammogram of 36 μM protamines at water/1,2-dichloroethane interfaces as observed with a 5.3 μm-radius pipet. Dotted line is the background. Dashed line is the current integration of the solid line after subtracting the dotted line. Scan rate: 20 mV/s.

TEA^+ transfer. While the increase in the current is much sharper than that of TEA^+ transfer, the forward wave of protamine transfer is more broadened than predicted by eq 2 with $z = 20$, indicating that the current is limited by interfacial transfer of protamines. Indeed, analysis of the wave using the theory for quasi-reversible steady-state voltammetry[7] gives the standard rate constant, k^0, of 6.2×10^{-4} cm/s, the transfer coefficient, α, of 0.24, and $E^{0'} = -0.1556$ V (see Supporting Information). The rate constant is much smaller than those of TEA^+ (>0.5 cm/s) and surface active dyes ($\sim 10^{-2}$ cm/s),[8] whereas the diffusion coefficient of protamines is only several times smaller than that of these ions. The transfer coefficient is also smaller than the typical value of 0.5 ± 0.1,[9] suggesting that most of the charges on protamines are screened by ion-pairing with counteranions, a small fraction of the interfacial potential is effectively involved, or both (see below).[10]

When less polar 1,2-dichloroethane ($\epsilon = 10.42$; for nitrobenzene, $\epsilon = 35.6$) is used, protamine transfer is followed by their interfacial adsorption (Figure 2). The current integration of the background-subtracted voltammogram (dashed line in Figure 2) indicates that most protamines transferred across the interface during the forward scan were stripped into the water phase during the backward scan. This result confirms that the transferred protamines accumulate near

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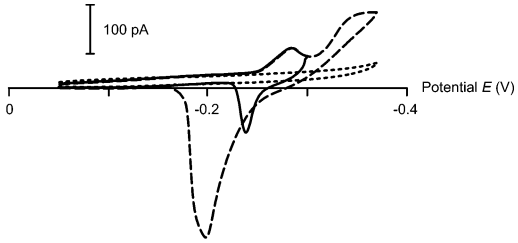

Figure 3. Cyclic voltammograms of 36 μM protamines at water/1,6-dichlorohexane interfaces as observed with a 5.8 μm-radius pipet. Dotted line is the background. Scan rate: 40 mV/s.

the interface rather than diffuse away into the bulk organic phase. The forward and backward waves were analyzed as those of irreversible reactions involving adsorption/desorption processes (see Supporting Information).[11] Importantly, in contrast to the electrode/electrolyte solution interfaces, the potential drop across ITIES develops at both sides of the interface.[12] Therefore, the irreversible voltammogram is characterized not only by the transfer coefficient ($\alpha' = 0.50$) but also by the fraction of the potential drop between the aqueous phase and the adsorption site with respect to the overall phase boundary potential ($\beta' = 0.18$).[10b,13] Interestingly, β' thus obtained is close to the fraction of the potential at the aqueous side of the water/1,2-dichloroethane interfaces (0.22; for more discussion, see Supporting Information).[14]

Use of another nonpolar solvent 1,6-dichlorohexane ($\epsilon = 8.60$)[15] results in a prepeak in addition to the main wave similar in shape to that at water/1,2-dichloroethane interfaces (the dashed line in Figure 3). The main wave is due to the irreversible transfer of protamines followed by interfacial adsorption ($\alpha' = 0.64$ and $\beta' = 0.24$; see Supporting Information). The prepeak current was proportional to the scan rate ($R^2 = 0.992$ between 10 and 40 mV/s), indicating that it is due to interfacial adsorption processes. The integration of the prepeak for the solid line in Figure 3 results in 1.3×10^{-10} C (2.1×10^{-10} mol/cm^2 with $z = 20$), which is in the typical range for monolayer adsorption.[11b] The charge thus accumulated was completely released into the water phase during the backward scan.

Assuming a Faradic process,[16] we determined that the pair of prepeaks is due to protamine transfer coupled with adsorption processes. In analogy to electrode reactions with the products strongly adsorbed,[17] such prepeaks can be assigned to protamine transfer followed by interfacial adsorption. Assignments of pre- and postpeaks at ITIES on the basis of the concept of electrode reactions, however, are vague.[10,13] Indeed, another possibility is the protamine transfer between adsorption sites at the aqueous and organic sides of the interface.[10] In either mechanism, the large peak width agrees with that of the irreversible reaction with $\alpha' = 0.30$ and $\beta' = 0.30$ (see Supporting Information). The knowledge about the location of the adsorbed molecules, however, is required to identify the transfer mechanism and interpret the meaning of the parameters.[10,13]

In summary, we have demonstrated for the first time that the polarizable ITIES allow for the study of the phase transfer of biological polyions by voltammetry. The direction of the reactions can be externally controlled by the applied potential, and quantitative analysis of the diffusion-limited steady-state current was straightforward, which can be envisioned as advantages of amperometric polyion sensors over the potentiometric counterpart.[3a] The charge number of the transferred protamines thus determined from i_{ss} indicates that the shape of the voltammograms is controlled by the interfacial ion-transfer processes. The extremely broadened waves are due to the effectiveness of a small fraction of the applied potential as assumed in our analysis, the smaller effective charge of protamines by ion-pairing, or both. Interestingly, apparently a smaller charge number of DNA molecules during the transfer process was also observed recently in their potential-driven transport through single channel hemolysin embedded in the bilayer lipid membrane.[18] Such similarity implies that ITIES may serve as a model of artificial[3a,19] and biological[18] membranes for understanding potential-driven transport of biological macromolecules.

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Supporting Information Available: Details of the voltammetric measurements and data analysis (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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