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Ion-transfer voltammetry of streptothricin antibiotics with differently sized lysine oligomers at a nitrobenzene | water interface

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ABSTRACT

The transfer of streptothricin antibiotics containing a β-lysine monomer (ST-F), trimer (ST-D), and tetramer (ST-C) at a polarizable nitrobenzene (NB) | water (W) interface was studied by cyclic voltammetry. Under acidic conditions, the streptothricins existed in fully protonated forms, as ST-F^{2+}, ST-D^{4+} and ST-C^{5+} cationic species. Within the potential range tested, ST-D^{4+} and ST-C^{5+} ions gave reversible voltammograms due to their transfer across the NB | W interface. The formal potentials, $\Delta_O^W\phi^0{}_{ST}'$, of ST-D^{4+} and ST-C^{5+} ions were determined. In the presence of a neutral ligand (L), dibenzo-18-crown-6, in NB, ST-F^{2+}, ST-D^{4+} and ST-C^{5+} ions gave reversible voltammograms due to their transfer assisted by the formation of ST-FL_2^{2+}, ST-DL_4^{4+} and ST-CL_5^{5+} complexes with the ligand in NB. Using the $\Delta_O^W\phi^0{}_{ST}'$-values, association constants between the NH_3^+-groups of ST-D^{4+} and ST-C^{5+} ions and the crown ether in NB were determined. The association constants were in agreement with each other. By assuming that the association constant of ST-F^{2+} was equal to those of the ST-D^{4+} and ST-C^{5+} ions, the $\Delta_O^W\phi^0{}_{ST}'$ of the ST-F^{2+} ion was estimated. Interestingly, the determined $\Delta_O^W\phi^0{}_{ST}'$-value was increasingly negative in the order of ST-F^{2+} (~0.24 V) > ST-D^{4+} (0.199 V) > ST-C^{5+} (0.194 V), indicating that the lysine oligomer affected the phase-transfer characteristics of streptothricin.

1. Introduction

Recently liquid–liquid electrochemical studies on interfacial phenomenon for multivalent ion have been widely undertaken. The multivalent ions investigated contain not only synthetic ionic polymer [1,2] and dendrimer [3,4], but also natural products such as protein [5–11], and heparin [12–14]. These studies have shown that the electrochemistry at the interfaces between two immiscible electrolyte solutions is a useful methodology to investigate the interfacial behavior of the multivalent ion and complex formations of multivalent ion with counter ion and ionic surfactants as well as to detect for target multivalent ions in analytical applications [14,15].

In a previous paper [16], we reported on the ion-transfer voltammetric behavior of ε-poly-L-lysine at a polarized nitrobenzene (NB) | water (W) interface when the W-phase was buffered at acidic pH and the lysine polymer existed in polycationic form. Unlike monomeric lysine, the polymer gave well-defined current-potential curves due to its transfer across the NB | W interface, suggesting that the polymerization of lysine affected the phase-transfer characteristics. In addition, the voltammetric data was compared with those of synthetic cationic polymers [1], and the difference in hydrophobicity/hydrophilicity of functional groups in the polymer backbone could be discussed.

In the presence of dibenzo-18-crown-6 (DB18C6), the lysine polymer gave a well-defined voltammetric wave due to its ion-transfer assisted by the formation of a complex, in which the oxyethylene group of DB18C6 coordinates to the NH_3^+ groups of the lysine residue. The voltammetric data could be analyzed in a manner similar to the DB18C6-assisted transfer of protonated amino acids and peptides [17–19]. Thus, the voltammetric technique would also provide quantitative information on the phase transfer characteristics of biologically interesting substances containing lysine oligomers.

Streptothricin antibiotics, produced by *Streptomyces* bacteria, contain streptothrisamine and β-lysine oligopeptide (Fig. 1) [20]. The antimicrobial activity of streptothricin is much higher than that of streptothrisamine or the lysine oligomer alone. In addition, the activity increases with the lysine oligomer size, *n*. Therefore, the effect of *n* on the physicochemical properties—including the phase transfer characteristics—is also interesting from the viewpoint of antibiotics to elucidate the role of lysine oligomers in the antibiotics mechanism.

In this study, we investigated the voltammetric behavior of streptothricins having different values of *n*, that is, having a monomer (ST-F), trimer (ST-D) and tetramer (ST-C) in the absence or presence of DB18C6 at a NB | W interface. As described in Section 3, these streptothricins would be protonated in the β-lysine group, and exist in a fully protonated form (ST^{z+}, $z = n + 1$), that is, as ST-F^{2+}, ST-D^{4+}, and ST-C^{5+} ions, under the acidic condition. From the voltammetric data, the formal potential of the ST^{z+} ion, $\Delta_O^W\phi^0{}_{ST}'$, and the stoichiometry and association constants of the ST^{z+}-DB18C6 complex were determined.

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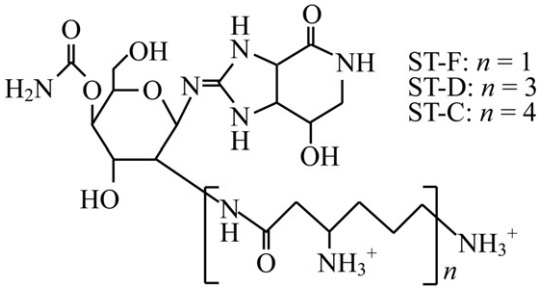

Fig. 1. Chemical structure of the streptothricin containing a β-lysine oligopeptide in the fully protonated form.

In addition, the effect of a supporting electrolyte anion on the voltammetric wave due to the transfer of ST^{z+} ions was investigated.

2. Experimental

The streptothricins, ST-F, ST-D, and ST-C, were synthesized and purified according to the previously reported method [20–22]. The preparation of tetrapentylammonium tetraphenylborate (TPnATPB) and the purification of tetrapentylammonium chloride (TPnACl) and NB were described elsewhere [23]. Other chemicals were of reagent grade and used as received.

The transfer of the streptothricins across the 0.1 M TPnATPB (NB) | 0.1 M CH_3COOH and 0.05 M $MgCl_2$ (W, pH 3.0) interface was studied by cyclic voltammetry using a three-electrode system, consisting of two reference electrodes for the W- and NB-phases (RE1 and RE2, respectively) and a counter electrode for the NB-phase (CE). The electrochemical cell is represented by cell-I:

Phase I	Phase II	*	Phase III	Phase IV	
Pt	0.1 M TPnATPB	0.1 M TPnATPB	x mM streptothricin 0.1 M CH_3COOH 0.05 M $MgCl_2$	0.05 M $MgCl_2$	AgCl | Ag
(CE)	(NB)	(NB)	(W, pH 3.0)	(W)	(RE1)

Phase V	Phase VI	
0.02M TPnACl 0.1 M $MgSO_4$	0.1 M TPnATPB	Ag | AgCl
(W)	(NB)	(RE2)

where M = mol dm^{-3}. The polarized NB | W interface, that is, the test interface, is indicated by the asterisk. The electrolytic cell used in this study was essentially the same as that described elsewhere [24]. The surface area of the test interface, A, was 0.126 cm^2.

The applied potential, E, is defined as the terminal potential of RE1 referred to that of RE2. The solution resistance between the two reference electrodes was about 3 kΩ for cell-I. The E was controlled by a potentiostat furnished with a positive feedback iR compensation circuit. The E is related to the Galvani potential difference at the NB | W interface, $\Delta_O^W\phi$, as given by

$$E = \Delta_O^W\phi + \Delta E_{ref}, \quad (1)$$

where ΔE_{ref} is a constant which depends only on the reference electrode system. The reversible half-wave potential for the transfer of tetramethylammonium (TMA^+) ion across the NB | W interface was determined to be ${}_rE_{1/2,TMA} = 0.380 \pm 0.005$ V with cell-I, in which phase III was replaced by 0.50 mM TMACl, 0.05 M $MgCl_2$ (W). The ${}_rE_{1/2,TMA}$ is related to the standard potential, $\Delta_O^W\phi^0{}_{TMA}$, as given by

$${}_rE_{1/2,TMA} = \Delta_O^W\phi^0_{TMA} + (RT/F)\ln\left[\left(\gamma^O_{TMA}/\gamma^W_{TMA}\right)\right] + (RT/F)\ln\left[\left(D^W_{TMA}/D^O_{TMA}\right)^{1/2}\right] + \Delta E_{ref}, \quad (2)$$

where $\gamma^\alpha{}_{TMA}$ and $D^\alpha{}_{TMA}$ are the activity coefficient and diffusion coefficient, respectively, of the TMA^+ ion in α-phase (α = W or O). Here and in the following, R, T, and F have the usual meanings. By taking that $\Delta_O^W\phi^0{}_{TMA} = 0.035$ V [25], $\gamma^O{}_{TMA}/\gamma^W{}_{TMA} = 1$, and $D^W{}_{TMA}/D^O{}_{TMA} = 2.07$ [26], ΔE_{ref} was estimated to be 0.367 V with cell-I. In this paper, applied potential in CVs is reported in terms of $\Delta_O^W\phi$. All of the experiments were performed at 25 ± 1 °C.

3. Results and Discussion

3.1. Non-assisted Transfer of Fully Protonated Streptothricin ST^{z+}

Curve a in Fig. 2 shows a CV at the 0.1 M TPnATPB (NB) | 0.1 M CH_3COOH and 0.05 M $MgCl_2$ (W, pH 3.0) interface, that is, the base current, recorded with a scan rate of $v = 0.1$ V s^{-1}. Curve b in the figure shows the CV at the NB | W (pH 3.0) interface in the presence of 0.1 mM ST-D in the W-phase. As shown by curve c in the figure, the voltammetric current after correction for the base current exhibits a pair of well-defined peaks. The potential of these peaks was almost independent of pH of the W phase, as described in the latter section. Thus the voltammetric current was attributed to the transfer of ST-D ionic species (not to a facilitated transfer of proton by ST-D species). The positive current peak corresponds to the transfer of ST-D from W to NB, and negative current peak corresponds to the back transfer to W.

The height of the positive peak current, i_{pa}, was proportional to the bulk concentration of streptothricin in the W-phase, ${}^*c^W{}_{ST}$, at $v = 0.1$ V s^{-1}, and to $v^{1/2}$ at ${}^*c^W{}_{ST} = 0.1$ mM, as shown in Fig. 3. The equation of i_{pa} of reversible CV for the transfer of the ST^{z+} ion is given by [27]

$$i_{pa} = 0.4463zFA(zF/RT)^{1/2}\left(D^W_{ST}\right)^{1/2}v^{1/2}{}^*c^W_{ST}, \quad (3)$$

where $D^W{}_{ST}$ is the diffusion coefficient of the ST^{z+} ion in the W-phase and z the charge of ST^{z+}.

To estimate the z-value of ST^{z+}, we conducted a pH titration measurement for the free base of ST-F with HCl. From the amount of HCl required to reach the equivalence point, the ST-F was considered to be diprotic base, that is, only the β-lysine parts of ST-F was protonated. By assuming $z = 4$ for ST-D^{z+}, the $D^W{}_{ST}$ was determined to be $(1.7 \pm 0.4) \times 10^{-6}$ cm^2 s^{-1} using Eq. (3).

The separation of peak potential, defined by $\Delta(\Delta_O^W\phi_p) = \Delta_O^W\phi_{pa} - \Delta_O^W\phi_{pc}$ ($\Delta_O^W\phi_{pa}$ and $\Delta_O^W\phi_{pc}$ being the positive and negative peak potentials, respectively) was $\Delta(\Delta_O^W\phi_p) = 0.022 \pm 0.003$ V at ${}^*c^W{}_{ST} = 0.1$ mM

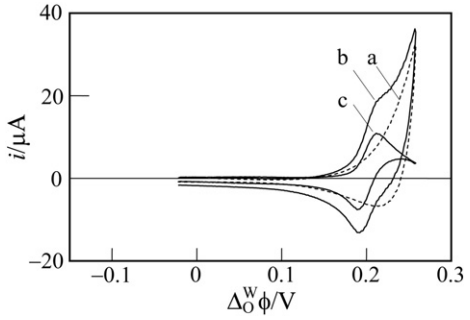

Fig. 2. Cyclic voltammograms (CVs) at the 0.1 M TPnATPB (NB) | 0.1 M CH_3COOH and 0.05 M $MgCl_2$ (W, pH 3.0) interface in the (a) absence and (b) presence of 0.1 mM ST-D in the W-phase. Curve c: b–a. Scan rate, $v = 0.1$ V s^{-1}.