

Ion transfer voltammetry of tryptamine, serotonin, and tryptophan at the nitrobenzene|water interface

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Abstract

The transfer of the cationic forms of tryptamine and serotonin across the nitrobenzene (NB)|water (W) interface was studied by cyclic voltammetry. Well-defined voltammetric waves were observed within the potential window. The standard potentials of the transfer were determined from the midpoint potentials of the voltammograms. The transfer of the cationic form of tryptophan across the NB|W interface was also observed using an acidic aqueous solution.

Key words: Ion transfer; Liquid|liquid interfaces; Tryptamine; Serotonin; Tryptophan

1. Introduction

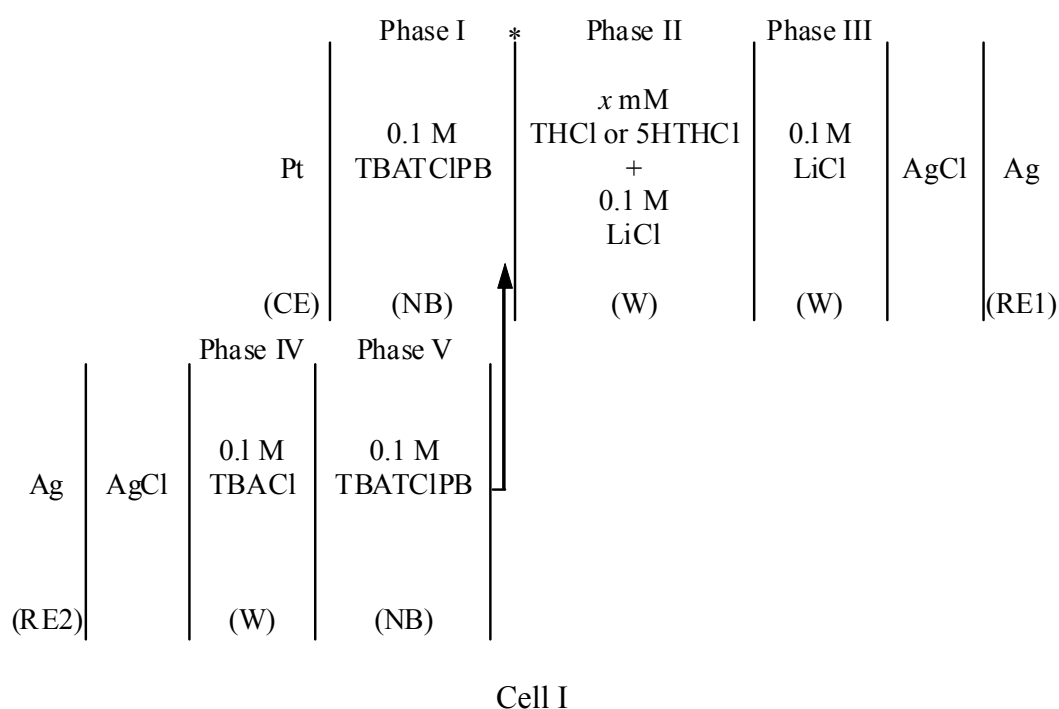
Tryptamine (indole-3-ethylamine, T) and serotonin (5-hydroxytryptamine, 5HT) are biologically active indole amines which are synthesized from tryptophan (Trp) in organisms. 5HT acts as a neurotransmitter in the central nervous system of animals. The interaction between 5HT and phospholipid membranes has become of interest in recent years, and has been studied by calorimetry [1-3], fluorometry [4], and theoretical calculations [5,6]. The researchers in this field are requiring a reliable value of the hydrophobicity of the cationic form of 5HT, because 5HT is protonated at physiological pH. However, an appropriate method to evaluate it has not been known.

Ion transfer voltammetry of aromatic amines, including amino acids, dipeptides, neurotransmitters, and anesthetics, at the organic solvent (O)|water (W) interface has been studied by many authors [7-18]. Much attention has been paid to the hydrophobicity of protonated cations of the aromatic amines, as determined by the reversible half-wave potentials of the voltammograms, and to the relationship to their biological or pharmacological activities. In the present study, we observed the transfer of the cationic forms of T, 5HT, and Trp across the nitrobenzene (NB)|W interface by the use of cyclic voltammetry, in order to evaluate the hydrophobicity of these ions. The standard potentials of the transfer were determined from the midpoint potentials of the voltammograms. The dependence of the midpoint potential on pH of the W phase was also examined.

2. Experimental

Tryptamine hydrochloride was prepared by mixing tryptamine (Aldrich) with equimolar amount of HCl. Reagent-grade chemicals of 5-hydroxytryptamine hydrochloride and L-tryptophan (Nacalai) were used as received. Tetrabutylammonium tetrakis(4-chlorophenyl)borate (TBATCIPB), tetrabutylammonium chloride (TBACl), and NB were prepared and purified according to the reported method [19,20]. Other chemicals were of reagent grade, and used as received.

The transfer of the protonated tryptamine (TH^+), serotonin (5HTH^+), and tryptophan (TrpH^+) across the NB|W interface was studied by cyclic voltammetry. The electrochemical cell for TH^+ and 5HTH^+ is represented by Cell I.



To examine the pH dependence of the voltammograms of TH^+ and 5HTH^+ , 0.1 M acetate buffer (pH 5–6), 0.1 M phosphate buffer (pH 6–8), 0.1 M ammonia buffer (pH 8–10), or 0.1 M carbonate buffer (pH 10–11) was used in place of 0.1 M LiCl in Phase

II. For the measurement of TrpH^+ , Phase II was replaced by x mM Trp + 0.1 M HCl to produce the cationic form. In Cell I, the interface between Phases I and II, indicated by an asterisk, is the test interface. The geometric area of the test interface was 0.126 cm^2 . The applied potential (E) is defined as the terminal potential of RE1 referred to that of RE2. The E was controlled by a potentiostat with a positive feedback IR compensation circuit (Hokuto, HA1010mM1A). In this paper, the E -values in voltammograms are reported against the midpoint potential of the transfer of tetramethylammonium ion across the NB|W interface ($E_{m,\text{TMA}}$). In cyclic voltammograms, the current was recorded after subtraction of the background current from the experimental current. All of the experiments were performed at 25 ± 1 °C.

3. Results and Discussion

Figure 1 shows the cyclic voltammograms of the transfer of TH^+ and 5HTH^+ across the NB|W interface. Well-defined voltammetric waves with anodic and cathodic peaks, which correspond to the transfer of TH^+ and 5HTH^+ from W to NB and from NB to W, respectively, were observed within the potential window shown in the curve c of Fig. 1. The anodic peak currents (I_{pa}) were proportional to the concentrations (c) of TH^+ and 5HTH^+ between 0.05–0.50 mM (Fig. 2A), and also to the square root of the scan rate (ν) between 0.005–0.2 V s^{-1} (Fig. 2B). The anodic and cathodic peak potentials shifted slightly to more positive and negative potentials, respectively, with increasing ν . The peak potential separations were 58 mV for both TH^+ and 5HTH^+ , when ν is extrapolated to zero. The midpoint potentials (E_{m}) remained constant independently of c as well as ν , and were -0.005 ± 0.002 V and 0.092 ± 0.001 V (vs. $E_{m,\text{TMA}}$), respectively, for TH^+ and

5HTH⁺. These results indicate that the voltammograms can be assigned to reversible waves of monovalent cation transfer. The pH of the test solutions was 6.5, which was much lower than the reported p*K*_a value of 10.2 for TH⁺ and 10.0 for 5HTH⁺ [21], indicating that the presence of the deprotonated neutral forms T and 5HT were negligible. Thus, based on the assumption that the difference between the reversible half-wave potential ($E_{1/2}^r$), as determined from the E_m of the reversible cyclic voltammogram, and the standard potential ($\Delta_{NB}^W\phi^\circ$) of the transfer of ion across the NB|W interface is equal to that of TMA⁺, that is,

$$\Delta_{NB}^W\phi^\circ = \Delta_{NB}^W\phi^\circ_{TMA} + E_{1/2}^r - E_{1/2,TMA}^r, \quad (1)$$

the $\Delta_{NB}^W\phi^\circ$ values of TH⁺ and of 5HTH⁺ were determined by taking $\Delta_{NB}^W\phi^\circ_{TMA} = 0.035$ V [22]. The values obtained are summarized in Table 1. The standard Gibbs energies of transfer from NB to W ($\Delta_{tr}G^{\circ,NB \rightarrow W}$) and the standard partition coefficient ($\log P^\circ$) [17] were calculated from the $\Delta_{NB}^W\phi^\circ$, and are also listed in Table 1. The $\Delta_{NB}^W\phi^\circ$ of 5HTH⁺ was more positive than that of TH⁺ by 97 mV, indicating that 5HTH⁺ is more hydrophilic than TH⁺ by the addition of hydroxyl group. Dvořák et al. have reported cyclic voltammetry of the transfer of β -phenylethylammonium ions across the NB|W interface [8], and shown that the addition of hydroxyl group to the aromatic ring led to the shift of $E_{1/2}^r$ by 77 mV. Our result may be compared with it. From the slopes of the regression lines in Fig. 2B, the diffusion coefficients of TH⁺ and 5HTH⁺ in W were estimated to be $(8.8 \pm 0.8) \times 10^{-6}$ cm² s⁻¹ and $(5.7 \pm 0.2) \times 10^{-6}$ cm² s⁻¹, respectively. The value obtained for 5HTH⁺ agrees well with the reported value of $(5.4 \pm 0.2) \times 10^{-6}$ cm² s⁻¹ in 0.1 M phosphate buffer (pH 7.4) [23].

Figure 3 shows the dependence of $E_{1/2}^r$ of TH⁺ and 5HTH⁺ on the pH of W phase.

The $E_{1/2}^r$ values of TH^+ and 5HTH^+ kept constant at neutral pH, whereas positive shifts of the $E_{1/2}^r$ values were observed at higher pH range. When the kinetics of protonation and deprotonation of amines in W phase are sufficiently fast, the $E_{1/2}^r$ can be expressed by [11]

$$E_{1/2}^r = E_{1/2}^{r'} + \frac{RT}{F} \ln \left[1 + \frac{K_a}{[\text{H}^+]} \left(1 + \sqrt{\frac{D^O}{D^W} K_p} \right) \right], \quad (2)$$

where $E_{1/2}^{r'}$ is the reversible half-wave potential at sufficiently low pH, K_a is the dissociation constant of the protonated form, K_p is the partition coefficient of the neutral form, D^α is the effective diffusion coefficient of the amine in α ($=$ O or W) phase [12], and R , T , and F are used in the usual meanings. The broken curves in Fig. 3 represent the calculated values of $E_{1/2}^r$ by Eq. (2), on assuming $\text{p}K_a = 10.2$ for TH^+ or 10.0 for 5HTH^+ [21], $\log K_p = 1.55$ for T or 0.21 for 5HT (reported for octanol|water [24]), and $D^O/D^W = 0.5$. The bending at lower pH in the plot of TH^+ can be explained by the larger $\log K_p$ value of T than that of 5HT.

The solid curve a in Fig. 4 shows the cyclic voltammogram of the transfer of TrpH^+ across the interface of 0.1 M TBATClPB (NB) | 0.1 M HCl (W). The anodic wave of TrpH^+ overlapped with the final rise of the background current shown in the curve b of Fig. 4. Although the anodic peak was not clear, the shapes of the voltammograms can be reproduced well by theoretical calculation of reversible waves [25], as shown by the circles on the curve a. We fitted the theoretical curves to the experimental results and obtained $E_{1/2}^r = 0.098 \pm 0.005$ V (vs. $E_{m,\text{TMA}}$). The term $(RT/F) \ln \left[1 + (K_a/[\text{H}^+]) \left(1 + (D^O/D^W)^{1/2} K_p \right) \right]$ in Eq. (2) was estimated to be 1 mV at pH 1.0 , on assuming $\text{p}K_a = 2.43$ [26], $\log K_p = -1.06$ [24], and $D^O/D^W = 0.5$. Thus, the $\Delta_{\text{NB}}^W \phi^\circ$, $\Delta_{\text{tr}} G^{\circ, \text{NB} \rightarrow \text{W}}$, $\log P^\circ$ values of TrpH^+ were determined and are summarized in Table 1.

We are now extending our study to examine the interaction of serotonin and other indole amines with a phospholipid monolayer formed at the NB|W interface. In addition, it will be interesting to study the interaction of these ions with other biomaterials, such as proteins and saccharides, by voltammetry at O|W interfaces.

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Table 1

Standard potentials of transfer, standard Gibbs energies of transfer, and standard partition coefficients of protonated indole amines at NB|W interface (25 °C)

Indole amine	$\Delta_{\text{NB}}^{\text{W}}\phi^{\circ} / \text{V}$	$\Delta_{\text{tr}}G^{\circ, \text{NB} \rightarrow \text{W}}$ / kJ mol^{-1} a)	$\log P^{\circ}$ b)
TH ⁺	0.030±0.002	-2.9±0.2	-0.51±0.03
5HTH ⁺	0.127±0.001	-12.3±0.1	-2.15±0.02
TrpH ⁺	0.132±0.005	-12.7±0.5	-2.23±0.08

a) $\Delta_{\text{tr}}G^{\circ, \text{NB} \rightarrow \text{W}} = -zF\Delta_{\text{NB}}^{\text{W}}\phi^{\circ}$, and b) $\log P^{\circ} = -(zF/2.303RT)\Delta_{\text{NB}}^{\text{W}}\phi^{\circ}$, with the charge $z = +1$.

Figure captions

Fig. 1. Cyclic voltammograms of the transfer of 0.40 mM (a) TH^+ and (b) 5HTH^+ at the 0.1 M TBATCIPB (NB) | 0.1 M LiCl (W) interface. The broken curve (c) is the background current. Scan rate, $\nu = 0.1 \text{ V s}^{-1}$.

Fig. 2. Plot of (A) I_{pa} vs. c at $\nu = 0.1 \text{ V s}^{-1}$ and (B) I_{pa} vs. $\nu^{1/2}$ at $c = 0.40 \text{ mM}$ for (a) TH^+ and (b) 5HTH^+ . The solid lines are the regression lines.

Fig. 3. Plot of E_{m} vs. pH for (a) TH^+ and (b) 5HTH^+ . Measurements were carried out in 0.1 M acetate buffer (pH 5–6), 0.1 M phosphate buffer (pH 6–8), 0.1 M ammonia buffer (pH 8–10), or 0.1 M carbonate buffer (pH 10–11). The broken curves were calculated by Eq. (2) with the parameters given in text.

Fig. 4. (a) Cyclic voltammograms of the transfer of 0.40 mM TrpH^+ at the 0.1 M TBATCIPB (NB) | 0.1 M HCl (W) interface. The circles on the curve (a) were calculated theoretically [25] with $E_{1/2}^{\text{r}} = 0.098 \text{ V}$ (vs. $E_{\text{m,TMA}}$). The broken curve (b) is the background current. Scan rate, $\nu = 0.1 \text{ V s}^{-1}$.

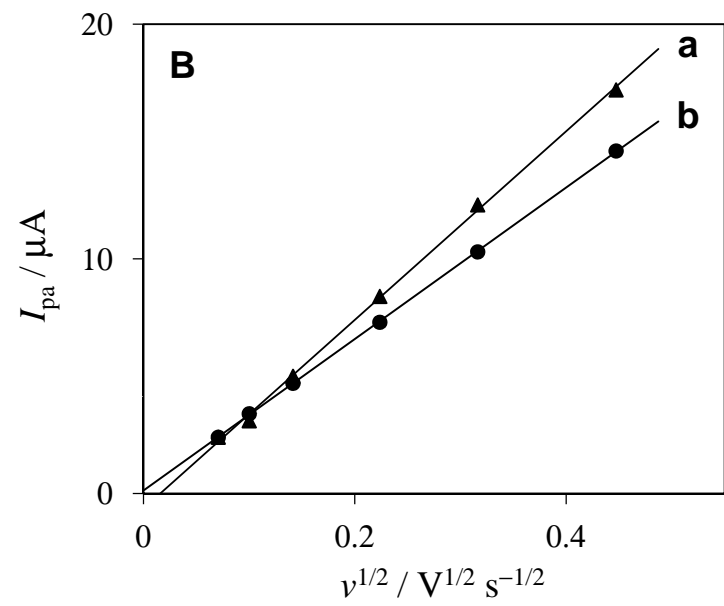
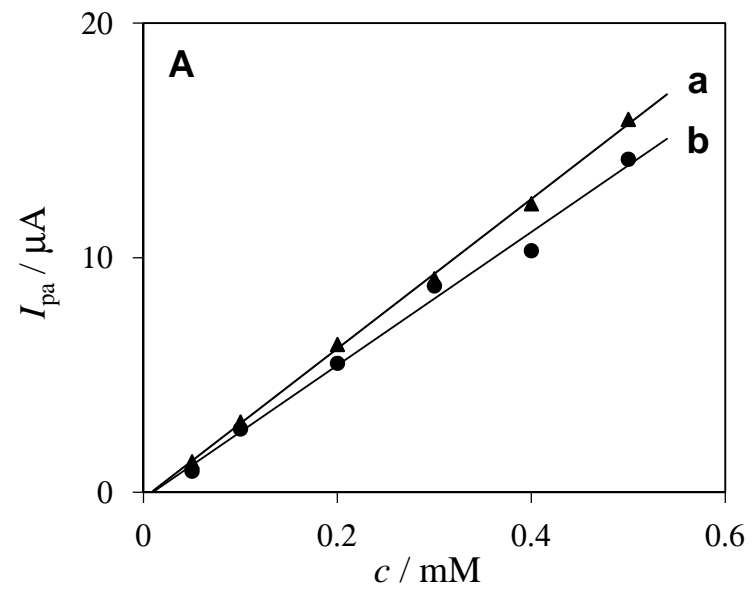


Fig. 2

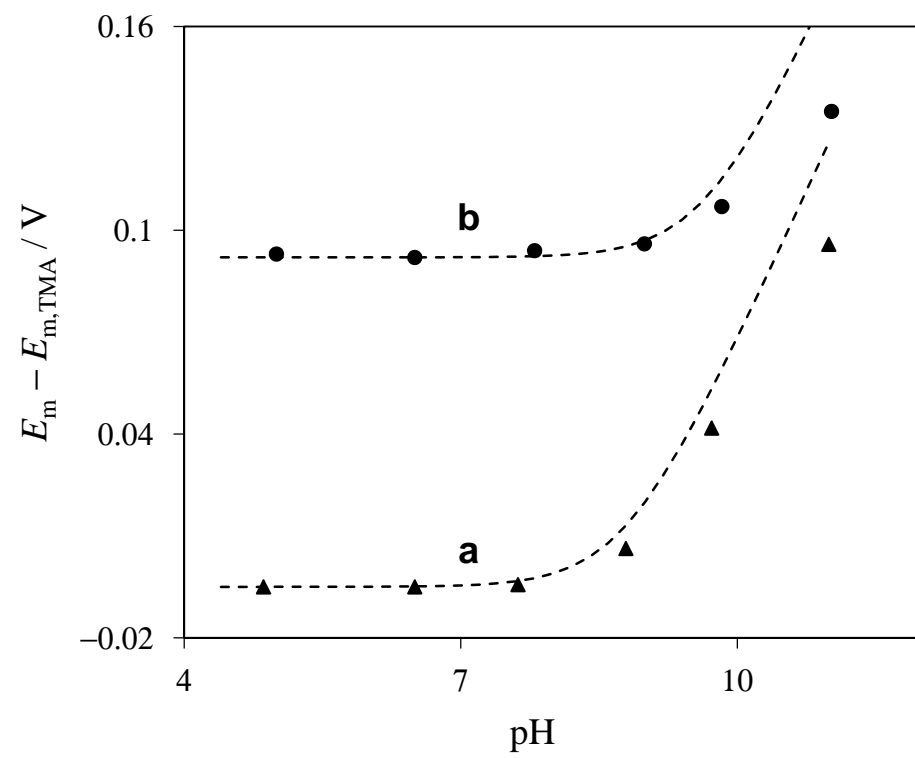


Fig. 3

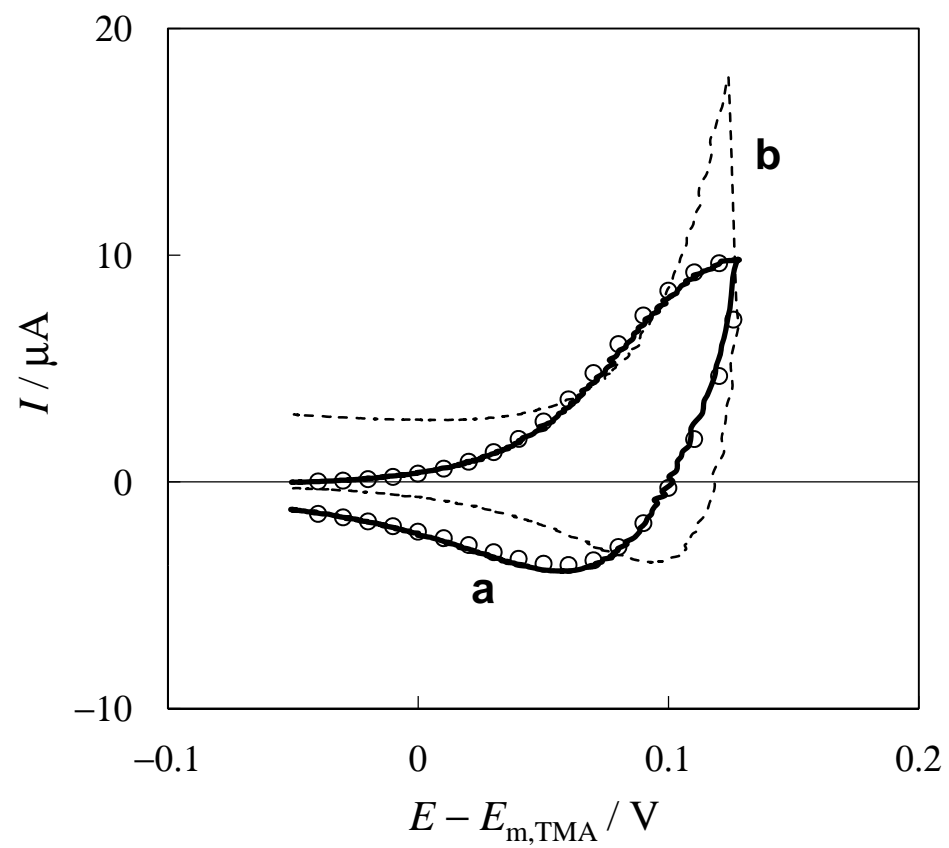


Fig. 4