Voltammetric Determination of Tryptophan and Serotonin at Glassy Carbon Paste Electrodes in a Potential Region Free from Interfering Substances

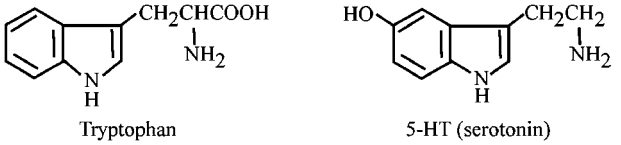
Kazufumi Inada, Katsuhiko Nishiyama,* and Isao Taniguchi
Department of Applied Chemistry and Biochemistry, Kumamoto University, 2-39-1 Kurokami, Kumamoto 860-8555

(Received April 8, 2009; CL-090347; E-mail: nishiyama@kumamoto-u.ac.jp)

Voltammetric determination of tryptophan (Trp) and serotonin (5-HT) was successfully conducted. To do this, we analyzed the redox peaks of their oxidation products at glassy carbon paste electrodes (GCPEs) in phosphate buffer solutions in the presence of interfering substances. The oxidation products of Trp and 5-HT provided redox couples at more negative potentials than for that of the interfering substance: fivefold tyrosine (Tyr) did not hinder the determination of Trp, and 200-fold uric acid (UA) and acetaminophen (APAP) did not hinder the determination of 5-HT.

Tryptophan (Trp, shown in Scheme 1) is an essential amino acid, an important building block for the construction of proteins and serves as a precursor for biosynthesis of biologically significant molecules such as serotonin (5-HT, shown in Scheme 1). As such, 5-HT availability in the brain should depend on blood Trp levels.[1] Blood 5-HT has been recognized as a useful indicator for a variety of clinical diagnoses. Specifically, the mean fasting blood 5-HT concentration in normal subjects has been shown to range from sub-μM to low-μM, while patients with diseases such as carcinoid syndrome display markedly elevated levels of blood 5-HT.[2,3] Based on above reasons, reliable, efficient, inexpensive analytical methods are valuable for the determination of Trp and 5-HT in blood or foods.

Voltammetry constitutes an interesting procedure as an alternative to separation-based methods for the analysis of biomolecules due to its sensitivity and simplicity. Catalytic effects have been observed for Trp and 5-HT on certain electrodes, including hemin-[4] and acetyl choline-modified[5] electrodes. Voltammetric determinations especially suffer from interference that exist in vivo in high concentrations and have oxidation potentials close to that of Trp and 5-HT. Tyr often limits this analytical technique for this reason. Due to the proximal isoelectric points of Trp (5.89) and Tyr (5.66), it is difficult to detect only one species of interest selectively and simply by utilizing differences in their charges. Several papers have reported for the determination of Trp in the presence of Tyr. Large peak separation between Trp and Tyr was obtained on a diamond electrode in an alkaline solution.[6] Trp was selectively determined by a stripping voltammetric technique in the presence of Tyr on an overoxidized polypyrrole film-modified electrode.[7] Similarly, UA and APAP have oxidation potentials close to that of 5-HT, and as such often limit its analysis. In a healthy human being, typical concentrations of UA in the blood are 120–450 μM. APAP has been widely used as an analgesic and antipyretic agent. Although the interfering substance of UA was eliminated or improved on several electrodes, including a DNA-,[8] a Nafion/ruthenium-oxide-,[9] and a carbon-nanotube coated-electrode,[10] the influence of APAP has not been discussed to date.



Tryptophan 5-HT (serotonin)

Scheme 1.

When indoles such as Trp and 5-HT are oxidized, new redox couples are observed in their voltammograms, and the peak potentials of the new redox couples are significantly more negative than that of Trp and 5-HT. Herein, we report the successful determination of Trp and 5-HT levels by implementation of novel redox couples in the presence of large amounts of interfering substances. Since the electrode surface would be deactivated by the irreversible adsorption of oxidation products, disposable electrodes have distinct advantages compared to other types of electrodes. Carbon paste electrodes (CPEs) were employed in the present work due to their ease of preparation and facile reuse.

Trp, 5-HT, Tyr, UA, and APAP were purchased from Sigma. Glassy carbon powder (1–12 μm), mineral oil, and poly(sodium 4-styrenesulfonate) (PSS) were purchased from Aldrich. Milli-Q water ($\rho = 18\,\mathrm{M\Omega\,cm^{-1}}$) was used for the preparation of all the solutions. A 0.1 M phosphate buffer solution (pH 7.0) was employed as a supporting electrolyte. Differential pulse voltammetry (DPV) was performed with a BAS 100 analyzer. A platinum (Pt) wire and Ag/AgCl (3 M NaCl, BAS) were used as a counter and a reference electrode, respectively. All experiments were conducted at room temperature ($22 \pm 2\,^{\circ}\mathrm{C}$). Unmodified glassy carbon paste electrodes (GCPEs) were prepared by mixing 90 wt % GC powder with 10 wt % mineral oil. The mixture was introduced into a Teflon tube (1-mm diameter) with a stainless wire used for electrical contact. PSS-modified GCPEs were prepared by grinding GC powder with 0.5 wt % PSS prior to the addition of mineral oil. The electrode surface was renewed by cutting the top of the electrode.

Selective determination of Trp in the presence of Tyr is often difficult because of the close peak potentials of Trp and Tyr. As shown in Figure 1 by the solid line, Trp provided the single oxidation peak O at 0.62 V in the first anodic sweep and the single reduction peak R′ at 0.11 V in the cathodic sweep. These voltammetric behaviors can be described by the equations:

$$\mathrm{Trp(red)} - \mathrm{e} \rightarrow \mathrm{Trp(ox)} \quad \mathrm{(O)}$$
$$\mathrm{Trp(ox)} \rightarrow \mathrm{Trp(ox)'}$$
$$\mathrm{Trp(ox)'} + \mathrm{e} \rightarrow \mathrm{T(red)'} \quad \mathrm{(R')}$$

As shown by the dotted line in Figure 1, Tyr provided the clear oxidation peak O at 0.64 V in the first anodic sweep. However, no clear peaks were observed in the cathodic scan for Tyr. Therefore, we can detect Trp selectively in the presence of Tyr by an-

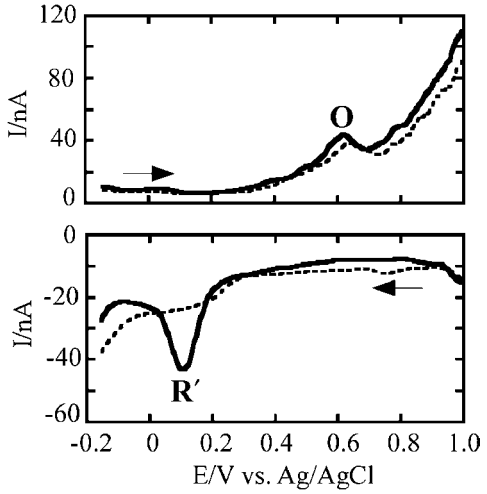


Figure 1. DPVs of 2 μM Trp (solid line) and 5 μM Tyr (dotted line) at a GCPE in a PB solution for 1st anodic (top) and the cathodic scan (bottom). Quiet time: 60 s.

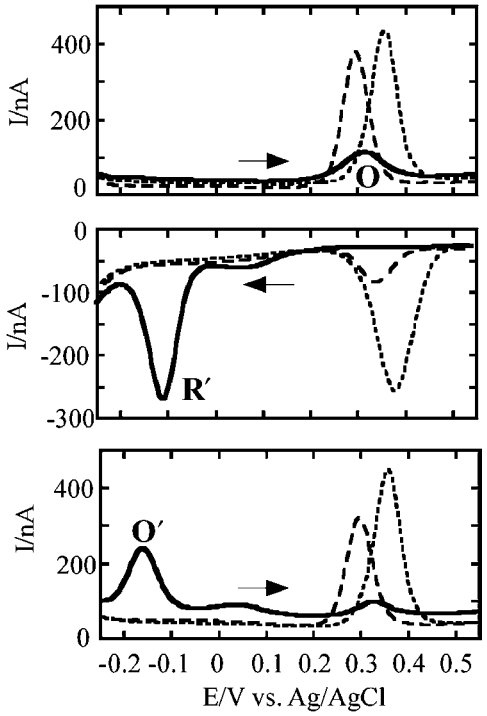


Figure 2. DPVs of 2 μM 5-HT (solid), 50 μM UA (broken), 50 μM APAP (dotted line) at a PSS-modified GCPE in a PB solution for 1st anodic (top), the cathodic (middle), and 2nd anodic scan (bottom). Preconcentration time: 5 min. Quiet time: 20 s.

alyzing the reduction peak of the oxidation product of Trp. Trp levels were selectively determined in the presence of a fivefold excess of Tyr.

Since 5-HT is cationic under neutral conditions (pK_a = 9.97), GCPE was modified by PSS as a cation exchanger to enhance the sensitivity of 5-HT. The solid line in Figure 2 shows the DPV of 5-HT at a PSS-modified GCPE. 5-HT provided the oxidation peak O at 0.32 V on the first anodic sweep, but the corresponding reduction peak was not observed for the cathodic scan. The reduction peak R′ at −0.11 V and reoxidation peak O′ at −0.16 V were observed on the cathodic sweep and second anodic sweep, respectively. When UA and APAP were present in the solution, large oxidation peaks were observed in the potential range between 0.2 to 0.5 V, as shown by the broken and dotted lines in Figure 2; the peak O overlapped with those of the interfering substances. On the other hand, the peak potential of R′ and O′ was significantly more negative than those of the interfering substances. As such, 200-fold UA and APAP did not affect the peak current of 5-HT at R′ and O′.

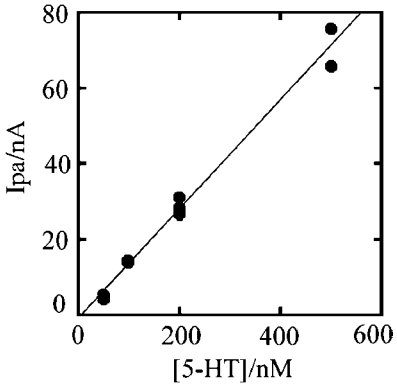


Figure 3. Calibration plot of 5-HT using O′ on DPVs.

For Trp, although the sensitivity decreased as the concentration increased, 2 μM Trp showed almost twofold larger peak current than that of 1 μM Trp. For 5-HT, the detection limit should be below 50 nM, and the calibration curve was linear up to 500 nM as shown in Figure 3.

In summary, we succeeded in selective detection of Trp and 5-HT by using redox reaction of oxidation products of samples in the presence of interfering substances.

References

1 I. González-Burgos, E. Olvera-Cortés, A. R. Del Angel-Meza, and A. Feria-Velasco, *Neurosci. Lett.* **1995**, *190*, 143.
2 E. Pussard, N. Guigueno, O. Adam, and J.-F. Giudicelli, *Clin. Chem.* **1996**, *42*, 1086.
3 I. P. Kema, W. G. Meijer, G. Meiborg, B. Ooms, P. H. B. Willemse, and E. G. E. de Vries, *Clin. Chem.* **2001**, *47*, 1811.
4 C. G. Nan, Z. Z. Feng, W. X. Li, D. J. Ping, and C. H. Qin, *Anal. Chim. Acta* **2002**, *452*, 245.
5 G.-P. Jin, X.-Q. Lin, and J. Gong, *J. Electroanal. Chem.* **2004**, *569*, 135.
6 G. Zhao, Y. Qi, and Y. Tian, *Electroanalysis* **2006**, *18*, 830.
7 Z. Chen, K. Okamura, M. Hanaki, and T. Nagaoka, *Anal. Sci.* **2002**, *18*, 417.
8 X. Jiang and X. Lin, *Anal. Chim. Acta* **2005**, *537*, 145.
9 J.-M. Zen, I.-L. Chen, and Y. Shih, *Anal. Chim. Acta* **1998**, *369*, 103.
10 K. Wu, J. Fei, and S. Hu, *Anal. Biochem.* **2003**, *318*, 100.

Published on the web (Advance View) June 6, 2009; doi:10.1246/cl.2009.686