Selective Determination of Tryptophan by Using a Carbon Paste Electrode Modified with an Overoxidized Polypyrrole Film

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We report on the selective determination of tryptophan, using a carbon paste electrode coated with an overoxidized polypyrrole film. Out of 21 protein amino acids, only tryptophan and tyrosine exhibited an oxidative voltammetric response with this electrode. Tryptophan, which was preferentially concentrated to the electrode under an open circuit condition, was determined by the stripping voltammetric technique with a linear response range of 10–100 μM. For the determination of 10 μM tryptophan, interference from a 15-fold excess of tyrosine gave an positive error of 6%, while the other amino acids did not exhibit any detectable interference.

(Received October 9, 2001; Accepted January 24, 2002)

Determination of amino acid has been attracting much attention due to increasing demands for biomedical and food applications. Currently, HPLC and capillary electrophoresis techniques most frequently have been used for such applications owing to their high performances in separation efficiencies. Although electrochemical techniques have some important advantages over these techniques in that on-site and real time monitoring are possible, only a few amino acids, such as tyrosine, tryptophan and cysteine, can be directly detected in a modest potential range at either conventional or chemically modified electrodes.1-4 To apply electrochemical techniques to a much wider range of amino acids, aiming at application to HPLC detectors, metallic substrates, such as copper, have been used as working electrode materials and the use of derivatizing agents has also been reported.5-9

The narrow detection spectrum, on the other hand, makes direct electrochemical techniques suitable for sensor applications, where selectivity as well as sensitivity is a matter of interest. In the present work, we discuss a highly selective carbon paste electrode modified with an overoxidized polypyrrole (OPPy) film for the detection of tryptophan. L-Tryptophan is an essential amino acid and is a precursor to the neurotransmitter serotonin. It has been reported that inadequate serotonin levels in human brains are in part responsible for depression and obsessive/compulsive behavior and that L-tryptophan is now being prescribed for a much wider range of disorders, such as anxiety, insomnia, addiction and obesity.

We have coated a carbon paste electrode with an OPPy film to concentrate tryptophan into the film, focusing on its cation accumulative property. It has been pointed out that nucleophilic attack of hydroxide ion to polypyrrole upon overoxidation destroys its conjugated structure by the insertion of carbonyl groups on pyrrole rings.10-13 A typical reaction scheme presented so far is shown below:

\[\text{NH}_2 + \text{H}_2\text{O} \rightarrow \text{OH}^{-} + \text{H}_2\text{O} \]

Scheme 1

The accumulation of cationic compounds has been assigned to the introduction of the electron-rich oxygen groups, such as carbonyl groups11-13 and carboxyl groups.14 In this study, we prepared a carbon paste electrode coated with an OPPy film for the selective determination of tryptophan, expecting an enhanced accumulative effect from an OPPy film.

Experimental

Chemicals

Reagent grade pyrrole and (+)-10-camphorsulfonic acid were purchased from TCI Chemicals (Tokyo). All the amino acids used were supplied from TCI and Wako Chemicals (Wako, Japan). Carbon in particle form (ICB-3020, nominal diameter 20 mm) was purchased from NIPPON CARBON (Tokyo). Paraffin oil was obtained from Ishizu Chemical (Osaka). AMIZET B, a medicinal solution for total parenteral nutrition of amino acid was purchased from TERUMO (Tokyo). The nominal content of each amino acid in mg ml⁻¹ was isoleucine (8.500), leucine (13.50), lysin (8.000), methionine (3.900),
phenylalanine (7.700), threonine (4.800), tryptophan (1.600),
valine (9.000), cysteine (1.000), tyrosine (0.500), arginine
(11.10), histidine (4.700), alanine (8.600), aspartic acid (0.500),
glutamic acid (0.500), glycine (5.500), proline (6.400) and serine
(4.200). Milli-Q water was used throughout. All the other
chemicals were of reagent grade and were used without further
purification.

**Apparatus and procedures**

Electrochemical experiments were performed with an EG&G
Model 263A potentiostat at ambient temperature (25±1°C). A
carbon paste electrode (diameter, 3 mm) and a platinum wire
(1 mm x 5 cm) served as the working and counter electrodes.
All the electrochemical measurements were referred to an
Ag[AgCl] saturated KCl|| electrode. To prepare a carbon paste
electrode (diameter 3 mm), a carbon/paraffin weight ratio of
5.6/1 was used, unless otherwise stated. One potentiodynamic cycle, starting from 0.0 V and being switched
back at 1.2 V at 60 mV s⁻¹, was applied to coat the electrode
with a thin polypyrrole film in an aqueous solution containing
0.1 M pyrrole and 0.1 M camphorsulfonic acid. The
polypyrrole film was then overoxidized in aqueous 0.1 M
NaOH by applying five voltammetric cycles at 20 mV s⁻¹
between -0.5 V and 1.2 V. In anodic stripping voltammetry
(ASV), tryptophan in pH 2 HCl-0.1 M KCl was
preconcentrated to an OPPy-modified carbon paste electrode
for 8 min under an open circuit condition, unless otherwise
stated. The electrode was then transferred to an aqueous 0.1
M KCl solution (pH 2.0, adjusted with HCl) for the
voltammetric measurement.

To study the porosity of an OPPy film, polypyrrole was
deposited on a glassy carbon electrode (diameter, 3 mm) from
an aqueous solution containing 0.10 M pyrrole and 0.10 M
dopant in the same manner as the carbon paste electrode.
The polypyrrole film was then overoxidized potentiodynamically
(scan range, from -0.1 V to +1.2 V; 5 cycles; 20 mV s⁻¹) in 0.1
M NaOH. The molecular volume of the dopant was evaluated
with WinMOPAC (V2.0, Fujitsu) and Free Wheel (v. 0.57T,
Butch Software).

**Results and Discussion**

**Optimization of fabrication parameters**

Among 21 protein amino acids, only tryptophan and
tyrosine exhibited voltammetric responses at almost the same
potential, though observed as totally irreversible single
oxidation waves (Table 1). The sensitivity of the carbon
paste electrodes was higher than those for the glassy carbon
electrodes. However, these two electrodes exhibiting similar
peak currents for reduction of 1.0 mM ferrocyanide (15 μA
and 14 μA for the glassy carbon and carbon paste electrodes)
indicated that the sensitivity increase was not due to the
difference in the surface roughness between the two
electrodes. The reason for the increase is not clear yet, but
the extraction of amino acid into the impregnated paraffin
phase after deprotonation of cationic amino acid might be
responsible.

The carbon paste electrode showed current increases for
both tryptophan and tyrosine in similar degrees, but, in
contrast, an OPPy-coated electrode preferentially
concentrated tryptophan, leading to better selectivity for this
amino acid (Table 1). To utilize this feature in voltammetric
analysis of tryptophan, optimization of an OPPy film was
carried out by controlling the permeability and thickness of
an OPPy film. Monitoring the oxidation current of tryptophan
in linear sweep voltammetric experiments revealed that the
permeability of tryptophan across an OPPy film was
dependent on the molecular volume of dopant (Fig. 1). It has
been known that dopant plays an important role in
characterization of conducting polymers, 15-18 and the results
in the figure suggest a correlation between film porosity and
dopant size. A dopant expelled from a polypyrrole film upon
overoxidation is expected to leave a dopant-sized nano-pore
and, indeed, camphorsulfonic acid gave the highest current
for the oxidation of tryptophan (0.134 nm³), which is the
closest to camphorsulfonic acid (0.134 nm³) in the molecular
volume. The permeability should also be dependent on film
thickness and morphology as well as on pore size, but this
figure shows that camphorsulfonic acid gives the highest

**Table 1** Voltammetric characteristics of tryptophan and tyrosine at
glassy carbon, carbon paste and OPPy-coated carbon paste
electrodes in 0.10 M KCl–HCl (pH 2.0) ⁴⁻

<table>
<thead>
<tr>
<th>Electrode</th>
<th>0.10 mM Tryptophan</th>
<th>0.20 mM Tyrosine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E ox / V</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I p / mA</td>
<td>E ox / V</td>
</tr>
<tr>
<td>Glassy carbon</td>
<td>1.02</td>
<td>1.04</td>
</tr>
<tr>
<td>Carbon paste</td>
<td>1.06</td>
<td>1.05</td>
</tr>
<tr>
<td>OPPy-coated carbon paste</td>
<td>1.03</td>
<td>1.04</td>
</tr>
</tbody>
</table>

a. Scan rate, 100 mV s⁻¹; diameter of the electrodes, 3 mm.
b. Peak potential for oxidation.
c. Peak current for oxidation.

**Fig. 1** Tryptophan (Trp) oxidation current (0.2 mM) at an OPPy-
modified glassy carbon electrode as a function of dopant size.
Dopant: (a) perchloric acid, (b) benzenesulfonic acid, (c)
toluensulfonic acid, (d) 1,5-naphthalendisulfonic acid, and (e)
10-camphorsulfonic acid.
content. Such an increase is expected from the results observed for the carbon and carbon paste electrodes (Table 1). The decrease after the maximum can be explained in terms of the increased thickness of the paraffin layer, which may cause the decrease in the electrical conductivity on the carbon particles. Thus, the content of 15% was selected in the following experiments. Figure 2B shows the sensitivity of an OPPy-coated carbon paste electrode as a function of the deposition time of polypyrrole. Microscopic examination of the electrode surface allowed us to confirm that a thicker film was formed when a slower scan rate was selected. The maximum in the current can be explained in terms of two factors acting counter to each other; as the film becomes thicker, the number of the cation exchange sites created in an OPPy film increases, while permeability of tryptophan through the film decreases. Hereafter, the scan rate of 60 mV s⁻¹ was selected for the preparation of an OPPy-coated electrode.

**ASV analysis of tryptophan**

Figure 3 shows selective preconcentration of tryptophan at an OPPy-modified carbon paste electrode. In contrast to the voltammetric experiment, tyrosine did not show any detectable peak in ASV experiments. Table 1 suggests that little affinity of tyrosine to the overoxidized polypyrrole film, as compared to tryptophane, would be the main reason for the absence of the stripping peak. The reason for the little affinity is not very clear yet, but looser fitting of tyrosine with the film pores due to its molecular volume (0.117 nm³) being smaller than that for tryptophane (0.134 nm³) might be responsible. Thus, tryosine preconcentrated in the film, though not taken up as much as tryptophan, can be easily extracted back to the solution phase when the electrode was immersed into a new electrolyte solution for stripping current monitoring.

As shown in Fig. 4, the saturation of the film indicates that tryptophan at a concentration smaller than 0.1 mM can be
accurately determined as long as a preconcentration time of less than 8 min is applied.

Figure 5 shows the effect of the pH of a sample solution on the stripping current. The highest current was found at pH 2, while 80% of the current was still observed even at pH levels above 4. The main equilibrium component of tryptophan at pH 4 is an electroneutral bipolar ion and only less than 5% of tryptophan exists as cations (Scheme 2). It has been reported that the introduction of electron-rich oxygen groups in an overoxidized polypyrrole film accumulates cationic compounds, but the results obtained here indicate that a substantial amount of the neutral amino acid is taken up into the film. The reason for the concentration is not very clear yet, but a dipole-dipole interaction between the introduced carbonyl group and the bipolar ion might be responsible. In addition, some of the uptake could be ascribed to the extraction of undissociated amino acid (H_2N–CHR–COOH) into the impregnated paraffin. It is interesting to note here that the increase in uptake occurred at pH levels much higher than the pK_a of tryptophan (2.4; Scheme 2). If cationic tryptophan is taken up into an OPpy film, then the increase should occur at pH values lower than the pK_a. The shift might have occurred due to a strong interaction between tryptophan cation and the cation exchange sites in the film.

It should be noted that the peak current of tryptophan observed in ASV analysis is not much different from that for direct voltammetric analysis (cf. Table 1 and Fig. 4), probably due to saturation of both the films with the amino acid. For an OPpy-modified electrode poised at a spontaneous potential (ca. 0 V), it takes about 8 min until the film is saturated with the amino acid, while the uptake in the direct voltammetric experiments seems to be established almost instantaneously during potential scanning. In the latter case, the amino acid in the film is forced to redistribute through the film upon application of the potential at the film/electrode interface, leading to the fast uptake equilibrium in the voltammetric experiment. In conclusion, the ASV technique improves the selectivity for tryptophan by accumulating it slowly but preferentially.

Figure 6 illustrates the dependence of stripping current on tryptophan concentration. A linear dependence was found in the range 5–100 μM and the detection limit estimated by linear sweep voltammetry was 1 μM for an S/N ratio of 2. Biomedical and food samples frequently contain 21 protein amino acids, indicating that each analysis technique employed must be highly selective to the target amino acid. To see if our technique meets this selectivity requirement, we studied interference from other amino acids. As is listed in Table 2, a 20-fold excess of any other amino acid except for tyrosine virtually did not interfere with the determination of tryptophan and a 15-fold excess of tyrosine showed a positive error of as small as 6%.

The modified electrode exhibited excellent stability and reproducibility for the determination of tryptophan and was easily reactivated by applying five repeated potentialdynamic cycles between 0.7 and 1.2 V in aqueous 0.1 M KCl–HCl (pH 2). Variation in the peak current was less than ±1.5% for 5 consecutive determinations and an appreciable decrease in the peak current was not found in 20 experiments over a week. Further, a variance in the current for five freshly prepared electrodes was less than ±2%. We applied this technique to the determination of tryptophan in amino acid nutrition solution, AMIZET B, which contained 18 different protein amino acids (see the experimental section for contents of each). The AMIZET solution, diluted 100 times with pH 2 HCl, was subjected to ASV analysis to give a tryptophan
Table 2  Interference with 10.0 µM tryptophan from other protein amino acids in ASV analysis

<table>
<thead>
<tr>
<th>Interfering amino acid</th>
<th>Relative current</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td>Alanine</td>
<td>1</td>
</tr>
<tr>
<td>Arginine</td>
<td>1</td>
</tr>
<tr>
<td>Asparagine</td>
<td>1</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>1</td>
</tr>
<tr>
<td>Cysteine</td>
<td>1</td>
</tr>
<tr>
<td>Cystine</td>
<td>1</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>1</td>
</tr>
<tr>
<td>Glutamine</td>
<td>1</td>
</tr>
<tr>
<td>Glycine</td>
<td>1</td>
</tr>
<tr>
<td>Histidine</td>
<td>1</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>1</td>
</tr>
<tr>
<td>Leucine</td>
<td>1</td>
</tr>
<tr>
<td>Lysine</td>
<td>1</td>
</tr>
<tr>
<td>Methionine</td>
<td>1</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>1</td>
</tr>
<tr>
<td>Proline</td>
<td>1</td>
</tr>
<tr>
<td>Serine</td>
<td>1</td>
</tr>
<tr>
<td>Threonine</td>
<td>1</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>1</td>
</tr>
<tr>
<td>Valine</td>
<td>1</td>
</tr>
</tbody>
</table>

a. Preconcentration, 8 min in pH 2.0 HCl–0.10 M KCl; stripping, 100 mV s⁻¹ in pH 2.0 HCl–0.10 M KCl.
b. Concentration of coexisting amino acid in µM.

concentration of 7.60±0.10 mM (n=3), which agreed well with the nominal concentration of 7.83 mM. From these results, we can safely conclude that the ASV technique developed here has excellent selectivity and stability for practical applications to medicinal samples.

Conclusions

The technique described in this paper provides a highly selective and stable tryptophan sensor with applicability to medicinal samples. At least 10-fold excess of other protein amino acids virtually did not interfere with the determination of tryptophan. A linear response for tryptophan was found over a range of 5.0–100 µM and the detection limit for an S/N ratio of 2 was estimated to be 1 µM. The accumulation time would be made shorter by employing a more sensitive voltammetric technique such as differential pulse voltammetry. The high selectivity of the electrode towards tryptophan can be explained in terms of molecular size recognition arising from a nano-porous structure of the oxidized polypyrrole film.

References