Electrochemical Detection of Melatonin, a Time-Keeping Hormone, with Alkanethiol-Modified Gold Electrodes

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1 INTRODUCTION

Melatonin (N-acetyl-5-methoxytryptamine) is an indole hormone which is synthesized in pineal gland and eye. This indole compound has been considered as a time-keeping hormone at the biological clocks, because its levels in vivo are photoperiod-dependent and exhibit circadian rhythms under the constant dark conditions1,2). Melatonin in the biological samples can be detected by the combination of a HPLC system and an electrochemical detector at the present day3). Recently the development of the more simple monitoring systems for melatonin in unpurified samples has been requested from the basic and applied researches of the biological rhythms. But it is difficult to detect this hormone selectively by electrochemical oxidation without separation process, because biological sample such as blood contains many interfering substances. These substances such as catecholamines, indoleamines and ascorbate are oxidized easier than melatonin. Then, for the purpose of the accomplishment of the separation-free detection of melatonin, we paid attention to the point that melatonin is hydrophobic, while most interfering substances are hydrophilic. We expected that the electrode surfaces modified with hydrophobic groups could be used to catch and oxidize melatonin selectively by the hydrophobic interaction. It is well known that alkanethiols are self-assembled onto gold surface to make the densely packed, hydrophobic monolayer4). Takehara et al. reported recently the permeation of a few biological hydrophobic compounds, such as FAD and ubiquinone, into the n-alkanethiol monolayer formed on a gold electrode5). Here we tried to apply n-alkanethiol-modified gold electrodes to detect melatonin selectively. The effect of alkyl chain length on the selective oxidation of melatonin is also discussed.

2 EXPERIMENTAL

Gold electrodes were cleaned with hot chromic acid mixture and rinsed with distilled water and ethanol. Then the electrodes were immersed into the ethanol solutions containing 20 mM (M=mol dm⁻³) of various n-alkanethiols (CₙSH, n=4,8,12,16) for 15 to 20 min to modify the gold electrode surface by the hydrophobic chains (the modified electrodes are represented as Cₙ/Au electrodes). n-Alkanethiols were purchased from Wako Pure Chem. Ind., Ltd. After rinse with ethanol and distilled water, the electrodes were set into the 50mMNa₂SO₄, 0.1M phosphate buffer solutions (pH 7.0), in which 0.5mM indoleamine or catecholamine were dissolved. A guaranteed reagents of melatonin, serotonin, dopamine were used without further purification. Differential pulse voltammograms were measured to characterize electrochemical detection of these amines on a bare and modified gold electrodes, with a polarographic analyzer (Yanaco P-1100).

3 RESULTS AND DISCUSSION

Differential pulse voltammetry of melatonin was carried out at a bare gold electrode and at C₄/Au electrodes to investigate the modification effects on the oxidation of melatonin. Oxidation peak potentials and...
currents in voltamograms of melatonin are arranged in Table 1. At a bare gold electrode, oxidation peak for melatonin was observed at a potential of 0.7V vs Ag/AgCl. The oxidation wave of melatonin at a C4/Au electrode was almost similar to that at a bare gold electrode. However, the oxidation waves at the C8, C12, C16/Au electrodes were significantly declined. That is, oxidation of melatonin was disturbed on the C8, C12, C16/Au electrodes. It may be explained that longer and densely packed alkyl chains prevented migration of melatonin to the gold electrode surface.

<table>
<thead>
<tr>
<th>Electrodes</th>
<th>Au</th>
<th>C4/Au</th>
<th>C8/Au</th>
<th>C12/Au</th>
<th>C16/Au</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidation peak potential (V vs Ag/AgCl)</td>
<td>0.7</td>
<td>0.7</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Oxidation peak current (μA)</td>
<td>2.4</td>
<td>2.2</td>
<td>0.8</td>
<td>0.2</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Figure 1 shows the differential pulse voltamograms of electrolyte (phosphate buffer containing Na2SO4), melatonin, serotonin and dopamine, respectively on a bare gold and a C4/Au electrode. The oxidation peaks for serotonin and dopamine at a bare gold electrode were observed at the potentials of 0.35V and 0.2V vs Ag/AgCl, respectively. On the other hand, no oxidation peaks for serotonin and dopamine were observed at a C4/Au electrode. The oxidation of serotonin and dopamine is clearly suppressed at a C4/Au electrode, though oxidation of melatonin is smoothly performed. These results support that a C4-modified electrode may be used to detect melatonin selectively. It is suggested that suitable hydrophobicity and barrier length of alkyl groups at the alkanethiol-modified electrodes are important factors to achieve the selective electrochemical detection of the melatonin.

The level of melatonin in blood is several nM at midnight. A few other hydrophobic metabolites may also be detected with a C4-modified electrode. The detailed selectivity and the detection limit of melatonin with this modified electrode are now on study.

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REFERENCES