Effect of Electrode Modification by Ionic Polymer Adsorption on Electrochemiluminescence of Luminol

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The Chemiluminescence of luminol is useful for the high sensitive quantitative analysis of biochemicals that can be converted into hydrogen peroxide by an oxidase. Conveniently, luminol is inexpensive and possesses low toxicity. However, the method has stability problems because reactant-mixing conditions influence the results.

Electrochemiluminescence (ECL) is generated by electrolytic reaction instead of catalysis. ECL of luminol can be performed on completely mixed solutions of luminol and hydrogen peroxide. Especially, the characteristic of ECL is applicable for micro total analytical system (μTAS). However, the optimal pH for the ECL reaction of luminol is near 11, far from intensity at physiological pH.

In this work, we expected that cationic polymer modification onto the electrode would be increased pH and anionic polymer modification onto the cationic polymer layer would block permeation of the anion by electrostatic repulsion. The ITO was soaked for 15 min in an aqueous solution of 1 wt% polyethyleneimine (PEI, MW: 70,000) buffered by 0.1 M borate at pH 8.0. The treated ITO is designated PEI-ITO. The PEI-ITO was soaked in an aqueous solution of 1 wt% polyacrylic acid (PAA, MW: 25,000) for 15 min. The treated ITO is designated PAA-PEI-ITO. The ECL intensity at the ITO electrode in a mixed solution of 1.0 mM luminol and 10-30 μM hydrogen peroxide buffered by phosphate or Tris-HCl (pH 7.4) was measured. The electrode potential was applied in alternate pulses of 0.00 V versus Ag/AgCl for 3 s and 1.00 V for 3 s. Photons emitted from the ECL flow cell were detected by photomultiplier tube connected to a digital ammeter.

The X-ray photoelectron spectroscopy (XPS) show that treatment with PEI decrease the relative surface content of indium and tin, while dramatically increase those of carbon and nitrogen(**Table 1**). Furthermore, PAA treatment clearly decreases the contents of indium, tin, and nitrogen. These results support the formation of an ionic polymer layer on the ITO. The thickness of the polymer-adsorption layer was less than 10 nm, which limited depth of detection by XPS, because the emitted electrons from indium and tin were detectable on the treated ITO.

The relation between PMT current at the electrodes and hydrogen peroxide concentration is shown in **Figure 1**. A high degree of linearity existed between current intensity and hydrogen peroxide concentration in the range of 0-30 μM.

We were concerned that permeation of strongly buffering phosphate ion into the adsorbed PEI layer might reduce the effect of PEI on pH at the electrode surface and on ECL intensity. However, the ECL was enhanced by PEI more dramatically in phosphate buffered solution than in Tris-HCl buffered solution although the phosphate is more strong buffer anion than Cl⁻.

At the body fluid specimen in sample solution, we were apprehensive that anionic reducer into the PEI layer might inhibit the ECL of luminol. The slope of the line is a suitable parameter to estimate sensitivity of ECL to hydrogen peroxide concentration. The slopes are listed in **Table 2**. Unfortunately, ECL *via* the PEI modified

electrode is seriously inhibited by anionic reducing agent. Removal of the reducing agents by enzymatic pre-reactor may enable analysis of biochemicals in body fluids using ECL at a PEI-ITO electrode.

Table 1 Surface elemental composition of the unmodified and modified ITO, determined by XPS

ITO electrode	Surface elemental composition[%]				
	In	Sn	O	C	N
Unmodified-	20.9	3.0	36.4	37.9	1.8
PEI -	8.3	1.3	27.9	59.0	3.5
PAA-PEI-	3.0	0.4	28.1	65.6	2.8
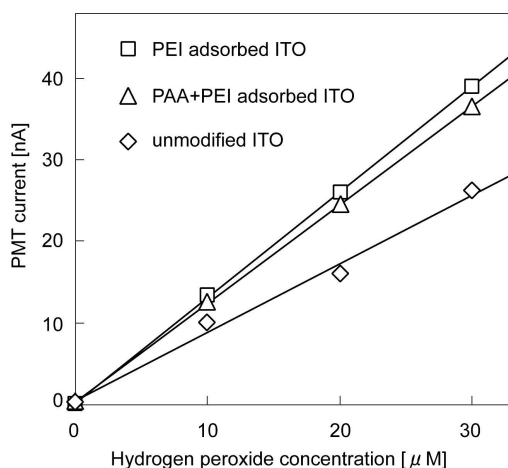
Figure 1 Electrochemiluminescent intensity by the unmodified or the polyion adsorbed ITO electrode with 0.1 M Tris-HCl buffer solution

Table 2 Slope of PMT current as a first-order function of hydrogen peroxide concentration in the absence or presence of 100 μ M ascorbic acid with 0.1 M Tris-HCl buffer solution [mA/M]

ITO electrode	Ascorbic acid	
	absence	presence
Unmodified-	0.84	0.52
PEI -	1.29	0.24
PAA-PEI-	1.24	0.26