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# Differential pulse adsorptive stripping voltammetry of osmium-modified peptides

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**Abstract**

Complexes of osmium tetroxide with nitrogen ligands were developed and used in our laboratory as probes of the DNA structure. Here, we show that the complex of osmium tetroxide with 2,2′-bipyridine (Os,bipy) can be used for modification and electrochemical detection of proteins at neutral pH. Salmon luteinizing hormone (SLH) containing two tryptophan (Trp) residues and human luteinizing hormone (HLH) containing one Trp were modified by Os,bipy and measured by differential pulse adsorptive stripping voltammetry (DPAdSV) at a hanging mercury drop electrode (HMDE). The intensity of the DPAdSV catalytic signals corresponded to the number of Trp residues in the peptide molecule. Decreasing pH of the background electrolyte from 6.6 to 3.8 led to the increase of DPAdSV signals, suggesting that at pH 3.8, the DPAdSV detection limit might be well below 1 ng/ml. Our results suggest that Os,bipy is potentially useful for chemical modification of proteins. 

*Keywords:* Chemical modification of peptides; Osmium tetroxide,2,2′-bipyridine; Luteinizing hormones; Differential pulse adsorptive stripping voltammetry; Mercury electrode

## 1. Introduction

In recent years, chemical probes have been increasingly applied to study DNA in vitro and in vivo [1,2]. Among them, single-stranded-selective probes such as diethyl pyrocarbonate (DEPC), bromo- and chloroacetaldehyde (BAA, CAA) and osmium tetroxide, pyridine (Os,py) have been especially useful in the studies of supercoil-stabilized DNA structures [3]. At the beginning of the 1980s, we showed that Os,py could be applied as a probe of DNA structure, reacting preferentially with single-stranded (ss) and distorted DNA regions [4]. Moreover, DNA-Os,py adducts can be determined by means of differential pulse polarography using the mercury drop electrode (DME) and voltammetry using the hanging mercury drop electrode (HMDE); these adducts produce, in addition to the catalytic signal at about −1.2 V, also smaller faradaic signals at more positive potentials [5–7]. Later, it was shown that DNA might be destabilized by the relatively high concentration of pyridine [2,5,8–10]. Therefore, pyridine was replaced by 2,2′-bipyridine (bipy) and other ligands suitable for probing of the DNA structure, such as *N*,*N*,*N*′,*N*′-tetramethylethylendiamine (TEMED) or bathophenanthroline disulfonic acid (BPDS).

Application of these complexes as chemical probes of protein structure may appear quite interesting provided that these complexes would react with amino acid side chains under conditions close to physiological. Most chemical reagents applied for modification of proteins can be used only under conditions remote to ones in cells, e.g. the chemical modification of tryptophan residues in proteins by *N*-bromosuccinimide is carried out at pH around 4.0, otherwise the specificity of the reagent to tryptophan and degree of tryptophan modification is decreased [11]. Therefore, chemical probes reacting with proteins under physiological conditions are sought. Deetz and Behrman [12] showed that complex of Os,py with tryptophan derivatives formed bis(pyridine) osmate esters (Fig. 1) similar to thymidine-Os,py adducts. In this paper, we modified two peptides differing in their tryptophan content by Os,bipy and tested their electrochemical activity at the HMDE. We found that these peptides produced signals similar to those

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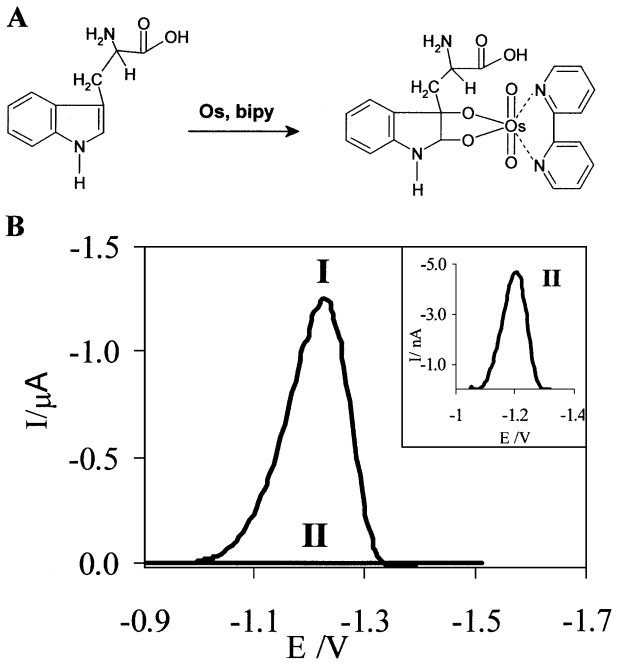


Fig. 1. (A) Formation of the ester between osmium tetroxide,2,2′-bipyridine (Os,bipy) and tryptophan. (B) Inset, differential pulse adsorptive stripping voltammograms of Os,bipy-modified salmon luteinizing hormone (SLH). Two hundred nanograms per milliliter of SLH in 0.1 M Britton–Robinson buffer, (I) pH 3.8, (II) pH 6.6, inset pH 6.6—zoom. Pulse amplitude 50 mV/s, scan rate 10 mV/s, time of accumulation 120 s, initial potential −0.10 V, stirring, moving average baseline correction.

of DNA-Os,bipy adducts and the intensity of these signals reflected the tryptophan content in the peptide molecules.

## 2. Experimental

### 2.1. Materials and methods

Salmon luteinizing hormone (SLH, $M_r = 1182.3$) and human luteinizing hormone (HLH, $M_r = 1212.3$) (Scheme 1) were obtained from Sigma. Osmium tetroxide was purchased from JMC (England). All other chemicals were of analytical grade. Nitrocellulose membranes were obtained from Millipore. Solutions were prepared from triple-distilled water. The supporting electrolyte used was 0.1 M Britton–Robinson buffer. The reaction mixture contained SLH or HLH at a concentration of 500 μg/ml, 10 mM Tris–HCl (pH 7.0), 2 mM $OsO_4$ and 2,2 mM 2,2′-bipyridine (Os,bipy). The reaction was carried out at 26 °C for 8 h, dialysed for 5 h at 4 °C against 0.1 M Tris–HCl (pH 7.0) using nitrocellulose membranes.

SLH: pGlu-His-**Trp**-Ser-Tyr-Gly-**Trp**-Leu- Pro-Gly-$NH_2$

HLH: pGlu-His-**Trp**-Ser-Tyr-Gly- Leu-Arg-Pro-Gly-$NH_2$

Scheme 1.

### 2.2. Apparatus and procedures

Differential pulse adsorptive stripping voltammetry (DPAdSV) measurements were performed with an Autolab analyzer (Eco Chemie, The Netherlands) in connection with VA-Stand 663 (Metrohm, Zurich, Switzerland). Three-electrode system was used consisting of Ag/AgCl/3M KCl electrode as a reference and platinum wire as an auxiliary electrode. The working electrode was the hanging mercury drop electrode (HMDE) with an area of 0.4 $mm^2$. All measurements were done at room temperature.

## 3. Results and discussion

Earlier we showed that DNA-Os,bipy adducts produced polarographic peak at about −1.2 V due to the catalytic hydrogen evolution [5,8,9]. The height of this signal increased with decreasing pH of the background electrolyte and the signal showed characteristics of catalytic electrode processes [4–6,13,14]. Because we were interested in whether peptides modified by Os,bipy would behave in a similar way, we treated two luteinizing hormones (differing

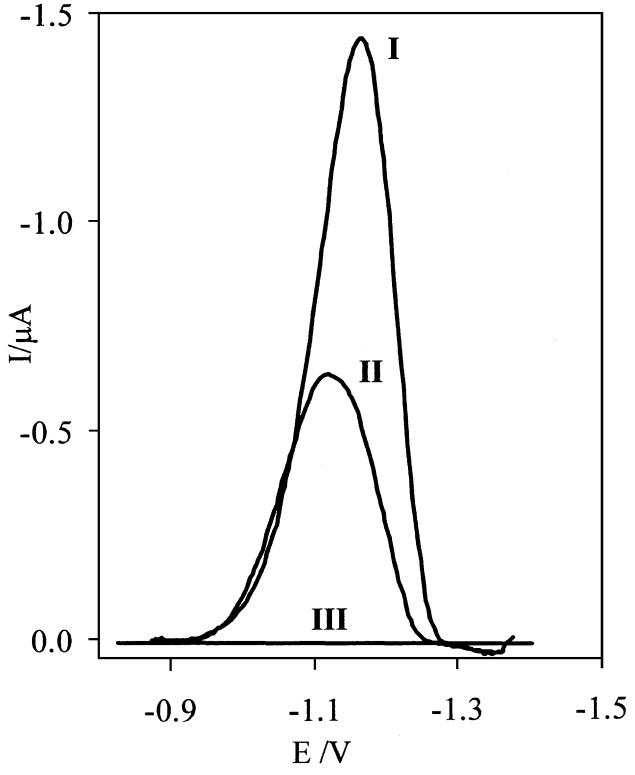


Fig. 2. Differential pulse adsorptive stripping voltammograms of Os,bipy-modified luteinizing hormones: (I) SLH, (II) human (HLH) and (III) unmodified SLH. Five hundred nanograms per milliliter SLH or HLH in 0.1 M Britton–Robinson buffer, pH 4.0. Other details as in Fig. 1.

from each other by the number of tryptophan residues Scheme 1) with 2 mM Os,bipy using the procedure previously applied for DNA modification [1]. In comparison to unmodified SLH (Fig. 2), this peptide produced, after the Os,bipy treatment, a well-developed DPAdSV peak at about −1.2 V. While in the 0.1 M Britton–Robinson buffer, pH 6.6, only a small peak was obtained, the peak observed at pH 3.8 was by almost three orders of magnitude higher (Fig. 1), showing a similar pH dependence observed with the same peak produced by DNA-Os,bipy adducts.

We compared SLH containing two tryptophan residues with HLH containing only one tryptophan residue. Both modified hormones produced well-developed peaks but the area of HLH peak corresponded to 50% of that of SLH peak, suggesting that tryptophan residues are responsible for the observed peaks (Fig. 2). The measurements were performed at relatively high peptide concentration (500 ng/ml) where the peak height depended little on the peptide concentration (not shown). Our results are in agreement with Deetz and Behrman [12], who showed the formation of bis(pyridine) osmate esters in tryptophan derivatives similar to thymidine-Os,py adducts. Using DPAdSV at acid pH, we were able to detect Os,bipy-modified peptides at concentration down to 1 ng/ml at $t_A = 120$ s. It can be expected that substantially lower concentrations can be determined at longer accumulation times.

## 4. Conclusion

In this paper, we show that treatment of peptides with Os,bipy at neutral pH results in DPAdSV peaks at about −1.2 V at HMDE. The tests we have so far performed (unpublished results), including the dependence on pH mentioned in this paper, suggest that this peak is most probably due to the catalytic hydrogen evolution. More details will be published elsewhere. Considering our previous results obtained with DNA-Os,bipy adducts, studies of Os,py modification of tryptophan [12,15] and our results (Fig. 2), we can conclude that for the peak at −1.2 V produced by the studied peptides, tryptophan residues are probably responsible. Using acidic background electrolytes, subnanomolar concentrations of Os,bipy-modified peptides can be determined.

The d.c. polarographic presodium wave [16] and Brdička double-wave [17] have been known for decades as the only protein signals due to the catalytic hydrogen evolution. Brdička polarographic waves observed in presence of cobalt ions have been widely applied in medicine [18] and also in molecular–biological studies [19]. Recently, it has been shown that peptides [20] and proteins [21] produce constant current chronopotentiometric "peak H" at highly negative potentials that is an analogy of the d.c. polarographic presodium wave (known to be poorly developed and of little analytical use) [18]. Peak H is well developed and can be used for the determination of peptides and proteins at very low concentrations. Here, we demonstrate a new catalytic reaction of proteins that combine high sensitivity of the electrochemical determination with the power of the chemical probes. Our preliminary results of Os,bipy modification of the tumor suppressor protein p53 are in good agreement with the results in this paper, suggesting that Os,bipy might be useful for chemical modification of peptides and proteins.

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