Enzyme-modified carbon fiber microelectrode for the specific and sensitive quantification of choline using fast scan cyclic voltammetry

Lugo-Morales LZ, Corder AK, Sombers LA*
Department of Chemistry, North Carolina State University, Raleigh, NC 27613, USA
(*lasomber@ncsu.edu)

Introduction

There is widespread interest in studying the mesolimbic dopamine (DA) pathway because it is highly implicated in reward and motivated behavior [1]. Glutamate release in the ventral tegmental area (VTA) can modulate the DA neurons that reside there, causing them to fire in bursts and inducing transient extracellular DA fluctuations in the nucleus accumbens (NAc) [2-3]. Significant evidence suggests that a cholinergic afferent from the laterodorsal tegmentum might enable DA neurons in the VTA to respond to glutamatergic inputs to the region [4]. However, direct evidence characterizing this modulation in intact animals is lacking, due largely to the absence of novel strategies for monitoring in vivo acetylcholine (ACh) at sufficiently high spatial and temporal resolution. Rapid fluctuations of electrochemically active molecules can be studied in real-time using fast-scan cyclic voltammetry (FSCV) at carbon fiber microelectrodes (CFMEs). This electrochemical approach is well suited for real-time measurements of chemical changes in the brain, due to electrochemical selectivity in the face of interferents, and the excellent temporal and spatial resolution afforded by the technique. Choline is the biosynthetic precursor to and breakdown product of ACh [5]. In presence of the oxidoreductase enzyme choline oxidase (ChOx), the substrate choline is converted into betaine and hydrogen peroxide (H$_2$O$_2$). This work reports on the development and characterization of a novel voltammetric detection method for choline using enzyme-modified CFMEs based on the encapsulation of ChOx within electrodeposited chitosan hydrogel at the CFME surface. In our laboratory this enzyme immobilization technique was previously developed to fabricate glucose oxidase (GOx) microbiosensors that were characterized both in vitro and in vivo for the real-time detection of extracellular glucose fluctuations in living brain tissue. This approach eliminates the need for spatial signal subtraction schemes, the addition of redox mediators, or chemically-selective coatings that are required with the traditional use of enzyme-modified electrodes. Thus, it will enable the sensitive and specific quantification of rapid fluctuations of choline at discrete locations in brain tissue, and will enable countless new experiments that require the real-time detection of ACh.

Methods

To fabricate the enzyme-modified microelectrodes, CFMEs were electrochemically pretreated using a triangular waveform ranging from -0.4 V to +1.4 V and cleaned in purified isopropyl alcohol. These CFMEs were lowered into an aqueous solution of 1% chitosan containing 1 mg/mL ChOx (from
Alcaligenes sp.), 1mg/mL bovine serum albumin (BSA) and 0.5% Triton X-100 and its pH was adjusted to 5.0. A DC power supply was used to apply -3.0 V (vs Ag/AgCl), a potential sufficient to reduce protons in solution to H₂ gas. Thus, a pH gradient was generated at the microelectrode surface. When the local pH rose above chitosan’s pKa (~6.3), chitosan was deprotonated and electrodeposited as a film on the microelectrode surface, locally encapsulating ChOx. GOx/chitosan modified electrodes were fabricated using the same electrodeposition technique but without the addition of BSA and TritonX-100 as stabilizers. All in vitro data were collected in a flow-injection apparatus.

Stereotaxic surgery was performed on male Sprague-Dawley rats implanted with a jugular vein catheter under urethane anesthesia (n = 5) for in vivo characterization of GOx/chitosan-modified microelectrodes. A GOx-modified electrode was implanted into the caudate putamen (CPu, +1.2 mm A/P, +3.6 mm M/L, -4.5-6.0 mm D/V) with a Ag/AgCl reference electrode in the contralateral hemisphere. All electrochemical measurements for the detection of glucose and choline were made every 100 msec by applying a triangular waveform (+0.1V to +1.4V, 400 V/s). Data was background subtracted and digitally filtered.

Results and Discussion

We have developed a genuinely new, fast-scan voltammetric detection scheme for H₂O₂ [6]. Using this approach, we have quantitatively and selectively monitored pharmacologically-evoked glucose fluctuations with unprecedented chemical and spatial resolution (Figure 1). Furthermore, this novel biosensing strategy should generalize to the encapsulation of any H₂O₂-producing enzyme, enabling broad application to chemical monitoring with FSCV.

Here, we have used it at CFMEs modified with an electrodeposited chitosan matrix that confines ChOx. At ChOx/chitosan-modified electrodes, conversion of choline to betaine generates 2 molecules of H₂O₂ that are detected on the electrode surface and identified by way of a characteristic cyclic voltammogram. Voltammograms collected in vitro (Fig. 1a) show a single peak at ~1.2 V, correlating with H₂O₂ [6]. Micromolar range of choline concentrations generated a linear response (n = 1, r² = 0.98, three replicated injections for each concentration, Fig. 1b). The entire data set is plotted as a color plot (Fig. 1c), allowing the presence of specific substances to be discriminated from electrical or chemical noise, and statistically verified [7].

Figure 1. In vivo performance of GOx/chitosan-modified microelectrodes.
Glucose concentration traces collected in the CPu of a representative animal one
min. after intravenous injection of glucose infusion (purple) compared to saline (black).

![Graph](image)

Figure 2. Preliminary data collected from a ChOx/chitosan-modified microelectrode. (a) Background-subtracted voltammograms for H$_2$O$_2$ enzymatically generated in response to choline. (b) Linear response to micromolar concentrations of choline ($n = 1$). Error bars, ± s.e.m represent replicated injections for each concentration. (c) Current (color scale) generated in response to choline (arrow) plotted with respect to the applied potential (y axis) and collection time (x axis).

References