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Journal of Colloid and Interface Science 296 (2006) 165–177
JOURNAL OF Colloid and Interface Science
www.elsevier.com/locate/jcis

Synthesis and characterization of an electroactive surface that releases γ-aminobutyric acid (GABA)

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Received 21 May 2005; accepted 16 August 2005

Available online 15 September 2005

Abstract

We report the synthesis and characterization of a new electroactive surface capable of releasing the neurotransmitter γ-aminobutyric acid (GABA) upon reduction. The GABA was anchored to an alkanethiol via electrochemically active quinone (abbreviation, TM-GABA). The quinone unit, upon reduction to the hydroquinone, cyclizes to release GABA into solution. The half-life is 99 s. The self-assembled monolayer (SAM) of TM-GABA on gold was prepared and characterized with several surface sensitive techniques. X-ray photoelectron spectroscopy (XPS) explored the SAM formation of TM-GABA on Au surfaces. Cyclic voltammograms showed the ability to electrochemically control the quinone unit at the distal end of the chain. GABA was selectively released upon electrochemical reduction at a potential of −700 mV. The functional GABA terminal group was detected by surface plasmon resonance measurements of anti-GABA antibody binding.

Keywords: Self-assembled monolayers (SAMs); XPS; SPR; Cyclic voltammogram; Electrochemical reduction; GABA

1. Introduction

Advances in the science and engineering of bioactive surfaces, as well as the broader areas of micro- and nanofabrication, make possible the design and assembly of ligand-modified surfaces with chemically defined composition and structure, and with bioactivity toward specific ligands and physiologically active tissues [1–6]. One challenging and exciting possible application of such bioactive surfaces is their use as a prosthetic device at chemical synapses in diseased neural tissue. For example, retinal degenerative diseases such as age-related macular degeneration (AMD) and retinitis pigmentosa (RP) involve the deterioration of rod and cone photoreceptors but are thought in certain cases to preserve the functionality of post-photoreceptor retinal neurons [7–9]. In such cases, an implantable device that, in response to an external signal (e.g., light), presented neurotransmitter to post-synaptic membrane receptors of remaining healthy neurons could restore stimulus-dependent function of the diseased tissue (e.g., in AMD and RP, light-dependent activity of post-photoreceptor retinal neurons) [10,11]. The present study was undertaken to develop a prototype surface preparation capable of releasing free neurotransmitter in response to an electrical signal. As the test neurotransmitter to be incorporated in this preparation, we chose γ-aminobutyric acid (GABA), the native neurotransmitter of multiple classes of chemical synapses found in retina and other tissues of the central nervous system.

The release of neurotransmitters from a surface at an appropriate moment in response to an electrical signal is becoming an important objective, considering its applications [12–15]. However the strategy for designing a well-controlled interface that releases neurotransmitter instantly has not yet been well-documented. Previous studies have suggested the use of specific

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[1] These authors contributed equally to the work. W. Matsuda conducted all syntheses and C. Yan carried out all surface analyses.

0021-9797/$ – see front matter
doi:10.1016/j.jcis.2005.08.029

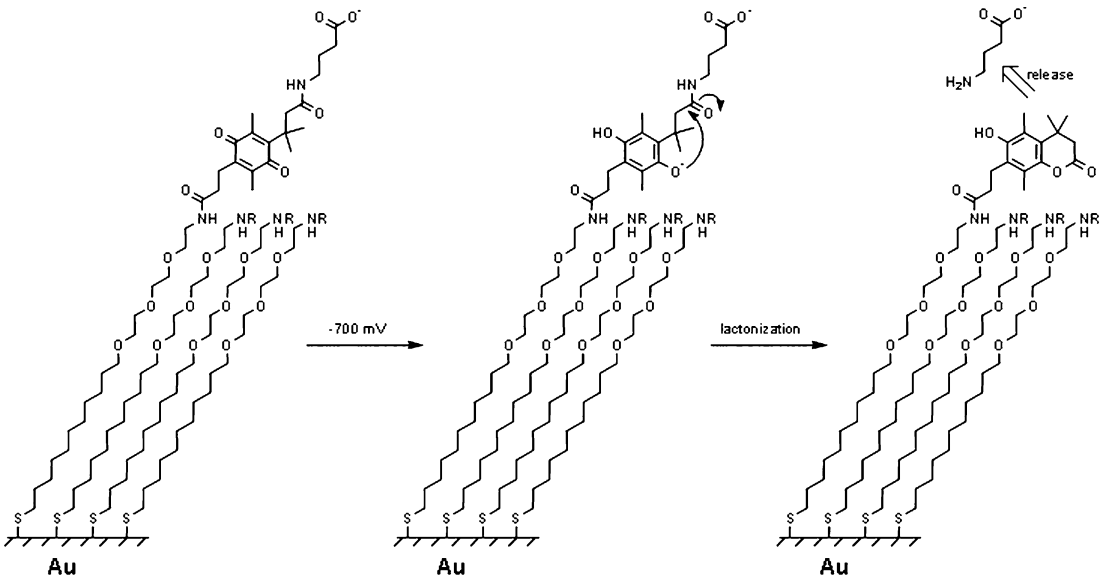
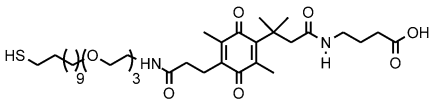


Fig. 1. Electrochemical reduction of a self-assembled TM-GABA monolayer on gold. The alkanethiol linked to gold surface organizes the TM-GABA into a close packed self-assembled monolayer structure while the triethylene glycol units provide a hydrophilic layer near the aqueous surface. The electroactive quinone unit releases GABA upon reduction. The overall design follows that pioneered by Mrksich and co-workers (see text).

design elements. For example, Mrksich and colleagues [1,3–5] tethered various electroactive units to gold via chains containing both a polymethylene segment and a PEO (polyethylene oxide) segment to achieve electrochemical control of redox reactions at the chain's distal end. The polymethylene segments of the attached chains collectively form a well-defined SAM structure near the gold surface [16–18], whereas the PEO units create a hydrophilic layer in contact with the aqueous solution. The latter was shown to inhibit protein absorption [19].



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Our approach for transducing an electrical signal into the release of the neurotransmitter GABA is shown in Fig. 1. The actual compound studied, **1**, borrows from a biotin releasing surface recently reported by Hodneland and Mrksich [5], which in turn, built upon the earlier protecting group chemistry and the pro-drug approach developed by Carpino, Wang, and Borchardt [20–22]. Herein we describe the synthesis of **1** and the preparation of self-assembled monolayers (SAMs) of this material on gold surfaces. The molecular composition, electrochemical behavior, and the biological activity of the SAM were characterized by XPS, SPR and cyclic voltammetry. The aggregate evidence supports the electrochemically stimulated release of GABA. We also tested monolayers comprising a mixture of TM-GABA (**1**) and an oligo(ethylene glycol) (OEG)-terminated alkanethiol on gold in order to achieve additional control of the chain packing density. To our knowledge, this is the first report of the synthesis of TM-GABA (**1**) and the characterization of TM-GABA monolayers using different surface-sensitive techniques.

2. Experimental

2.1. General

Reagents and solvents used in reactions were obtained from commercial sources and were used without further purification except as follows: tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl. *N*,*N*-dimethylformamide (DMF) was dried over 4 Å molecular sieves. Methylene chloride (CH_2Cl_2) and triethylamine were distilled from CaH_2. All reactions were monitored by TLC using silica gel 60 F_{254} glass plates (Merck). Flash chromatography was performed with 32–63 μm silica gel (Merck).

^{1}H NMR and ^{13}C NMR data were obtained on either 400 or 500 MHz Varian U400 and U500 instruments in chloroform-*d* ($CDCl_3$) unless otherwise noted. ^{1}H NMR spectra obtained in $CDCl_3$ were referenced to 7.26 ppm, and those obtained in D_2O were referenced to 4.79 ppm. ^{13}C NMR spectra obtained in $CDCl_3$ or D_2O were referenced to 77.00 ppm. Chemical shifts are reported in parts per million (ppm) and coupling constants are reported in hertz (Hz). IR spectra were collected on a Mattson FTIR 5000 with major bands reported in cm^{-1}. UV/vis spectra were obtained using a Shimadzu UV-2501PC recording spectrophotometer. FAB-MS and EI-MS data were collected by the mass spectrometry service at the University of Illinois at Urbana-Champaign (UIUC). Elemental analysis was performed in the microanalytical laboratory at UIUC.

The mixed triethylene glycol alkanethiol, $HO(CH_2CH_2O)_6(CH_2)_{11}SH$ (EG6) was custom synthesized by Obiter Research, LLC, Urbana, USA. Rabbit IgG (purified immunoglobulin reagent grade) and monoclonal anti-γ-aminobutyric acid (clone GB-69, abbreviation: anti-GABA) were purchased from Sigma, St. Louis, MO. GABA-OBu^t [23], 2-[2-(2-undec-10-enyloxyethoxy)ethoxy]ethanamine **8** [24], **3** [22], and **7** [20–22] were prepared according to known methods.