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Binding and potential-triggered release of L-glutamate with molecularly imprinted polypyrrole in neutral pH solutions

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ABSTRACT

Molecularly imprinted polymers (MIPs) are interesting for potential-regulated trafficking of molecules. In this study, we investigate the binding properties of L-glutamate (Glu) in molecularly imprinted polypyrrole (MIPPy) in neutral pH solutions. We prepared MIPPy by electrochemically depositing Glu-doped polypyrrole and subsequently overoxidizing the polymer. By means of immunofluorescence microscopy analysis of MIPPy using specific anti-Glu antibodies, we demonstrate binding of Glu in MIPPy. Visual imaging of the samples allows us to easily discern between selective and non-selective interactions. Significant fluorescence is observed from MIPPy incubated in solutions containing Glu, while control MIPPy samples imprinted with Cl^- and subsequently exposed to Glu do not yield detectable fluorescence signals. To quantify the binding interactions, we use electrochemical quartz microbalance (EQCM) measurements. Glu uptake by MIPPy occurs in the absence of an applied electrical potential. From the change in mass of MIPPy versus the Glu concentration in solution, we determine a binding free energy of -6.0 ± 0.2 kJ/mol. Based on these results, the binding interactions are discussed in terms of hydrogen bonding combined with electrostatic interactions between the polymer and the neurotransmitter. Glu release from MIPPy is triggered at low potentials (-0.3 V (Ag/AgCl)). These properties make MIPPy interesting for applications such as drug delivery and chemical implants.

1. Introduction

Molecularly imprinted polymers (MIPs) are being widely investigated in the literature for applications related to sensing, catalysis, and drug delivery [1–5]. MIPs have great potential for the development of sophisticated biomedical devices, such as chemical implants, which regulate biomolecular concentrations in solution via selective uptake and release processes. The reliability of MIP functionality, however, is often limited by factors such as the poor accessibility of binding sites within the polymer matrix [6], heterogeneous [7] or non-selective binding [1,3], interactions of the anion with polar solvents such as water [8], and the poor long-term stability for molecular recognition [9]. For this reason, materials which meet the strict demands for molecular recognition applications are required.

Electrochemically synthesized MIPs offer much potential for realizing flexible and novel applications [10–12]. Polypyrrole (PPy) is a stable, electroactive polymer which was widely investigated in the 1980s and 1990s and has recently re-emerged as a highly relevant material for MIP applications [10] due to its stability and interesting redox properties. PPy can be easily synthesized electrochemically. During synthesis, anions from the electrolyte are incorporated into the polymer backbone, resulting in conductive, doped polymer films which adhere to electrode surfaces [13]. The dopant anion influences the PPy properties [14] and can be chosen to achieve desired functionalities [15]. Deore et al. [16] initially showed that overoxidizing PPy results in molecular selectivity for the templating ion. Since then molecularly imprinted PPy (MIPPy) has been investigated for the potential-regulated, selective uptake of cationic amino acids, such as L-Glu [17] and L-aspartate [18], in low pH solutions. Recently, Mehdini et al. [19] demonstrated a novel synthesis route allowing for increased surface area for

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improved recognition of ascorbic acid by MIPPy in aqueous solutions.

As L-Glu is the most abundant neurotransmitter in the central nervous system, the selective regulation of L-Glu concentrations in physiological pH solutions would be highly interesting in the fields of biology and medicine. This, however, has been difficult to realize to date. We recently demonstrated the proof-of-concept of potential-regulated enantioselective uptake and *release*, i.e. molecular trafficking, of anionic L-Glu from MIPPy in neutral pH solutions [20].

Selective uptake of amino acids by MIPPy has been attributed to electrostatic interactions between the charged molecule and electrode combined with shape recognition [17,18,20,21]. The exact mechanisms, however, have not been confirmed. Generally, molecular recognition occurs due to specificity, e.g. shape recognition, between the target molecule and binding site combined with non-covalent interactions, e.g. electrostatic, hydrogen bonding or hydrophobic interactions [22,23]. In the case of molecular recognition with MIPs, interactions have been additionally attributed to Van der Waals and charge-transfer interactions [1]. While shape recognition has been clearly demonstrated for the uptake of amino acids with MIPPy, the nature of the binding interactions remains unclear. In order to develop molecular trafficking devices, where the target molecule is subsequently released via an optical or electrical trigger, it is of key importance to identify and quantify these interactions.

In this study, we investigate the binding interactions of Glu in MIPPy in neutral pH solutions. A protocol for the fabrication of MIPPy is described. Immunofluorescence microscopy using a specific anti-Glu antibody is applied to confirm binding of the neurotransmitter in MIPPy. Electrochemical quartz microbalance (EQCM) measurements are used to quantify the binding energy between the neurotransmitter and the polymer. The results from the fluorescence studies and EQCM data indicate that uptake of Glu by MIPPy is dominated by hydrogen bonding combined with electrostatic interactions. Release of Glu from MIPPy is triggered by applying a low negative potential to the MIPPy electrode. Finally, potential-regulated trafficking of Glu with MIPPy is demonstrated.

2. Experimental

2.1. Materials

Pyrrole (Py) (99%, extra pure and 98% FCC) was purchased from Acros Organics and from Sigma-Aldrich, and glutamic acid monosodium salt monohydrate (NaGlu) and sodium chloride, both ≥99% from Sigma-Aldrich. Water was purified by a Milli-Q water system ($R = 18.2 \times 10^6\ \Omega$ cm). Py was vacuum distilled (10^{-2} to 10^{-3} bar) before use and stored under Ar atmosphere at −10 °C. All other chemicals were used as-received.

2.2. Electrochemical synthesis of Glu-templated PPy

Py (0.4 M) and NaGlu (0.5 M) or NaCl (0.5 M) were added to Milli-Q water ($R = 18 \times 10^6\ \Omega$ cm) and mixed in an ultrasonic bath to produce electrolyte solutions for polymer electrosynthesis. To avoid oxidation of Py during polymer growth, the solutions were flushed with Ar for at least 5 min before use. For the polymer deposition and cyclic voltammetric measurements, an electrochemical workstation (Model 660C) from CH Instruments with accompanying software Version 6.28 was used. Glass electrochemical cells with fixed electrode geometry (three electrodes) were used for the experiments. The reference electrode for all experiments was Ag/AgCl in 3 M KCl (aq) (CH Instruments). The counter-electrode was a Pt wire from CH Instruments. Au (150 nm)-coated glass substrates with a Cr (5 nm) adhesion layer were used as working electrodes and substrates for polymer deposition. All experiments were carried out at room temperature.

PPy was potentiostatically deposited at +0.6 V and the current during deposition was monitored to determine the film thickness. The films used for the scanning electron microscopy studies were 40 ± 5 nm thick. The films used for fluorescence and EQCM studies were approximately 20 ± 5 nm thick. The films were well rinsed with Milli-Q water after deposition to remove unbound Glu and Py from the sample surface. To create Glu-templated PPy (MIPPy), the films were galvanostatically overoxidized in a phosphate buffer at pH 7 with a constant current density of $I = 0.025$ mA/cm^2 until the potential reached the value of 1.2 V [18] and subsequently washed in water.

2.3. Investigations of polymer structure and morphology

The thickness and roughness of the polymer films were determined using a Veeco Dektak 6 M profilometer. The morphology of the synthesized films was investigated using a Helios NanoLab 600i scanning electron microscope (SEM) from FEI.

2.4. Glu detection in MIPPy: analysis and fluorescence image acquisition

The distribution of Glu in MIPPy was analyzed by means of indirect immunofluorescence microscopy using anti-Glu antibodies. The polymer samples were washed in 0.1 M sodium phosphate buffer (PB) with pH 7.4. The samples were then incubated with rabbit anti-Glu antibodies (1:500; Sigma-Aldrich) in PB at room temperature for 2 h. The samples were washed repeatedly with PB to remove unbound antibodies and subsequently incubated with the secondary antibody Alexa Fluor 488-conjugated goat anti-rabbit IgG (Invitrogen) at room temperature for 1 h to visualize anti-Glu–Glu complexes in the MIPPy samples. After washing with PB, polymers were mounted with Vectashield (Vector Laboratories) to prevent fading of the fluorescence signals. Anti-Glu–Glu complexes in MIPPy were verified by means of fluorescence imaging performed with a Leica TCS SL confocal microscope using a 63×/1.32 oil immersion objective with a resolution of 512 × 512 pixels. The fluorescence intensities of single pixels from the images were analyzed with the program *Fiji* [24].

2.5. Electrochemical quartz crystal microbalance (EQCM) measurements

For EQCM investigations, a Maxtex RQCM Quartz Crystal Microbalance Research System from Inficon with accompanying software was used. MIPPy was electrochemically synthesized on Au-coated Inficon 5 MHz crystal working electrodes. The exposed area was 137 mm^2 and the active area was 34.19 mm^2. As a retention system for the crystal, the CHC-15 crystal holder and the GC-15 Glass Cell from Inficon (45 mL) were used.

The conversion of the resonant frequency shift f to the mass change m was performed according to the Sauerbrey equation [25]:

$$\Delta m = -\frac{\Delta f}{C_f},$$

where $C_f = 0.056$ Hz/ng/cm^2 is the sensitivity factor of the quartz resonator. The measurement resolution of the EQCM setup is 0.4 ng/cm^2.