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Label-free histamine detection with nanofluidic diodes through metal ion displacement mechanism

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ABSTRACT

We design and characterize a nanofluidic device for the label-free specific detection of histamine neurotransmitter based on a metal ion displacement mechanism. The sensor consists of an asymmetric polymer nanopore fabricated via ion track-etching technique. The nanopore sensor surface having metal–nitrilotriacetic (NTA–Ni^{2+}) chelates is obtained by covalent coupling of native carboxylic acid groups with N_{α},N_{α}-bis(carboxymethyl)-L-lysine (BCML), followed by exposure to Ni^{2+} ion solution. The BCML immobilization and subsequent Ni^{2+} ion complexation with NTA moieties change the surface charge concentration, which has a significant impact on the current–voltage (I–V) curve after chemical modification of the nanopore. The sensing mechanism is based on the displacement of the metal ion from the NTA–Ni^{2+} chelates. When the modified pore is exposed to histamine solution, the Ni^{2+} ion in NTA–Ni^{2+} chelate recognizes histamine through a metal ion coordination displacement process and formation of stable Ni-histamine complexes, leading to the regeneration of metal-free NTA groups on the pore surface, as shown in the current-voltage characteristics. Nanomolar concentrations of the histamine in the working electrolyte can be detected. On the contrary, other neurotransmitters such as glycine, serotonin, gamma-aminobutyric acid, and dopamine do not provoke significant changes in the nanopore electronic signal due to their inability to displace the metal ion and form a stable complex with Ni^{2+} ion. The nanofluidic sensor exhibits high sensitivity, specificity and reusability towards histamine detection and can then be used to monitor the concentration of biological important neurotransmitters.

1. Introduction

Neurotransmitters are considered as chemical messengers that transmit neurological information in the form of electrical signals within the cellular system of living organisms [1]. Up to date, more than 100 neurotransmitters have been identified by the scientists and are classified into different groups based on their chemical structures: i) biogenic amines (histamine, dopamine, serotonin, acetylcholine, etc.), ii) amino acids (glycine, glutamic acid, gamma-aminobutyric acid, etc.), iii) peptides (neurotensin, vasopressin, somatostatin, etc.), and iv) gaseous species (H_2S, NO and CO). A disturbance in the level of neurotransmitter content can adversely affect the transmission process, leading to depression, schizophrenia, drug dependence and degenerative diseases in human beings [2]. Histamine (Hm) naturally occurs in human body in trace amounts and plays a key role in physiological functions controlled by brain, for example, neurotransmission, sleep, memory storage, thermoregulation, inflammation, secretion of hormones and gastric acid, food intake and cardiovascular control [3–6]. An excess of Hm level in the body can cause Alzheimer's disease, abnormal arousal, asthma, allergies and some other neuropsychiatric disorders [7,8]. Moreover, Hm is also present in some types of fish (e.g., tuna fish, sardine and mackerel, etc.) and cheeses where Hm concentrations $\geq$ 50 mg per 100 g can cause food poisoning [9,10].

To date, different methods have already been developed for the detection of this biologically important amine, including gas chromatography [11], high-performance liquid chromatography

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(HPLC) [12,13], capillary zone electrophoresis [14,15], spectrofluorimetry [16–18], enzymatic assay [19], and flow immunoassay [20]. For example, Yamaguchi and co-workers have demonstrated selective and sensitive Hm sensing with HPLC coupled with fluorescence detection techniques [13]. Recently, Hm detection has been achieved via the modulation of fluorescence signals based on ligand exchange mechanisms using metal ion complexes [17,18,21]. To this end, Oshikawa et al. used a metal complex of cyanine dye for the determination of Hm released during the mast cell degranulation via a coordination displacement process [21]. Similarly, Seto et al. have also demonstrated the Hm detection based on specific coordination displacement between metal–iminodiacetic acid complexes and Hm analyte, leading to changes in fluorescence signal [17,18]. The above techniques can be employed for the sensitive detection of various neurotransmitters but most of them need expensive instrumentation and are time consuming. It is of interest to design simple and low-cost detection techniques with fast response times and we demonstrate here a new label-free Hm detection method using a nanofluidic device.

During the recent years, asymmetric nanopores have been widely used for the miniaturization of (bio)chemical sensing devices because of their unique transport properties (e.g., permselectivity, gating, and current rectification) [22–27]. The sensing capability of these devices can be evidenced from the changes in the electronic read-out of the pore caused by the transport of an analyte under an applied voltage or the ligand-receptor interactions that occur upon the addition of analyte molecules in the bathing solutions. Previously, asymmetric nanopores have been successfully employed for the selective recognition of biomolecules [28–35], metal ions [36–41], anions [42–44], amino acid enantiomers [40,45–49], and small organic molecules [37,50]. However, there is still a room to expand the scope and potentialities of such tiny-sized pores by designing nanofluidic sensors for the detection of biogenic amines.

We present a nanofluidic sensing device for the label-free recognition of Hm. For this purpose, the pore surface is chemically decorated with nitrilotriacetic (NTA) moieties through the functionalization of N_α,N_α-bis(carboxymethyl)-L-lysine (BCML) chains. Subsequently, the formation of NTA–Ni^{2+} chelates on the pore surface is achieved by exposing the BCML-modified pore to a Ni^{2+} ion solution. Upon metal ion complexation, the nanopore current-voltage (*I–V*) characteristics are modified due to changes in the surface charge density. In the present case, the sensing principle is based on the displacement of Ni^{2+} from the NTA moieties. Due to the regeneration of NTA groups, the pore can be restored to the initial *I–V* characteristics. Among the various neurotransmitters examined in this study, only Hm is able to displace the metal ion from the NTA groups because of the formation of stable Ni-Hm complexes. The nanopore-based sensor exhibits high sensitivity, specificity and reusability towards Hm detection.

2. Materials and methods

2.1. Materials

The irradiation of 12 μm thick polyethylene terephthalate (PET) membranes (Hostaphan RN 12, Hoechst) was achieved at the linear accelerator UNILAC (GSI, Darmstadt) using single swift heavy ions (Au) of kinetic energy 11.4 MeV/nucleon.

All the chemicals and reagents were of analytical grade and used as received without further purification. *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDC), pentafluorophenol (PFP), N_α,N_α-bis(carboxymethyl)-L-lysine hydrate (BCML), glycine (Gly), serotonin (5-HT), gamma-aminobutyric acid (GABA), dopamine (DA) and histamine (Hm), sodium hydroxide and potassium chloride were purchased from Sigma-Aldrich, Schnelldorf, Germany.

2.2. Fabrication of single asymmetric nanopores

Before chemical etching of latent ion tracks, the swift heavy ion irradiated polymer membranes were sensitized with soft UV light. For this purpose, the tracked polymer membranes were exposed to UV irradiation on each side for 60 min. The UV sensitized latent ion damage tracks were converted into conical nanopores through the asymmetric track-etching technique reported by Apel and co-workers [51]. The chemical track-etching process was performed in a custom-made conductivity cell having three chambers. This conductivity cell was employed for the simultaneous fabrication of single-pore and multipore membranes. To achieve this goal, a single-shot (1 ion hitting the foil) membrane and a membrane irradiated with 10^7 ions per cm^2 were placed on both sides of the middle compartment of the conductivity cell and clamped tightly. An etching solution (9 M NaOH) was filled in the middle compartment having apertures on both sides. In this way the chemical etchant was in direct contact with both the membranes. The two compartments on either side of the middle chamber were filled with a stopping solution (1 M KCl + 1 M HCOOH). To monitor the etching process, gold electrodes were inserted on both sides of the single-ion irradiated membrane and a potential of −1 V was applied across the membrane. The etching process was carried out at room temperature. The current remained zero as long as the etchant had not permeated the whole length of the membrane. After the breakthrough (a point at which the etchant pierced the membrane), an increase in the ionic current flowing through the nascent pore was observed. The etching process was stopped when the current reached a certain defined value. Then, the membranes were thoroughly washed with stopping solution in order to neutralize the etchant, followed by deionized water. The etched membranes were then dipped in deionized water overnight in order to remove the residual salts. This process resulted in polymer samples containing approximately conical single pores with carboxylic groups (COOH) generated on the inner pore walls due the hydrolysis of ester bonds in the back-bone of polymer chains.

2.3. Functionalization of nanopore surface

The COOH groups on the pore surface were first activated by exposing the single pore membrane to an ethanol solution of *N*-(3-dimethyl-aminopropyl)-*N*-ethylcarbodiimide (EDC; 100 mM)/pentafluorophenol (PFP; 200 mM) for 1 h at room temperature. A solution of N_α,N_α-bis(carboxymethyl)-L-lysine hydrate (BCML) was prepared in an ethanol/water (8/2) mixture. The solution was neutralized by the addition of triethylamine. Then, the activated single pore membrane was exposed to BCML (25 mM) solution and the reaction was allowed to occur overnight. Finally, the functionalized membrane was washed several times with ethanol followed by deionized water.

2.4. NTA-Ni(II) complexation

For the complexation of Ni(II) ion with NTA moieties, a solution of $NiSO_4$ (100 mM) was prepared in deionized water. The pH of the $NiSO_4$ solution was adjusted to pH 10 with dilute NaOH. A polymer membrane containing a single BCML-modified pore was first washed with water (pH 10) in order to obtain fully ionized carboxylate groups. Then, the single pore membrane was immersed in a $NiSO_4$ solution for four hours at room temperature to achieve NTA–Ni(II) chelates on the pore surface.