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Enantioselective Recognition in Biomimetic Single Artificial Nanochannels

Cuiping Han,[†] Xu Hou,[∥] Huacheng Zhang,[‡] Wei Guo,[‡] Haibing Li,[*,†] and Lei Jiang[*,‡,§]

[†]Key Laboratory of Pesticide and Chemical Biology (CCNU), Ministry of Education, College of Chemistry, Central China Normal University, Wuhan 430079, P. R. China

[‡]Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, P. R. China

[§]Beijing University of Aeronautics and Astronautics, Beijing 100191, P. R. China

[∥]National Center for Nanoscience and Technology, Beijing 100190, P. R. China

Supporting Information

ABSTRACT: Efficient enantiomer discrimination with a convenient system remains a challenge in the fields of biochemistry, medical science, and pharmaceutics. Here we report a simple enantioselective sensing device based on a single artificial β-cyclodextrin-modified nanochannel system. This nanodevice shows highly selective recognition of histidine enantiomers through monitoring of ionic current signatures.

Chiral discrimination is a prominent feature of the living world. The body is amazingly chiral-selective, exhibiting different physiological responses to different enantiomers.[1] One isomer may produce the desired therapeutic activities, while the other may be inactive or produce unwanted effects. Amino acids are important bioactive substances. Research on enantiomeric recognition of amino acids can provide important information leading to a better understanding of chiral recognition in biological systems and furthering the development of useful molecular devices in biochemical and pharmaceutical studies.[2] Although progress in chiral discrimination has been achieved during the past decades,[3] the design of structurally simple yet efficient systems for the enantioselective recognition of amino acids still remains as a challenging task.[4] Recently, sensing with biomimetic nanochannels has drawn enormous research attention because of their simplicity and potential applications in mimicking the biological process.[5] α-Hemolysin nanochannels have been used experimentally for the detection of drug enantiomers.[6] While this is a promising chiral-sensing paradigm, the fragility of the lipid bilayer membranes limits its practical application.

Here we report a convenient and robust nanochannel-based chiral analysis system for highly chiral-specific sensing of L-histidine (L-His). Artificial nanochannels fabricated in track-etched polymer membranes are considered as promising candidates for biological sensing applications because of their mechanical and chemical stability.[7] Single conical artificial nanochannels modified with proper functional groups have proven to be a novel sensing platform for the detection of DNA,[8] proteins,[9] and organic molecules[10] on the basis of ion transport. Chiral discrimination with this nanochannel system, which was not achieved in the earlier studies, is present in our work.

The chemical groups incorporated on the inner walls of the channel play crucial roles in the sensing performance.[8−10] To achieve the goal of chiral recognition, functionalization of the channel wall with an appropriate chiral ligand is critical and essential. As seminatural artificial receptors made up of cyclic oligosaccharides, β-cyclodextrin (β-CD) and its derivatives have been successfully used in the enantioselective recognition of L- and D-amino acids because of their fascinating intrinsically chiral cavity.[11] Within this framework, the integration of β-CD molecules into solid-state nanochannels would lead to the creation of robust signal-responsive devices for analysis of chiral amino acids.

Scheme 1 shows the fabrication and operating principle of the chiral-responsive system. The single conical nanochannel was prepared by asymmetric chemical etching of a 12 μm thick polyethylene terephthalate (PET) membrane containing a single ion track in the center.[12] The diameter of the large opening (base) of the conical nanochannel was ∼640 nm, while that of the narrow opening (tip) at the opposite face was ∼11 nm. After the chemical etching process, carboxyl groups were exposed on the nanochannel surface, and mono-6-amino-β-CD molecules were immobilized in the channel by a classical EDC/NHS coupling reaction (see the Supporting Information). The functionalized nanochannel exhibited excellent chiral recognition capability toward L-His, which was manifested via the changes in the ionic current flowing through the nanochannel. Upon exposure of the β-CD-modified nanochannel to a solution of L-His, selective binding of L-His to the channel wall occurred inside the confined geometry. This effect induced a decrease in the transmembrane ionic current. In contrast, no significant changes in the ionic current were found when the modified channel was exposed to solutions of D-His or other aromatic amino acids.

The functionalized nanochannel was characterized by measuring current−voltage ($I-V$) curves. The bare nanochannel rectified the ionic current in an environment at neutral pH as a result of the presence of anionic carboxylate ($-COO^-$) groups.[13] After functionalization, the neutral β-CD diminished the negative charge of the channel surface, leading to a decrease of ∼40% in the rectified ionic current (Figure S2 in the Supporting Information). The changes in the $I-V$ characteristics prior to and after modification confirmed the success of the β-CD immobilization

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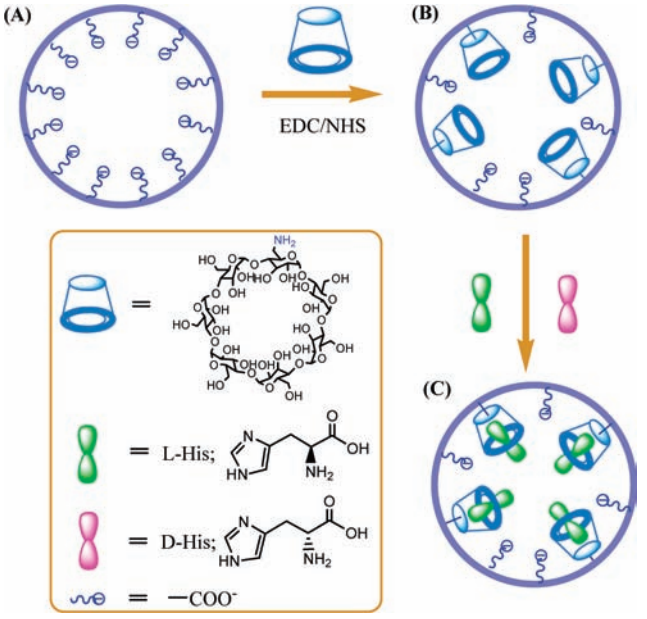
Scheme 1. Biomimetic Chiral-Responsive Single-Nanochannel System[a]



[a] (A) Single conical nanochannel fabricated using an asymmetric track-etching technique. (B) Covalent attachment of NH_2-β-CD with carboxyl groups via carbodiimide coupling chemistry. (C) Principle of chiral recognition of His. The modified nanochannel works as a chiral receptor for His, as selective binding of L-His to the inner wall of the nanochannel disturbs the channel surface, resulting in a significant change in the transmembrane ionic current.

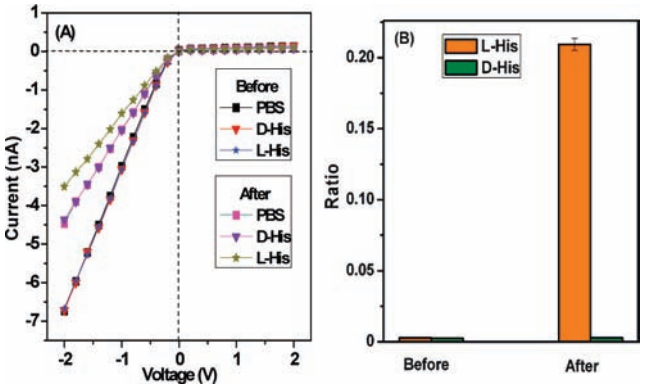


Figure 1. (A) $I-V$ curves for the single nanochannel before and after β-CD modification in 50 mM PBS (pH 7.2) without or with the addition of 1 mM L-His or D-His, as labeled. (B) Current change ratios for the single nanochannel before and after β-CD modification in 50 mM PBS (pH 7.2) upon addition of 1 mM L-His or D-His. Chiral discrimination of the His enantiomers was well-realized using the modified nanochannel via the change in the ionic current.

on the inner wall of the channel. In addition, the results of contact angle measurements and X-ray photoelectron spectroscopy (XPS) analysis (Figure S1 and Tables S1 and S2) also confirmed that the β-CD was modified on the surface of PET film successfully.

The responsive His transport properties of this nanochannel system were evaluated by measuring the ionic current across the

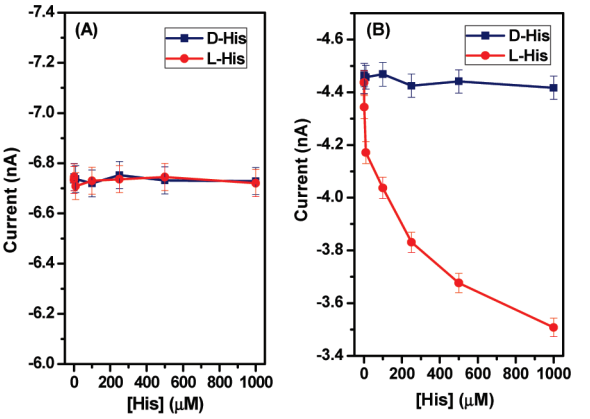


Figure 2. Current−concentration ($I-C$) properties of the single nanochannel (A) before and (B) after β-CD was attached to the inner channel wall. The large difference in the enantiomeric ionic currents makes the β-CD-modified nanochannel practically useful as a sensor for the enantioselective recognition of L-His.

channel in 50 mM phosphate buffered saline (PBS, pH 7.2). Figure 1A depicts $I-V$ curves of the single nanochannel before and after β-CD modification in the presence of 1 mM L- or D-His. When the bare channel was exposed to the electrolyte containing 1 mM L-His or D-His, no significant change in the ionic current was found. After modification, there was a remarkable difference: a significant decrease in the transmembrane ionic current was observed in the presence of 1 mM L-His, while the current stayed nearly unchanged in the presence of 1 mM D-His. To quantify the current changes, the current change ratio at −2 V [defined here as the absolute value of the current change ratio, i.e., $(I - I_0)/I_0$] was determined. Figure 1B shows the ionic current change ratios before and after β-CD modification of the nanochannel in the presence of 1 mM L- or D-His. It is evident that the modified channel displayed good chiral selectivity to L-His. The immobilized β-CD molecules on the channel wall serve as chiral receptors for His.

Previous studies have shown that β-CD can selectively bind L-His relative to D-His to form stable β-CD/L-His complexes.[14] In the channel system, this chiral selectivity can be transduced into an electronic signal provided by the ionic flux through the channel. The β-CD/L-His complexes formed on the inner wall of the channel shield the surface charge through the electrostatic interaction between the positively charged His[15] and negatively charged channel, which in turn results in a significant decrease in the transmembrane ionic current.

This deduction was strongly supported by further pH-dependent studies (Figure S3). At pH 10, the addition of His did not induce any significant change in the $I-V$ curve because both the channel and His are negatively charged, causing an electrostatic repulsion between them. Close to the isoelectric point of His (~7.6), the His molecules are neutral and did not show any effect on the rectification behavior of the channel. At pH 3.5, the channel behaves as neutral (pI 3.8), which results in a linear $I-V$ curve. At such an acidic pH, the His is positively charged. L-His molecules selectively adsorb on the natural channel wall and impart positive charge to the channel surface, resulting in inversion of rectification as expected for a positively charged surface. Meanwhile, the $I-V$ curve stayed nearly unchanged in the presence of 1 mM D-His.

Figure 2 describes the current changes in the nanochannel before and after β-CD modification at −2 V upon the addition of

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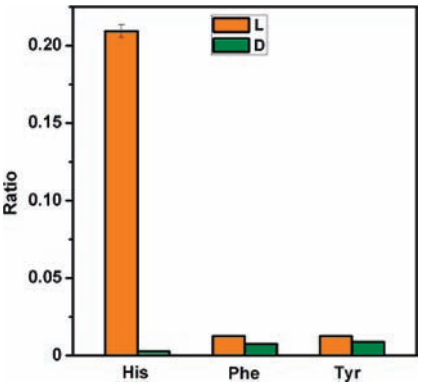

Figure 3. Current change ratios for the β-CD-modified single nanochannel in 50 mM PBS (pH 7.2) upon addition of 1 mM L- or D-His, L- or D-Phe, and L- or D-Tyr, respectively.

L-His or D-His solutions with different concentrations under the same conditions. The transmembrane ionic currents for the unmodified channel exhibited no obvious changes upon treatment with L-His or D-His in the concentration range 0−1 mM (Figure 2A). When these measurements were repeated with the β-CD-modified channel, a clear difference in the results for the two enantiomers was observed (Figure 2B). The ionic currents decreased gradually with increasing L-His concentration from 0 to 1 mM, whereas the current remained almost constant for D-His. The corresponding $I-V$ curves are shown in Figures S4 and S5. The binding strength constants calculated on the basis of the Langmuir model[16] were 1.01 for D-His and 16.66 for L-His, indicating that the modified channel produced stronger adsorption of L-His than D-His (Figure S6).

One important aspect of sensing platforms is the detection selectivity. In order to show that this approach is valid for creating a chiral His sensing platform inside the nanochannel, we repeated the same experiments using two other aromatic amino acids, phenylalanine (Phe) and tyrosine (Tyr), under the same conditions. Although β-CD molecules have been reported as chiral receptors for L- and D-Phe[11c] and L- and D-Tyr,[11c,d] those phenomena were not observed in our channel system. As Figure S7 shows, the ionic currents in the nanochannel were nearly unaffected by Phe or Tyr. This behavior can be explained by electrostatic interactions between the amino acids and the channel surface. The isoelectric points of Phe and Tyr are 5.48 and 5.89,[15] respectively. At pH 7.2, these two amino acids are negatively charged. An electrostatic repulsion therefore existed between the amino acids and the negatively charged channel that prevented the amino acids from adhering to the inner wall of the channel, leaving the channel surface undisturbed. Therefore, we did not observe any influence on the rectification behavior of the channel after the introduction of Phe or Tyr molecules. Figure 3 shows the current change ratios for the modified nanochannel in the presence of 1 mM L- or D-His, L- or D-Phe, and L- or D-Tyr. It is obvious that the β-CD-modified nanochannel displayed His-specific chiral discrimination. The reproducibility of the proposed method, evaluated as the standard deviation of three replicates of the modified nanochannel with a sample containing 1 mM L-His, was ~2% (Figure S8).

In summary, we have described the construction of a chiral sensing nanodevice based on a single conical nanochannel fabricated in a PET membrane. Chiral recognition elements (β-CD molecules) were incorporated into the channel by directly exploiting the carboxyl groups generated during the track-etching process. The modified nanochannel provided a novel sensing platform to discriminate chiral His on the basis of rectified ionic currents. Further variation of the chiral sensor system could be achieved by modifying the nanochannel with other chiral ligands, thus enabling the design of sensors for other chiral substrates. This successful study is a potential step toward the ability to simulate the process of chiral recognition in living organisms. On the basis of these findings, we believe that artificial nanochannel systems offer real promise for preparing practical chiral-sensing devices that could be employed in a biological environment.

■ ASSOCIATED CONTENT

Supporting Information. Synthetic procedures, contact angle measurements, XPS analysis, $I-V$ properties of the single nanochannel, and pH-dependent experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
lhbing@mail.ccnu.edu.cn; jianglei@iccas.ac.cn

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