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Bioinspired Target-Specific Crystallization on Peptide Nanotubes for Ultrasensitive Pb Ion Detection

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Peptides are versatile components of the bionanotechnology toolbox, whose chemical compositions and three-dimensional (3D) structures can be optimized to function as catalysts,^[1] sensors,^[2] and building blocks for supramolecular architectures.^[3] Among these cutting-edge applications, the utilization of peptides to mimic the catalytic growth of 3D crystals by biomineralizing organisms is gaining attention for the fabrication of exotic nanoparticles and nanowires.^[4] For example, to synthesize metal nanowires, a suitable template can be modified with peptides that have high affinity for the metal ion precursor and can catalyze the crystallization of the material after applying a metallization stimulus.^[5] Here, we combine this bioinspired crystal growth concept with the peptide nanotube detection platform for the development of ultra-sensitive sensors for lead ions. Heavy metals such as Pb, Hg, and As are highly toxic environmental pollutants.^[6] Since the risk of poisoning by these heavy metals is high even at very low concentrations,^[7] there is a growing interest in developing new sensing strategies that can detect ultralow levels of heavy metal ions rapidly. Furthermore, high specificity for a particular metal is desirable in order to avoid interference from other ions in field samples. If peptides with high affinity and specificity to bind target metal ions were implemented with electrochemical transducers, the metallization of these ions on the peptide could increase the conductivity between electrodes, and hence this conductivity change could be used as the signal for the detection of the heavy metal ion.

In this communication we report the fabrication of Pb ion biosensors by integrating electrochemical transducers with peptide nanotubes that can bind Pb ions specifically and template the growth of Pb crystals via molecular recognition (see Scheme S1 in the Supporting Information). A key functional building block in this bioinspired sensor is the peptide DHHTQQHD, referred to as TAR-2-Asp, and the part of this peptide sequence, DHHTQQH (= TAR-2), has high affinity for lead salts as demonstrated by applying stringent genetic engineering protocols.^[8] For the integration of the peptide into the sensor platform, the TAR-2-Asp peptide was physisorbed on the peptide nanotubes and the resulting Pb-binding peptide assemblies were positioned to bridge a pair of electrodes separated by an approximately 1 μm gap via positive dielectrophoresis (Figure 1a).^[9] After incubation with Pb ion and a reduction step, the metallization of the nanotubes induces changes in the electrical properties of the transducer (Figure 1b and Figure S1 in the Supporting Information). To enhance the detection limit, we applied the conventional electrical signal amplification via supportive Ag deposition,^[10] and then we could detect 0.01 nM of Pb^{II} , which is at least 10^4 times lower than the detection limit of previous DNA/peptide-based sensors that use optical probes for the heavy metal ion detection.^[11]

To examine our hypothesis that the peptide nanotubes can be metalized by target Pb ions to template the growth of Pb nanowires in ultralow concentrated Pb^{II} solutions, the surfaces of the peptide nanotubes were imaged by a transmission electron microscope (TEM) after the nanotube solutions were incubated with Pb ion. When the TAR-2-Asp-conjugated peptide nanotubes were incubated with $\text{Pb}(\text{NO}_3)_2$ for 1 h and the reduction was assisted by NaBH_4 (10 mM) for 15 min, the nanotubes showed dark contrast in the TEM micrograph, thus indicating that the nanotubes templated the growth of Pb crystals (Figure 2a). The electron diffraction pattern also matches the interplanar distances of (420), (511), and (531) faces of a cubic Pb crystal (Figure 2a inset, JCPDS-04-0686). In the same condition, when the peptide nanotubes without the TAR-2-Asp peptide were incubated in the Pb ion solution, the resulting nanotubes were transparent in the TEM images, and no clear diffraction patterns were obtained even after the concentration of Pb^{II} was increased 10^4 times (Figure 2b). These results demonstrate the high affinity of the TAR-2-Asp peptide for Pb ions and its biomimetic function to nucleate Pb crystals, therefore validating the hypothesis that this peptide can be applied as the biorecognition element for the proposed Pb-ion biosensor. To evaluate the specificity of the peptide for the target ion, the TAR-2-Asp-conjugated nanotubes were incubated with other divalent cations such as Zn^{II} , Cu^{II} , Co^{II} , and Hg^{II} whose concentrations were 10^4 times higher than the Pb^{II} concentration. TEM images of the resulting peptide nanotubes in Figure 2a showed no contrast after reduction, and this observation demonstrates that the TAR-2-Asp binds Pb ions specifically (Figure S2 in the Supporting Information).

After demonstrating that the TAR-2-Asp-conjugated peptide nanotubes can template Pb nanowires in ultralow-concentrated Pb ion solutions, the electrochemical detection of this process was examined as illustrated in Figure 1. As a blank measurement, the sensors were incubated in solutions without Pb^{II} and the electrical properties of the circuit were queried with a peak-to-peak excitation voltage of 10 mV and frequencies between 10^3 and 10^5 Hz. In this experiment, a purely capacitive response was obtained even after depositing Ag, which is very similar to the same measurement before incubating with Pb^{II} and adding the

Ag enhancement agent (Figure 3a and Figure S3). This result indicates that conductive nanowires were not synthesized in the absence of Pb^{II} and the Ag deposition alone did not connect the electrodes under this condition. Since this protocol for the electrochemical detection of heavy metal ions, ion adsorption, reduction, and signal enhancement was validated by this control experiment, we applied the protocol to Pb ion detection. When the sensors were incubated with Pb^{II} , even at concentrations as low as 0.01 nM, the electric property of the nanotube that bridged the electrodes changed dramatically, as shown in the impedance spectrum (Figure 3b); between 10^3 and 10^4 Hz the impedance modulus ($|Z|$) is less dependent on the frequency and phase values (θ) are also closer to 0, which indicates the presence of a resistor in parallel with residual capacitive components of the circuit.^[12] These results not only demonstrate the ultrasensitive detection of Pb^{II} but also show the potential for the assay from the measurement of the electrical property of Pb nanowires based on the transduction mechanism.

To determine the concentration of the heavy metal ion and dynamic range of the Pb^{II} detection, we studied the correlation between the electrical signal and the concentration of Pb^{II} as well as the specificity of the biosensor for the target heavy metal ion. Here, several sensors were incubated with solutions containing Pb^{II} at different concentrations or other heavy metals ions with the high concentration of 100 nM. In these experiments, each measurement in different concentrations of Pb^{II} was performed with a different sensor platform. After reduction and Ag deposition, the conductance between the electrodes was extracted by fitting the spectra to the equivalent circuits in Figure 1 with the Zview software (Figure 3c).^[13] For the Pb ion assay, the conductance of the nanowires increased linearly with the concentration of Pb^{II} between 0 and 1 nM with the sensitivity of 2.25 μS per decade (Figure S4 in the Supporting Information). Above the Pb^{II} concentration of 1 nM, the binding sites on the nanotube sensor were saturated and the only fluctuation of signal in this regime was observed due to random error factors depending on the device-to-device discrepancy. When the biosensors were incubated with Hg^{II} , Zn^{II} , Co^{II} , or Cu^{II} with the concentration of 100 nM, no conductance was observed, which is consistent with the results in Figure S2 that no metal coatings were grown on the nanotubes due to the specificity of the TAR-2-Asp peptide for Pb ions. Moreover, the sensor with the peptide nanotube that was not conjugated with TAR-2-Asp did not register any conductance between the electrodes even when incubated with 100 nM Pb^{II} and after adding the Ag enhancement agent (“control” in Figure 3c), as expected from the results in Figure 2b.

In conclusion, we integrated the peptide nanotube detection platform with the biomimetic Pb crystallization process for the development of Pb ion biosensors. In this sensor configuration, peptides immobilized on the nanotubes bind the target heavy metal ion and nucleate Pb crystals, and then the electric signal via the metallization is transduced as current flowing through the metalized nanotube. The exceptional sensitivity and selectivity is the strength of this sensor, and these features could be due to the molecular recognition and the biomimetic crystallization function of the peptide, which increases the signal-to-noise ratio and the specificity as it is observed in enzymatic biosensors. The weakness of this sensor is the reusability, however this reusability issue of the peptide nanotube detection platform is addressed by circulating nonimmobilized peptide nanotubes in sample solutions.^[14] By this way, an array of interdigitated electrodes successfully detected the binding

events between the nanotubes and analytes with the change in the impedance of the transducer, and this device configuration is also applicable to this Pb sensor platform. This concept could be adapted to detect various heavy metal ions provided that peptides that can bind them with high affinity and specificity are available for the fabrication of the sensor. The compact design and inexpensive fabrication of the sensor combined with the electrochemical transduction for an uncomplicated integration with the circuitry make this sensor a promising alternative for the in-field detection of ultralow levels of Pb^{II}.

Experimental Section

Peptide nanotubes that bind Pb^{II} were fabricated by incubation with the TAR-2-Asp peptide (DHHTQQHD, 1 mg mL⁻¹ in water, Genscript) overnight. The TAR-2-Asp peptide contains a sequence with high affinity for Pb (DHHTQQH)^[8] and an Asp amino acid added to the C-terminus to increase its solubility in water. After washing with deionized water twice by centrifugation (1 h, 14 000 rpm), the nanotubes were immobilized between the electrodes as previously described.^[9] Briefly, the nanotubes were directed to gold electrodes, fabricated by common photolitho-graphic steps and separated by approximately 1 μ m distance, by positive dielectrophoresis (5 V peak-to-peak voltage, 10 Hz frequency). After the resulting peptide nanotube detection platforms were immersed in solutions containing heavy metal ions for 1 h, the reduction of the metal on the nanotube was assisted by NaBH₄. To enhance the signal and improve the contact between the nanotube and the transducers, AgNO₃ (10 mM) and hydroquinone (50 mM) were added for 3 min twice.^[10] To examine the selectivity of the sensor, the TAR-2-Asp-conjugated nanotube platform was immersed in Hg^{II}, Zn^{II}, Co^{II}, and Cu^{II} solutions that were prepared from HgCl₂, ZnNO₃ 6H₂O, CoCl₂, and CuCl₂ of the highest purity available, respectively. TEM images were taken with a Zeiss EM 902 microscope (80 kV acceleration voltage) and diffraction patterns were obtained with a JEOL 1200EX TEM (100 kV acceleration voltage). Impedance spectra were obtained in dry conditions with a 1260 Solatron Impedance Analyzer

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Strategy for the detection of ultralow levels of Pb^{II} with the peptide nanotube detection platform: a) In the absence of Pb^{II} nanowires are not templated by the nanotubes and no conductance is measured between the electrodes. b) In the presence of Pb^{II} the peptide nucleates the crystallization of Pb; after signal enhancement by Ag reduction, the metallic Pb/Ag coating on the nanotube that bridges the electrodes appears as a resistor in the circuit and the conductance between the electrodes is detected as the signal for Pb^{II} .

Figure 2. TEM microscopy images and electron diffraction patterns (inset) of a) Pb nanowire templated by TAR-2-Asp-conjugated peptide nanotubes in 0.01 nM Pb^{II} and b) neat peptide nanotubes with no TAR-2-Asp after the same process in 100 nM Pb^{II}.

Figure 3.

Specific detection of ultralow levels of Pb(II): a) In the absence of Pb (II), a Pb nanowire is not templated by the peptide nanotubes and a pure parasitic capacitor of 50 pF is observed in the impedance spectrum even after adding the Ag enhancement agent. b) In the presence of 0.01 nM Pb (II), a nanowire is synthesized, and a resistor can be observed in parallel in the circuit (as shown in the inset of Figure 1b) after subtraction of the parasitic capacitance. c) Conductance (G) of the peptide nanotube between the electrodes after the incubation with different heavy metal ions with the concentration indicated on the bars. “Control” is the experiment in the presence of 100 nM Pb (II) without the TAR-2-Asp peptide conjugated with the peptide nanotube (corresponding to Figure 2b).