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COMMUNICATION

Enhancement of selective determination of the perfect match and mismatch of single nucleobases with a biosensing electrode based on surface-coarsened anatase TiO_2 nanobelts†

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An alkaline hydrothermal and acid etching process was used to prepare surface-coarsened anatase TiO_2 nanobelts (CTNs) with enhanced (001) facets. The CTNs were used for electrochemical selective determination of the perfect match and mismatch of single nucleobases at the physiological pH of 7.4. It is supposed that the structure and surface morphology of CTNs play important roles in the nature of the adsorption/bonding and packing density of single base pairs on the nanobelt surfaces. Within the present experimental context, the CTNs are considered to be promising candidates for biosensing of nucleic acids that will be of fundamental significance to diagnostic medicine and molecular biology research.

1. Introduction

The normal biological functions of nucleic acids, including replication, substrate recognition of enzymes, and construction of tertiary protein structures, are realized by selective hydrogen bonding interactions between nucleobases, such as adenine (A) and thymine (T), adenine (A) and uracil (U), and guanine (G) and cytosine (C).[1] Abnormality of biological processes arises when mutation occurs. For instance, the deamination of cytosine (C) to uracil (U) leads to a G–C pair to an A–T pair point mutation in the DNA molecule, which may cause leukemia and cancer.[2] Therefore, the selective pairing of complementary nucleic acid bases plays an important role in regulating biological processes and functions of nucleic acids. However, it is difficult to have a selective determination of the base pair interactions in aqueous solutions.[3]

Selectivity is one important factor in the determination of the merits of active sensing materials. At present, how to improve the selectivity of an active sensing material remains a great challenge.[4,5] Towards this end, low dimensional nanostructures, which exhibit unique characteristics of efficient transport of electrons, may allow highly selective multiplexed determination of biochemical species and thus may be exploited as effective biosensing materials.[6–9]

For electrochemical biosensors, the active sensing materials on the electrode act as catalysts that facilitate the reactions of biochemical compounds to obtain the output signals,[6] where catalytic activity is critical in the selection and development of sensing materials. TiO_2 is a well-known multifunctional material. There are currently four natural TiO_2 polymorphs that include rutile, anatase, brookite, and TiO_2 (B).[10] Their functions strongly depend on their crystalline and morphological structures.[11] Anatase titanium dioxides, in particular, exhibit high-purity single crystals with a high percentage of reactive (001) facets that may lead to enhanced catalytic activity and selectivity among these four phases.[12–14] Thus, high-purity anatase single crystals have been proposed to be promising candidates for biosensor applications.

A practical method for the production of nanostructures should enable simultaneous control of the dimensions, properties, and morphology.[6] For instance, an alkaline hydrothermal and acid etching process has been used to prepare surface-coarsened anatase TiO_2 nanobelts (CTNs), which not only exhibited a quasi-one-dimensional nanostructure, but also manifested a high specific surface area and catalytic sensing performance due to their roughened surfaces and pure anatase structure with enhanced (001) facets. In a previous study, we showed that the CTNs might be employed as biosensing materials which exhibited enhanced electrocatalytic activities in the oxidation of nucleobases in a 0.1 M phosphate buffer solution (PBS, pH = 7.4).[15] More importantly, in the current work, we found that the CTNs could selectively detect the perfect match and mismatch of single nucleobases at the physiological pH of 7.4. At this pH, the selective determination of single nucleobase paring is of particular interest for electrochemical sensor applications in physiological media.[16,17] To the best of our knowledge, there has been no report on electrochemical selective determination of the perfect match and mismatch of nucleobase pairing at the physiological pH of 7.4 by using TiO_2 nanobelts as the biosensing materials. Within the present experimental context, the CTNs are considered to be promising candidates in the sensitive detection of nucleic acids that will be of fundamental significance to diagnostic medicine and molecular biology research.

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For comparison, TiO_2 nanobelts were also prepared *via* an alkaline hydrothermal method without the acid etching process (the experimental details are included in the ESI†). Analysis of the XRD patterns (Fig. 1a, curve 1) of the uncauterized TiO_2 nanobelts (TNs) confirms that these samples are mixed phases of anatase and TiO_2 (B) (JCPDS 46-1238). TiO_2-B has a metastable monoclinic structure, which is a crystalline form of titania with a looser structure than anatase and rutile.[18] In contrast, the cauterized TiO_2 nanobelts (CTNs) are pure anatase (JCPDS 21-1272), as manifested in curve 2 in Fig. 1a. Additionally, the (004) diffraction peak of the CTNs became much stronger and sharper than that of the TNs. This result indicates that nanobelts are elongated along the [001] direction with preferred anisotropic growth along the *c*-axis of the anatase lattice.[19] The high-purity anatase with a high percentage of reactive (001) facets would have promising applications in (bio)sensing due to their high catalytic reactive activity.[12,13] Panels b and c of Fig. 1 show representative SEM micrographs for the uncauterized and cauterized TiO_2 nanobelts. Both samples exhibit a width of 50 to 200 nm, and length of up to hundreds of micrometres. The TNs exhibited a smooth surface (Fig. 1b), while the CTNs showed a rough surface (Fig. 1c). Obviously, the CTNs would possess higher specific surface and provide higher interface activity. This result implies the CTNs would have a higher catalytic and determination ability. In the following section, biosensing properties of the TiO_2 nanobelts were examined by electrochemical measurements of nucleobase interactions.

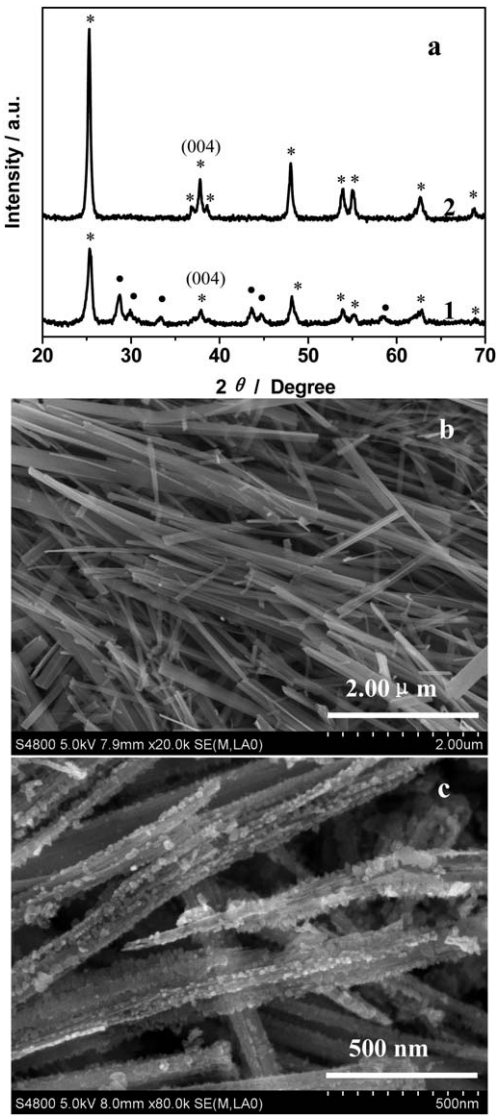

Fig. 1 Determination of skeleton crystal structure and morphology of TiO_2 nanobelts. (a) XRD patterns of (1) TiO_2 nanobelts without cauterization, (2) surface-coarsened TiO_2 nanobelts with cauterization. *, anatase TiO_2; ○, monoclinic TiO_2; and SEM images of (b) TiO_2 nanobelts without cauterization, and (c) TiO_2 nanobelts with cauterization.

Nucleobase pairing is the contributor not only to sequence-dependent recognition of nucleic acids, genetic information storage, and high fidelity of DNA polymerase replication, but also to RNA polymerase transcription where, for instance, wobble base pairs (such as U/G) are involved in RNAs.[19,20] So it is important to have a selective and sensitive determination of the base pair interactions. Huang found that the 2-Se substitution largely increases the specificity of base pair recognition.[19]

Here, a sensing electrode was prepared by depositing the TiO_2 nanobelts onto a glassy carbon electrode (GCE) surface with a conductive adhesive (CA) (see ESI† for details). Fig. 2 shows the voltammograms of the TiO_2 nanobelts-modified electrodes in 0.1 M PBS (pH 7.4) in the presence of 0.1 mM single nucleobases. It can be seen that at the TNs/CA/GCE electrode, the control experiment in a blank PBS supporting electrolyte manifested only a featureless voltammetric profile between +0.5 and +1.2 V. By contrast, two irreversible oxidation peaks appeared at +0.62 V and +0.89 V in the presence of 0.1 mM guanine and adenine (Fig. 2a). The first peak can be ascribed to the electro-oxidation of guanine, and the second one to adenine.[15] Similar features were observed in the presence of thymine, uracil (or cytosine), where two weak and broad oxidation peaks appear at +1.1 and +1.25 V (Fig. 2b), as a result of the electro-catalytic oxidation of the pyrimidine bases. Note that these peak potentials were virtually invariant at both TNs/CA/GCE electrode and CTNs/CA/GCE electrode.

Interestingly, electrochemical responses of single base-pair interactions were different at the TiO_2 nanobelts modified electrodes in PBS at pH 7.4 (Fig. 3). The SWV results show the surface-coarsened TiO_2 nanobelts (CTNs) can selectively detect the perfect match or mismatch of single nucleobases (Fig. 3c and d). For example, compared to the electro-catalytic features of G–C perfect match and G–T mismatch, G–U mismatch leads to a negative shift of 30 mV of oxidation peak potential (Fig. 3c). In contrast, compared to the electro-catalytic oxidation features of A–C mismatch, A–U perfect match and A–T perfect match lead to a positive shift of 30 mV (Fig. 3d). Notably, under the same experimental conditions, there was no shift of the electro-catalytic profiles with the uncauterized TiO_2 nanobelts (TNs) (Fig. 3a and b). These results indicate that TiO_2 nanobelts have higher selectivity after acid etching. It is well-known that the structure and surface morphology of nanocrystalline semiconductors are known to be important factors in influencing the nature of the bonding and the packing density of sensitizing molecules and compounds.[11,21] The selectivity difference for the determination of the perfect match and mismatch of single nucleobases may be ascribed to the crystal structure and surface morphology of the TiO_2 nanobelts. XRD studies showed that the CTNs exhibited pure anatase structures with enhanced [001] facets, whereas TNs only displayed a mixed phase of TiO_2 (B) and anatase. The difference of

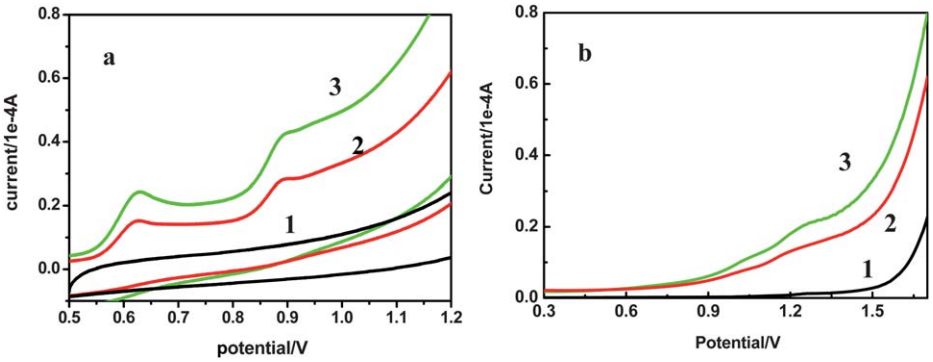

Fig. 2 Voltammetric detection of single nucleobase at TiO_2 nanobelts modified electrodes. (a) Cyclic voltammetric detection: (1) TNs (or CTNs)/CA/GCE in 0.1 M PBS of pH 7.4, (2) TNs/CA/GCE and (3) CTNs/CA/GCE in 0.1 M PBS, pH 7.4 containing 0.1 mM guanine and adenine. (b) Square wave voltammetry (SWV) detection: (1) TNs (or CTNs)/CA/GCE in 0.1 M PBS of pH 7.4, (2) TNs/CA/GCE and (3) CTNs/CA/GCE in 0.1 M PBS of pH 7.4 containing 0.1 mM thymine, uracil (or cytosine).

the crystal structure for TiO_2 nanobelts after acid etching may result in different catalytic active sites for the adsorption/bonding of nucleobases and hence electrocatalytic reactivity for nucleobases pairing.[11–13] Further, the SEM results show that the CTNs manifested a rough surface. This indicated acid-treated TiO_2 nanobelts would have a higher specific surface area with an enhanced catalytic sensing activity. In other words, the structure and surface morphology of acid-treated TiO_2 nanobelts may affect the nature of the adsorption/bonding and packing density of single base pairs on the electrode surface, and hence a shift of the electrocatalytic potential and enhancement of the selective determination for the perfect match and mismatch of single nucleobases. Further work on the catalytic mechanism of surface-coarsened anatase TiO_2 nanobelts for the selective determination of nucleobases interactions will be carried out in the near future.

In conclusion, surface-coarsened anatase TiO_2 nanobelts with enhanced [001] facets were synthesized by using a combination of an alkaline hydrothermal and acid etching process. The results of XRD and SEM show that the resulting sample consists of pure anatase phase with enhanced (001) facets and quasi-one-dimensional nanostructure with roughened surfaces. The surface-coarsened anatase TiO_2 nanobelts exhibited selective determination of the perfect match and mismatch of single nucleobases. It is likely that the enhanced biosensing performance is due to the unique surface morphology of surface-coarsened anatase TiO_2 nanobelts with a pure anatase structure and enhanced (001) facets that affected catalytic adsorption/bonding of nucleobase pairs onto the nanobelt surface. Within the present experimental context, surface-coarsened anatase TiO_2 nanobelts with enhanced (001) facets can be used as promising candidates in nucleic acid biosensing and the present work might open a door for

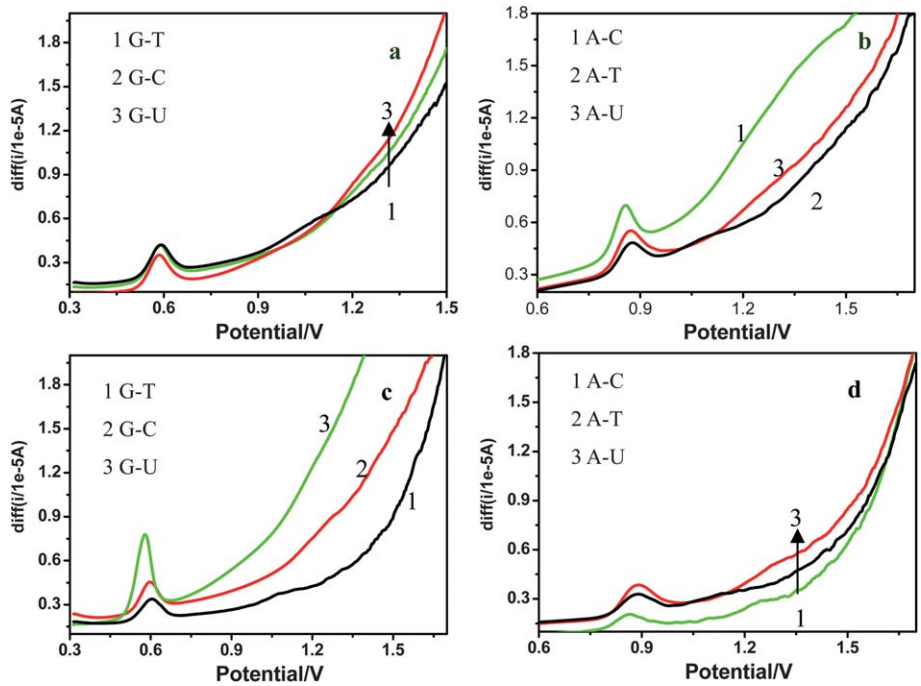

Fig. 3 SWV of single base-pair matches: (a), (b) TNs/CA/GCE; (c), (d) CTNs/CA/GCE in 0.1 M PBS (pH 7.4) containing 0.1 mM nucleobases.

developing a novel application area of TiO_2 nanobelts. Further work on the catalytic mechanism of surface-coarsened anatase TiO_2 nanobelts for the selective determination of nucleobases interaction will be pursued in the near future.

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