Photocatalytical reduction of disulphide bonds in peptides on Ag-loaded nano-TiO_2 for subsequent derivatization and determination

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Experimental Section

Chemicals and Materials. Tetrabutyl titanate (chemical pure grade) was obtained from Sinopharm Chemical Reagent Co. (Shanghai, China). Sylgard 184 silicone elastomer base and its curing agent were obtained from Dow Corning Corporation (Midland, MI); 1,4,7,10-tetraazacyclododecane-1,4,7-trisacetic acid-10-maleimidoethylacetamide (MMA-DOTA) was purchased from Macrocyclics (Dallas, TX); vasopressin (≥ 97%), oxidized glutathione (GSSG, ≥ 99%), insulin (from bovine pancreas, ≥ 27 units mg^{-1}), tri-n-butylphosphine (TBP, ≥ 99%) and 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB, ≥ 99%) were all purchased from Sigma-Aldrich (St. Louis, MO, USA); trifluoroacetic acid (TFA) used as a component of the HPLC mobile phase was also purchased from Sigma-Aldrich (St. Louis, MO, USA); and methanol and acetonitrile of HPLC grade were purchased from Merck (Darmstadt, Germany). All other chemicals were of analytical reagent grade and were purchased from Sinopharm Chemical Reagent Co. (Shanghai, China). Ultrapure water with a resistivity of 18 MΩ (Millipore, Bedford, MA, USA) was used throughout this

study.

Apparatus. HPLC was performed with a Shimadzu LC-2010A system (Kyoto, Japan) equipped with a quaternary pump, an autosampler (10-100μL), a thermostated column compartment and a vacuum degasser and a Shimadzu SPD-10Avp UV-Vis detector (190-600 nm) as well. The system was controlled using Shimadzu Class-VP software. Separations were carried out with a Shimadzu VP-ODS C18 reversed phase column (2.0 mm I.D. ×250 mm in length; particle size, 5μm; porosity, 80Å). A 40-W low-pressure Hg-lamp (253.7 nm, 25 mm O.D. × 130 mm in length) was used as an illumination source. Scanning electron microscopy (LEO 1530, Oberkochen, Germany) was used to image the morphology of Ag-deposited TiO_2. The HPLC was coupled to an Esquire-LC ESI ion trap mass spectrometer (Bruker Daltonics, Bremen, Germany) in a post-column splitting ratio of 3:1. The ESI ion trap mass spectrometer was used in the positive mode. The operational parameters were as follows: nebulizer, 55 psi; dry gas, 8 L min^{-1}; dry temperature, 300 °C; capillary, -3500 V; endplate offset, -500V; skim 1, 35.0 V; skim 2, 6.0 V; capillary exit offset, 60.0 V; octopole, 2.80 V; lens 1, -5.0 V; lens 2, -60.0 V; trap drive, 55.0; and max accumulation time, 50.00 ms.

Preparation of nanoTiO_2 and Ag-loaded nanoTiO_2 as well as coating of them onto the surface of a glass fibre. The TiO_2 nanoparticle was prepared as follows: a homogeneous yellow sol was prepared by adding 5% (v/v) HCl in ethanol dropwise to a mixture of 40% (v/v) tetrabutyl titanate in ethanol and agitating at room temperature for 2 h. After being allowed to age for 3 days, the resulting product was dried at 80 °C. This process was followed by calcination at a gradually increasing temperature

(1.5 °C min^{-1}) to 450 °C before keeping constant for 1 hour, allowing that an antase TiO_2 was formed.

The preparation of the Ag-loaded TiO_2 nanoparticle was carried out in accordance with the above procedure, but 100 mmol L^{-1} $AgNO_3$ solution was added to the homogeneous yellow sol solution.

NanoTiO_2 and Ag-loaded TiO_2 (Ag/TiO_2) were deposited on the glass fibre using the sol-gel coating method.[1, 2] NanoTiO_2 was stabilized on the surface of a glass fibre by dipping the glass fibre into and pulling it out of a sol-gel solution which was prepared with tetrabutyl titanate and ethanol (pH 3.5, adjusted by diluted HCl). The Ag/TiO_2 film on the surface of a glass fibre was prepared in the same way but adding 0.1 mol L^{-1} $AgNO_3$ in the sol-gel solution. The dip-coated glass fibres were dried at 80 °C for 30 min and then calcined at a gradually increasing temperature (1.5 °C min^{-1}) to 450 °C before keeping constant for 1 hour.

Fabrication of UV-Ag/TiO_2-HCOOH reduction unit. The on-line fluidic channel device consisted of two polydimethylsiloxane (PDMS) layers. A master mould was made of polytetrafluoroethylene, and the curved protuberance was 1 mm in diameter and 300 mm in length. Sylgard 184 silicone oligomer and cross-linking agent (Sylgard 184 silicone elastomer curing agent) were thoroughly mixed at 10:1 (w/w) ratio and poured over the master mould. After curing at 65°C for 1 h, the cured PDMS was carefully cut and peeled off from the master so as to get a PDMS layer with a curved microchannel (1.0 I.D. × 300 mm in length). TiO_2 or Ag/TiO_2 coated glass fibre (0.3 mm O.D.) was put simply in the channel, and then another freshly made

PDMS membrane (~ 0.5 mm) was bound at 65 °C for 2 h to cover it to get the device. It should be noted that the covering membrane prepared should be at a suitable mature-stage to guarantee the strong adhesive strength avoiding the leak of solution while not to clog the flow of the solution. The final device was rinsed with 10× the total volume (217 μL) of the device with ultrapure water using a syringe pump before use.

Reduction of GSSG, vasopressin and insulin under UV-TiO_2-HCOOH and UV-Ag/TiO_2-HCOOH as well as subsequent determination at 412 nm of DTNB derivatives. Appropriate amount of GSSG (or vasopressin or insulin) and HCOOH were added into the TiO_2 or Ag/TiO_2 solution (5 mL) containing an appropriate amount of HCOOH. After N_2 gas bubbling for 20 min, UV-irradiation (253.7 nm, a 40 W UV-light source) was continued for 1 min, 2 min, 3 min, 4 min, 5 min, 10 min, 30 min and 1 h. After centrifuging for 5 min at 8000 rpm, 150 μL supernatant was taken for determination at 412 nm with 3 mmol L^{-1} DTNB derivatization in 0.3 mol L^{-1} sodium phosphate buffer (pH 7.88) containing 15 mmol L^{-1} EDTA. An electron or hole scavenger must be used to prevent the rapid recombination when an oxidation or reduction process is desired using the photocatalytical nanoTiO_2. To improve the reduction efficiency of the peptides on Ag/TiO_2, various hole-scavengers including methanol, glycerol, formaldehyde, acetone, formic acid, sodium formate and triethanolamine were investigated (Fig. S1). The ratio of Ag (0-1 wt.%) loaded on nanoTiO_2 and HCOOH concentration (0.18-7.92 vol.%) added in the solution as well as different h^+ scavengers were also investigated, and the results for GSSG are

illustrated in Fig. S1.

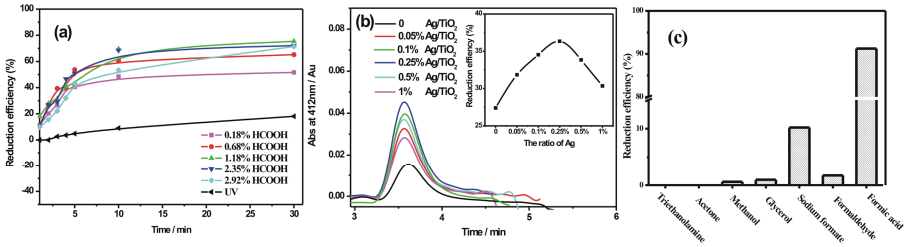

Fig. S1 GSSG reduction on TiO_2 (a) and Ag/TiO_2 nanoparticles (b and c). Reduction efficiency under different HCOOH concentration with 1 g L^{-1} TiO_2 (a); on nanoTiO_2 loaded with different amounts of Ag (1.18% HCOOH) under 1 min of irradiation (b); different 1.5% hole scavengers (c). Flow-injection mode, flow rate at 0.15 mL min^{-1}. Derivatization conditions: 3 mmol L^{-1} DTNB in 15 mmol L^{-1} EDTA and 0.3 mol L^{-1} Na_3PO_4 buffer (pH 7.88) at a flow rate of 0.05 mL min^{-1}; detection wavelength, 412 nm.

In order to verify the reduction efficiency, MMA-DOTA was used to block the nascent sulphydryls, and then the reduced and MMA-DOTA blocked peptides were determined using ESI-MS.[3] The results are shown in Fig. S2.

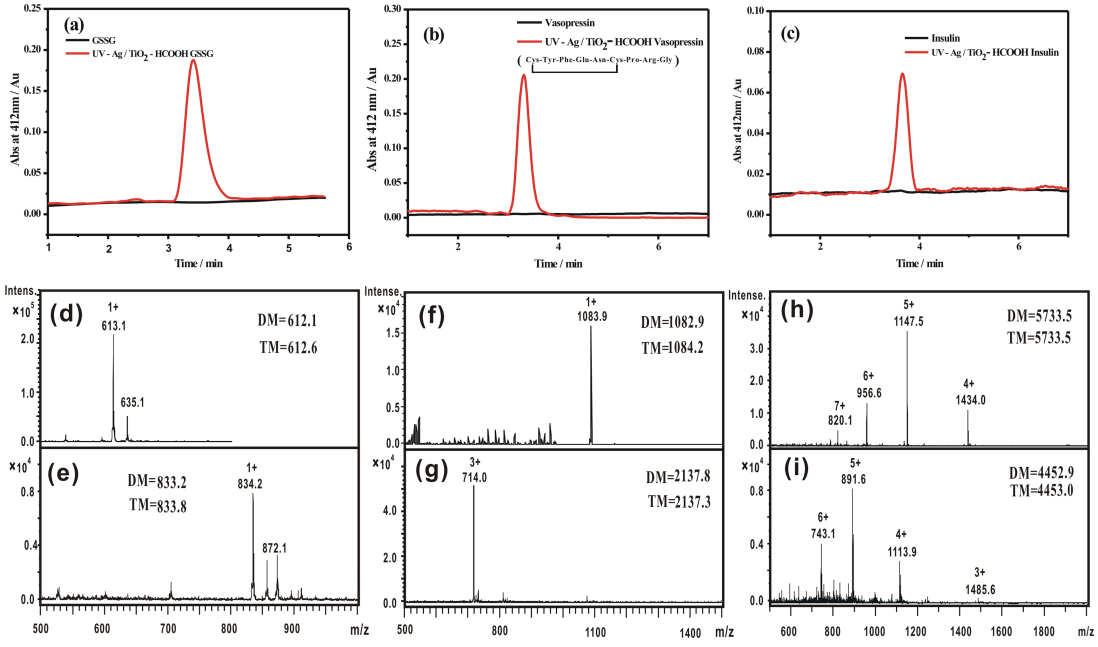

Fig. S2 Comparative signals of GSSG (a), vasopressin (b) and insulin (c) detected with DTNB before and after Ag/TiO_2 photocatalytical reduction. ESI-MS spectra of GSSG (d), MMA-DOTA labelled GSH (e), intact vasopressin (f), MMA-DOTA labelled vasopressin (g), intact insulin (h) and the MMA-DOTA labelled B chain of insulin (i). Flow-injection mode, flow rate, 0.15 mL min^{-1}. Nascent sulphydryl blocking conditions: MMA-DOTA was added to conjugate the nascent free sulphydryl groups directly at 37 °C for 2 h (pH 7.2). DM and TM in the MS spectra denote deconvolution and theoretical molecular weights, respectively.

An online reduction experiment was carried out as shown in Scheme 1. The peptide and 0.25% HCOOH were combined and flowed through an Ag/TiO_2 photocatalytical reduction reactor at a flow rate of 0.3 mL min^{-1}. The reduced peptide effluent was followed by DTNB derivatization and determined at 412 nm. The results without and with the on-line reduction and those of different concentrations of the peptides are shown in Fig. S3 and S4.

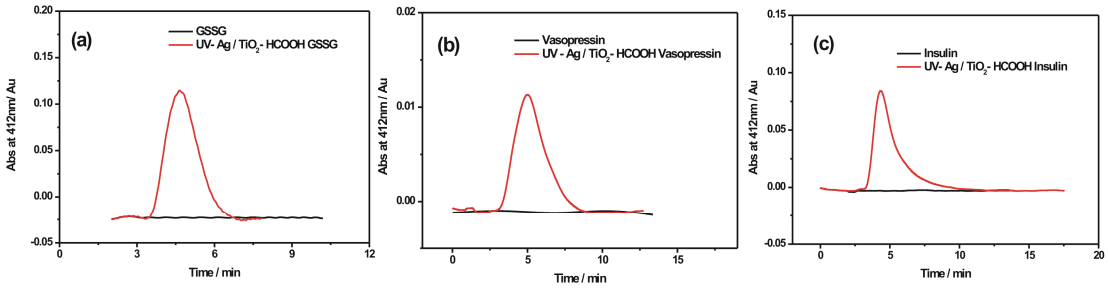

Fig. S3 Comparative signals of GSSG (a), vasopressin (b) and insulin (c) detected with DTNB before and after UV-Ag/TiO_2-HCOOH online reduction. Flow-injection mode, flow rate, 0.15 mL min^{-1}. Flow rate of HCOOH was 0.15 mL min^{-1}. Derivatization conditions: 3 mmol L^{-1} DTNB in 15 mmol L^{-1} EDTA and 0.3 mol L^{-1} Na_3PO_4 buffer (pH 7.88) at a flow rate of 0.1 mL min^{-1}.

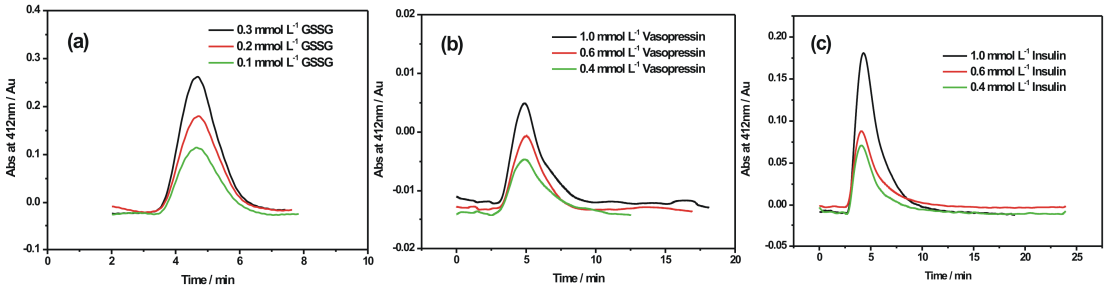

Fig. S4 On-line reduction of GSSG (a), vasopressin (b) and insulin (c). Flow-injection mode, flow rate, 0.15 mL min^{-1}. Flow rate of HCOOH was 0.15 mL min^{-1}. Derivatization conditions: 3 mmol L^{-1} DTNB in 15 mmol L^{-1} EDTA and 0.3 mol L^{-1} Na_3PO_4 buffer (pH 7.88) at a flow rate of 0.1 mL min^{-1}.

Extraction and analysis of phytochelatins (PCs). *Phaeodactylum tricornutum (P. tricornutum)* culture and Cd-stress experiments were carried out as described in our previous study.[4] *P. tricornutum* was obtained from the Center for the Collection of Marine Bacteria and Phytoplankton in the State Key Laboratory of Marine Environmental Science, Xiamen University. *P. tricornutum* cells exposed for 4 days to $CdCl_2$ (0.1 mg L^{-1}) were collected, then re-suspended in ice-cold and N_2 saturated HCl, and homogenized in a sonication ice bath, followed by centrifugation. A 500 μL aliquot of the supernatant was subjected to being reduced with 0.25%-Ag/TiO_2 nanoparticle and a 1 % HCOOH solution under UV irradiation. For comparison of samples, another 500 μL aliquot of the supernatant was subjected to reaction with TBP after neutralization using NaOH. An appropriate amount of HCl was then added to adjust the pH to 1, and finally the homogenate was centrifuged. The peaks in the chromatograms obtained using TBP and UV-Ag/TiO_2-HCOOH reduction were identified by ESI-MS (Fig. S5), indicating that GSH, PC_2, PC_3, PC_4 and PC_5 were induced under Cd-stress.

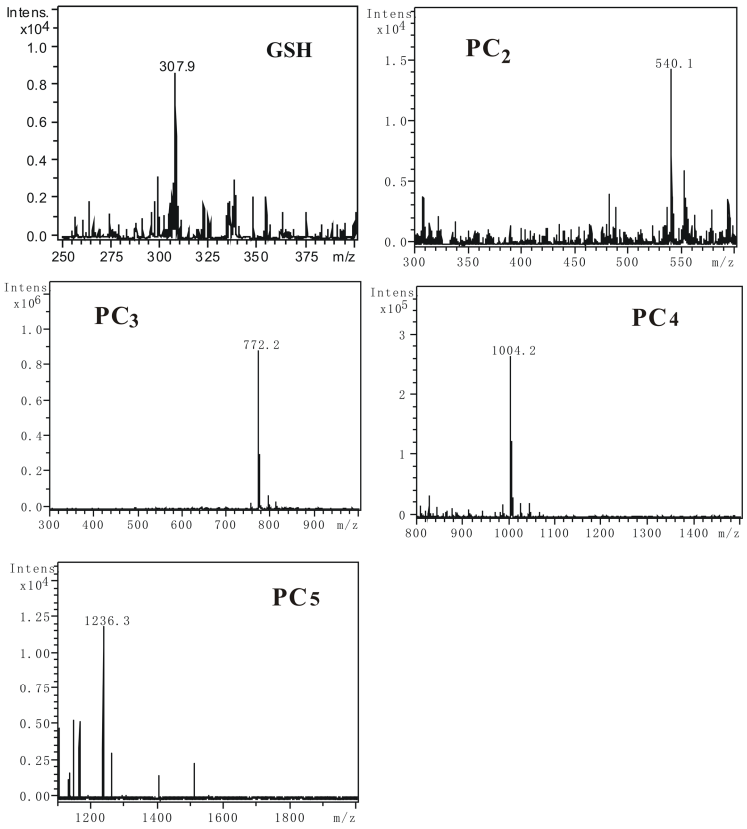

Fig. S5 The PCs induced by Cd in *P. tricornutum* identified using HPLC-ESI-MS. Mobile phase A, 0.02% TFA-H_2O; mobile phase B, 0.02 % TFA-ACN; elution program: 0-25 min, B 2-25%; 25-30min, B 25%; flow rate, 0.15 mL min^{-1}. ESI-MS conditions were described in the Apparatus section.

References

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