Supplementary Material (ESI) for Chemical Communications

Electronic Supplementary Information (ESI) for

Electrochemical detection of kinase-catalysed thiophosphorylation using gold nanoparticles

Kagan Kerman and Heinz-Bernhard Kraatz*

Department of Chemistry, University of Western Ontario, 1151 Richmond Street, London, Ontario, N6A 5B7 Canada.

Tel: +1 (519) 661-2111, ext. 81561; *E-mail: hkraatz@uwo.ca

EXPERIMENTAL

Reagents

Colloidal Au nanoparticles (5 nm) were purchased from Sigma (Japan). The biotinylated protein kinase C_ζ peptide (Biotin-SIYRRG***S***RRWRKL) was purchased from Calbiochem (EMD Biosciences, USA). The modified protein kinase C_ζ pseudosubstrate sequence contains Ser_{119} instead of Ala_{119}.[1] Protein kinase C from rat brain (E. C. 2.7.1.37) was purchased from Sigma in 50% glycerol containing 20 mM Tris, 0.5 mM EDTA, 0.5 mM EGTA, 5 mM DTT, 100 mM NaCl, 0.02% Tween 20, and 1 μg/mL leupeptin. One unit (U) of PKC will transfer 1 nanomole of phosphate from ATP into histone H1 per min at 30ºC.[2,3] Streptavidin from Streptomyces avidinii (1 U will bind 1 μg biotin) and Tween® 20 (Polyethylene glycol sorbitan monolaurate) were purchased from Sigma. Other reagents were purchased from Merck. All solutions were prepared and diluted using ultra-pure water (18.3 MΩ-cm) from the Millipore Milli Q system.

Instruments

Square wave voltammetry (SWV) was performed using a CHInstruments 660 system (Austin, TX). The disposable screen-printed carbon electrodes (SPCEs) were kindly donated by Professor Eiichi Tamiya in Osaka University and BioDevice Technology Ltd. (Ishikawa, Japan). The total length of a SPCS was 11 mm, and the geometric area of the working electrode was 2.64 mm^2. Scanning electron microscopy (SEM) images of Au nanoparticles were obtained using a JEOL 840A at an acceleration voltage of 8 kV. Transmission electron microscopy (TEM) was performed using Philips CM200 UT high-resolution transmission electron microscope at an acceleration voltage of 110 kV.

Procedure

In order to avoid rapid evaporation of the solutions on the surfaces, the SPCEs were incubated in petri-dishes at room temperature throughout the preparatory steps. The electrochemical measurements were performed for three times for each condition (n=3), except otherwise stated. The results show the average of three measurements with the error bars indicating the relative standard deviation (RSD).

Electrode pre-treatment

An electrode pre-treatment was carried out before the modifications with biomolecules with the aim of improving the sensitivity and reproducibility of the results as described by Díaz-González et al.[4] Briefly, an aliquot of 0.1 M H_2SO_4 (20 µL) was dropped on SPCEs and an anodic current of 25 µA was applied for 2 min. Then, the electrodes were washed using 0.1 M Tris HCl buffer (TBS, pH 7.2).

Immobilization of the biotinylated substrate peptides on streptavidin-modified SPCEs

An aliquot of 10 µM streptavidin solution (5 µL) was allowed to coat the working electrode of the pre-treated SPCEs and kept overnight at 4°C. The following incubation steps were performed dropping 25 µL aliquots of the solutions on the SPCS surface at room temperature. After each incubation step the electrodes were washed with 0.05% (w/v) Tween 20 in TBS. Free surface sites of the streptavidin-modified SPCSs were blocked with 1.0% (w/v) Tween 20 in TBS. Then, the biotinylated substrate peptides (50 µM) were immobilised, incubating an aliquot (5 µL) of 3.0 µg/mL solution for 90 min on the streptavidin-coated surface. After this incubation step, the SPCSs were rinsed with blank TBS.

PKC-catalyzed thiophosphorylation on the SPCE surface

Kinase assay buffer included 20 mM Tris, 0.5 mM EDTA, 10 mM $MgCl_2$, 500 µg/mL phosphatidyl serine (pH 7.5). The concentrations of ATP-S and PKC were varied according to the optimum experimental conditions. The aliquots (200 µL) of the optimized assay buffer including 100 U/mL PKC and 75 µM ATP-S were pipetted into 1.5-mL vials. Substrate peptide-immobilized SPCEs were placed in the vials incubated at 30ºC for 1 h in a heating block (VWR Scientific, USA). After 1 h of incubation, the SPCEs were removed and washed with blank TBS. Then, 20 µL of Au nanoparticle solution (5 nm, A_{520}=0.5) was placed on the surface and the SPCEs were incubated for 1 h in a petri-dish at room temperature. Afterwards, the SPCEs were washed with blank TBS and placed in the electrochemical workstation.

Electrochemical measurement on SPCE surface

Electrochemical detection was performed by spotting 20 µL of 0.1 M HCl onto the surface of Au nanoparticle-modified SPCE at room temperature. Square wave voltammetry (SWV) involved the oxidation of Au nanoparticles by applying 1.20 V for 60 s. After this oxidation procedure, the oxidized ions were reduced by sweeping the potential from 1 to 0 V with an amplitude of 25 mV at 15 Hz frequency.

References:

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2. U. Kikkawa, A. Kishimoto and Y. Nishizuka, *Ann. Rev. Biochem.*, 1989, **58**, 31-41.
3. G. M. Walton, P. J. Bertics, L. G. Hudson, T. S. Vedvick and G. N. Gill, *Anal. Biochem.*, 1987, **161**, 425-437.
4. M. Díaz-González, D. Hernández-Santos, M. B. Gonzáles-Garcia, A. Costa-Garcia, *Talanta*, 2005, **65**, 565-573.

ESI Fig. 1 TEM image of Au nanoparticles after PKC-catalyzed thiophosphorylation of the substrate peptides under the given experimental conditions as described above. Large aggregates of Au nanoparticles were observed, indicating the high affinity of thiophosphorylated peptides towards Au nanoparticles.