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Electronic Suppementary Information

A Method for Highly Efficient Catalytic Immobilisation of Glucose Oxidase on the Surface of Silica

Yong-Kyun Sim, Jung-Woo Park, Bo-Hyeong Kim, Chul-Ho Jun*

Department of Chemistry, Yonsei University, Yonsei-ro 50, Seodaemoon-gu, Seoul, 120-749 Korea(South)

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I. Materials

Glucose oxidase (GOx, from Aspergillus niger, 147,000 U/g) was purchased from Sigma Aldrich and used after purification by gel-filtration (ÄKTAexplorer100, GE Healthcare). NHS-ester-functionalized methallylsilane **1** (2,5-dioxopyrrolidin-1-yl 1-(3-(methylbis(2-methylallyl)silyl)propyl)-1H-1,2,3-triazole-4-carboxylate) was prepared by using reported procedure.[1] $Sc(OTf)_3$ (**2**) were purchased from Lancaster and Sigma Aldrich, respectively. Silica employed in the GOx-immobilisation experiments (pore size: 30 nm, 10 μm, 90 m^2/g) was purchased from Fuji Silysia. Acetonitrile was distilled by reported procedure prior to use.[2]

II. Measurements

a. LC-MS/MS for GOx Analysis_Q-TOF.

Nano LC-MS/MS analysis was performed with a nano HPLC system (Agilent, Wilmington, DE, provided by Yonsei Proteome Research Center). A nano chip column (Agilent, Wilmington, DE, 150 mm × 0.075 mm) was used for peptide separation. The mobile phase A for LC separation was 0.1% formic acid in deionized water and the mobile phase B was 0.1% formic acid in acetonitrile. The chromatography gradient was designed for a linear increase from 5% B to 30% B in 25 min, 40% B to 60% B in 5 min, 90% B in 10 min, and 5% B in 15 min. The flow rate was maintained at 300 nL/min. Product ion spectra were collected in the information-dependent acquisition (IDA) mode and were analyzed by using Agilent 6530 Accurate-Mass Q-TOF using continuous cycles of one full scan TOF MS from 200-1500 *m*/*z* (1.0 s) plus three product ion scans from 50-1800 *m*/*z* (1.5 s each). Precursor *m*/*z* values were selected starting with the most intense ion, using a selection quadrupole resolution of 3 Da. The rolling collision energy feature was used, which determines collision energy based on the precursor

[1] U.-Y. Jung, J.-W. Park, E.-H. Han, S.-G. Kang, S. Lee and C.-H. Jun, *Chem. Asian J.* 2011, **6**, 638.
[2] *Purification of Laboratory Chemicals*, 4th edition (Eds: W. L. F. Armarego, D. D. Perrin), Butterworth-Heinemann, Oxford, 1996, pp. 77.

value and charge state. The dynamic exclusion time for precursor ion *m*/*z* values was 60 s. For Database Searching, the mascot algorithm (Matrixscience, USA) was used to identify peptide sequences present in a protein sequence database.

b. MALDI-TOF MS measurements for GOx and GOx-CMs

MALDI-TOF MS measurements were performed on a Bruker model LRF20 using sinapinic acid as a matrix. Elementary Analyses were provided by Yonsei University Center for Research Facilities (Elemental Analyzer, 2400 Series II CHNS/O, Perkin Elmer).

c. Determination of GOx Activity

Glucose oxidase (GOx) activity was determined using a GOx assay kit (from Stressgen Biotechnologies) according to instructions provided by manufacturer. GOx-immobilised silica (1 mg) was dispersed in PBS (1 mL), and the resulting solution was serially diluted. Then, aqueous D-glucose solution (1 μM) was added to a diluted solution. After the sample was incubated at room temperature in the dark for 30 minutes, the supernatant solution was used for determining the concentration of H_2O_2 generated. H_2O_2 solution (30 % solution, Calbiochem) was used as a standard solution. Then, the enzyme activity was determined from the standard curve.

d. Determination of GOx Loading

The concentration of immobilised GOx was determined using the Bradford method (Bio-Rad).[3] In this experiment, the reaction of an aqueous solution of **GOx-CM** with silica (20 mg) in the presence of **2** was carried out at 0 ^{o}C for 2 hours, and the resulting **GOx-CM@Si** was filtered, and washed with deionized water (100 mL). The resulting solution was concentrated to 4 mL by centrifugal filter (Amicon Ultra-15

[3] Bradford method (Bio-Rad) was used for determining the amount of immobilised enzyme according to the manufacturer's instructions. Bradford, M. M. *Anal. Biochem.* 1976, **72**, 248-254.

Ultracel-30k membrane) and the GOx concentration of the resulting solution was analyzed by Bradford method.

III. Experimental

a. Preparation of Chemically Modified GOx (GOx-CM). For preparing **GOx-CM(48)**, to an aqueous GOx solution (4.23 mg GOx, 5.288 x 10^{-5} mmol, 0.6 mL) in 1.5 mL eppendorf tube was added NHS-ester-functionalized methallylsilane **1** (1.03 mg, 48 equivalents based on the lysine residue). The mixture was stirred at 0 ^{o}C for 2 h. For the unpurified sample, **GOx-CM(48)**, the mixture was used without further purification process. Other modified GOx samples, **GOx-CM(16)**, **GOx-CM(32)** and **GOx-CM(64)**, were prepared by same procedure with using different amounts of **1** (0.34 mg for **GOx-CM(16)**, 0.69 mg for **GOx-CM(32)** and 1.37 mg for **GOx-CM(64)**).

b. Preparation of GOx-CM@Si. For preparing **GOx-CM(48)@Si**, to a 1.5 mL eppendorf tube was charged with the **GOx-CM(48)** sample prepared above, silica (particle size: 10 μm, pore size: 30 nm, 20 mg) and $Sc(OTf)_3$ (**2**, 0.125 mg (3 μL stock solution (concentration: 4.164 mg **2** / 100 μL H_2O))). Then, the reaction mixture was stirred at 0 ^{o}C for 2 h. After the reaction, the mixture was filtered and the precipitate was washed thoroughly with deionized water. The resulting silica was dried under reduced pressure to afford **GOx-CM(48)@Si**. Other GOx-immobilised silica samples, **GOx-CM(16)@Si**, **GOx-CM(32)@Si** and **GOx-CM(64)@Si**, were prepared by using the same procedure with using different amounts of **2** (1 μL stock solution for **GOx-CM(16)@Si**, 2 μL stock solution for **GOx-CM(32)@Si** and 4 μL stock solution for **GOx-CM(64)@Si**).

c. Preparation of GOx-G@Si (Figure 3b). A mixture of aqueous GOx solution (4.23 mg GOx, 5.288 x 10^{-5} mmol, 0.6 mL) and NHS-ester-functionalized silica (**NHS-grafted silica**, 20.0 mg) was stirred at 0 ^{o}C for 2 h. The mixture was then filtered and washed thoroughly with deionized water. The resulting silica was dried under reduced pressure to afford **GOx-G@Si**. **NHS-grafted silica** was prepared as

follows: To a mixture of triflic acid (TfOH, 11.1 μl (10 mol%)) and silica (pore size: 30 nm, 0.5 g) in 5 mL microreactor, compound **1** (505 mg (1.25 mmol)) and acetonitrile (3 mL) was added and the reaction mixture was stirred at room temperature for 12 h. After the reaction, the mixture was filtered and the precipitate was washed thoroughly with dichloromethane. The resulting silica was dried under reduced pressure to obtain sample **NHS-grafted silica**. The loading rate of NHS-ester group in **NHS-grafted silica** was determined to be 0.28 mmol/g NHS-ester groups, based on the N value of elemental analysis (Elemental analysis of **NHS-grafted silica**: C, 3.92; N, 1.54).

d. Q-TOF Mass Analysis of GOx

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  1 MQTLLVSSLV VSLAAALPHY IRSNGIEASL LTDPKEVAGR TVDYIIAGGG
 51 LTGLTTAARL TENPDITVLV IESGSYESDR GPIIEDLNAY GDIFGSSVDH
101 AYETVELATN NQTALIRSGN GLGGSTLVNG GTWTRPHKAQ VDSWETVFGN
151 EGWNWDSVAA YSLQAERARA PNAKQIAAGH YFNASCHGIN GTVHAGPRDT
201 GDDYSPIVKA LMSAVEDRGV PTKKDLGCGD PHGVSMFPNT LHEDQVRSDA
251 AREWLLPNYQ RPNLQVLTGQ YVGKVLLSQN ATTPRAVGVE FGTHKGNTHN
301 VYAKHEVLLA AGSAVSPTIL EYSGIGMKSI LEPLGIDTVV DLPVGLNLQD
351 QTTSTVRSRI TSAGAGQGQA AWFATFNETF GDYAEKAHEL LNTKLEQWAE
401 EAVARGGFHN TTALLIQYEN YRDWIVKDNV AYSELFLDTA GVASFDVWDL
451 LPFTRGYVHI LDKDPYLRHF AYDPQYFLNE LDLLGQAAAT QLARNISNSG
501 AMQTYFAGET IPGDNLAYDA DLSAWVEYIP YNFRPNYHGV GTCSMMPKEM
551 GGVVDNAARV YGVQGLRVID GSIPPTQMSS HVMTVFYAMA LKIADAILAD
601 YASMQ
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Figure S1. Sequence of GOx; 16 lysine (K) residues were found.

Mascot search against parameters: all entries (NCBInr database downloaded on July 4 2011, fixed modification; carboxyamidomethylated at cysteine residues; variable modification; oxidized at methionine residues, maximum allowed missed cleavage; 1, MS tolerance; 100 ppm, MS/MS tolerance; 0.1 Da). Only peptides resulting from trypsin digests were considered.