Supporting Information

Guanidyl-Functionalized Graphene as a Bi-function Adsorbent for Selective Enrichment of Phosphopeptides

Lin-Nan Xu, Li-Ping Li, Liang Jin, Yu Bai*, and Hu-Wei Liu

Beijing National Laboratory for Molecular Sciences, Key Laboratory of Bioorganic Chemistry and Molecular Engineering of Ministry of Education, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China. Tel: +86 10 6275 8198; E-mail: yu.bai@pku.edu.cn

Experimental Section

Materials

Graphene oxide (GO) was purchased from Nanjing XFNANO Materials Tech Co., Ltd. Thionyl chloride ($SOCl_2$) was purchased from Sinopharm Chemical Reagent Co., Ltd. 1,6-hexanediamine, O-methylisourea hemisulfate (OMIU), and 2,5-dihydroxybenzonic acid (DHB) were purchased from J&K Scientific Ltd. N,N-dimethylformamide (DMF) was from J. T. Baker. Trypsin, α-casein, β-casein, and acetonitrile (ACN) were purchased from Sigma-Aldrich. Ammonium bicarbonate (NH_4HCO_3) was purchased from Fluka. Trifluoroacetic acid (TFA) was obtained from Acros Organics. Acetic acid (HAc), phosphoric acid (H_3PO_4) and sodium hydroxide (NaOH) were from Beijing Chemical Works. Water used as solvent was from Wahaha Group Co., Ltd. Non-fat milk was purchased from a local supermarket.

Preparation of amino- and guanidyl-functionalized graphene

Synthesis of amino-functionalized graphene (AFG)

120 mg GO, 30 mL $SOCl_2$, and 1.5 mL DMF were mixed in a 100 mL flask. After sonification for sufficient dispersion of GO in solvent, the mixture was stirred at 70℃ for 24 h. The acylated intermediate was separated from solution by centrifugation, washed with dry DMF and directly transferred into 30 mL 1,6-hexanediamine heated at 55℃. The mixture was stirred at 55℃ for 48 h and then diluted with 25 mL ethanol. The product was washed with water and ethanol for several times and then dried under vacuum at room temperature.

Synthesis of guanidyl-functionalized graphene (GFG)

50 mg AFG, 3.0781 g OMIU, and 50 mL water were mixed in a 100 mL flask. After sonification for dispersion of AFG and dissolution of OMIU, the pH was adjusted to 11 by NaOH. The mixture was stirred at 60℃ for 24 h and the reaction was terminated by adding

1.65 mL TFA. After washing by water and ethanol, the product was dried under vacuum at room temperature.

Preparation of α-casein, β-casein, and non-fat milk digests

α-casein and β-casein were dissolved in 50 mM NH_4HCO_3 solution. After addition of trypsin with 1:40 (w/w) enzyme-to-protein ratio, the proteins were digested at 37℃ for 20 h.

30 μL non-fat milk was diluted with 970 μL 50 mM NH_4HCO_3 solution. The diluent was centrifuged under 14,000 rpm for 25 min. After denaturation of the supernatant at 100℃ for 5 min, 30 μg trypsin was added and the mixture was allowed for digestion at 37℃ for 20 h.

Enrichment of phosphopeptides

In general, the tryptic digests were diluted with loading buffer (buffer A: 50% ACN, 0.1 M HAc; or buffer B: 66.6% ACN, 0.05% TFA) to a certain concentration, and the adsorbent (AFG or GFG) was dispersed in water by sonification. 50 μL suspension of adsorbent (1 mg/mL-4 mg/mL) was added to 200 μL peptide diluent, and the mixture was gently shaked by a rotator. After centrifugation and removal of the supernatant, the adsorbent was washed by 100 μL loading buffer for three times and mixed with 5 μL matrix solution of ACN/H2O 1:1 (v/v) containing 30 mg/mL DHB and 5% H_3PO_4.

Instrumentation

Infrared spectrum was measured with KBr pellet by Bruker Tensor 27 FT-IR. Absorbance spectrum was acquired with resolution of 4 cm^{-1} and subtraction of background of air. Average spectrum of 32-time measurements was recorded.

Elemental composition was analyzed by Vario EL from Elementar Analysensysteme GmbH to acquire the content of C, H, N in weight

For TEM analyses, drops of dispersion of materials were deposited on 200 mesh copper grids equipped with micro-grids and coated with ultrathin carbon film and dried in air. The morphology of samples were observed by FEI Tecnai G2 T20 TEM operated at 120 kV.

MALDI-TOF MS spectra were acquired by a Bruker Daltonics ultraflex TOF mass spectrometer. Reflection mode was adopted and voltage parameters were set as followed in our work: ion source 1, 25.00 kV; ion source 2, 22.15 kV; lens, 10.55 kV; reflector, 26.30 kV; reflector 2, 14.10 kV. The laser frequency was set on 20 Hz. 1 μL of mixture of materials with peptides adsorbed and matrix solution was directly deposited on a plate and dried in air for MALDI-TOF MS analysis.

Figure and Table

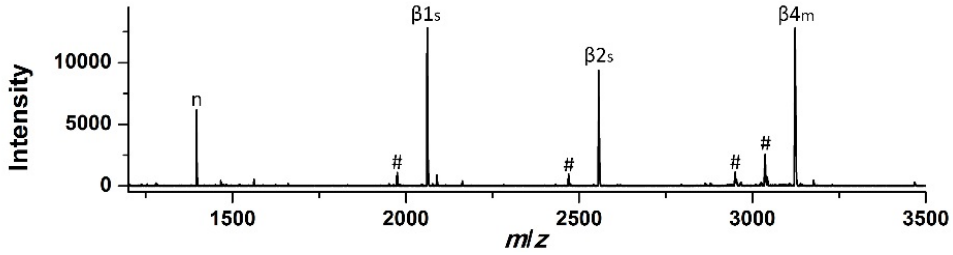

Figure S1 Enrichment of phosphopeptides from digest of β-casein at 4×10^{-7} M by AFG. Composition of loading buffer is 60% ACN with 0.1 M HAc. (s: monophosphopeptide; m: multiphosphopeptide; #: dephosphorylated residue of phosphopeptides; n: non-phosphorylated peptide).

Table S1 Detailed informations of phosphopeptides enriched from digests of α-casein, β-casein and non-fat milk

No.	*m/z*	phosphorylation sites	Sequences
α1	1237.0	1	TVDMEpSTEVF
α2	1661.0	1	VPQLEIVPNpSAEER
α3	1927.2	2	DIGpSEpSTEDQAMEDIK
α4	1943.1	2	DIGpSEpSTEDQAoMEDIK
α5	1952.3	1	YKVPQLEIVPNpSAEER
α6	2618.1	4	NTMEHV**pSpSpS**EESIIpSQETYK
α7	2635.1	4	NToMEHV**pSpSpS**EESIIpSQETYK
α8	2677.3	3	VNELpSKDIGpSEpSTEDQAMEDIK
α9	2703.3	5	pyroQMEAEpSI**pSpSpS**EEIVPNpSVEQK
α10	2719.5	5	QMEAEpSI**pSpSpS**EEIVPNpSVEQK
α11	2735.3	5	QoMEAEpSI**pSpSpS**EEIVPNpSVEQK
α12	2935.0	3	EKVNELpSKDIGpSEpSTEDQAMEDIK
α13	3007.8	4	NANEEEYpSIG**pSpSpS**EESAEVATEEVK
α14	3087.7	5	NANEEEYpSIG**pSpSpS**EEpSAEVATEEVK
β1	2061.6	1	FQpSEEQQQTEDELQDK
β2	2555.9	1	FQpSEEQQQTEDELQDKIHPF
β3	2965.8	4	ELEELNVPGEIVEpSL**pSpSpS**EESITR
β4	3122.1	4	RELEELNVPGEIVEpSL**pSpSpS**EESITR