Electronic Supplementary Material (ESI) for Chemical Communications

Supplementary Information

Tracking chemical reactions on the surface of filamentous phage by mass spectrometry

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Supplementary Figure

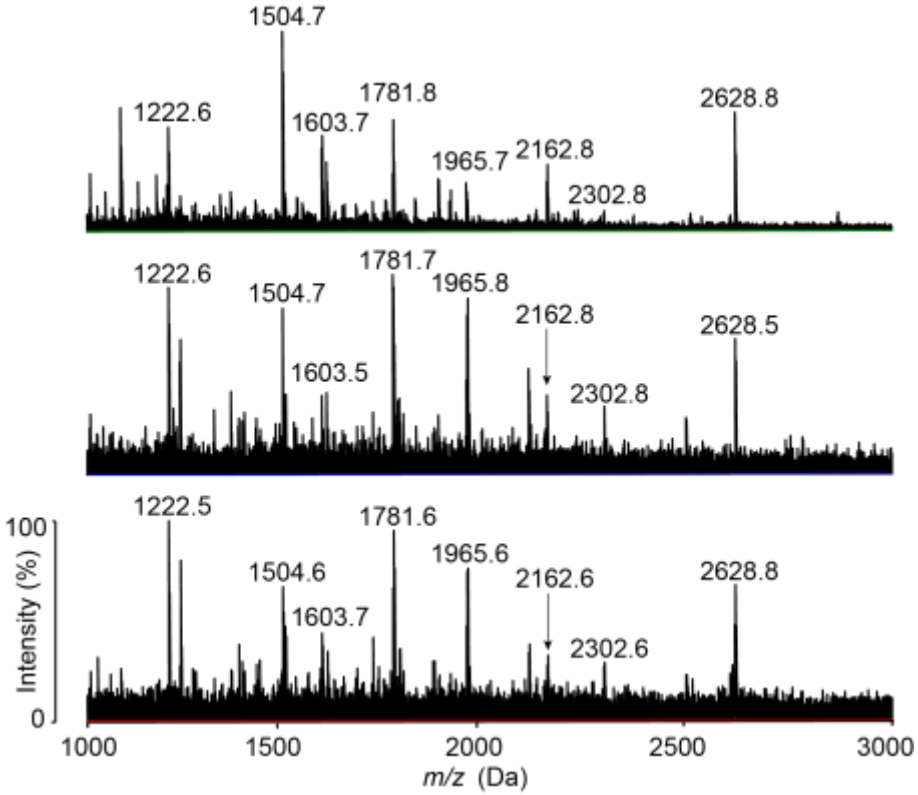

Figure S1. MALDI spectra of phage treated with trypsin. The three spectra were obtained with phage produced on different days. The masses of the peptides do not correspond to those expected for trypsinized M13 phage coat proteins but to *E. coli* proteins that were co-purified with phage.

Supplementary Table

Accession	Protein	Coverage	Unique peptides	Peptides
P04949	Flagellin	96.18	45	45
C4ZXE9	Glycine-tRNA ligase beta subunit	55.44	29	29
P24216	Flagellar hook-associated protein 2	62.39	15	15
P13035	Aerobic glycerol-3-phosphate dehydrogenase	52.3	18	18
P29744	Flagellar hook-associated protein 3	68.14	13	13
P0AEK2	3-oxoacyl-[acyl-carrier-protein] reductase FabG	57.38	12	12
P0A9M8	Phosphate acetyltransferase	32.35	16	16
P23843	Periplasmic oligopeptide-binding protein	34.25	14	14
P33235	Flagellar hook-associated protein 1	36.2	12	12
P09546	Bifunctional protein PutA	14.32	10	11

Table S1. Proteins co-purified with phage. Unmodified phage was trypsinized, analyzed by LC-MSMS and the results searched against a K12 *E.coli* database. The proteins with the ten highest searching scores are shown. The UniProtKB accession number, name of the protein, percent of protein covered by identified peptides, number of unique peptides and total number of peptides, are indicated.

Reagents and materials

2YT medium powder, chloramphenicol, ammonium bicarbonate, ethylenediaminetetraacetic acid (EDTA) and poly(ethylene glycol) average molecule weight 6000 (PEG6000) were purchased from AppliChem (Darmstadt, Germany). Sodium chloride was purchased from Carl Roth GmbH (Karlsruhe, Germany). tris(2-carboxyethyl)phosphine (TCEP), agar, iodoacetamide (IAA), 1,3,5-tris(bromomethyl)benzene (TBMB) and α-cyano-4-hydroxycinnamic acid (α-CHCA) were purchased from Aldrich (Buchs SG, Switzerland). Acetonitrile (HPLC grade) was purchased from Fisher Scientific UK (Leics, UK). MS grade trypsin (TRSEQZ) was purchased from Worthington Biochemical Corporation (Worthington, NJ, USA). Vivaspin 20 filter concentrator (100K MWCO PES) and Vivaspin 6 filter concentrator (30K MWCO PES) were purchased from GE Healthcare (Goettingen, Germany). Nanosep 10L Omega filters were purchased from PALL life science (Basel, Switzerland).

Apparatus

The bacteria culture was incubated in a Multitron II shaker from Blanclabo (Lonay, Switzerland). Samples were centrifuged in a Sorvall RC BIOS centrifuge equipped with a F8-6x1000y rotor or a Multifuge 3L-R centrifuge equipped with a Sorvall rotor from Thermo scientific (Geneva, Switzerland). Gel filtration was performed on a Bio-RAD BioLogic DuoFlow FPLC (Reinach BL, Switzerland) equipped with a Sephacryl S-500 column (HiPrep 16/60) from GE Healthcare (Glattbrugg, Switzerland). Masses were measured on an Axima-CFR plus MALDI-TOF mass spectrometer from Kratos Shimadzu Biotech (Manchester, UK).

Methods

Phage production

The phage displaying the peptide 'LCQLDCTWQCR' as fusion of pIII was previously isolated in phage selections from a library termed '3x3 library'.[1] This phage is based on the vector fdg3p0ss21[2] which was engineered to be deficient in three disulfide bridges domains D1 and D2 of pIII. 2 mL of an over-night culture of TG1 *E. coli* cells harboring the phage vector was used to inoculate 500 mL 2YT media containing chloramphenicol (30 μg/mL) in a 2 L shaking flask. The culture was shaken (220 rpm) at 30 °C for 16 hrs. Phage were purified by PEG-precipitation as described previously.[3, 4]

Purification of phage by size-exclusion chromatography

Phage were purified by size-exclusion chromatography using a Sephacryl S-500 column (HiPrep 16/60) and a Bio-RAD BioLogic DuoFlow FPLC system. Phage was run in buffer G as mobile phase (10 mM NH_4HCO_3 in mQ water; the pH was not adjusted and was measured to be 8.1) at 1 mL/min. The phage eluted as a broad single peak at around 45-50 mL. The absorption was monitored at 220, 260 and 280 nm.

Chemical modification of phage-displayed peptide

Phage in 10 mL buffer G was reduced with 1 mM TCEP at 42 °C for 1 hr. The concentration of TCEP was lowered as follows. Phage solution was centrifuged in Vivaspin 20 filter concentrators at 4 °C to reduce the volume to 1 mL (centrifugation at 4500 rpm in swing-out rotor; centrifugation can take more than 1 hr) and diluted with 3 mL of chilled buffer G. This step was repeated twice.

For the modification of phage peptide with the thiol-reactive linkers, the volume of the phage solution was adjusted to 8 mL with buffer G. The thiol-reactive linkers dissolved in ACN were added (2 mL) to achieve a final concentration of 20 μM TBMB, 150 μM TATA or 40 μM TBAB. The reaction tubes were inverted several times and incubated for 1 hr at 30 °C.

For the modification of phage peptide with IAA, the volume was adjusted to 5 mL with buffer G. IAA in 20 μL mQ water was added to achieve a final concentration of 2 mM IAA.

Mass spectrometric analysis of phage-displayed peptide (protocol A)

Modified phage (10 mL) was precipitated with 2.5 mL PEG solution (20% PEG 6000, 2.5 M NaCl) in a conical 15 mL falcon tube, centrifuged at 4500 rpm at 4 °C for 30 minutes. The phage pellet was resuspended in 0.5 mL buffer R (20 mM NH_4HCO_3, 5 mM EDTA, pH 8.0). The modified phage was further purified by gel filtration as described above. Eluted phage was concentrated in a Vivaspin 20 filter concentrator to 0.5 mL and diluted with 5 ml mQ water to decrease the salt concentration. This step was repeated once and the phage solution concentrated then to 0.5 mL. 5 μL trypsin (1 mg/mL) was then added to the phage in 0.5 mL and incubated at 37 °C for 24 hrs. The phage was removed by filtration of the reaction solution in a Nanosep 10L Omega filter. The flow-through with the peptide was lyophilized and resuspended in mQ water (10 μL). The peptide (0.5 μL) was mixed with α-CHCA matrix (0.5 μL saturated solution in 50% v/v acetonitrile and 50% v/v water with 0.1% v/v TFA) and loaded onto a carrier plate. The molecular masses of the peptides were determined with a MALDI-TOF mass spectrometer

Mass spectrometric analysis of phage-displayed peptide (protocol B)

The PEG-purified phage (0.5 mL) was purified by size-exclusion chromatography, reduced with TCEP and chemically modified as described above. The concentration of the alkylating reagent was reduced as follows. The phage solution was centrifuged in a Vivaspin 6 filter concentrator at 4 °C (4500 rpm in a swing-our rotor, around 30 minutes) to reduce the volume to 0.5 mL and 4 mL chilled buffer G was added to the phage. This step was repeated once. The phage solution was again centrifuged in the same Vivaspin 6 filter concentrator at 4 °C to reduce the volume to 0.5 mL and 4 mL chilled mQ water was added to the phage. This step was repeated also once. Finally, the phage solution was concentrated again in the same filter to 0.5 mL. The phage (0.5 mL) was treated with trypsin (5 μL at 1 mg/mL) at 37 °C for 24 hrs. The tryptic reaction (0.5 μL) was mixed directly with α-CHCA matrix (0.5 μL) and loaded onto a carrier plate. The molecular mass of the linear peptide was determined with a MALDI-TOF mass spectrometer.

Determination of phage infectivity

Phage (0.5 mL) in buffer G was incubated with or without trypsin (5 μL at 1 mg/mL) at 37 °C for 24 hrs. 12 serial dilutions (10-fold) of the phage were prepared using 2YT medium. 20 μL of each dilution were added to exponentially growing TG1 cells (180 μL, OD_{600} = 0.4) and incubated for 90 minutes at 37 °C. 10 μL of the infected TG1 cells were spot onto 2YT agar plates containing 30 μg/mL chloramphenicol. The number of colonies was counted the next day and the number of infective phage was calculated.

Liquid chromatography-tandem mass analysis and protein identification

The unmodified phage purified by gel filtration purified was trypsinized as described above. The digested phage solution was desalted through C18 Spin Tips (Pierce, Geneva, Switzerland) as described by the manufacturer and consecutively eluted with 10×20 μL of 60% acetonitrile. Eluted fractions were pooled, dried under vacuum, and dissolved in mQ water for further characterization.

Samples were analysed on a hybrid linear trap LTQ-Orbitrap XL mass spectrometer (Thermo Scientific, Bremen, Germany) interfaced with a nanoUPLC Ultimate 3000 system (Dionex). The peptide sample was run on a C18 reversed-phase nanocolumn with a gradient from 5 to 85% acetonitrile in water and 0.1% formic acid: 5 min at 0% of solvent B, from 0 to 25% of B in 35 min, 25 to 50% B in 15 min, 50 to 90% in 5 min, 90% B during 10 min, 90 to 0% in 5 min and 15 min at 0% (total time: 90 min). Acquired MS/MS spectra were analysed by Proteome Discoverer Software 1.4 (Fisher Scientific, Switzerland).

References

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