Supplementary Material

Substrate specificity and screening of the integral membrane protease Pla

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Bacterial strains and plasmids. A non-pigmented avirulent strain of *Y. pestis* KIM, substrain D28, expressing Pla and its pPCP-cured version, substrain D47, were grown on Heart Infusion (Difco, Detroit, MI) agar plates for 36 hours at 26ºC. *E. coli* strain BL21(DE3) (Invitrogen, Carlsbad, CA) was used as a host to express recombinant Pla. A 2.4-kb SmaI-EcoRI fragment of pPCP containing the *pla* gene with its own promoter and surrounding regions was cloned in pBluescript SK+ (Stratagene, LaJolla, CA) to produce a plasmid pBLA4L.

Preparation of recombinant Pla. A mature Pla containing histidine-tag on N-terminus was purified from inclusion bodies using Bug Buster (EDM Chemicals, Inc. San Diego, CA) followed by a single step of purification on Ni-NTA resin (Qiagen, Valencia, CA) in denaturing conditions as recommended by manufacturer. To obtain the active enzyme, denatured Pla was refolded as described previously for the refolding of OmpT.[1] The refolded Pla was activated by incubation in the presence of 100 μM of rough lipopolysaccharide (LPS) of *E.coli* (Sigma, St.Lpois, MO).

Peptide synthesis. PEGA, Fmoc-Gly-Wang, Fmoc-Ala-Wang, Sieber Amide and EDANS NovaTag resins were from NovaBiochem. PEGA 1900 resin was from Versamatrix. Fmoc-Glu(EDANS)-OH was from NovaBiochem. Fmoc-amino acids, HBTU, HOBt, DIEA, NMP were from AdvancedChemTech. HATU was from Oakwood Products, Inc. DBU, piperidine, TFA were from Aldrich. DMF and TIS were from Acros. EDT was from Fluka. Dabcyl-OH was from AnaSpec. A Model 90 Peptide Synthesizer by Aapptec was used to prepare properly substituted resins for library synthesis. Library synthesis was performed in a 96-well reactor of a Solution Parallel Synthesizer from Aapptec. LC/MS analysis was performed on a Surveyor system from ThermoFinnigan. Preparative HPLC

was done using a Gilson system equipped with 321 Pump, UV/Vis 155 Detector, FC 204 Fraction Collector and a Vydac 218TP1010 C18 Proteins & Peptides column.

The peptide coupling steps were done using 5 eq of Fmoc-amino acid or Fmoc-amino acid isokinetic mixture, HBTU, HOBt and 10 eq of DIEA in NMP for 1h. The attachment of first amino acid to NovaTag resin was done by treating the resin twice with 5 eq of Fmoc-amino acid, HATU, HOBt and 10 eq of DIEA in NMP for 30 min. Fmoc-deprotection was done by treating a resin twice with a DBU/piperidine/DMF (2:2:96) mixture for 5 min. For the cleavage from Sieber resin 1% TFA in DCM was used; for the cleavage from the other resins TFA/H_2O/EDT/TIS 94.0:2.5:2.5:1.0 was used.

To introduce a randomized position into a peptide an isokinetic mixture of amino acids was used, Fmoc-amino acid, mol %: Fmoc-Ala-OH, 3.58; Fmoc-Asp(OtBu)-OH, 3.70; Fmoc-Glu(OtBu)-OH, 3.84; Fmoc-Phe-OH, 2.66; Fmoc-Gly-OH, 3.04; Fmoc-His(Trt)-OH, 3.76; Fmoc-Ile-OH, 18.30; Fmoc-Lys(Boc)-OH, 6.56; Fmoc-Leu-OH, 5.23; Fmoc-Met-OH, 2.42; Fmoc-Asn(Trt)-OH, 5.64; Fmoc-Pro-OH, 4.56; Fmoc-Gln(Trt)-OH, 5.61; Fmoc-Arg(Pbf)-OH, 6.87; Fmoc-Ser(tBu)-OH, 2.93; Fmoc-Thr(tBu)-OH, 5.04; Fmoc-Val-OH, 11.90; Fmoc-Tyr(tBu)-OH, 4.36.

Fluorimetric assay. The kinetics of the hydrolysis of the substrates labeled with the fluorophore *[5 - ((2 - Aminoethyl)amino)naphthalene - 1 - sulfonic acid]* (EDANS) and the quencher 4-(dimethylaminoazo)benzene-4-carboxylic acid (Dabcyl) was determined by mixing 5 μg of recombinant Pla (or 5 x 10^6 bacterial cells expressing Pla) with 35 μM of the substrate in 100 mL reaction mixture in 10 mM Tris-HCl pH 8.0/150 mM NaCl in a microtiter plate. Fluorescence was detected using plate reader (Synergy HY, Bio-Tek Instrument Inc., Vermont) at excitation and emission wavelengths of 360 and 460 nm, respectively.

Fibrinolysin assay. A fibrin film lysis assay for plasminogen activator activity was performed as described by Beesley et.al.[2] Briefly, 10 mL of bovine fibrinogen (Sigma, St. Louis, Miss.) in borate buffer at a concentration of 2.5 mg/mL was clotted in a Petri dish with the addition of 50 U of bovine thrombin (Sigma). The activity was tested by spotting 4-μL samples onto film, followed by incubation at 26ºC or 37ºC for up to 24 hours and checking the samples periodically for the cleared areas.

1. Kramer, R. A., Zandwijken, D., Egmond, M. R. & Dekker, N. **2000**, *Eur. J. Biochem. 267*, 885-893.
2. Beesley, E. D., Brubaker, R. R., Janssen, W. A. & Surgalla, M. J. **1967**, *J. Bacteriol. 94*, 19-26.