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Chemical rescue of protein tyrosine phosphatase activity

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Supplementary Information:

Supplementary Figure 1:

Alignment of Cysteine-Enriched WPD-Loop PEST Mutants

Wild-type:	W	P	D	H	D	V	P	-	-	S	S	F
P198C:	W	C	D	H	D	V	P	-	-	S	S	F
D201C:	W	P	D	H	C	V	P	-	-	S	S	F
P203C:	W	P	D	H	D	V	C	-	-	S	S	F
S204C:	W	P	D	H	D	V	P	-	-	C	S	F
P198C/S204C:	W	C	D	H	D	V	P	-	-	C	S	F
CC-204:	W	P	D	H	D	V	P	C	C	S	S	F

Material and Methods

FlAsH Synthesis

FlAsH was synthesized as previously described. [1-3]

Cloning

A fragment encoding the catalytic domain of PTP-PEST (residues 1–307) was amplified and cloned into pET21b (resulting plasmid: pERB041) as described previously.[4]

Insertional Mutagenesis

PEST-CC-204: A plasmid encoding wild-type PEST (pERB041, ~50 ng), cloned *Pfu* 10× reaction buffer (5 μL), cloned *Pfu* DNA polymerase (1 μL, 2.5 U, Stratagene), 2 mM dNTP mix (5 μL), and water (36 μL) were combined with appropriate primers (1 μL of each at a concentration of 300 ng/μL), and placed in temperature cycler. The reaction mixture was subjected to 18 cycles of 95 ºC for 30 seconds, 55 ºC for 1 minute, and 68 ºC for 16 minutes. Sequences (5′ to 3′) of mutagenic primers are as follows: GACCATGATGTTCCTTGCTGCTCATCATTTGATTCTAT and ATAGAATCAAATGATGAGCAGCAAGGAACATCATGGTC. Following temperature cycling, 10 U (1 μL) of *Dpn* I restriction enzyme were added, and the reaction mixture was incubated at 37 ºC for 1 hour, ethanol-precipitated, and re-dissolved in 3 μL of water. The resulting DNA solution was used to transform DH5α competent cells. Plasmid DNA from an ampicillin-resistant colony was sequenced (Cornell Biotechnology Resource Center) over the PEST coding region to confirm the presence of the CC-204-encoding insertion (plasmid: pVLC001).

Protein Expression and Purification

BL21(DE3)-codonPLUS-RIL competent *E. coli* cells (Stratagene) were transformed with pERB041 (wild-type PEST) or pVLC001 (PEST-CC-204). Single colonies were picked and used to inoculate 500 mL LB cultures, which were grown to mid-log phase at 37 °C, and induced with 0.2 mM IPTG for 16 hours at 26 °C. Cells were pelleted and frozen at -80 °C. Cell lysis was achieved by incubation of the cell pellets with 10 mL of BPER (Pierce), and purifications of enzymes were carried out using

SwellGel Nickel Chelated Discs (Pierce) according to the manufacturer's instructions. The protein solutions obtained were concentrated with CentriPrep Centrifugal Filter Devices (Millipore) and exchanged into pH 7.0 buffer containing 50 mM 3,3-dimethylglutarate, 1 mM EDTA, 150 mM NaCl, and 1 mM dithiothreitol. The concentrated protein solutions were flash-frozen in liquid nitrogen and stored at -80 °C. Protein concentrations were determined by Bradford assay, and enzyme purities were estimated by SDS-PAGE.

PEST Activity Assays: General Conditions

PTP activity assays (see specific variations below and in Figure legends) were carried out at 22 °C in a total reaction volume of 200 μL containing *p*NPP and the appropriate PEST enzyme in 1×PTP buffer (50 mM 3,3-dimethylglutarate at pH 7.0, 1 mM EDTA, and 50 mM NaCl). Reactions were quenched by the addition of 40 μL of 5 M NaOH. The reaction mixtures (200 μL) were loaded onto a 96-well plate, and the absorbances at 405 nm were measured. Kinetic constants were determined by fitting the resulting data to the Michaelis-Menten equation using SigmaPlot 10.0.

PEST Activity Assays: Fixed FlAsH Concentration

To measure the effect of FlAsH on PEST activity, solutions of PEST (2.5 μM) in 1×PTP buffer were incubated in the absence (DMSO vehicle only) or presence of FlAsH (10 μM). After 2.5 hours at room temperature, the solution was diluted and assayed for PTP activity as described above (final concentration of PEST in the PTP assay: 500 nM).

PEST Activity Assays: Dose-Dependence of FlAsH-Induced Activation

FlAsH-concentration-dependence assays were carried out at 4 mM *p*NPP, 125 nM PEST-CC-204, and varying concentrations of FlAsH in 1×PTP buffer. After a 2.5 hour incubation of enzyme and FlAsH (or vehicle control), *p*NPP was added. The reactions were quenched and quantified as described above.

PEST Activity Assays: Time-Dependence of FlAsH-Induced Activation

The time course of PEST-CC-204 activation was determined as described above under "*PEST Activity Assays: Fixed FlAsH Concentration*" with the exceptions that aliquots were removed at various times during the pre-incubation and that PTP activity was measured under conditions approaching maximum PTP velocity (16 mM *p*NPP).

PEST Activity Assays: Crude Cell Lysates

Induction of wild-type PEST and PEST-CC-204 expression in BL21(DE3)-codonPLUS-RIL cells was carried out as described above. The resulting cultures were centrifuged at 4580 *g* for 20 minutes. The supernatants were discarded and the cell pellets were resuspended in PTP assay buffer (50 mM 3,3-dimethylglutarate pH 7.0, 50 mM NaCl, 1 mM EDTA). Cell suspensions were lysed with a Virsonic 475 Ultrasonic Cell Disrupter sonicator (The VirTis Company) using a 1/8″ microtip. Aliquots of 1500 μL resuspended cells were sonicated for 20 seconds, then chilled on ice for 30 seconds, then sonicated again for 20 seconds. All sonication was performed at between 8-10% maximal sonication. Lysates were clarified by centrifugation for 5 min at 13,400 rpm (16,000 g) at 4°C. The resulting pellet was discarded and the supernatant was analyzed for protein

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concentration (Bradford) and PTP activity as described above. 40 μL of clarified lysate was used for activity assays.

Fluorescence

Solutions containing either an absence or presence of PEST-CC-204 (125 nM) in the presence of FlAsH (1.25 μM) were incubated in 1×PTP buffer (50 mM 3,3-dimethylglutarate at pH 7.0, 1 mM EDTA, and 50 mM NaCl). After 2.5 hours, the FlAsH fluorescence values (excitation: 510 nm, emission: 540 nm) were measured on a Spectra Max M5 96-well fluorescence plate reader (Molecular Devices). The resulting data were normalized to the FlAsH-only controls. Error bars represent the standard deviations from three independent measurements.

References

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