

Supplementary material

Structural characterization of murine phosphodiesterase 5 isoforms and involvement of cysteine residues in supramolecular assembly.

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Supplementary Figure S1. Sequence coverage of MmPDE5A3 by MS after trypsin digestion. Residues in red are those covered by MS analysis. Cys residues were protected by iodoacetamide (IAM).

Supplementary Figure S2. Sequence coverage of MmPDE5A3 reacted with N-ethylmaleimide (NEM) by MS. Residues in red are those covered by MS analysis of tryptic peptides.

Supplementary Figure S3. Zoom of MS spectrum of the peptide 315-330 in MmPDE5A3 control and treated with NEM.

Supplementary Table S1. Localization of Cys residues mapped by MS on the homologous structures deposited in the PDB.

Supplementary Figure S1.

Sequence coverage of MmPDE5A3 by MS after trypsin digestion. Residues in red are those covered by MS analysis. The 25 Cys residues (yellow, bold, underlined) were protected by iodoacetamide (IAM). All the Cys, excepted 625 and 637, were visible. Cys 447, 522, 544 were oxidised to sulfenic acid.

MmPDE5A3 control

MVNAWFSE	RV	HNIPV	C	KEGI	RAHTES	C	S	C	LQQSPHADNT	TPGAPARKIS	ASEFDRPLRP	60
IVVKDSE	GT	SFLSDSGK	E	KE	QMLPTPPRF	D	S	D	EGDQ	C	SRL	120
FLHIHGLISA		DRYSLFLV	C	E	DSSKDKFLIS				RLFDVAEGST	LEEASNN	C	180
VAAFGEPLNI		KDAYEDPRFN			AEVDQITGYK				TQSIL	C	MPIK	240
GTFTEKDEKD		FAAYLAF	C	G	VLHNAQLYET				SLENKRNQV	LLDLASLIFE	E	300
KIAATIISFM		QVQK	C	TIFIV	DED	C	PDSFSR		VFHME	C	EEVG	360
QYVKN	TE	MEPL			FPWTNENMGH				VNTP	C	IGSLL	420
KMEENTGKIK		AFNQND	E	QFL	EAFVIF	C	GLG		IQNTQMYEAV	ERAMAKQMT		480
AEETRELQA		LSAAV	V	PSAQ	TLKITDFSFS				DFELSDLETA	L	C	540
EVL	C	RWILSV			KKNYRK	N	VAY		HNWRHAFNTA	Q	C	600
HDLDHRGVNN		SYIQRSEHPL			AQLY	C	HSIME		HHHFDQ	C	CLMI	660
LKIIKQAILA		TDLALYIKRR			GEFFELIRKN				QFSFEDPLQK	ELFLAMLMTA	C	720
PIQQRIAE	L	V			AAEFFDQ	G	DR		ERKELNMEPA	DLMNREKKNK		780
THVSED	C	CLPL			LNG	C	RKNRQK		WQALAEQQEK	MLLNGESSQG	KRD	823

Supplementary Figure S2.

Sequence coverage of MmPDE5A3 reacted with N-ethyl-maleimide (NEM) by MS. Residues in red are those covered by MS analysis of tryptic peptides.

MmPDE5A3 + NEM

MVNAWFSE	RV	HNIPV	C	KEGI	RAHTES	C	S	C	LQQSPHADNT	TPGAPARKIS	ASEFDRPLRP	60
IVVKDSE	GT	SFLSDSGK	E	KE	QMLPTPPRF	D	S	D	EGDQ	C	SRL	120
FLHIHGLISA		DRYSLFLV	C	E	DSSKDKFLIS				RLFDVAEGST	LEEASNN	C	180
VAAFGEPLNI		KDAYEDPRFN			AEVDQITGYK				TQSIL	C	MPIK	240
GTFTEKDEKD		FAAYLAF	C	G	VLHNAQLYET				SLENKRNQV	LLDLASLIFE	E	300
KIAATIISFM		QVQK	C	TIFIV	DED	C	PDSFSR		VFHME	C	EEVG	360
QYVKN	TE	MEPL			FPWTNENMGH				VNTP	C	IGSLL	420
KMEENTGKIK		AFNQND	E	QFL	EAFVIF	C	GLG		IQNTQMYEAV	ERAMAKQMT		480
AEETRELQA		LSAAV	V	PSAQ	TLKITDFSFS				DFELSDLETA	L	C	540
EVL	C	RWILSV			KKNYRK	N	VAY		HNWRHAFNTA	Q	C	600
HDLDHRGVNN		SYIQRSEHPL			AQLY	C	HSIME		HHHFDQ	C	CLMI	660
LKIIKQAILA		TDLALYIKRR			GEFFELIRKN				QFSFEDPLQK	ELFLAMLMTA	C	720
PIQQRIAE	L	V			AAEFFDQ	G	DR		ERKELNMEPA	DLMNREKKNK		780
THVSED	C	CLPL			LNG	C	RKNRQK		WQALAEQQEK	MLLNGESSQG	KRD	823

The long peptide (43 aa) containing Cys 625 and 637 was not visible neither in the control nor in the NEM-modified MmPDE5A3. Peptides with Cys highlighted in grey were not visible in the sample reacted with NEM.

Cys highlighted in cyan were not modified by NEM.

Cys highlighted in green are totally modified by NEM; Cys highlighted in yellow are partially modified by NEM; Cys highlighted in black were oxidized to sulfenic acid prior to react with NEM.

Table S1. Localization of Cys residues mapped by MS on the homologous structures deposited in the PDB. Localization of Cys within the regulatory (GAF-A and GAF-B) and catalytic (CAT) domains, based on the primary structure alignment of MmPDE5A3 (1-823) with the PDB deposited structures of MmPDE5A1 GAF-A (aa 154-302; PDB ID: 2k31 [1]), HsGAF-B (aa 346-508; PDB ID: 2xss [2]), HsCAT (aa 534-858; PDB ID: 5jo3) and HsPDE2, a cAMP phosphodiesterase (194-728, PDB ID: 3ibj [3]). NEM unequivocally modifies 3 Cys, one per domain, namely 258, 315, 711; it also modifies other 4 Cys, namely 117 on GAF-A, 447 on GAF-B, 522 and 544 in CAT. It is worth noticing that the last three Cys were already oxidised in the freshly purified sample prior to reaction with NEM, indicating a high reactivity despite their putative buried position (according to the homology structures).

Color code in Figure S2	Cys numbering according to MmPDE5A3	Position the homology structures
Cyan	16, 27, 29, 97	Possibly in GAF-A, not covered by any structure
Black	117	Possibly in GAF-A, Solvent exposed
Green	258	GAF-A, Solvent exposed
Yellow	139	GAF-A, Solvent exposed
Yellow	168	GAF-A, Solvent exposed
Grey	216	GAF-A, buried in a hydrophobic cavity
Green	315	GAF-B, buried
Cyan	324	GAF-B, solvent exposed
Yellow	336	GAF-B, solvent exposed
Grey	395	GAF-B, solvent exposed
Grey	401	GAF-B, buried
Grey	416	GAF-B, buried
Black	447 (sulfenic acid)	GAF-B, buried (same topological position as C216)
Black	522 (sulfenic acid)	CAT, buried (Ala in HsPDE2)
Black	544 (sulfenic acid)	CAT, buried (Ala in HsPDE2)
Grey	572	CAT, water filled cavity (Phe in HsPDE2)
invisible	625	CAT, solvent exposed (Asn in HsPDE5 and Arg in HsPDE2)
invisible	637	CAT, solvent exposed (Ala in HsPDE2)
Green	711	CAT, buried behind the catalytic Zn ²⁺
Grey	773	CAT, buried (Ala in HsPDE2)
Grey	787	CAT, buried (Ala in HsPDE2)
Grey	794	CAT, buried (Val in HsPDE2)

References:

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3. Pandit J, Forman MD, Fennell KF, Dillman KS, Menniti FS. Mechanism for the allosteric regulation of phosphodiesterase 2A deduced from the X-ray structure of a near full-length construct. *Proc Natl Acad Sci USA.* 2009; **106**(43): 18225-18230. DOI: 10.1073/pnas.0907635106.

Supplementary Figure S3. Zoom of MS spectrum which compares the peptide 315-330 between the control (top panel), where all the Cys are totally alkylated by IAM, and the NEM treated MmpPDE5A3 (bottom panel), where Cys315 is alkylated by NEM and Cys324 is alkylated by IAM. The confirmation is derived from MSMS analysis.

