An isozyme-specific redox switch in human brain glycogen phosphorylase modulates its allosteric activation by AMP

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Running title: Redox regulation of the human brain glycogen phosphorylase

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Supplementary Information

Supplementary Figure 1. Determination of the redox state of bGP cysteine residues by differential labeling and mass spectrometry

(a) Oxidized and reduced bGP were reduced by DTT following labelling of free cysteine residues by IAA, and then labelled using NEM. Proteins were then subjected to SDS-PAGE prior to extraction and trypsin digestion. Peptides were then separated on a capillary reverse phase column. Data for Cys318 only are represented.

Fragmentation of Cys318 and Cys326 labeled by IAA and NEM

Peptides containing Cys318 and Cys326 were separated on a capillary reverse phase column prior to MS/MS analysis. Mass spectrum corresponding to peptides containing Cys318 (b and c) and Cys326 (d and e) labeled by IAA and NEM respectively are presented.

(b) MS/MS spectra of the MH^{2+} (m/z 503.75) CAM-alkylated peptide FGCRDPVR

(c) MS/MS spectra of the MH^{2+} (m/z 537.76) NEM-alkylated peptide FGCRDPVR

(d) MS/MS spectra of the MH^{2+} (m/z 572.75) CAM-alkylated peptide TCFETFPDK

(e) MS/MS spectra of the MH^{2+} (m/z 572.75) NEM-alkylated peptide TCFETFPDK

Supplementary Figure 2. Structural model of Cys318-Cys326 disulfide bond

Ribbon representation of C_α trace of the dimer of bGP. The AMP-binding site is marked by the allosteric effector AMP (surface representation) and is located at the dimer interface (left panel). AMP-binding site is composed of the Cap loop from one subunit and the helices 2 and 8 as well as the adenine loop from the other subunit. Under oxidative conditions, Cys318 and Cys326, which belong to the adenine loop, are oxidized to form a disulphide bond that avoid the AMP-dependent activation of bGP.

Supplementary Figure 3. Cα-RMSF of loop residues for the wild-type and the structure with disulfide bond*

*Calculation was made for each MD replicate. Upper panel: monomer A, lower panel: monomer B.

Supplementary table 1. Distance separating the alpha carbons of each pair of Cysteine residues

$C\alpha_1/C\alpha_2$ (Å)	109	143	318	326	373	436	445	496	581	757	784	808
109		24,14	48,59	48,88	42,41	59,81	46,22	16,91	43,26	51,16	46,05	33,74
143	24,14		37,33	34,35	21	39,02	23,36	14,08	31,4	40,32	32,53	19,45
318	48,59	37,33		**7,86**	24,67	48,76	32,08	49,45	66,59	71,85	67,15	50,38
326	48,88	34,35	7,86		18,57	41,11	25,17	47,49	61,71	66,23	62,8	47,83
373	42,41	21	24,67	18,57		28,97	8,41	35,07	44,35	50	44,94	32,9
436	59,81	39,02	48,76	41,11	28,97		21,17	49,5	41,82	38,79	48,06	49,42
445	46,22	23,36	32,08	25,17	**8,41**	21,17		36,71	40,24	44,5	41,88	34,1
496	16,91	14,08	49,45	47,49	35,07	49,5	36,71		28,04	38,69	29,37	19,5
581	43,26	31,4	66,59	61,71	44,35	41,82	40,24	28,04		15,71	15,08	30,25
757	51,16	40,32	71,85	66,23	50	38,79	44,5	38,69	15,71		30,12	44,47
784	46,05	32,53	67,15	62,8	44,94	48,06	41,88	29,37	15,08	30,12		22,17
808	33,74	19,45	50,38	47,83	32,9	49,42	34,1	19,5	30,25	44,47	22,17	

The distance between Cα from each pair of cysteine was measured using Chimera Software and reported. Distances are expressed in Å. The pairs of cysteine residues with a Cα distance inferior to 10 Å were considered as close enough to be potentially involved in an intramolecular disulfide bond.

Supplementary table 2. Identification of oxidized cysteines by differential labeling followed by mass spectrometry

Cysteine ID	Residues Start-End	Peptide Sequence	m/z NEM	m/z CAM	Oxidized by H_2O_2
109	95 - 139	TLQNTMVNLGLQNA**C**DEAIYQLGLDLEELEEIEEDAGLGNGGLGR	NI	1223,59	nd
143	120 - 161	LAA**C**FLDSMATLGLAAYGYGIR	1201,6	778,725	YES
318	317 - 324	FG**C**RDPVR	NI	503,748	YES
326	325 - 333	T**C**FETFPDK	606,765	572,753	YES
373	371 - 387	KT**C**AYTNHTVLPEALER	691,013	1002	YES
436	428 - 439	RMSVIEEGD**C**K	696,317	662,305	YES
445	**439 - 458**	**RINMAHL<u>C</u>VIGSHAVNGVAR**	**561,547**	**544,54**	**NO**
496	-	-	NI	NI	nd
581	577-590	QLLN**C**LHVVTLYNR	905,986	871,972	YES
757	741 - 754	QAVDQISSGFFSPKEPD**C**FK	1178,05	763,028	YES
784	774 - 796	VFADYEAYMQ**C**QAQVDQLYRPK	969,113	946,438	YES
808	805 - 823	NIA**C**SGKFSSDR	561,774	544,765	nd

For each labeled peptide, m/z allowed the distinction between reduced (NEM) and oxidized (CAM) state (column m/z NEM for NEM labeling ; column "m/z CAM" for CAM labeling). Data were analyzed using Maxquant software. Relative quantification of NEM and CAM modified peptide are represented by NEM/CAM ratio in both reduced and oxidized condition. Decrease of NEM/CAM ratio between reduced and oxidized condition was considered as representative of the oxidation of the cysteine after treatment by H_2O_2.

Supplementary table 3. Contacts with AMP

Threshold (Å)	3		4		4.5		5		5.5		6	
	SS	No SS	SS	No SS	SS	No SS	SS	No SS	SS	No SS	SS	No SS
Average* Number of protein residues in contact with AMP												
	21	21	27	26	32	30	38	34	45	41	53	51
Average* Frequency of AMP-Loop residues contacts												
1-contact	1048	2348	1748	3580	1977	4014	2495	4279	3422	4084	4105	2972
2-contacts	174	121	268	369	554	785	864	1624	1143	2859	1636	4040
3-contacts	3	13	24	101	43	192	195	341	433	730	817	1596
4-contacts	0	0	0	3	0	19	0	72	1	164	15	338

* Average was calculated over the two monomers and the two MD replicates. The conformations were stored every 1ps, which corresponds to100000 frames for 100ns.

Upper part: Average number of protein residues having at least one atom below threshold distances from AMP simultaneously.

Lower part: Frequency of contacts made by loop residues (residues 314 to 334) calculated over the whole simulation (10000 frames). 1-contact means only one residue of the loop is contacting simultaneously, 2-contact means two residues of the loop are contacting AMP simultaneously, etc. For example for a 6 A threshold, two residues of the loops are contacting AMP molecule simultaneously roughly half of the time (4040 over 10000 frames)in the wild-type structure.

Supplementary Table 4. Oligonucleotides used for site-directed mutagenesis

Mutation	5' primer	3' primer
Cys 318 Ser	TCCAAGTTCGGC a GCCGGGACCCTGTGAGAA	GGGTCCCGGC t GCCGAACTTGGACGACTTG
Cys 326 Ser	CCTGTGAGAACC a GTTTCGAGACGTTCCCAGACA	ACGTCTCGAAAC t GGTTCTCACAGGGTCCCG

The cysteine residues were mutated into serine residues using primers containing the single point mutation to make. The lowercase base correspond to the mutated base.