Identification of Oxidative Modifications of Hemopexin and Their Predicted Physiological Relevance

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Supplemental Material

Data Availability

Refer to web versions in Pub Med Central for supplementary material. The MS proteomics data have been deposited in the Mass Spectrometry Interactive Virtual Environment (MassIVE) with a data set identifier (ftp://MSV000080394@massive.ucsd.edu). MassIVE is a community resource that was developed by an NIH-funded Center for Computational Mass Spectrometry at the University of California, San Diego, to promote the global and free exchange of MS data. The RAW data files and the MASCOT search file names for the MS/MS SRM analyses of peptides derived from human, rabbit and rat hemopexin deposited in MassIVE are summarized in Supplementary Table I.

Supplemental Table I

Table or Figure	Species of Hemopexin/ǂstate		Deposited .RAW and .DAT MASCOT search files	scan#	Peptide
Table I	Human	native	HuHPXTIF1FS3Yn199	2173	^{198}YY^{n}CFQGNQFLR^{208}
	Rabbit	native	RbtHPXTIFS1Yn201	3037	^{200}YY^{n}CFQGNQFLR^{210}
	Rat	native	RatHPXTIFS2Yn118	711	^{117}YY^{n}CFQGNK^{204}
Table II	Rabbit	native	RbtHPXTIIYn	2592	^{118}VWVY^{n}TSEK^{125}
				2715	^{228}DY^{n}FLSCPGR^{236}
				2720	^{316}LY^{n}LIQDTK^{323}
				2837	^{324}VY^{n}VFLTK^{330}
Table III	Rabbit	HOCl 1:10	RbtHPXTIIITIVHOCl_c	975	^{118}VW^{ox}VYTSEK^{125}
		HOCl 1:10	RbtHPXTIIITIVHOCl_c	3297	^{211}FNPVSGEVPPGY^{Cl}PLDVR^{227}

		HOCl 1:10	RbtHPXTIIITIVHOCl_c	1770	^{331}GGYTLVNGY^{Cl}PK^{341}
		tBuOOH 1:2.5	RbtHPXTIIITIVtBu_b	2685	^{200}YY^{tBu}CFQGNQFLR^{210}
		tBuOOH 1:1	RbtHPXTIIITIVtBu_a	1766	^{228}DY^{tBu}FLSCPGR^{236}
Table IV	Rabbit	HOCl 1:10	RbtHPXTIIITIVHOCl1_c	3392	^{211}FNPVSGEVPPGY$^{di\text{-}Cl}$PLDVR^{227}
		HOCl 1:10	RbtHPX TIIITIVHOCl1_c	1489	^{228}DY^{Cl}FLSCPGR^{236}
		HOCl 1:10	RbtHPX TIIITIVHOCl1_c	1682	^{228}DY$^{di\text{-}Cl}$FLSCPGR^{236}
		tBuOOH 1:1	RbtHPX TIIITIVtBu_a	3776	^{211}FNPVSGEVPPGY^{tBu}PLDVR^{227}
		tBuOOH 1:2.5	RbtHPX TIIITIVtBu_b	3543	^{211}FNPVSGEVPPGY^{tBu}PLDVR^{227}
		tBuOOH 1:10	RbtHPXTIVtBu_c	4021	^{211}FNPVSGEVPPGY^{tBu}PLDVR^{227}
Figure 1	Human	native	HuHPXTIF1FS3Yn199	2173	^{198}YY^{n}CFQGNQFLR^{208}
Figure 3	Human	N/MPO/GO t=0 a	HuHPXF3t0aYn119		^{198}YY^{n}CFQGNQFLR^{208}
		N/MPO/GO t=0 b	HuHPXF3t0bYn119		
		N/MPO/GO t=0 c	HuHPXF3t0cYn119		
		N/MPO/GO t=5min a	HuHPXF3t5aYn119		
		N/MPO/GO t=5min b	HuHPXF3t5bYn119		
		N/MPO/GO t=5min c	HuHPXF3t5cYn119		
		N/MPO/GO t=10min a	HuHPXF3t10aYn119		
		N/MPO/GO t=10min b	HuHPXF3t10bYn119		
		N/MPO/GO t=10min c	HuHPXF3t10cYn119		
		N/MPO/GO t=15min a	HuHPXF3t15aYn119		
		N/MPO/GO t=15min b	HuHPXF3t15bYn119		
		N/MPO/GO t=15min c	HuHPXF3t15cYn119		
		N/MPO/GO t=30min a	HuHPXF3t30aYn119		
		N/MPO/GO t=30min b	HuHPXF3t30bYn119		
		N/MPO/GO t=30min c	HuHPXF3t30cYn119		
		N/MPO/GO t=1h a	HuHPXF3t1haYn119		
		N/MPO/GO t=1h b	HuHPXF3t1hbYn119		
		N/MPO/GO t=1h c	HuHPXF3t1hcYn119		
		N/MPO/GO t=2h a	HuHPXF3t2haYn119		
		N/MPO/GO t=2h b	HuHPXF3t2hbYn119		
		N/MPO/GO t=2h c	HuHPXF3t2hcYn119		
Figure S1	Rabbit		RbtHPXTITIIFS1Yn201	3037	YY^{n}CFQGNQFLR
Figure S2	Rat		RatHPXTIFS2Y118t	711	YY^{n}CFQGNQFLR
Figure S3	Human		HuHPXTIF1FS3Yn199	2173	YY^{n}CFQGNQFLR
Figure S4	Human Std curve[a]	10µM a	HuHPXFS410a		
		10µM b	HuHPXFS410b		
		10µM c	HuHPXFS410c		
		20µM a	HuHPXFS420a		

20µM b	HuHPXFS420b
20µM c	HuHPXFS420c
30µM a	HuHPXFS430a
30µM b	HuHPXFS430b
30µM c	HuHPXFS430c
40µM a	HuHPXFS440a
40µM b	HuHPXFS440b
40µM c	HuHPXFS440c
50µM a	HuHPXFS450a
50µM b	HuHPXFS450b
50µM c	HuHPXFS450c
60µM a	HuHPXFS460a
60µM b	HuHPXFS460b
60µM c	HuHPXFS460c
70µM a	HuHPXFS470a
70µM b	HuHPXFS470b
70µM c	HuHPXFS470c
80µM a	HuHPXFS480a
80µM b	HuHPXFS480b
80µM c	HuHPXFS480c
90µM a	HuHPXFS490a
90µM b	HuHPXFS490b
90µM c	HuHPXFS490c

Supplemental Table I. File names for the MS/MS analyses of peptides derived from human, rabbit and rat hemopexin deposited via the MassIVE partner repository. [‡]Treatments: HOCl 1:10 (file code HOCl_c); tBuOOH 1:1, 1:2.5 or 1:10 (files codes tBu_a, _b and _c, respectively). [a]Celtek peptides for standard curve: each file name corresponds to data for each concentration of 1mM stock peptide (indicated) of native YYCFQGNQFLR and nitrated YY^{n}CFQGNQFLR peptides with 2 reference NFPSPVDAAFR peptide samples (carried out in triplicate: a,b,c).

SUPPLEMENTAL FIGURES

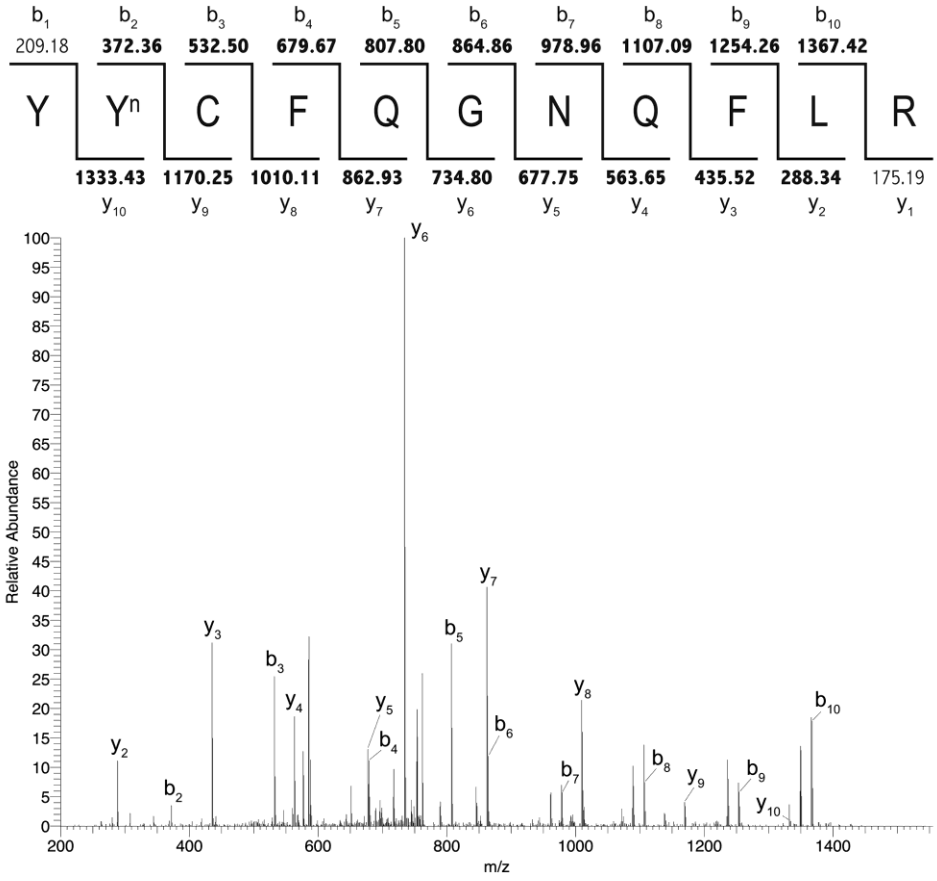

Figure S1. Nitration of rabbit hemopexin occurs *in vivo* at Y_{201}. Tyrosine nitration was detected by LC-MS/MS analysis of hemopexin isolated from rabbit plasma. Xcalibur BioWorks was used to identify Y^{n}_{201} on the tryptic peptide $YY^{n}CFQGNQFLR$ (see Table I for XCorr value). Matched b and y ions are shown in bold in the ions diagram (above) and are indicated next to corresponding peaks in the MS/MS spectrum.

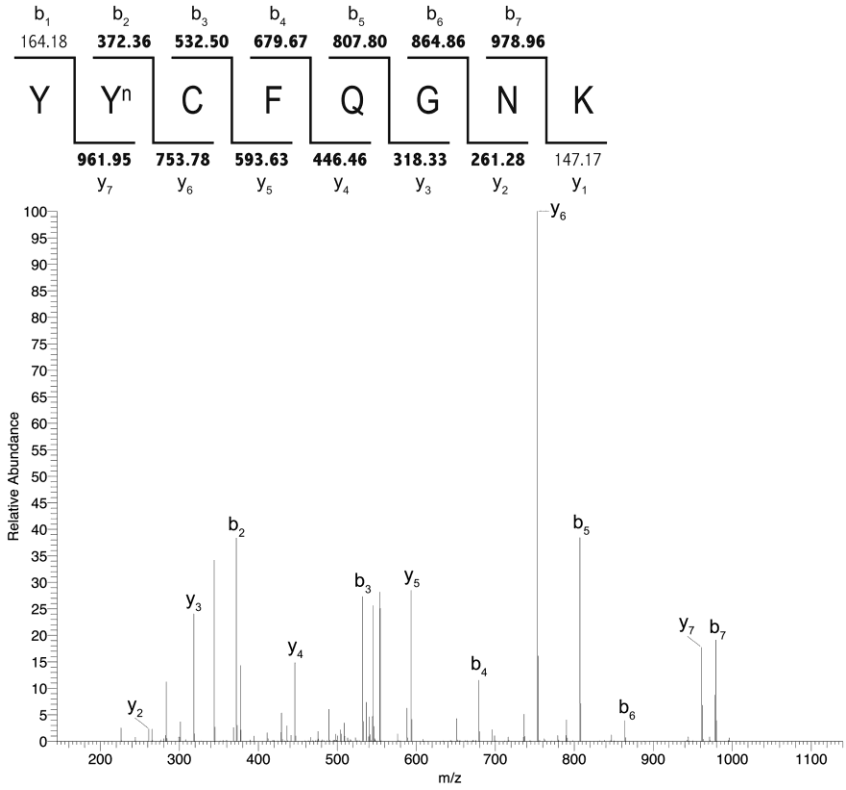

Figure S2. Nitration of rat hemopexin occurs *in vivo* at Y118.
Tyrosine nitration was detected by LC-MS/MS analysis of hemopexin isolated from rat plasma. Xcalibur BioWorks was used to identify Y^n118 on the tryptic peptide $YY^nCFQGNQFLR$ (see Table I for XCorr value). Matched b and y ions are shown in bold in the ions diagram (above) and are indicated next to corresponding peaks in the MS/MS spectrum.

#	b	b^{++}	b*	b^{*++}	Seq.	y	y^{++}	y*	y^{*++}	#
1	164.0706	82.5389			Y					11
2	***372.1190***	186.5631			Y	1377.6004	689.3039	1360.5739	680.7906	10
3	***532.1497***	266.5785			C	1169.5520	585.2797	1152.5255	576.7664	9
4	***679.2181***	340.1127			F	**1009.5214**	505.2643	992.4948	496.7511	8
5	***807.2767***	404.1420	790.2501	395.6287	Q	**862.4530**	431.7301	845.4264	423.2169	7
6	***864.2981***	432.6527	847.2716	424.1394	G	**734.3944**	367.7008	717.3679	359.1876	6
7	***978.3410***	489.6742	961.3145	481.1609	N	677.3729	339.1901	660.3464	330.6768	5
8	***1106.3996***	553.7034	1089.3731	545.1902	Q	**563.3300**	282.1686	546.3035	273.6554	4
9	***1253.4680***	627.2377	1236.4415	618.7244	F	**435.2714**	218.1394	418.2449	209.6261	3
10	***1366.5521***	683.7797	1349.5256	675.2664	L	**288.2030**	144.6051	271.1765	136.0919	2
11					R	175.1190	88.0631	158.0924	79.5498	1

Figure S3. MASCOT search results for nitration of human hemopexin *in vivo*.
The results of a recent MASCOT search of the same data file (05Dec1316.RAW and scan (2173), which identified the same peptide YY^{n}CFQGNQFLR. The monoisotopic mass of the neutral peptide is 1539.6565 Da.

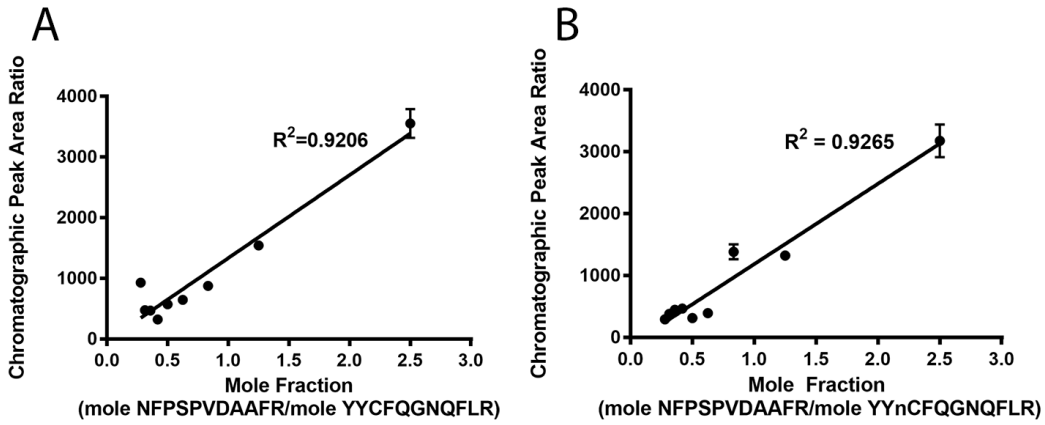

Figure S4. Calibration curves to determine the mole fraction of in-vitro nitrated Y^{199} in human hemopexin over the period of 120 minutes incubation with the myeloperoxidase/glucose oxidase system for the *in vitro* nitration of proteins.
The time course of nitration using SRM data were quantitated using the chromatographic peak areas of the modified peptide at each time point were corrected by the reference peptide. Calibration curves for the analysis of (A) YYCFQGNQFLR and (B) YY^{n}CFQGNQFLR peptides. Response ratios were obtained from calibration curves made with HPLC-purified, synthetic peptide standards. Stock solutions of each peptide were standardized with an assay for free amine groups using the amino acid arginine as the primary standard. These calibration curves allow the chromatographic peak area ratios determined in the LC/tandem MS analyses to be converted into mole fraction values for evaluating the stoichiometry of the reaction as shown in figure 3D.