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Supplementary Data

Mass Spectrometry of apoBS and holoBS Tryptic Fragments

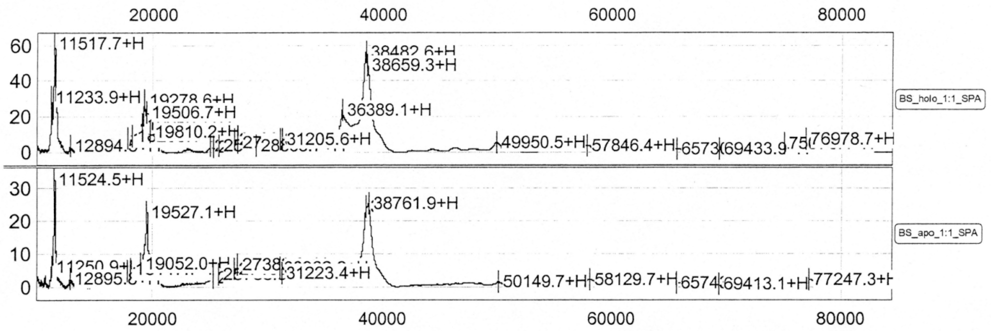
ApoBioB and 2Fe-BioB samples were treated with 0.001% trypsin for 30 minutes. These samples are identical to those shown in Figure 2, lane 6. Matrix-assisted laser-desorption ionization time-of-flight (MALDI-TOF) spectra were recorded on a Ciphergen PBSIIC mass spectrometer. Spectra were collected by averaging 100 spectra using a laser energy of 200 and the sensitivity set at 10. Digests were diluted 1:1, 1:5, 1:10, and 1:50 in either α-cyano-4-hydroxycinnamic acid (for peptide fragments from 1-9 kD) or sinnapinic acid (for fragments from 10-100 kD). The concentration of both matrices was 3.5 mg/mL (50:50:0.1 acetonitrile/water/TFA). In separate controls, samples were desalted using Millipore Ziptips using the manufacturers protocols, but this did not improve the results.

A kinetic analysis of the major bands in Figure 2 suggested that all of the major bands likely derive from a single cleavage of full length protein. We generated a table of all fragments that could result from either one or two tryptic cleavages of the full length sequence and compared these to the fragments detected by MALDI mass spectrometry. Assignments are tabulated below. There appears to be some cleavage of 2-3 residues from the N-terminus. The protein for these experiments was His$_6$-BioB with a TEV protease site that had been treated with TEV protease and repurified. The predicted N-terminal sequence (NH$_2$-GSAHRP…., M_{av} = 38661) appears to have been shortened by either 2 residues (NH$_2$-AHRP…., M_{av} = 38517) or three residues (NH$_2$-HRP…., M_{av} = 38446). The 2 residue shortened protein is identical to the native enzyme after in vivo removal of the N-terminal formyl-Met. Some of the tryptic fragments appear to match best to further cleavage of these 2-3 residue-truncated sequences. The major bands detected correspond best to cleavage at Arg66, Lys68, Arg169, Arg246, and Arg328.

Major Bands on SDS PAGE

	Rf	estim MW	assignment	sequence	Mass Spec	MS Intensity	calc avg M
Holo	0.565	39	1-347	GSAH --> AAAL	38659.3	48.6	38660.6
			2-347	AHRP-->AAAL	38482.6	56.1	38516.5
			3-347	HRP-->AAAL			38445.5
P1	0.578	36	2-328	AHRP-->QQQR	36389.1	23.9	36405.2
			3-328	HRP-->QQQR			36334.2
P2	0.616	30	66-347	YKTG-->AAAL	31205.6	7.3	31052.4
			68-347	TGLE-->AAAL	30953	7.6	30761.0
P3	0.638	27	1-246	GSAH-->DFIR	27357.8	5.8	27386.8
			3-246	HRP-->DFIR	27085.6	7.4	27171.7
P4	0.707	19	169-347	TYQE-->AAAL	19511.9	22.6	19564.3
P5	0.737	17	1-168	GSAH-->ITTR	19059.1	15.9	19096.2
P6	0.862	10	247-347	TIAV-->AAAL	11517.7	60.9	11273.8
Small Fragments	~1	≤7	1-67	GSAH-->SRYK	7901.4	11.4	7900.0
			1-65	GSAH-->QSSR	7602.2	16.6	7608.0

S2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49950.5+H
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6573
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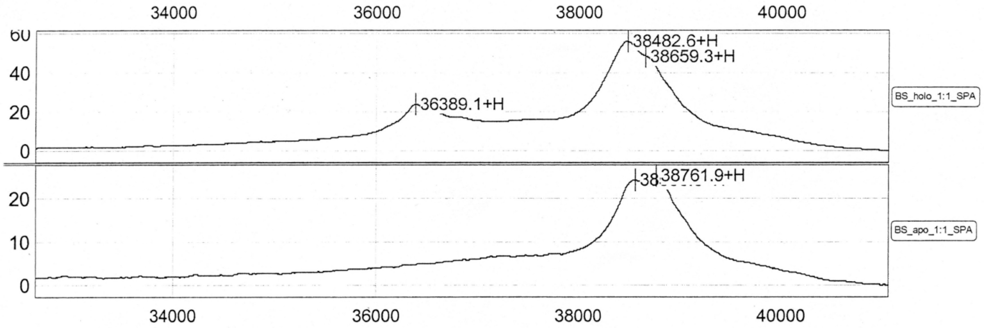
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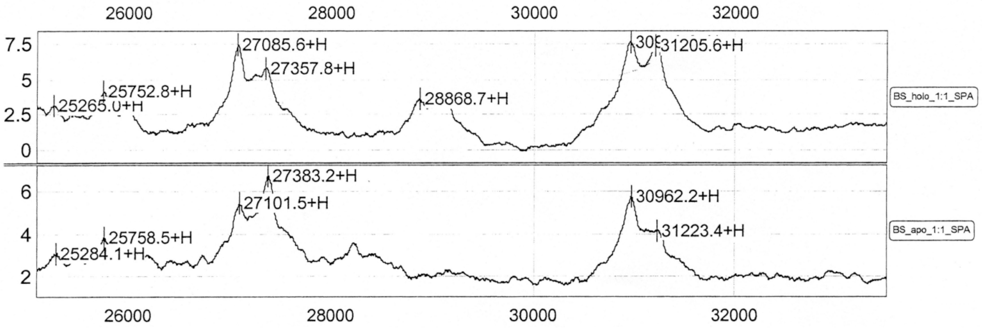
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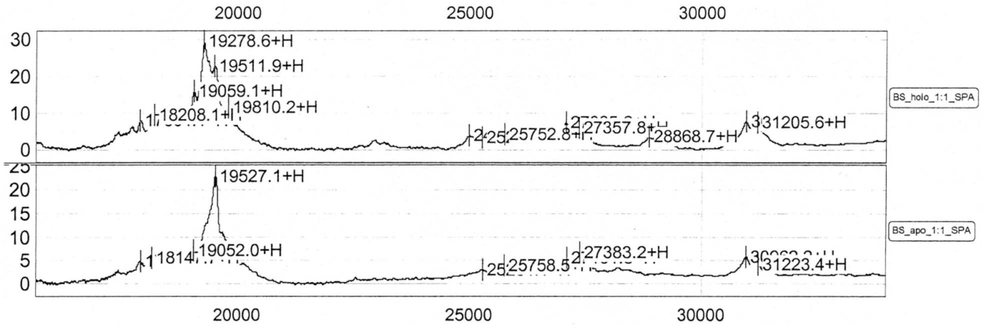
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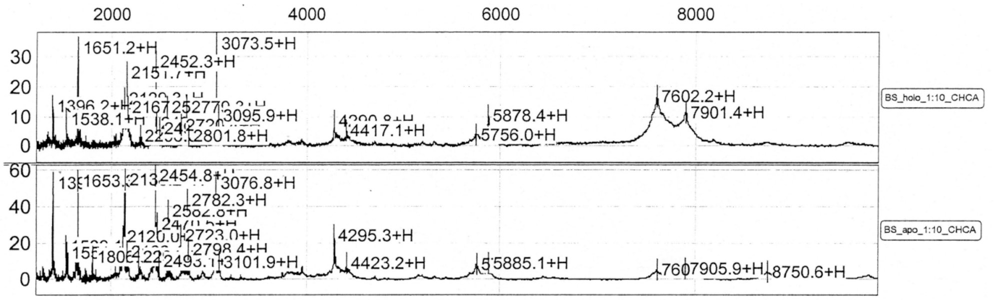
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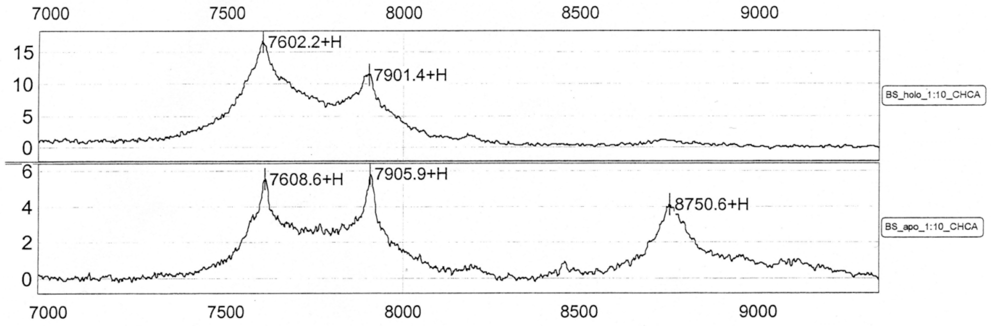
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