Analysis of Bovine Kappa-Casein Glycomacropeptide by Liquid Chromatography-Tandem Mass Spectrometry

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Table of Content

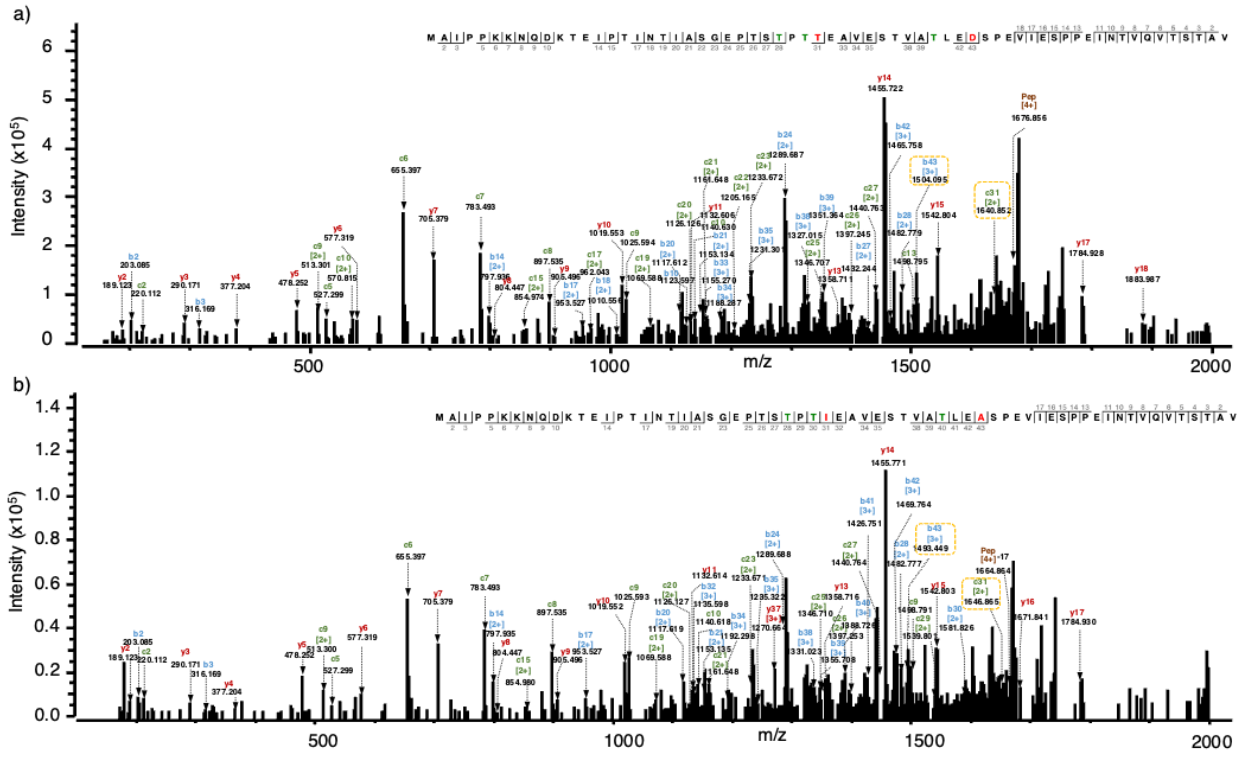

Figure S1. MS/MS spectra for intact aCMP with sequence A and B. a) Tandem MS spectra for the intact aCMP with sequence A, observed m/z 1341.661, [M+5H]5+, scan time = 21.90 min found in the CMP powder 2 sample. b) Tandem MS spectra for the intact aCMP with sequence B, observed m/z 1335.294[M+5H]5+, scan time = 22.23 min found in the CMP powder 2 sample.

m/z [M+H]$^+$	Composition	Structure
168.065	GalNAc-36	-2H & -2OH
186.076	GalNAc-18	-H & -OH
204.087	GalNAc	
274.092	NeuAc-18	-H & -OH
292.103	NeuAc	
366.140	GalNAcGal	
454.156	GalNeuAc	
495.182	GalNAcNeuAc	
639.225	GalNAcGalNeuAc-18	-H & -OH or -H & -OH
657.235	GalNAcGalNeuAc	or
948.330	$Gal_1NAcGal_1NeuAc_2$	

Figure S2. The representative oxonium ions identified from intact gCMPsand gCMP fragments (GalNAcGal, GalNAcGalNeuAc and $GalNAc_1Gal_1NeuAc_2$). Glycan symbols: yellow square, N-acetyl galactosamine; yellow circle, galactose; and purple diamond, N-acetyl neuraminic acid.

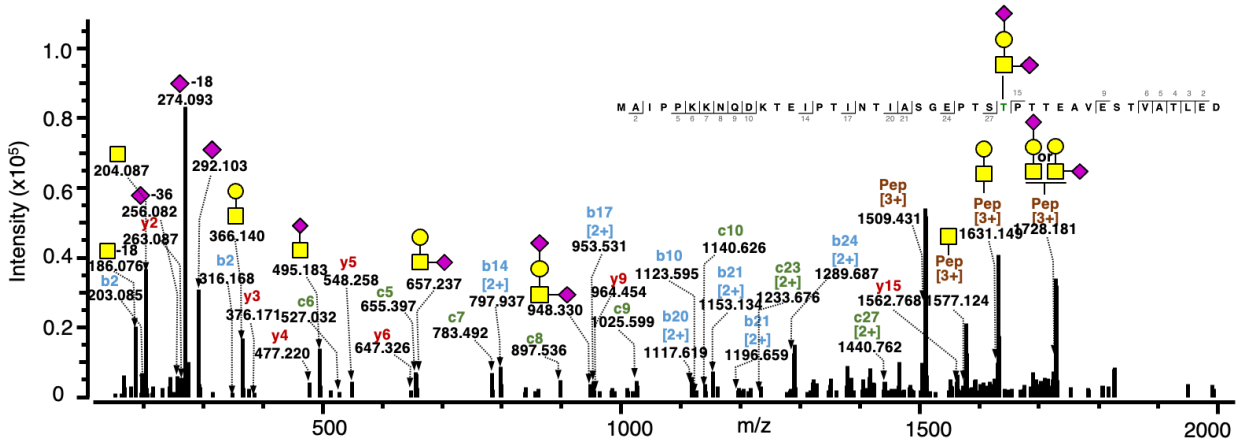

Figure S3. Annotated MS/MS spectra for gCMP fragment with sequence A one O-glycan. Tandem MS spectra for MAIPPKKNQDKTEIPTINTIASGEPTSTPTTEAVESTVATLED + GalNAc1Gal1NeuAc2 (m/z 1369.159, z=4) had a glycosylation site at Thr154 in the CMP standard sample. Glycan symbols: yellow square, N-acetyl galactosamine; yellow circle, galactose; and purple diamond, N-acetyl neuraminic acid.

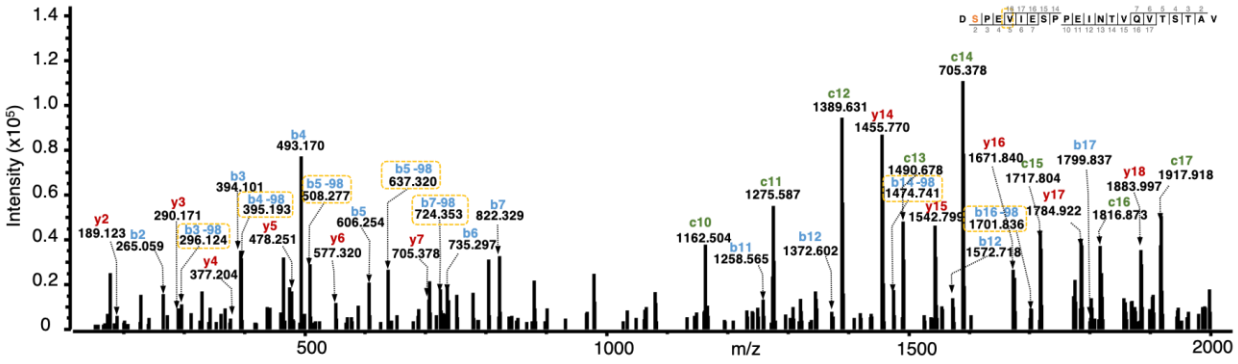

Figure S4. Annotated MS/MS spectra for aCMP fragment with sequence A with phosphorylation. Tandem MS spectra for DSPEVIESPPEINTVQVTSTAV (m/z 2276.082, z=2) had a phosphorylation site at Ser170 found in CMP powder 1 sample.

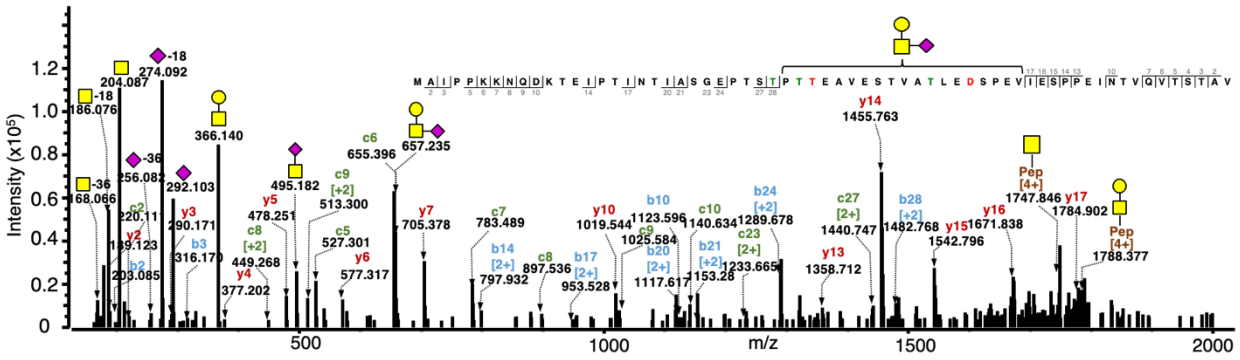

Figure S5. Annotated MS/MS spectra for intact gCMP with sequence A containing trisaccharide O-glycan. Tandem MS spectra for the intact gCMP with sequence B containing one O-glycan (GalNAcGalNeuAc) and one phosphate, observed m/z 1488.924 [M+5H]5+, scan time = 21.68 min found in the CMP standard sample. Glycan symbols: yellow square, N-acetyl galactosamine; yellow circle, galactose; and purple diamond, N-acetyl neuraminic acid.

Table S1. The 51 intact CMPs found in the four samples with peptide sequence, number of phosphorylations, number of oxidations, number of O-glycosylations, glycan structure, observed mass, calculated mass and error ppm.

*Excel S-1

Table S2. The 159 CMP fragments found in the four samples with peptide sequence, number of phosphorylations, number of oxidations, number of O-glycosylations, glycan structure observed mass, calculated mass and error ppm.

*Excel S-2

Table S3. The counts of intact gCMP containing 1, 2 and 3 O-glycans identified in the four samples (CMP STD, CMP powder 1, CMP powder 2 and WPI).

	CMP STD	CMP powder 1	CMP powder 2	WPI
1 *O*-glycan	17	18	18	18
2 *O*-glycans	14	15	16	15
3 *O*-glycans	9	8	9	9