Comparison of chicken light and dark meat using LC MALDI-TOF mass spectrometry as a model system for biomarker discovery

WP 651

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Introduction

Biomarker discovery is challenged by the need to distinguish subtle differences in components contained within complex samples. To date, most proteomic studies of muscle have been performed on human or rodent samples. However, chicken contains muscles distinguishable by eye, *i.d.* light and dark meat, which are expected to contain differences at the protein and protein isoform level. Presented here is a comparative study of light and dark meat as a model system for muscle proteomics. Our LC-MALDI analytical platform with high resolution TOF instrumentation (R>40,000) coupled with mass accuracy of <2 ppm across the peptides mass range allows for potential biomarkers to be determined at the MS level of analysis. The protein IDs are confirmed by MS/MS.

Methods

Work Flow

Chicken light and dark meat samples were obtained from a local supermarket. The sample preparation and characterization work flow is illustrated in **Fig. 1**.

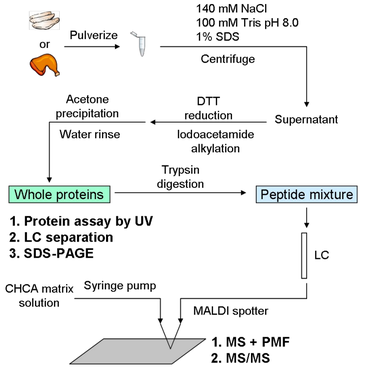
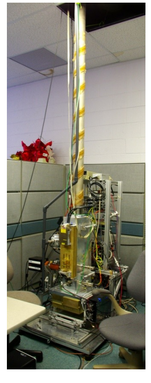


Fig. 1 Work flow chart



14.8 m flight path

Fig. 2 High performance mass spectrometer

Mass Spectrometry

All spectra were acquired on the high performance MALDI-TOF spectrometers built in our company. [1] (**Fig. 2**). The mass bin was set at 1 ns; average of 1000 laser shots; laser frequency 1 kHz; scan speed 1 mm/s; mass range 100-2700 Da; operation gas pressure 2×10^{-8} Torr.

PMF Search Conditions

A peak list consisting of masses and intensities was submitted to our own internal PMF program, [2] which iteratively identifies proteins starting from the most abundant. It calculates theoretical intensities from tryptic peptides and decreases protein scores for peptides that are not found. The database consists of ~14,000 sequences and was compiled from UniProt Swiss-Prot and TrEMBL for birds.

Results

Protein Characterization

Protein concentration: UV absorbance at 280 nm was calibrated using BSA standards. Based on the calibration function, the concentrations of chicken light and dark meat were estimated to be 1.13 mg/mL and 0.62 mg/mL respectively.

Protein digestion efficiency: SDS-PAGE separation of the whole proteins before and after trypsin digestion suggests the digestion is efficient and consistent as shown in **Fig. 3**.

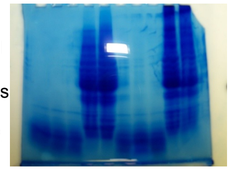


Fig. 3 SDS-PAGE gel of digested and whole chicken protein samples. Lanes are numbered from left to right. Lane 1-3 dark meat after digestion, 4-5 dark meat, 6-8 white meat after digestion, 9-10 white meat

Peptide Characterization

LC MALDI MS: MALDI MS analysis was performed on peptide mixture separated on LC with long gradient. **Fig. 4** shows the TIC of 3-hour separation of chicken dark meat peptides. **Fig. 5** gives an example of the MS spectrum showing high resolution of the instrument. PMF results are summarized in **Table 1**.

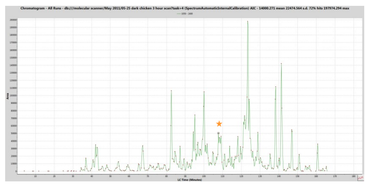


Fig. 4 3-hour LC chromatogram for chicken dark meat peptides

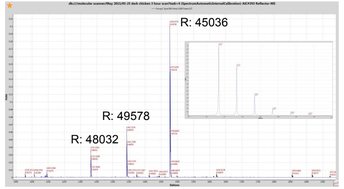


Fig. 5 High resolution MS spectrum of 1476 fraction (R>40,000)

	Dark			Light		
protein	1	2	3	1	2	3
ACTA	12(3)	11(1)	15(5)	15(2)	13(2)	16(2)
ACTN	20(7)	26(4)	27(2)	20(6)	21(6)	18(5)
AK1				7(7)		6(7)
ATP2A1	12(10)		12(8)	7(15)		
CRM	7(8)		16(6)	8(5)	11(4)	8(4)
CKMT2	11(6)					
GAPDH						5(16)
MYH1		75(3)				
MYH2	42(4)		47(3)			
MYH3			72(1)			
MYH4		10(2)				
MYH5				76(1)	85(1)	80(1)
MYH6	59(1)		33(15			
MYL1		6(5)	9(4)	9(3)	8(3)	8(6)
MYL2			4(9)			
MYLR				9(10)	8(7)	6(8)
PGR					8(5)	12(9)
TNNC					7(12)	
TNNT				7(9)		7(15)
TPM	9(2)		8(10)	11(4)	9(5)	11(3)
trypsin			3(7)		2(8)	
peaks for PMF	1079	553	686	803	859	809
total fractions	106	80	675	81	270	81
fr Diff (976.4 -1790.9)	29	32	221	30	106	29
msms	64	56	457	47	195	62
msms ids	30	28	78	39	63	37

Table 1 ID of myosin isoforms by PMF and MS/MS in 3 LC runs each. Number of peptides matched is listed with the protein rank in parentheses. Proteins specific to light or dark meat are color coded. Myosin heavy chain isoforms (MYH) are in pale blue.

- One dominant MYH in light meat, several alternative MYH in dark meat; PMF occasionally selects different MYHs as the best fit
- 2 MYL isoforms detected
- Green proteins associate with actin. Yellow proteins are general metabolic enzymes in light meat
- Heterogeneity of MYH in dark meat may suppress some proteins.

MS/MS: LC fractions having different peptide masses in MS scan were automatically picked for MS/MS analysis using the software developed by our company. **Fig. 6** shows an example of one MS/MS spectrum. Peptide masses that differ in light and dark meat were sequenced and tabulated in **Table 2**, which confirms the results from PMF.

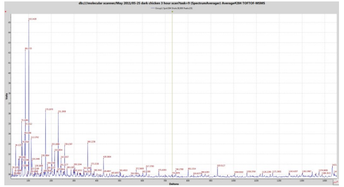


Fig. 6 MS/MS spectrum of m/z 1476

Table 2 MS/MS results indicate selected myosin isoform differences

mass	N-AA	sequence	isoform	dark	white
1187	759	AGLIGVLEEMR	MYH7	26	
1201	759	AGLLGLLEEMR	MYH		56
2214	887	NDLQLQVQAEADALADAEER	MYH	22	66
2198	887	NDLQLQVQAEADALADAEER	MYH	13	
1476	1117	TEELEEEIEAER	MYH6	60	
1488	1117	IEELEEEIEAER	MYH	84	75
1183	1282	LQTETGEYSR	MYH		72
1169	1282	LQTESGEYSR	MYH4	46	
1317	1308	QAFTQQIEELK	MYH3	29	
1303	1308	QGFTQQIEELK	MYH		34
1956	1406	NLQQEIADLTEQIAEGGK	MYH		23
1972	1406	NLQQEISDLTEQIAEGGK	MYH	38	
1659	1423	LQTEIEDLSVDLER	MYH8	36	
1688	1423	LQNEVEDLMVDVER	MYH		61
1702	1423	LQNEVEDLMIDVER	MYH3	73	55
1398	1680	ANLLQAEIEELR	MYH3	70	
1386	1680	ANLLQAETEELR	MYH6	50	
1384	1680	ANLLQAEVEELR	MYH	11	63
1377	1703	VAEQELMDASER	MYH3	68	
1373	1703	VAEQELLDASER	MYH		67
1359	1703	VAEQELLDASER	MYH5	25	
1401	1703	LAEQELLEATER	MYH8	29	
2348	1732	LETDIVQIQSEMEDTIQEAR	MYH		39
2319	1732	LETDLAQIQSEMEDTIQEAR	MYH3	93	
2321	1732	LESDISQIQSEMEDTIQEAR	MYH5	78	27
2347	1732	LETDIVQIQSEMEDTIQEAR	MYH		74
1342	1851	ELTYQAEEDR	MYH		70
1269	1851	ELTYQREEDR	MYH4	51	
1382	1901	IQHELEAAEER	MYH	60	57
1340	1901	VQHELDDAEER	MYH8	20	

Conclusion

- MS followed by PMF can quickly identify potential biomarkers
- MALDI-TOF MS/MS instrument helps confirm PMF ID
- LC separation increases MS/MS IDs but has little effect on PMF IDs
- Chicken light and dark meat differ by MYH isoforms

Reference

[1] Vestal, M. L. and Hayden, K. *Int. J. Mass Spectrom.* 2007, 268, 83-92

[2] Parker, K. C.; Garrels, J. I.; Hines, W.; Butler, E. M.; McKee, A. H. Z.; Patterson, D.; Martin, S. *Electrophoresis*, 1998, 19(11), 1920-1932

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