Identification of Proteins from Biological Samples by MALDI Peptide Mass Fingerprinting Without Protein Separation

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TP389

Introduction

Peptide Mass Fingerprinting (PMF) is a proven technique for protein identification, especially following separation by 2-dimensional electrophoresis. Here, we explore using PMF on crude biological material. In our model chicken biomarker discovery experiments, we compare dark to white meat, looking for specific masses. We show here that when high mass accuracy MALDI mass spectrometry is used on certain unseparated samples, PMF is able to identify some of the most abundant proteins, as well as candidate peptide isoform biomarkers.

Methods

PMF Analysis

- Get protein-containing sample
  - -from chicken breast (white) or leg (dark); homogenize
  - - from home-brewed beer sediment
  - - from saliva (supernatant or pellet)
  - -from oil-painting in museum (prepared by Dan Kirby, Harvard U.)
  - -from contaminated lab stock solution
- Reduce and alkylate in SDS
- Acetone Precipitate
- Trypsin Digest
- Collect MS Spectra
  - Use 'combo' machine-> 20,000 resolution
  - Use 'elite' machine, 14.8 m flight path-> 45,000 resolution
  - 1 or 2 kHz laser (up to 4 khZ possible, starts failing at 5kHz)
  - Collect 4000-10000 shots
- Prepare Peak list with 100 -~1000 masses, density filter if desired.
- Perform PMF Vs. combined SwissProt / Trembl database, keyed to taxon
- Confirm as needed with LC MALDI MSMS (MSMS machine).
- Prepare Peak List from LC-MALDI composite spectrum or from LC-MALDI peak list (also used to select precursors for MS-MS)
- Perform PMF on these peak lists as well.
  - Nearly equivalent results obtained as from unseparated digest
  - Useful for testing calibration across LC run.
  - Best results with LC-MALDI peak list from 45 K resolution!

Abbreviations: myosin heavy chain -> MYH

Grocery store food (chicken thigh and breast meat)

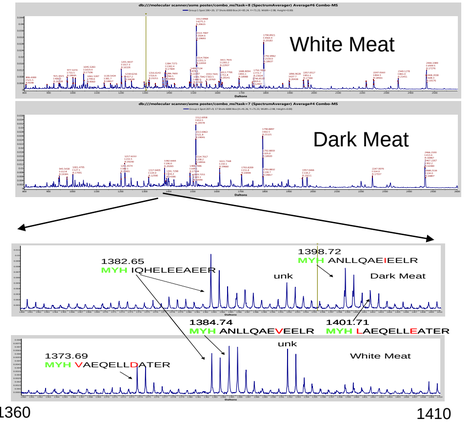
Home-brewed beer (from daughter's boyfriend)

saliva

Generic Art (oil / tempera)

Perform PMF on un-purified protein

Chicken dark and white meat is a model system for characterizing human muscle biopsy samples.
What differences can be observed without protein separation?



Several MYH isoforms are evident that differ between white and dark meat.

Typical LC-MALDI PMF results from chicken

I	protein	Leng	name	#Obs	#Obs_I	TrSM	%CM	%IM
1	MYH	1939	Myosin heavy chain	80	80	520885	33.9	16.5
2	ACTA1	377	Actin	16	16	96607	41.2	8.7
3	TPM	283	tropomyosin	11	12	47697	32.4	1.7
4	CKM	381	Creatine kinase	8	8	31986	29.6	1.5
5	ACTN2	897	Alpha-actinin-2	18	20	31834	24.6	1.3
6	MYL3	150	Myosin light chain	8	8	27931	48.8	3.2
7	AK1	194	Adenylate kinase	6	6	19119	37.8	0.6
8	MYLR	168	Myosin regulatory light	6	7	7887	29.3	1.6

#Obs -the # of peptides matched, after subtraction.
#Obs_I -the initial number of peptides matched
%CM -related to coverage, but weighted toward Arg-containg peptides.
%IM - percentage intensity matched.
All these proteins confirmed by LC-MALDI MSMS.
27 replicate digests of chicken meat (13 white, 14 dark)

N	gene	p	sequence	mass	ppm	Sc	#white	#dark	inten	% white
1				1890.83			0	14	9932	0.0
2	MYH3	y	qAPTQQIREELK	1317.64	13.8	29	1	14	13967	2.3
3	MYH6	y	TEELEEEIEAER	1476.68	3.9	60	1	14	9539	2.7
4	MYH5	y	VARQELLDAERR	1359.68	2.0	25	2	14	8967	6.7
5	MYH3	y	LGNEVEDLMIDVER	1702.83	0.2	73	11	14	37242	8.9
6	MYH5	y	LESDISQLQSEMEDTIQEAR	2322.10	11.0	78	9	14	10143	10.7
7	MYH4	y	NDLQLQVQAEADALADAEER	2199.07	18.8	13	9	14	13490	11.4
8	MYL	y	DTGTYEDFVEGLR	1501.71	16.9	37	13	14	48606	18.5
9	MYLK	q	SMTPQTPIQEFK	1501.71	4.0	28	13	14	48606	18.5
10	MYH8	y	LAEQELLEATER	1401.75	13.0	29	9	13	14277	19.2
11	CKM		ILTnPSNLGTGLR	1401.75	7.6	22	9	13	14277	19.2
										0.0
12				1553.75			13	14	20578	66.5
13	GAPDH		LVSWYDNEFGYSNR	1749.79	2.9	46	13	14	36273	66.6
14	VIM		LGDLYEEEPRELR	1618.81	0.1	13	13	14	14892	67.4
15				1778.93			13	12	11908	67.9
16				1744.84			13	13	19536	68.8
17	MYH	y	NDLQLQVQAEADSLADAEER	2215.06	1.6	66	13	14	19174	70.0
18	MYH	m	ANSEVAQWR	1060.53	11.0	49	13	14	31932	70.5
19	MYH2	y	SELQASLEEAEASLEHEEGK	2186.03	7.1	53	13	13	8447	72.8
20				3067.54			13	13	8398	80.7
21	MYH	y	LQNEVEDLMVDVER	1688.83	8.9	61	13	9	24964	87.8
22				1591.79			13	3	9088	89.6
23	MYH	y	ANLLQAEVEELR	1384.74	1.0	63	13	1	22607	98.4
24	MYH	y	LETDIYQIQSEMEDTIQEAR	2348.12	0.1	74	13	5	30540	99.1
25	MYH	m	LSGSIVDSTQYR	1530.79	1.7	45	13	0	15053	100.0
26	MYH	y	VAEQELLDATER	1373.69	2.2	67	13	0	16084	100.0
100			maximum possible				13	14	264131	

- The 100 most intense masses found in the 27 replicates. 26 masses were enriched in dark or white meat, and mostly matched to isoform-specific peptides from MYH of chicken.
- The most intense peptide was mapped to actin, and was expressed ~equally, as were all other actin peptides.
- Sc refers to the Mascot Score, searched using MSMS spectra from LC-MALDI experiments Vs. the NCBI vertebrate database.
- p -the peptide was identified by MSMS in at least 2 variant forms.
- m- the peptide was identified by MSMS in only one form, but the sequence is variable in the MYH isoforms that were identified by other peptides.

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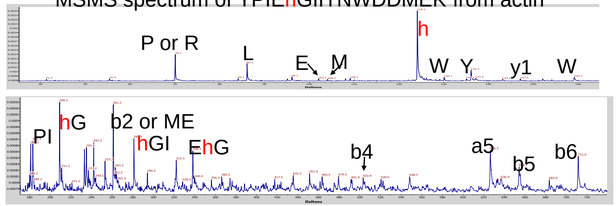
MSMS spectrum of YPIEhGIITNWDDMEK from actin



h indicates methyl histidine, with strong proton affinity

Saliva-> (by PMF only so far.)
Amylase (22% intensity), cystatin, IgA (supernatant)
- keratin 4, 13, 6A (pellet) .

Beer->
- yeast glycolytic enzymes
- barley ~protease inhibitors (at least 3 isoforms each) by both PMF and MS-MS
- PMF IDs marginal compared to the other systems, probably due to incomplete database issues, or protein degradation

Painting ->
- egg white (ovalbumin, lysozyme) from pigeon or duck.
- work supplied by and corroborated independently of us by Dan Kirby and Katherine Phillips, Harvard U.

Reagent contamination->
- identifies EfTu, porin from Burkholderia species.
- Accomplished by starting from SwissProt Bacteria database, then working through Trembl using smaller phylogenetic categories.

Conclusions:

- PMF is successful in identifying proteins from many crude samples.
- At top level, MYH isoforms distinguish chicken white meat from dark.
- Annotating database sequences with quantitative modifications (like actin h shown above) aids PMF.
- Protein degradation is often less serious than expected.
- Confirmation of PMF by MSMS requires LC separation (of homologous sample).

References:

1.) **Parker KC.** Scoring Methods in MALDI Peptide Mass Fingerprinting: ChemScore and the ChemApplex Program. JASMS 2002;13:22-39.
2.) See Poster WP651 for more details.

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