

Proteomic Analysis of Novel Marine Bacteria Using Multidimensional Chromatography and MALDI Mass Spectrometry

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Overview

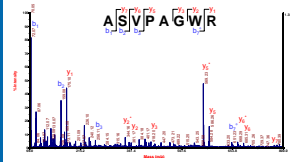
- Goals:
 - To develop mass spectrometric protocols to search for proteins involved in the conversion of light into energy in marine bacteria
 - To identify as many membrane proteins as possible from small cultures of marine bacteria
- Methods
 - Membrane preparation
 - LC-ESI and LC-MALDI MS/MS
 - Multidimensional Chromatography with LC-MALDI
- Results
 - Targeted proteins identified
 - 408 Proteins identified

Marine Bacteria Studied

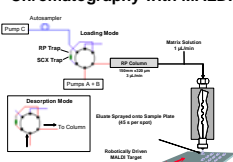


- SAR11**
 - Alphaproteobacteria *Pelagibacter ubique*
 - Possibly most abundant organism in oceans
 - Plays key role in the carbon cycle
 - ~1.3 million bp genome sequenced
 - Proteorhodopsin gene discovered (light dependent proton pump)
- Gammaproteobacteria**
 - Aerobic Anoxygenic Phototroph (AAP)
 - Strain investigated makes up 2-5% of bacteria in coastal Pacific Ocean
 - Photosynthetic gene clusters found

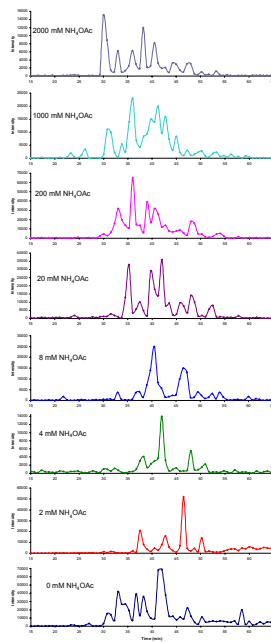
MS/MS of Proteorhodopsin Peptide by MALDI TOF/TOF



Method: Multidimensional Chromatography with MALDI



2D-LC MALDI-MS Base Peak Chromatograms



Results

- 372 Proteins Identified in SCX Experiment
 - 27% of proteome
 - Highly enriched in membrane proteins
 - 2.58 Peptides per protein
- 408 Total Proteins Observed
 - 30% of proteome
- Proteorhodopsin protein from SAR11 observed in Pacific Ocean off Oregon coast

Conclusions

- Identifying biologically relevant proteins out of small samples containing whole proteomes is possible, but only with correct choices in sample preparation and ionization technique
- Simple membrane preparation used to isolate targeted proteomes
- Proteorhodopsin only identified with LC-MALDI due to prevalence of arginine-ending peptides
- Multidimensional chromatography easily used with LC-MALDI

Acknowledgements

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References

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Introduction

- Samples consisted of cell pellets from SAR11 and a novel gammaproteobacterium
- Genes for targeted proteins detected by cloning-sequencing^{1,2}
- MALDI and ESI are complementary techniques, especially in regards to tryptic peptides^{3,4}
- Sample preparation and choice of ionization technique were tailored to identify the targeted proteins out of whole proteomes
- Multidimensional chromatography used with MALDI to identify as many membrane proteins as possible

Targeted Proteins

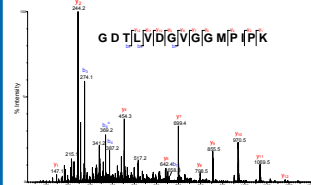
Proteorhodopsin from SAR11

MEKLELPAITAVALNDVGVANRITLLASIDGPGVIGIPWLVNALLASTA₁₋₃₂
 FFFIERSVDAQGRVSTVAGLVTGIAPIHIVNRYWVWNTQSPSTVYV₃₃₋₆₂
 IDWLITVPLMLGPFYVLAANRANSQIFRMLIQTILVGLDGLGADY₆₃₋₉₂
 INTYLPYIQAGNPFYLVYFFSQRAGKQRAKSGRGLATVPAQAMKIVT₉₃₋₁₂₂
 VQALVYIPLVYFQVDTQDGLKSLVYVRAQFLKIAFLDILVAAKQD₁₂₃₋₁₅₂
 DGRAK

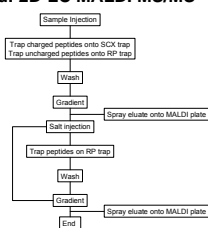
Photosynthetic Reaction Center H (PRC-H) from AAP

MITGALITQYIDVAQVALVYVNIFFPFLVFLHRLRSGRERQMLDGLDGGD₁₋₃₂
 LVQVQDMPIDKTYELANQGSSTLQDAPAVYELKANSITQPSAGYVPE₃₃₋₆₂
 YQSNWQDQANWATREPKSCLTIDKSRPVLVQVDSRYVNRSGRER₆₃₋₉₂
 GQAVLADCGDQGGTVDLNVFASRPFKFRVAVVQVDDGLLPMTHARVSK₉₃₋₁₂₂
 DDTYVQVSIIRSDQYIQVPLKLANPQVLTQKSKITAFYGGTLYATPERK₁₂₃₋₁₅₂
 EPEL

MS/MS of PRC Peptide by ESI Q-TOF



Method: 2D-LC MALDI MS/MS



Results for Targeted Proteins

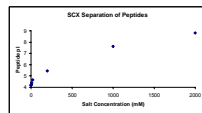
Proteorhodopsin from SAR11

MEKLELPAITAVALNDVGVANRITLLASIDGPGVIGIPWLVNALLASTA₁₋₃₂
 FFFIERSVDAQGRVSTVAGLVTGIAPIHIVNRYWVWNTQSPSTVYV₃₃₋₆₂
 IDWLITVPLMLGPFYVLAANRANSQIFRMLIQTILVGLDGLGADY₆₃₋₉₂
 INTYLPYIQAGNPFYLVYFFSQRAGKQRAKSGRGLATVPAQAMKIVT₉₃₋₁₂₂
 VQALVYIPLVYFQVDTQDGLKSLVYVRAQFLKIAFLDILVAAKQD₁₂₃₋₁₅₂
 DGRAK 12% Coverage

PRC-H from AAP

MITGALITQYIDVAQVALVYVNIFFPFLVFLHRLRSGRERQMLDGLDGGD₁₋₃₂
 LVQVQDMPIDKTYELANQGSSTLQDAPAVYELKANSITQPSAGYVPE₃₃₋₆₂
 YQSNWQDQANWATREPKSCLTIDKSRPVLVQVDSRYVNRSGRER₆₃₋₉₂
 GQAVLADCGDQGGTVDLNVFASRPFKFRVAVVQVDDGLLPMTHARVSK₉₃₋₁₂₂
 DDTYVQVSIIRSDQYIQVPLKLANPQVLTQKSKITAFYGGTLYATPERK₁₂₃₋₁₅₂
 EPEL 20% Coverage

Peptide pI Increases with Salt Concentration



Methods

- Cell pellets (<10 mg) from two species of marine bacteria cultured in sterile sea water
- Centrifugation used to isolate membranes
- Membranes solubilized with dodecyl maltoside
- In-solution tryptic digestion
- No reduction or alkylation
- LC-ESI performed on a Q-TOF Global Ultra
- LC-MALDI spotted with a MALDIprep³ device
- MALDI data collected on a 4700 Proteomics Analyzer
- Mascot⁴ used for data analysis

Theoretical Tryptic Digests

Proteorhodopsin from SAR11

Peptide	Mass (Da)
AEVPAQGR	843.41
INQSPVSR	855.41
SLYAFQAMR	1038.50
QVAVR	1050.50
DAFLR	1051.50
SENVNLTGAPQVSR	2344.20
QVNLVPLVLAQK	1077.51

Mostly peptides ending with R
 → Use MALDI

PRC-H from AAP

Peptide	Mass (Da)
LPLPIMR	932.51
LSRANQSR	938.51
SDQYQVSR	1081.50
ESWPLDQK	1129.50
YDQVQDGR	1181.50
TYELANQGSR	1195.51
PLKFRPSTKEL	1199.51
AGDYLTAWVSR	1348.51
SENLQVQAGPVR	1405.50
LAMPVQLGELR	1599.51
EPAPYDQGLYATPR	1716.50
ITGATVYDQVQVAVYVWVFFFLVFLHRL	1746.50
MSTIANITQVQAVQVAVWVWVFFFLVFLHRL	1878.50

Peptides ending with K and R
 → Use MALDI or ESI