Centre for Proteome Research

UNIVERSITY OF LIVERPOOL

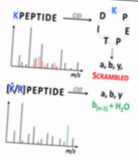
J. Am. Soc. Mass Spectrom. (2014)
DOI: 10.1007/s13361-014-0968-y

RESEARCH ARTICLE

Peptide Scrambling During Collision-Induced Dissociation is Influenced by *N*-terminal Residue Basicity

Ross Chawner,[1,2] Stephen W. Holman,[1,4] Simon J. Gaskell,[3] Claire E. Eyers[1,4]

[1]Michael Barber Centre for Mass Spectrometry, School of Chemistry, Manchester Institute of Biotechnology, University Manchester, Manchester, M1 7DN, UK
[2]Waters Corporation, Stamford Avenue, Wilmslow, SK9 4AX, UK
[3]Queen Mary University of London, London, E1 4NS, UK
[4]Department of Biochemistry, Institute of Integrative Biology, University of Liverpool, Liverpool, L69 7ZB, UK



Abstract. 'Bottom up' proteomic studies typically use tandem mass spectrometry data to infer peptide ion sequence, enabling identification of the protein whence they derive. The majority of such studies employ collision-induced dissociation (CID) to induce fragmentation of the peptide structure giving diagnostic b-, y-, and a- ions. Recently, rearrangement processes that result in scrambling of the original peptide sequence during CID have been reported for these ions. Such processes have the potential to adversely affect ion accounting (and thus scores from automated search algorithms) in tandem mass spectra, and in extreme cases could lead to false peptide identification. Here, analysis of peptide species produced by Lys-N proteolysis of standard proteins is performed and sequences that exhibit such rearrangement processes identified. The effect of increasing the gas-phase basicity of the *N*-terminal lysine residue through derivatization to homoarginine toward such sequence scrambling is then assessed. The presence of a highly basic homoarginine (or arginine) residue at the *N*-terminus is found to disfavor/inhibit sequence scrambling with a coincident increase in the formation of $b_{(n-1)}+H_2O$ product ions. Finally, further analysis of a sequence produced by Lys-C proteolysis provides evidence toward a potential mechanism for the apparent inhibition of sequence scrambling during resonance excitation CID.

Key words: Collision-induced dissociation, b-ion rearrangement, Peptide scrambling, Lys-C, Lys-N

Received: 17 April 2014/Revised: 14 July 2014/Accepted: 16 July 2014

Contents lists available at ScienceDirect
Hormones and Behavior
journal homepage: www.elsevier.com/locate/yhbeh

From sexual attraction to maternal aggression: When pheromones change their behavioural significance

Ana Martín-Sánchez [a], Lynn McLean [b], Robert J. Beynon [b], Jane L. Hurst [b], Guillermo Ayala [c], Enrique Lanuza [a], Fernando Martínez-Garcia [a,*]

[a] Laboratori de Neuroanatomia Funcional Comparada, Departments of Functional Biology and of Cell Biology, Faculty of Biological Sciences, Univ. Valencia, C. Dr. Moliner, 50, 46100 Burjassot, Spain
[b] Institute of Integrative Biology, University of Liverpool, Liverpool L69 7ZB, UK
[c] Department of Statistics and Operative Research, Faculty of Mathematics, Avda. Vicent Andrés Estellés, 1, 46100 Burjassot, Spain

The role of proteomics in studies of protein moonlighting

Robert J. Beynon*[1], Dean Hammond*, Victoria Harman* and Yvonne Woolerton*

*Centre for Proteome Research, Institute of Integrative Biology, University of Liverpool, Crown Street, Liverpool L69 7ZB, U.K.

Abstract

The increasing acceptance that proteins may exert multiple functions in the cell brings with it new analytical challenges that will have an impact on the field of proteomics. Many proteomics workflows begin by destroying information about the interactions between different proteins, and the reduction of a complex protein mixture to constituent peptides also scrambles information about the combinatorial potential of post-translational modifications. To bring the focus of proteomics on to the domain of protein moonlighting will require novel analytical and quantitative approaches.

Contents lists available at ScienceDirect
International Journal of Mass Spectrometry
journal homepage: www.elsevier.com/locate/ijms

Gas-phase intermolecular phosphate transfer within a phosphohistidine phosphopeptide dimer

Maria-Belen Gonzalez-Sanchez [a,1], Francesco Lanucara [a,b,1], Gemma E. Hardman [b], Claire E. Eyers [a,b,*]

[a] Michael Barber Centre for Mass Spectrometry, School of Chemistry, Manchester Institute of Biotechnology, University of Manchester, 131 Princess Street, Manchester M1 7DN, UK
[b] Institute of Integrative Biology, University of Liverpool, Crown Street, Liverpool L69 7ZB, UK

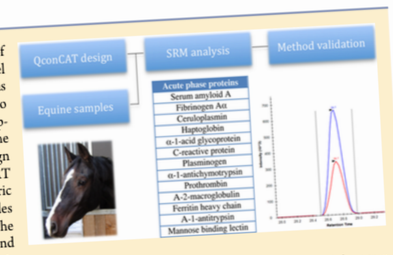
Journal of proteome research
Article
pubs.acs.org/jpr

Development of a Method for Absolute Quantification of Equine Acute Phase Proteins Using Concatenated Peptide Standards and Selected Reaction Monitoring

Louise Bundgaard,*[,†,‡] Stine Jacobsen,[†] Thomas F. Dyrlund,[‡] Mette Aa. Sørensen,[†] Victoria M. Harman,[§] Robert J. Beynon,[§] Philip J. Brownridge,[§] Lars J. Petersen,[∥,⊥] and Emøke Bendixen[‡]

[†]Department of Large Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Højbakkegaard Allé 5, Taastrup 2630, Denmark
[‡]Department of Molecular Biology and Genetics, Faculty of Science and Technology, Aarhus University, Gustav Wieds Vej 10c, Aarhus 8000, Denmark
[§]Protein Function Group, Institute of Integrative Biology, University of Liverpool, Crown Street, Liverpool L69 7ZB, United Kingdom
[∥]Department of Nuclear Medicine, Clinical Cancer Research Center, Aalborg University Hospital, Hobrovej 18-22, Aalborg 9000, Denmark
[⊥]Department of Clinical Medicine, Aalborg University Hospital, Sdr. Skovvej 11, Aalborg 9000, Denmark

Supporting Information



ABSTRACT: The aim of this study was the development of a quantitative assay that could support future studies of a panel of acute phase proteins (APPs) in the horse. The assay was based on a quantification concatamer (QconCAT) coupled to selected reaction monitoring methodology. Thirty-two peptides, corresponding to 13 putative or confirmed APPs for the *Equus caballus* (equine) species were selected for the design of a QconCAT construct. The gene encoding the QconCAT was synthesized and expressed as an isotope-labeled chimaeric protein in *Escherichia coli*. The QconCAT tryptic peptides were analyzed on a triple-quadrupole instrument, and the quantotypic properties were assessed in equine serum, wound tissue, and wound interstitial fluid. Reasonable quantotypic performance was found for 12, 14, and 14 peptides in serum, wound tissue, and interstitial fluid, respectively. Seven proteins were quantified in absolute terms in serum collected from a horse before and after the onset of a systemic inflammatory condition, and the observed protein concentrations were in close agreement with previous data. We conclude, that this QconCAT is applicable for concurrent quantitative analysis of multiple APPs in serum and may also support future studies of these proteins in other types of tissues and body fluids from the horse.

KEYWORDS: *QconCAT, acute phase proteins, veterinary proteomics, selected reaction monitoring, quantotypic peptides, wound healing, equine, validation*

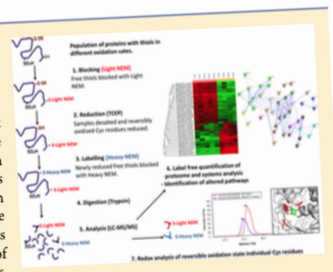
Journal of proteome research
Article
pubs.acs.org/jpr

Differential Cysteine Labeling and Global Label-Free Proteomics Reveals an Altered Metabolic State in Skeletal Muscle Aging

Brian McDonagh,*[,†] Giorgos K. Sakellariou,[†] Neil T. Smith,[†] Philip Brownridge,[‡] and Malcolm J. Jackson[†]

[†]MRC-Arthritis Research UK Centre for Integrated Research into Musculoskeletal Aging (CIMA), Skeletal Muscle Pathophysiology Research Group, Institute of Ageing and Chronic Disease, [‡]Protein Function Group, Institute of Integrative Biology, University of Liverpool, Liverpool L69 3GA, United Kingdom

Supporting Information



ABSTRACT: The molecular mechanisms underlying skeletal muscle aging and associated sarcopenia have been linked to an altered oxidative status of redox-sensitive proteins. Reactive oxygen and reactive nitrogen species (ROS/RNS) generated by contracting skeletal muscle are necessary for optimal protein function, signaling, and adaptation. To investigate the redox proteome of aging gastrocnemius muscles from adult and old male mice, we developed a label-free quantitative proteomic approach that includes a differential cysteine labeling step. The approach allows simultaneous identification of up- and downregulated proteins between samples in addition to the identification and relative quantification of the reversible oxidation state of susceptible redox cysteine residues. Results from muscles of adult and old mice indicate significant changes in the content of chaperone, glucose metabolism, and cytoskeletal regulatory proteins, including Protein DJ-1, cAMP-dependent protein kinase type II, 78 kDa glucose regulated protein, and a reduction in the number of redox-responsive proteins identified in muscle of old mice. Results demonstrate skeletal muscle aging causes a reduction in redox-sensitive proteins involved in the generation of precursor metabolites and energy metabolism, indicating a loss in the flexibility of the redox energy response. Data is available via ProteomeXchange with identifier PXD001054.

KEYWORDS: *Redox proteomics, aging, skeletal muscle metabolism, Grp78, aconitase and sirtuin1*

nature chemistry
REVIEW ARTICLE
PUBLISHED ONLINE: 21 MARCH 2014 | DOI: 10.1038/NCHEM.1889

The power of ion mobility-mass spectrometry for structural characterization and the study of conformational dynamics

Francesco Lanucara[1,2], Stephen W. Holman[1,2], Christopher J. Gray[2] and Claire E. Eyers[1,2]*

Mass spectrometry is a vital tool for molecular characterization, and the allied technique of ion mobility is enhancing many areas of (bio)chemical analysis. Strong synergy arises between these two techniques because of their ability to ascertain complementary information about gas-phase ions. Ion mobility separates ions (from small molecules up to megadalton protein complexes) based on their differential mobility through a buffer gas. Ion mobility-mass spectrometry (IM-MS) can thus act as a tool to separate complex mixtures, to resolve ions that may be indistinguishable by mass spectrometry alone, or to determine structural information (for example rotationally averaged cross-sectional area), complementary to more traditional structural approaches. Finally, IM-MS can be used to gain insights into the conformational dynamics of a system, offering a unique means of characterizing flexibility and folding mechanisms. This Review critically describes how IM-MS has been used to enhance various areas of chemical and biophysical analysis.

Contents lists available at ScienceDirect
Animal Behaviour
journal homepage: www.elsevier.com/locate/anbehav

Special Issue: Biochemistry & Animal Communication

Sex pheromones are not always attractive: changes induced by learning and illness in mice

Enrique Lanuza [a,*], Ana Martín-Sánchez [a], Pau Marco-Manclús [a], Bernardita Cádiz-Moretti [a], Lluís Fortes-Marco [a], Adoración Hernández-Martínez [a], Lynn McLean [b], Robert J. Beynon [b], Jane L. Hurst [b], Fernando Martínez-García [a]

[a] Laboratori de Neuroanatomia Funcional i Comparada, Departaments de Biologia Cel·lular i de Biologia Funcional, Facultat de Ciències Biològiques, Universitat de València, València, Spain
[b] Institute of Integrative Biology, University of Liverpool, Liverpool, U.K.

Research Article
Received: 25 January 2014 Revised: 27 February 2014 Accepted: 27 February 2014 Published online in Wiley Online Library
Rapid Communications in Mass Spectrometry
Rapid Commun. Mass Spectrom. **2014**, *28*, 1107–1116
(wileyonlinelibrary.com) DOI: 10.1002/rcm.6884

Probing the exposure of the phosphate group in modified amino acids and peptides by ion-molecule reactions with triethoxyborane in Fourier transform ion cyclotron resonance mass spectrometry

Francesco Lanucara[1,2]*, Simonetta Fornarini[3], Claire E. Eyers[2] and Maria Elisa Crestoni[3]

[1]Manchester Institute of Biotechnology, School of Chemistry, University of Manchester, Manchester M1 7DN, UK
[2]Institute of Integrative Biology, University of Liverpool, Liverpool L69 7ZB, UK
[3]Dipartimento di Chimica e Tecnologie del Farmaco, 'La Sapienza Universita' di Roma, P. le Aldo Moro 5, 00185 Roma, Italy

RATIONALE: Intramolecular hydrogen bonds between a phosphate group and charged residues play a crucial role in the chemistry of phosphorylated peptides, driving the species to specific conformations and affecting the exposure of the phosphate moiety. The nature and extent of these interactions can be investigated by measuring the reactivity of phosphate groups toward selected substrates in the gas phase.
METHODS: We used Fourier Transform Ion Cyclotron Resonance (FT-ICR) mass spectrometry (MS) to perform a systematic study on the gas-phase ionic reactivity of phosphorylated amino acids and peptides with triethoxyborane (TEB). Ions of interest were generated by electrospray ionization (ESI), isolated in the cell of the FT-ICR mass spectrometer, and allowed to react with a stationary pressure of TEB. The temporal evolution of the reaction was monitored and thermal rate constants were derived. The structure of the ionic products was confirmed by Collision-Induced Dissociation (CID) tandem mass spectrometry (MS/MS).
RESULTS: TEB was found to react with the phosphate of protonated phosphorylated amino acids and peptides by an addition-elimination pathway. The kinetic efficiency of the reaction showed a positive correlation with the charge state of the reagent ion, suggesting the existence of charge-state-dependent exposure of the phosphate groups towards the incoming neutral during the reaction. Isomeric phosphorylated peptides, only differing for the position of the modified serine residue, showed markedly different kinetic efficiencies.
CONCLUSIONS: The ability of a phosphorylated species to react with TEB depends on the ease of access to the phosphate moiety in the corresponding gaseous ion. Measuring the kinetic efficiency of such reactions can represent a valuable tool to explore the accessibility of phosphate groups in biomolecules. Copyright © 2014 John Wiley & Sons, Ltd.

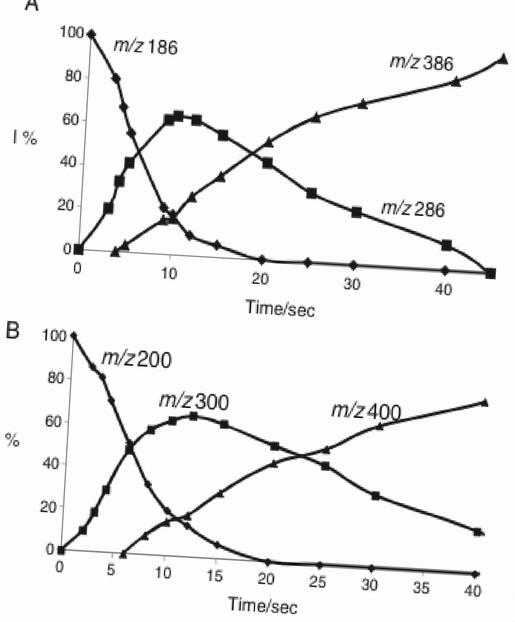


Figure 1. Temporal profiles of the relative ion abundances (I%) when (A) phosphoserine (*m/z* 186) and (B) phosphothreonine (*m/z* 200) are allowed to react with triethoxyborane at a pressure of 4.3×10^{-8} mbar.

The major urinary protein system in the rat

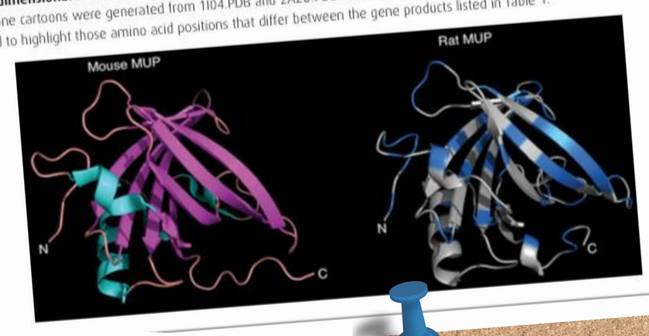
Guadalupe Gómez-Baena*[1], Stuart D. Armstrong*, Marie M. Phelan†, Jane L. Hurst‡ and Robert J. Beynon*

*Protein Function Group, Institute of Integrative Biology, University of Liverpool, Crown Street, Liverpool L69 7ZB, U.K.
‡Mammalian Behaviour and Evolution Group, Institute of Integrative Biology, University of Liverpool, Crown Street, Liverpool L69 7ZB, U.K.
†NMR Centre, Institute of Integrative Biology, University of Liverpool, Crown Street, Liverpool L69 7ZB, U.K.

Abstract

The genomes of rats and mice both contain a cluster of multiple genes that encode small (18–20 kDa) eight-stranded β-barrel lipocalins that are expressed in multiple secretory tissues, some of which enter urine via hepatic biosynthesis. These proteins have been given different names, but are mostly generically referred to as MUPs (major urinary proteins). The mouse MUP cluster is increasingly well understood, and, in particular, a number of roles for MUPs in chemical communication between conspecifics have been established. By contrast, the literature on the rat orthologues is much less well developed and is fragmented. In the present review, we summarize current knowledge on the MUPs from the Norway (or brown) rat, *Rattus norvegicus*.

Figure 3 | Three-dimensional structures of mouse and rat MUPs
Backbone cartoons were generated from 1I04.PDB and 2A2U.PDB for mouse and rat respectively. For the rat structure, blue is used to highlight those amino acid positions that differ between the gene products listed in Table 1.



OPEN ACCESS Freely available online
PLOS ONE

The Structure, Stability and Pheromone Binding of the Male Mouse Protein Sex Pheromone Darcin

Marie M. Phelan[1], Lynn McLean[2], Stuart D. Armstrong[2], Jane L. Hurst[3], Robert J. Beynon[2], Lu-Yun Lian[1]*

1 NMR Centre for Structural Biology, Institute of Integrative Biology, University of Liverpool, Liverpool, United Kingdom, **2** Protein Function Group, Institute of Integrative Biology, University of Liverpool, Liverpool, United Kingdom, **3** Mammalian Behaviour & Evolution Group, Institute of Integrative Biology, University of Liverpool, Leahurst Campus, Neston, United Kingdom

Abstract

Mouse urine contains highly polymorphic major urinary proteins that have multiple functions in scent communication through their abilities to bind, transport and release hydrophobic volatile pheromones. The mouse genome encodes for about 20 of these proteins and are classified, based on amino acid sequence similarity and tissue expression patterns, as either central or peripheral major urinary proteins. Darcin is a male specific peripheral major urinary protein and is distinctive in its role in inherent female attraction. A comparison of the structure and biophysical properties of darcin with MUP11, which belongs to the central class, highlights similarity in the overall structure between the two proteins. The thermodynamic stability, however, differs between the two proteins, with darcin being much more stable. Furthermore, the affinity of a small pheromone mimetic is higher for darcin, although darcin is more discriminatory, being unable to bind bulkier ligands. These attributes are due to the hydrophobic ligand binding cavity of darcin being smaller, caused by the presence of larger amino acid side chains. Thus, the physical and chemical characteristics of the binding cavity, together with its extreme stability, are consistent with darcin being able to exert its function after release into the environment.

Citation: Phelan MM, McLean L, Armstrong SD, Hurst JL, Beynon RJ, et al. (2014) The Structure, Stability and Pheromone Binding of the Male Mouse Protein Sex Pheromone Darcin. PLoS ONE 9(10): e108415. doi:10.1371/journal.pone.0108415

Editor: Paulo Lee Ho, Instituto Butantan, Brazil

Received April 3, 2014; **Accepted** August 26, 2014; **Published** October 3, 2014

Data Availability: The authors confirm that all data underlying the findings are fully available without restriction. All relevant data are within the paper, its Supporting Information files and protein structure coordinates are found in the Protein Data Bank under the accession codes 2L9C and 2LB6.

Funding: This work was funded by BBSRC LOLA grant [BB/J002631/1] to JLH, RJB and LYL. The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* Email: Lu-Yun.Lian@liverpool.ac.uk

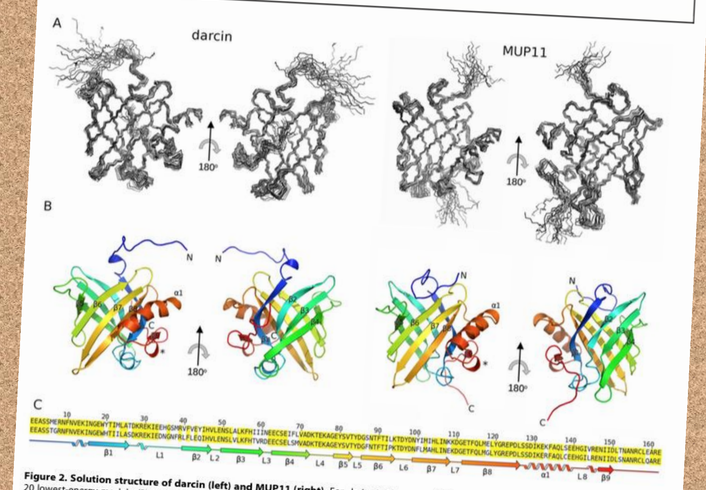


Figure 2. Solution structure of darcin (left) and MUP11 (right). For clarity 180° representations are shown. (A) The ensembles each comprise 20 lowest-energy models. (B) For each ensemble a representative closest-to-mean structure was selected and shown as a cartoon representation of the structural elements. Marked in asterisk is the conserved 3_{10}-helix between α1 and β9. (C) Alignment of the primary sequence of darcin (top) with MUP11 (bottom) with conserved residues highlighted in yellow. The structural schematic for darcin is coloured to correlate with the colouring on the cartoon representation shown in (B), from N to C terminus as blue to red. In (C), the S-S bridge between C64 and C157 is indicated as black lines linking the two residues.
doi:10.1371/journal.pone.0108415.g002

Carcinogenesis vol.35 no.8 pp.1798–1806, 2014
doi:10.1093/carcin/bgu086
Advance Access publication April 7, 2014

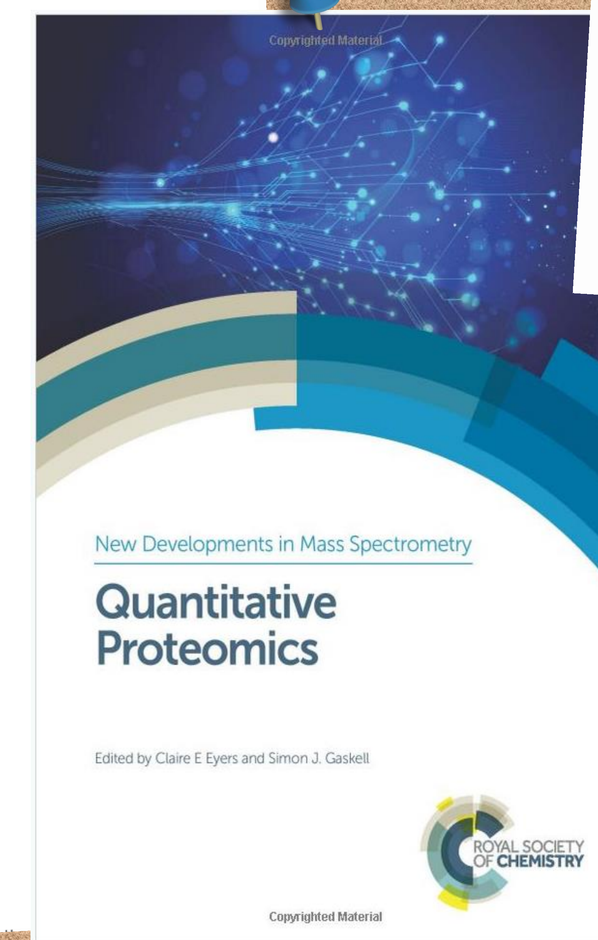
The neuroendocrine phenotype of gastric myofibroblasts and its loss with cancer progression

Silvia Balabanova[1], Chris Holmberg[1], Islay Steele[1], Bahram Ebrahimi[2], Lucille Rainbow[2], Ted Burdyga[1], Cathy McCaig[1], Lazso Tiszlavicz[3], Nantaporn Lertkowit[1], Olivier T.Giger[1], Simon Oliver[1], Ian Prior[1], Rod Dimaline[1], Deborah Simpson[2], Rob Beynon[2], Peter Hegyi[4], Timothy C.Wang[5], Graham J.Dockray[1] and Andrea Varro[1,6,*]

[1]Department of Cellular and Molecular Physiology, Institute of Translational Medicine, University of Liverpool, Liverpool, L69 3BX UK, [2]Institute of Integrative Biology, University of Liverpool, Liverpool, L69 3BX UK, [3]Department of Pathology and [4]Department of Medicine, University of Szeged, Szeged, H-6701 Hungary, [5]Department of Medicine, Columbia University, New York, NY 10032-3802, USA and [6]Department of Molecular and Clinical Cancer, Institute of Translational Medicine, University of Liverpool, Liverpool, L69 3BX UK

*To whom correspondence should be addressed. Departments of Molecular and Cellular Physiology and Molecular and Clinical Cancer Medicine, Institute of Translational Medicine, University of Liverpool, Crown Street, Liverpool L69 3BX, UK. Tel: +44(0) 151 794 5331; Fax: +44(0) 151 794 5315;
Email: avarro@liv.ac.uk

Stromal cells influence cancer progression. Myofibroblasts are an important stromal cell type, which influence the tumour microenvironment by release of extracellular matrix (ECM) proteins, proteases, cytokines and chemokines. The mechanisms of secretion are poorly understood. Here, we describe the secretion of marker proteins in gastric cancer and control myofibroblasts in response to insulin-like growth factor (IGF) stimulation and, using functional genomic approaches, we identify proteins influencing the secretory response. IGF rapidly increased myofibroblast secretion of an ECM protein, TGFβig-h3. The secretory response was not blocked by inhibition of protein synthesis and was partially mediated by increased intracellular calcium (Ca^{2+}). The capacity for evoked secretion was associated with the presence of dense-core secretory vesicles and was lost in cells from patients with advanced gastric cancer. In cells responding to IGF-II, the expression of neuroendocrine marker proteins, including secretogranin-II and proenkephalin, was identified by gene array and LC-MS/MS respectively, and verified experimentally. The expression of proenkephalin was decreased in cancers from patients with advanced disease. Inhibition of secretogranin-II expression decreased the secretory response to IGF, and its over-expression recovered the secretory response consistent with a role in secretory vesicle biogenesis. We conclude that normal and some gastric cancer myofibroblasts have a neuroendocrine-like phenotype characterized by Ca^{2+}-dependent regulated secretion, dense-core secretory vesicles and expression of neuroendocrine marker proteins; loss of the phenotype is associated with advanced cancer. A failure to regulate myofibroblast protein secretion may contribute to cancer progression.



Comparative study of the molecular variation between 'central' and 'peripheral' MUPs and significance for behavioural signalling

Marie M. Phelan*[1], Lynn McLean†, Jane L. Hurst‡, Robert J. Beynon† and Lu-Yun Lian*

*NMR Centre for Structural Biology, Institute of Integrative Biology, University of Liverpool, Liverpool L69 7ZB, U.K.
†Protein Function Group, Institute of Integrative Biology, University of Liverpool, Liverpool L69 7ZB, U.K.
‡Mammalian Behaviour and Evolution Group, Institute of Integrative Biology, University of Liverpool, Liverpool L69 7ZB, U.K.

Abstract

MUPs (major urinary proteins) play an important role in chemical signalling in rodents and possibly other animals. In the house mouse (*Mus musculus domesticus*) MUPs in urine and other bodily fluids trigger a range of behavioural responses that are only partially understood. There are at least 21 *Mup* genes in the C57BL/6 mouse genome, all located on chromosome 4, encoding sequences of high similarity. Further analysis separates the MUPs into two groups, the 'central' near-identical MUPs with over 97% sequence identity and the 'peripheral' MUPs with a greater degree of heterogeneity and approximately 20–30% non-conserved amino acids. This review focuses on differences between the two MUP sub-groups and categorizes these changes in terms of molecular structure and pheromone binding. As small differences in amino acid sequence can result in marked changes in behavioural response to the signal, we explore the potential of single amino acid changes to affect chemical signalling and protein stabilization. Using analysis of existing molecular structures available in the PDB we compare the chemical and physical properties of the ligand cavities between the MUPs. Furthermore, we identify differences on the solvent exposed surfaces of the proteins, which are characteristic of protein-protein interaction sites. Correlations can be seen between molecular heterogeneity and the specialized roles attributed to some MUPs.

Behaviour Meets Biochemistry: Animals Making Sense of Molecules Making Scents

A joint Biochemical Society–Association for the Study of Animal Behaviour Focused Meeting held at Charles Darwin House, London, U.K., 18–20 February 2014. Organized and Edited by Rob Beynon and Jane Hurst (University of Liverpool, U.K.).

The complexity of protein semiochemistry in mammals

Robert J. Beynon*[1], Stuart D. Armstrong*, Guadalupe Gómez-Baena*, Victoria Lee*, Deborah Simpson*, Jennifer Unsworth* and Jane L. Hurst†

*Protein Function Group, Institute of Integrative Biology, University of Liverpool, Liverpool L69 7ZB, U.K.
†Mammalian Behaviour and Evolution Group, Institute of Integrative Biology, University of Liverpool, Leahurst Campus, Neston, Cheshire CH64 7TE, U.K.