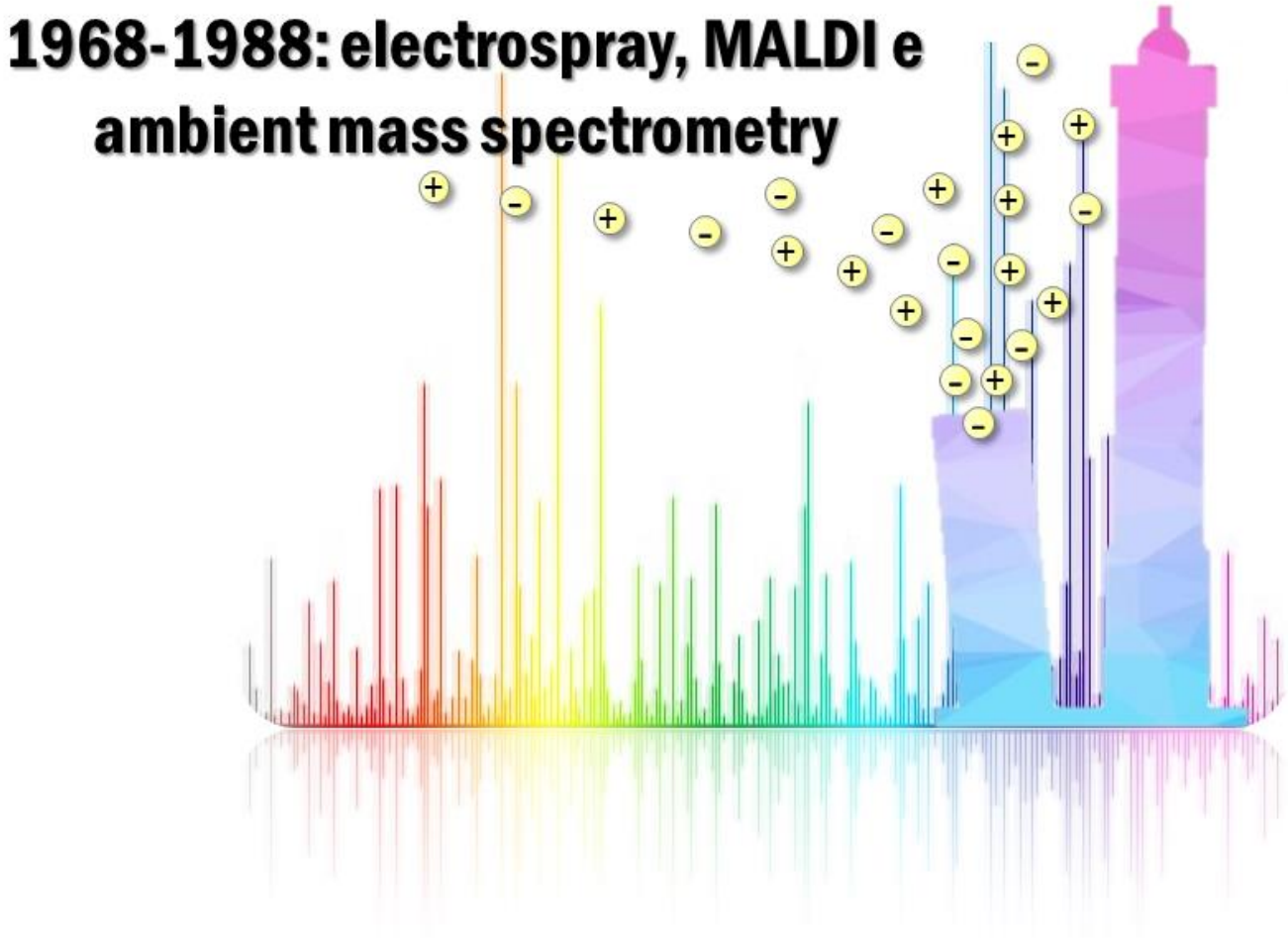
Società Chimica Italiana
Divisione di Spettrometria di Massa

1968-1988: electrospray, MALDI e ambient mass spectrometry



11 dicembre 2018

Aula "Giorgio Prodi"

Università degli Studi di Bologna

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50 anni fa Dole, precorrendo i tempi dell'electrospray, propose il 'Charged Residue Model' nella produzione di macroioni e venti anni dopo, nel 1988, ebbe inizio lo studio di grandi molecole, come le proteine, da parte di John Fenn mediante electrospray e di Michal Karas, Franz Hillenkamp e Koichi Tanaka mediante la ionizzazione MALDI.

THE JOURNAL OF CHEMICAL PHYSICS VOLUME 49, NUMBER 5 1 SEPTEMBER 1968

Molecular Beams of Macroions

MALCOLM DOLE, L. L. MACK, AND R. L. HINES*
Materials Research Center and Department of Chemistry, Northwestern University, Evanston, Illinois
AND
R. C. MOBLEY,† L. D. FERGUSON, AND M. B. ALICE
Bendix Research Laboratories, Southfield, Michigan
(Received 19 April 1968)

By means of electrospraying a dilute polymer solution into an evaporation chamber, negative macroions can be produced and a molecular beam formed by sampling the gaseous mixture of macroions, solvent, and nitrogen molecules with a nozzle-skimmer system of the Kantrowitz–Gray type. The macroion current can be detected by a Faraday cage after the light ions have been repelled from the beam by negative voltages on a repeller grid. Theoretical repeller voltages which best agree with the observed are those calculated by assuming a macroion velocity within 2% of the estimated supersonic beam velocity of 743 m sec^{-1}. Polystyrene macroions of 51 000 weight-average amu tend to form dimers and trimers in the beam while larger polystyrene macroions of 411 000 weight-average amu appear mostly to be multiply charged single species. The results demonstrate that definite mass/charge states can be formed by the electrospray technique, that a considerable monochromatization of macroion velocities in the beam takes place, and that the macroions become highly concentrated relative to low-molecular-weight solvent and nitrogen ions during the transit time in the supersonic beam.

Z. Phys. D – Atoms, Molecules and Clusters 10, 361–368 (1988)

Atoms, Molecules and Clusters
Zeitschrift für Physik D

Of protons or proteins

"A beam's a beam for a' that." (O.S. Burns)

C.K. Meng, M. Mann, and J.B. Fenn
Department of Chemical Engineering, Yale University, New Haven, CT 06520-2159, USA

Received 5 July 1988; final version 28 July 1988

Mass analyses have been carried out on ions produced by an Electrospray (ES) source from dilute solutions of protein molecules with molecular weights (M) in the range from 5000 to nearly 40000. Each spectrum comprises a sequence of peaks corresponding to multiply charged intact parent species. The ions of each peak differ from those of their adjacent neighbors by one unit charge, H^+ in these experiments. The maximum number of charges per ion generally increases with the molecular weight of the parent molecule, reaching a value of 45 in the case of alcohol dehydrogenase, at $M = 39830$ the largest species in this study. Thus the resulting values of m/z are within reach of a simple quadrupole mass filter whose nominal upper mass limit is 1500 daltons! The immediate application for the ES source is in mass spectrometric analysis of large fragile molecules of biochemical importance. But the multiply charged ions it produces are newcomers to the laboratory scene that constitute interesting subjects for study.

PACS: 07.75; 87.80; 82.80

Protein and Polymer Analyses up to *m/z* 100 000 by Laser Ionization Time-of-flight Mass Spectrometry

Koichi Tanaka†, Hiroaki Waki, Yutaka Ido, Satoshi Akita, Yoshikazu Yoshida and Tamio Yoshida
Shimadzu Corporation, Nishinokyo-Kuwabaracho, Nakagyo-ku, Kyoto 604, Japan

SPONSOR REFEREE: T. Matsuo, Osaka University, Osaka, Japan

Hitherto, ^{252}Cf plasma desorption mass spectrometry (PDMS) has been used to study peptides and proteins in the molecular weight range from 1 kDa to 35 kDa.[1,2] Fast atom bombardment mass spectrometry (FABMS) and secondary ion mass spectrometry (SIMS) have been applied to the analyses of proteins and polymers molecules.[3,4] On the other hand, in the area of laser desorption time-of-flight (TOF) mass spectrometry (MS), though there have been many papers on analyses of organic compounds, the molecular weight of these compounds has been relatively low.[5]

Two TOF systems were constructed. The first system utilized a digital wave memory and accumulation circuits. This system could accumulate the spectrum data of 8 K words within 1 ms. In the first place, a "one shot" TOF spectrum was stored into the wave memory, in the subsequent accumulating circuits the spectrum was accumulated in sequence. The second system utilized a constant fraction discriminator (CFD) and a multi-stop time-to-digital converter (TDC). The time intervals between "start" and "stop" pulses were measured with a time resolution of 1 ns. In these experiments, the first

Tanaka K., Waki H., Ido S. et al. *Rapid Commun. Mass Spectrom.*, 151–153 (1988)

CORRESPONDENCE

Laser Desorption Ionization of Proteins with Molecular Masses Exceeding 10 000 Daltons

Sir: Until recently the desorption of ions of bioorganic compounds in the mass range above 10 000 daltons seemed to be exclusively the domain of plasma desorption mass spectrometry (PDMS) (*1–4*). In 1987 Tanaka et. al. (*5*) reported laser desorption of protein molecular ions up to a mass of 34 000 daltons. Oligomers of lysozyme containing up to seven monomeric units have also been observed by this group, using a pulsed N_2 laser and a matrix of a metal powder, finely dispersed in glycerol. Fast atom bombardment or liquid secondary ion mass spectrometry (SIMS) data on compounds above 10 000 molecular weight show weak signal intensities and poor signal-to-noise (S/N) ratios. The only exception is the results reported on the analysis of small proteins in the range of 10–24000 daltons obtained with a 30-keV cesium ion source in a conventional double-focusing mass spectrometer (*6*), but sample amounts in the 10-μg range were necessary

energy of 3 keV and the postacceleration potential was limited to a maximum of 9 kV. The ion velocity of lysozyme (molecular weight 14 306), e.g. at the conversion electrode, amounts to a value of only 1.1×10^4 m/s and is even lower for the higher mass compounds. Note that a minimum velocity of ca. 1.7×10^4 m/s was determined for insulin ion detection in PDMS (*2*). Signals were registered by a Biomation transient recorder with an aquisition memory of only 2048 channels resulting in a very low time resolution in the high mass range (for details see results). Currently this also limits the accuracy of mass calibration, which is done with the sodium and matrix signals in the low mass range. Thus all results have to be understood as a documentation of the general feasibility of the technique for the generation of high molecular mass ions.

RESULTS AND DISCUSSION

Spectra of four proteins are reported here: lysozyme (from chicken egg white, molecular weight 14 306), β-lactoglobulin

Karas M., Hillenkamp F. . *Anal. Chem.* **60**, 2299–2301 (1988)

Da quegli anni molta strada è stata fatta!!

Innovazioni strumentali, perfezionamento di tecniche esistenti, introduzione di nuovi metodi di ionizzazione, molti dei quali ambient, si susseguono senza sosta.

COMITATO SCIENTIFICO

Gianluca Bartolucci, Università di Firenze

Cecilia Bergamini, ARPAE Emilia Romagna

Giuliana Bianco, Università della Basilicata

Maria Fiorenza Caboni, Università di Bologna

Donatella Caruso, Università di Milano

Roberta Galarini, IZSUM, Perugia

Gianluca Giorgi, Università di Siena

Fulvio Magni, Università di Milano Bicocca

Giorgio G. Mellerio, Università di Pavia

Michele Suman, Barilla Spa

Con il patrocinio di

Organizzato in collaborazione con:

PROGRAMMA SCIENTIFICO

9:30 Registrazione dei partecipanti e coffee break

10:00 Saluto
Intervento delle aziende

10:20-12:30 **Sessione 1**

Chairs: Donatella Caruso, Fulvio Magni

10:20-11:00 **ALI PER ELEFANTI MOLECOLARI**

Giorgio Giacomo Mellerio

Università degli Studi di Pavia

11:00-11:20 **NEW FRONTIERS IN PATHOLOGY: MALDI-MS IMAGING FOR DIAGNOSIS OF THYROID FINE NEEDLE ASPIRATION BIOPSIES**

Isabella Piga [1,2], ***Giulia Capitoli*** [3], ***Vanna Denti*** [1], ***Andrew Smith*** [1], ***Clizia Chinello*** [1], ***Stefania Galimberti*** [3], ***Fulvio Magni*** [1], ***Fabio Pagni*** [2]

[1] Department of Medicine and Surgery, University of Milano-Bicocca, Clinical Proteomics and Metabolomics Unit, Vedano al Lambro; [2] Department of Medicine and Surgery, University of Milano-Bicocca, Section of Pathology, Monza; [3] Department of Medicine and Surgery, University of Milano-Bicocca, Centre of Biostatistics for Clinical Epidemiology, Vedano al Lambro

11:20-11:40 **INTEGRATIVE MS-BASED PLATFORM FOR BIOENGINEERING & CELL THERAPY: LABEL-FREE NLC-MS/MS AND MALDI IMAGING FOR MOLECULAR AND CELLULAR CHARACTERIZATION OF HUMAN PLATELET LYSATE AND HYPS SCAFFOLDS**

Clizia Chinello [1], ***Federica Re*** [2], ***Camillo Almici*** [3], ***Andrew James Smith*** [1], ***Francesca Paolella*** [4], ***Gina Lisignoli*** [4], ***Isabella Piga*** [1], ***Vanna Denti*** [1], ***Luciana Sartore*** [5], ***Domenico Russo*** [2], ***Fulvio Magni*** [1]

[1]Department of Medicine and Surgery, University of Milano-Bicocca, Clinical Proteomics and Metabolomics Unit, Vedano al Lambro; [2]Unit of Blood Diseases and Stem Cell Transplantation, DPT of Clinical and Experimental Sciences, Brescia University, ASST Spedali Civili Brescia; [3]Laboratory for Stem Cells Manipulation and Cryopreservation, Department of Transfusion Medicine, ASST Spedali Civili of Brescia; [4]SC Laboratorio di Immunoreumatologia e Rigenerazione Tissutale, IRCCS Istituto Ortopedico Rizzoli, Bologna; [5]Department of Mechanical and Industrial Engineering, University of Brescia

11:40-12:00 **L'EVOLUZIONE, NEGLI ANNI, DELLA SORGENTE ESI**

Marco Biglietto

Sciex, Milano

12:00-12:30 **DSM: presente e futuro**

Donatella Caruso

Università degli Studi di Milano

12:30-14:30 Buffet lunch

14:30-17:00 **Sessione 2**

Chairs: Cecilia Bergamini, Gianluca Giorgi

14:30-15:00 **AMBIENT MASS SPECTROMETRY: RECENT DEVELOPMENTS IN IONIZATION SOURCES AND MASS SPECTROMETRY FOR ENVIRONMENTAL, FORENSIC AND MEDICAL APPLICATIONS**

Mario Francesco Mirabelli

Department of Chemistry and Applied Biosciences, ETH Zurich, 8093 Zurich, Switzerland ; CTC Analytics AG, 8006 Zurich, Switzerland

15:00-15:20 **DART & iKnife: RECENTE APPROCCIO PER RISPONDERE ALLE RICHIESTE DI RAPIDITÀ D'ANALISI NEL SETTORE ALIMENTARE**

Andrea Perissi

Waters, Sesto San Giovanni (MI)

15:20-15:40 **APPLICATION OF UHPLC-ESI-QTOF TO CHARACTERIZE *MINIMAS*, A NATURAL COMPLEX FOOD SUPPLEMENT**

Giada Fodaroni, Enrico Flamini, Sara Tamimi, Silvia Bedont, Denise DeCarli, Michela Burico, Anna Gaetano, Luisa Mattoli

Aboca, Sansepolcro (AR)

15:40-16:00 **DEFINIZIONE DEL RUOLO DEGLI ACIDI GRASSI NELLA REGOLAZIONE INTRINSECA DEL DIFFERENZIAMENTO NEURONALE MEDIANTE LC-ESI-MS/MS**

Matteo Audano, Silvia Pedretti, Maurizio Crestani, Emma De Fabiani, Donatella Caruso, Nico Mitro

Dipartimento di Scienze Farmacologiche e Biomolecolari (DiSFeB), Università degli Studi di Milano, Milano

16:00-16:20 **DIRECT ANALYSIS REAL-TIME–HIGH-RESOLUTION MASS SPECTROMETRY FOR TRITICUM SPECIES AUTHENTICATION**

Brunella Miano [1], Roberto Piro [1], Laura Righetti [2], Chiara Dall'Asta [2], Gianni Galaverna [2], Silvia Folloni [3], Michele Suman [4]

[1]Istituto Zooprofilattico Sperimentale delle Venezie, Viale dell'Università 10 – 35020 Legnaro; [2]Department of Food and Drug, University of Parma, via delle Scienze - 43100 Parma; [3]OPENFIELDS; [4]Barilla Food Research Labs, via Mantova 166 - 43122 Parma

16:20-16:40 **DRUG PLASMA STABILITY STUDY OF MULTIDRUG RESISTANCE INHIBITORS BY LC-MS/MS ANALYSIS**

Marta Menicatti, Matilde Maggini, Francesco Caponi, Donato Squillaci, Laura Braconi, Silvia Dei, Elisabetta Teodori, Gianluca Bartolucci

Dipartimento di Neuroscienze, Psicologia, Area del Farmaco e Salute del Bambino Sezione Scienze Farmaceutiche e Nutraceutiche, Università di Firenze, Via U. Schiff 6, 50019 Sesto F.no (FI)

16:40-17:00 **AROMATIC PROFILE DETERMINATION OF DIFFERENT ITALIAN PEACH AND NECTARINE CULTIVARS BY SPME-GC-MS**

Silvia Marzocchi [1], Sara Marziali [2], Federica Pasini [3], Roberto Gregori [1], Claudio Buscaroli [4], Silviero Sansavini [1], Maria Caboni [1]

[1] Department of Agricultural and Food Sciences and Technologies, University of Bologna, Piazza Goidanich 60, 47521 Cesena (FC)

17:00 **Assemblea dei soci DMS**

17:30 **Chiusura dei lavori e AUGURI!!**

ABSTRACTS

Ali per elefanti molecolari

Giorgio G. Mellerio

Dipartimento di Chimica, Università degli Studi di Pavia, via Taramelli, 10 27100 Pavia

Il titolo della comunicazione è tratto dalla Nobel Lecture (2002) di John B. Fenn (1917-2010) pubblicata nel 2003 su *Angew. Chem. Int. Ed*. **42** (33), 3871, (2003) e vuole indicare come prima del 1988 l'idea di rendere "volatili" e analizzabili dalla spettrometria di massa proteine e polimeri fosse reputata improbabile come vedere un elefante volare. In realtà Malcom Dole (1903-1990) comunicò nel 1968 su *Molecular Beams of Macroions* dando origine agli studi che portarono alla elettronebulizzazione e nello stesso 1968 Viktror Tal'rose (1922-2004) cercò di ottenere un sistema LC-MS (*Zhur. Fiz. Khim*., **42**, 3104,3112, (1968) iniziando una lunga serie di ingegnosi tentativi di accoppiamento tra le due tecniche. Gli sviluppi (ESI-MS) dovuti al gruppo di Fenn culminarono nel 1988 nelle applicazioni di identificazione di polipeptidi e proteine di massa molecolare di 40 kDa. Nel 1984 oltre ai lavori di Fenn, si ebbe la pubblicazione dei risultati della ricerca portata avanti dal 1979 dal gruppo russo guidato da Lidija Gall (nata nel 1934) (M.C. Aleksandrov *et al., Dokl. Akad. Nauk*. SSSR, **277**, 379, (1984) ripreso in lingua inglese su *Rapid Comm. Mass Spectrom.,* **22**, 267, (2008) "Extraction of ions from solutions under atmospheric pressure as a method for mass spectrometric analysis of bioorganic compound")

Un cammino altrettanto ingegnoso, intrapreso da diversi gruppi negli anni '80 del secolo scorso, ha assicurato lo sviluppo della tecnica di ionizzazione per desorbimento laser assistita dalla matrice tramite principalmente gli studi di Michael Karas (nato nel 1952) e Franz Hillenkamp (1936 – 2014, inventore del *laser microprobe mass analyzer* LAMMA) culminati nel 1988 e le applicazioni della *Soft Laser Desorption* (SLD) da parte di Koichi Tanaka (Nobel per la chimica nel 2002, nato nel 1959). L'acronimo MALDI fu utilizzato comunemente in anni successivi.

New frontiers in pathology: MALDI-MS Imaging for diagnosis of thyroid fine needle aspiration biopsies

Isabella Piga [1,2], ***Giulia Capitoli*** [3], ***Vanna Denti*** [1], ***Andrew Smith*** [1], ***Clizia Chinello*** [1], ***Stefania Galimberti*** [3], ***Fulvio Magni*** [1], ***Fabio Pagni*** [2]

[1] *Department of Medicine and Surgery, University of Milano-Bicocca, Clinical Proteomics and Metabolomics Unit, Vedano al Lambro*
[2] *Department of Medicine and Surgery, University of Milano-Bicocca, Section of Pathology, Monza*
[3] *Department of Medicine and Surgery, University of Milano-Bicocca, Centre of Biostatistics for Clinical Epidemiology, Vedano al Lambro*

Fine Needle Aspiration biopsy (FNAB) is the gold standard procedure to determine the malignant nature of thyroid nodules. However, approximately 20% of FNABs are diagnosed as "indeterminate for malignancy", and these patients undergo diagnostic thyroidectomy, often unnecessary. MALDI-MSI represents an ideal tool to explore the spatial distribution of proteins directly *in-situ*, integrating molecular and cytomorphological information. This enables the discovery of potential diagnostic markers in thyroid cytopathology. However, contamination of FNAB samples with red blood cells is problematic, given that large amounts of haemoglobin suppress other protein signals in the sample. The first aim of the study was to standardise the sample preparation of *ex-vivo* and *in-vivo* thyroid FNABs for proteomic MALDI-MSI analysis, in order to minimise haemoglobin interference. Three protocols were compared using *ex-vivo* biopsies collected from the same thyroid: (i) conventional air-dried smear; (ii) cytological smear immediately fixed in consecutive washes of 70%-90%-95% EtOH;(iii) liquid based cytological preparation. Then, we investigated the proteomic stability of the samples. *In-vivo* thyroid FNABs were collected from 14 patients (San Gerardo Hospital, Monza, Italy) and transferred into *CytoLyt* solution, centrifuged and re-suspended in *PreservCyt* solution. Cytospin spots have been positioned onto ITO-conductive slides and MALDI-MSI intact proteins analysis was performed using an ultrafleXtreme MALDI-TOF/TOF. Each FNAB was split into several samples in order to evaluate the experimental repeatability (intra-day and inter-day) of the proteomics analysis and the cytological samples stability after 7, 14 days and 2 months in preservative solution at 4°C. Results showed that liquid-based preparation efficiently remove hemoglobin, allow to preserve the proteomic integrity of the sample, and to store sample at 4°C for 14 days in the preservative solution before deposing it onto the conductive slide.
This pilot study represents the first example of MALDI-MSI being applied to *ex-vivo* and *in-vivo* thyroid FNABs, which have been prepared using the liquid-based cytological preparation, for proteomic analysis. Moreover, this protocol allows simple sample collection and shipment to be used not only for the proteomic MALDI-MSI analysis of thyroid FNABs but also for other biological liquid based specimens. Therefore, this study represents a step forward towards the implementation of MALDI-MSI, combined with a trustworthy and robust methodology, into the pathology unit.

This work was funded thanks to AIRC (Associazione Italiana per la Ricerca sul Cancro) MFAG GRANT 2016- Id.18445, FAR 2013–2016.

Integrative MS-based platform for boengineering & cell therapy: label-free nLC-MS/MS and MALDI imaging for molecular and cellular characterization of human platelet lysate and HyPS scaffolds

Clizia Chinello [1], ***Federica Re*** [2], ***Camillo Almici*** [3], ***Andrew James Smith*** [1], ***Francesca Paolella*** [4], ***Gina Lisignoli*** [4], ***Isabella Piga*** [1], ***Vanna Denti*** [1], ***Luciana Sartore*** [5], ***Domenico Russo*** [2], ***Fulvio Magni*** [1]

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[3]*Laboratory for Stem Cells Manipulation and Cryopreservation, Department of Transfusion Medicine, ASST Spedali Civili of Brescia*
[4]*SC Laboratorio di Immunoreumatologia e Rigenerazione Tissutale, IRCCS Istituto Ortopedico Rizzoli, Bologna*
[5]*Department of Mechanical and Industrial Engineering, University of Brescia*

Introduction:
Hydrogel-forming polymeric scaffolds (HyPS) provides a favourable 3D biomimetic and biodegradable environment for Human Mesenchymal Stromal Cells (hMSCs) [1]. Recently, in HyPS engineered with hMSCs, human platelet lysate (hPL) has been shown as highly effective to stimulate the hMSCs cell-growth and osteo-chondral differentiation [2-4]. Despite its proven beneficial effect on cell propagation, the proteins responsible in stimulating these multiple key signaling pathways have been not yet clarified and will be herein investigated by label-free nLC-MS/MS. Moreover, *in situ* proteomic evaluation of *in vitro* models such as HyPS scaffolds bioengineered with chondrogenic differentiated mesenchymal stem cells grown in presence of hPL, was also provided .

Methods:
Quali-quantitative evaluation of hPL (n=4) compared to Platelets Poor Plasma (PPP)(n=4) was achieved by label-free *shotgun* proteomics. IgG and albumin depleted and not depleted samples were trypsinized [5] and analysed by nLC-UHRTOF. Data was submitted to PEAKS studio and Mascot.
Proteomic images of chondrogenic differentiated hMSCs from bone marrow (BM), grown in HyPS scaffolds for 14 days with hPL, were obtained by RapifleX MALDI TissueTyper™ [6].

Results:
About 500 protein IDs for each plasma derivative were identified. An increase in identification power in depleted samples respect that the not depleted counterparty ranging from 15% to 20% was noticed. 59 of them resulted specific of hPL and not present in PPP independently from depletion, representing a possible panel of factors involved in cell stimulating effect of this supplement. Relative quantification highlighted 7 and 23 proteins significantly altered in their abundances comparing hPL vs PPP in not depleted and in depleted samples (fold change≥1.5; $p<0.05$ in at least 4 replicates; ≥2unique peptides). 3 of these differences were in common, including a critical proliferation regulator. The functions and networks of the proteins of interest were investigated and compared. *In situ* proteome evaluation of thin sections of PFA embedded

scaffolds bioengineered with BM- hMSCs, was also provided. Histology evaluation was also performed with H&E. The molecular images of trypsinized samples were co-registered with the stained counterparties and co-localization of specific peptide signals with differentiated cells were enlightened.

Conclusions:
Shotgun analysis and label-free quantification of hPL and PPP allowed to 'proteomically' characterize the supplement and to highlight possible candidates responsible for the beneficial effect onto the proliferation and differentiation of in vitro models. MS imaging analysis of seeded scaffold permitted also to recognize proteomic profiles specific of chondrogenic differentiated cells for *in situ* monitoring cell expansion and maturation.

References:

1. Dey K., Agnelli S., *et al*. *Int. J. Polym. Mater. Polym. Biomat*. (2018).
2. Orlandi C, *et al.Exp Dermatol.* (2018) Mar 31.
3. F. Re *et al*., Abstract, *International Translational and Regenerative Medicine Conference*, 25-27 April (2018), Rome
4. F. Re *et al*., Abstract number: B338, *44th Annual meeting of the European Society for Blood and Marrow Transplantation*, 18-21 March (2018), Lisbon.
5. Raimondo F, *et al. Mol Biosyst*. (2015) Jun;11(6):1708-16.
6. Galli M, *et al. Biochim Biophys Acta.* Jul;1865(7):817-827 (2017)

Fundings:

The research leading to these results has received funding from FAR 2014–2017, Fondazione Gigi & Pupa Ferrari Onlus, UniBs Health & Wealth Project and Fondazione Comunità Bresciana.

Ambient mass spectrometry: recent developments in ionization sources and mass spectrometry for environmental, forensic and medical applications

Mario Francesco Mirabelli

Department of Chemistry and Applied Biosciences, ETH Zurich, 8093 Zurich, Switzerland; CTC Analytics AG, 8006 Zurich, Switzerland

Ambient mass spectrometry certainly represents one of the most important development in the field of analytical chemistry in general, and mass spectrometry in particular. The "ambient revolution" opened new possibilities for the analysis of complex samples (solid, liquid and gasses), with little to no sample preparation and with a much higher throughput compared to conventional chromatographic approaches. Thanks to ambient ionization sources developed in recent years, such as DART and DESI, the direct mass spectrometric analyses of various samples rapidly spread, the driving force behind this interest being the possibility to perform real time sample characterization, skipping chromatographic separation, and greatly simplifying and shortening the whole analytical procedure.

Because of the diversity of the analytes of interest and the sample matrices that need to be investigated, the improvement of the quantitation capabilities of ambient mass spectrometry can only be achieved if several aspects are considered: improvement of current ionization sources and development of novel ones; use of fast sample cleanup strategies to reduce matrix effects; use of smart mass spectrometric techniques to increase specificity.

With this in mind, two flow-through ionization sources, DBDI (dielectric barrier discharge ionization) and cAPPI (capillary atmospheric pressure photoionization) were developed and directly interfaced with solid-phase microextraction (SPME), a very convenient sampling technique which extracts and enriches the analytes of interest from a sample. By using this unique combination of sampling and ionization strategies, chromatography could be avoided in many cases where the complexity of the sample matrix is not extremely high, especially when adequate pre- and post-extraction procedures are employed.

Strategies to reduce ionization suppression effects and a comprehensive outlook of the potential of these and other novel ambient approaches for food, environmental, medical and forensic analyses will be discussed.

Application of UHPLC-ESI-QTOF to characterize *Minimas*, a natural complex food supplement

***Giada Fodaroni*, Enrico Flamini, Sara Tamimi, Stella Bedont, Denise DeCarli, Michela Burico, Anna Gaetano, Luisa Mattoli**

Aboca, Sansepolcro (AR)

Keywords: MiniMas, mass spectrometry, food supplement

Introduction

Food supplements are intended to support, maintain or optimize physiological parameters, therefore the organism's homeostasis. The complex mechanisms that contribute to the maintenance of homeostasis have brought researchers to move from a reductionist to a holistic vision of physiology, biology and medicine. In this *panorama,* natural complex food supplements play a key role as all the compounds presents forms a natural complex system ready to interact at various level with the complexity of human organism [1].

Methods

To reach the global characterization of *Minimas*, different chromatographic methods based on high resolution mass spectrometry have been used. Organic polar and apolar compounds have been analysed using UHPLC combined with electrospray ionisation (ESI) and quadrupole time-of-flight (qTOF) MS and identified by in-house high-purity standard reference compounds database.

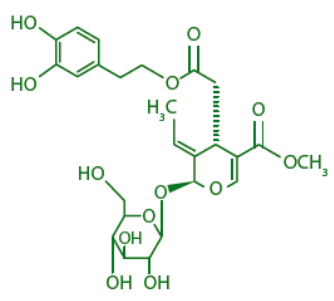
Results

The quality assurance of all natural complex food supplements is a very important goal particularly that of *Minimas,* a food supplement that helps to maintain the homeostasis of blood pressure and cardiac functions.

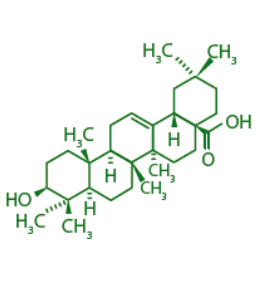
The main ingredients of this natural complex food supplement are Lycium barbarum fruits freeze-dried extract, Olea europaea leaves powder and freeze-dried extract, Crataegus monogyna top flowered powder and freeze-dried extract. In this work, plant dried-extracts ingredients of *Minimas* and *Minimas* itself have been analyzed by UHPLC-ESI-QTOF-MS methods. All the data collected have been matched with an *in-house* library containing more than 1000 natural compounds providing the identification of several compounds, that than have been quantified [2].

Some representative compounds identified and quantified are:

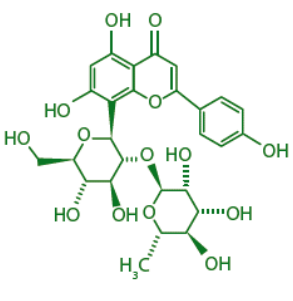
- Oleuropein, (glycosylated seco-iridoid), from Olea europaea
- Oleanolic acid, (phytosterol), from Olea europaea
- Vitexin-2''-O-rhamnoside, (Glycosyl-flavonoid), from of Crataegus monogyna.



Oleuropein



Oleanolic acid



Vitexin 2″-O-rhamnoside

Conclusions

The potency of the developed analytical approach applied to the natural complex food supplement *Minimas* led to its comprehensive characterization, getting a quali-quantitative identity card of its complexity. The analysis performed by different mass spectrometry methods allow to check the presence of all *Minimas* ingredients, opening new perspective to the routine quality control of natural complex food supplements.

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Definizione del ruolo degli acidi grassi nella regolazione intrinseca del differenziamento neuronale mediante LC-ESI-MS/MS

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Introduzione

La neurogenesi è un processo di fondamentale importanza per il corretto sviluppo del sistema nervoso centrale e periferico. Il differenziamento neuronale si svolge in diverse fasi, durante le quali le cellule progenitrici neuronali (NPCs) escono da uno stato di quiescenza dividendosi e acquisendo caratteristiche di cellule neuronali più mature, meglio note come neuroblasti che infine differenziano a neuroni maturi. Diversi studi hanno dimostrato come durante la maturazione neuronale le cellule assumono caratteristiche significativamente differenti a seconda dello stadio differenziativo. Tra esse, il metabolismo energetico rappresenta una variabile significativa: è infatti largamente accettato che le NPCs abbiano un metabolismo caratterizzato principalmente dal consumo di acidi grassi, da un basso consumo di glucosio e ridotta attività mitocondriale. Cellule più mature come i neuroblasti mostrano un metabolismo più ossidativo, basato sul consumo sia di acidi grassi sia di glucosio, mentre i neuroni maturi consumano prevalentemente glucosio mediante un metabolismo altamente ossidativo. Tuttavia, nonostante la grande mole di dati disponibili, il ruolo del metabolismo energetico come fattore di controllo intrinseco della staminalità e del differenziamento neuronale rimane tuttora poco caratterizzato.

Metodi

Lo studio è stato condotto utilizzando neuroblasti murini (N2A), tecniche di biologia cellulare, biologia molecolare e spettrometria di massa accoppiata a cromatografia liquida (LC-ESI-MS/MS). In particolare, è stato utilizzato un triplo quadrupolo API4000 (AB Sciex) accoppiato ad HPLC (Agilent) e ad un autocampionatore CTC-PAL HTS (PAL System) per condurre analisi di metabolomica e flussomica mediante l'uso di metaboliti marcati (U-$^{13}C_6$-glucosio, U-$^{13}C_5$-glutammina and U-$^{13}C_{16}$-palmitato).

Risultati

I nostri risultati dimostrano che cellule con una trascrizione del DNA mitocondriale insufficiente (controllo positivo) hanno una capacità differenziativa minore rispetto alle cellule controllo e basano il proprio metabolismo energetico principalmente sul consumo di acidi grassi. Sorprendentemente, cellule wild type esposte ad acido palmitico mostrano caratteristiche simili a cellule con un metabolismo energetico compromesso.

Conclusioni

Il metabolismo energetico, specie degli acidi grassi, è un processo intrinseco fondamentale per regolare lo stato differenziativo dei precursori neuronali.

Direct analysis real-time-high-resolution mass spectrometry for Triticum species authentication

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Due to favourable climate condition, Italy is a prominent producer of different wheat varieties. Several wheat baked goods are produced, but the most typical Italian foods, like pasta, pizza and bread, are made of durum and common wheat flour. Because of the great importance of wheat in the Italian food market, authenticity represents an essential quality parameter not only for the producers and regulatory bodies but also for consumers.
The aim of our study was to test the effectiveness of an unconventional non-targeted method for the discrimination of Triticum species using direct analysis real time–high-resolution mass spectrometry (DART–HRMS).
For this purpose, 60 wheat samples including durum, common and hulled wheat varieties were collected over two consecutive harvest years. Chemometric evaluation revealed an optimal sample clustering according to the wheat species and the presence of 18 significant markers able to discriminate the groups. The discrimination power obtained is promising since the use of DART–HRMS can significantly reduce the analysis time compared to chromatographic techniques.
A plausible future commercial and industrial scenario could see the application of this analytical approach especially to evaluate the risk of substitution of higher value wheat species with lower value flours.

Drug plasma stability study of multidrug resistance inhibitors by LC-MS/MS analysis

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The Multidrug Resistance (MDR), considered in this study, can be defined as the acquired resistance to the action of chemotherapeutic drugs, not correlated either by their chemical structures or by action mechanisms. The classic MDR is associated with the over-expression of P-glycoprotein (P-gp), an extrusion pump, expressed in many tissues, that eject a variety of antineoplastic drugs from the cells, lowering their concentrations below the necessary for anticancer action. In order to limit this effect the modulation or inhibition of the functions of P-gp with a new class of substances named MDR inhibitors is necessary. The aim of my thesis is the study of the chemical stability of a series of active compounds on MDR. The studied compounds show many common structural characteristics, they are constituted by two lateral aromatic moieties linked to a basic nitrogen atom by two alkylic chains of different length. The aromatic rings used in this series of compounds were: the trimethoxycinnamic residue, common to all the molecules, while the other aromatic portion can be constituted by a structure between the carboxiantracenic or the trimethoxybenzoic acids. Unfortunately the studied compounds contain ester groups in their structure that may be susceptible to hydrolysis by the plasma enzymes, modifying their bioavailability. Therefore, stability tests were carried out for all the new MDR inhibitors in phosphate-buffered solutions (PBS) and human plasma. The PBS solution was included in this study to evaluate the spontaneous hydrolysis suffered to the studied compounds by pH or the ionic strength of the physiological solution. The experiments of *Drug Stability* were carried out by the addition of each analyte to 100 µL of PBS or human plasma samples (1 mM final concentration). The obtained solutions were incubated at 37°C for different times (0, 30, 60 and 120 min) and analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS) method. The LC-MS/MS methods are considered the best choice for the quantitative determination of drugs and their metabolites in biological matrices in terms of sensitivity and specificity. In order to follow the degradation profiles of these MDR inhibitors, it was monitored the variation of the analyte concentration to the different incubation times. Furthermore, to verify that the degradation of compound was due to a hydrolysis mechanism by the esterase enzymes, the related alcohols were searched. This investigation is operated through MS/MS experiments such as *Precursor Ion Scan* and *Multiple Reaction Monitoring*.

Aromatic profile determination of different italian peach and nectarine cultivars by SPME-GC-MS

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Italy has always produced great quality peaches and nectarines. These fruits could be with white or yellow flesh and have very distinct sensory profile. For this reason, the aim of this work was a screening and comparison of different peach and nectarine cultivars of the Emilia-Romagna area, in order to give a pattern recognition of these varieties from their volatile compounds.
In particular, four white peaches varieties ("Buco Incavato", "Bella di Cesena", "Rosa del West", "S. Anna Balducci"), two white nectarines varieties ("Magique" and "Romagna Red") and one yellow nectarine variety ("Big Top") were analysed. The aromatic compounds of the investigated cultivars were extracted by headspace solid phase microextraction (SPME), using a DVB/CAR/PDMS fiber and identified with a gas chromatograph (GC) coupled to a mass spectrometer (MS). Seventeen characteristic peach aroma compounds were identified and quantified using 1-octanol as internal standard. Both in white and yellow nectarines, 2-hexenal was the preponderant compounds, followed by hexanal, 1-hexanol, 3-hexenyl acetate, 2-hexenol and 2-hexenyl acetate. On the other hand, in white peaches the most abundant compound was hexanal, followed by 2-hexenal, 3-hexenyl acetate and 2-hexenyl acetate. Bella di Cesena was the only variety that showed a considerable linalool concentration. Besides, for "Buco Incavato" and "Big Top" the effect of ripening on the volatile components was also evaluated. The results show that maturation affects the volatile profile of peaches and nectarines: both in "Buco Incavato" and "Big Top" the aroma compound concentration decreased from first to the last harvest time. In particular, in the "Big Top" sample was observed an increase of the aroma profile at second harvest time, before the decrease at third and final harvest time.

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