International Journal of Mass Spectrometry 289 (2010) 54–57

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

journal homepage: www.elsevier.com/locate/ijms

Short communication

Top-down protein sequencing by CID and ECD using desorption electrospray ionisation (DESI) and high-field FTICR mass spectrometry

Adam A. Stokes*, David J. Clarke, Stefan Weidt, Pat Langridge-Smith, C. Logan Mackay

SIRCAMS, School of Chemistry, The University of Edinburgh, West Mains Road, Edinburgh EH9 3JJ, Scotland, United Kingdom

ARTICLE INFO

Article history:
Received 13 August 2009
Received in revised form 31 August 2009
Accepted 2 September 2009
Available online 11 September 2009

Keywords:
Top-down
CID
ECD
FTICR-MS
DESI

ABSTRACT

We report high resolution spectra for the medium molecular weight proteins myoglobin and cytochrome-*c* obtained using a custom desorption electrospray ionisation (DESI) source coupled to a Bruker Daltonics 12 T Apex Qe Fourier transform ion cyclotron resonance mass spectrometer (FTICR-MS). The DESI source was designed for accurate alignment and reproduction of critical geometric variables. A two axis motorised stage was included to enable automated rastering of the sample under the DESI plume. Spectra for the intact proteins have been obtained under single-acquisition conditions and a top-down analysis of cytochrome-*c* was performed using both collision induced dissociation (CID) and electron capture dissociation (ECD) of the isolated $[M+15H]^{15+}$ charge state. The sequence coverage is comparable to that obtained using electrospray ionisation, demonstrating the utility of top-down protein analysis by DESI FTICR-MS.

Desorption electrospray ionisation (DESI) is one of the most exciting developments in mass spectrometry in recent years. It allows the ionisation of solid phase samples deposited on almost any surface and at ambient temperature and pressure. DESI was invented in the labs of Professor R. Cooks at Purdue University [1] with subsequent development and improvement by a number of groups around the world, including our own. Many groups have investigated the use of DESI for analysis of small inorganic molecules [2] and although some research has been conducted into analysis of biomolecules this has, for the most part, been confined to lipids and peptides [3–5]. The possibility of analysing biomolecules ranging in molecular weight from hundreds to tens of thousands of Daltons and at biologically important temperatures and pressures means that DESI is potentially a very important and powerful technique for analysing proteins. This analysis could be simple measurement of the molecular mass or by using MS/MS techniques, elucidation of the amino acid sequence and identification of sites of post-translational modifications. Recent publications using ambient pressure ionisation techniques include; DART [6], ASAP [7], MALDESI [8–10], LAESI [11] and IR-LADESI [12] and each of these techniques allows ionisation at ambient pressure with little or no sample preparation.

As described in this paper, we have designed, built and tested a versatile DESI source for top-down protein analysis using a 12 T Bruker Daltonics Apex Qe FTICR mass spectrometer. This has enabled direct analysis of intact proteins to be performed with minimal sample preparation. In a previous study, Takats et al. [13] reported a top-down DESI FTICR analysis of a synthetic polypeptide containing 36 residues. Our work presented here on myoglobin builds on the work by Basile and co-workers [14] and demonstrates that it is possible to produce a DESI signal from an intact protein with sufficient magnitude over an extended period of time (~10 min) such that top-down sequencing by DESI FTICR can be performed on proteins with a molecular weight of at least 12 kDa. We report single acquisition DESI FTICR mass spectra for both myoglobin and cytochrome-*c* that correspond to picomole sample consumption. In addition, for cytochrome-*c* we present top-down DESI FTICR mass spectra obtained using both collision induced dissociation (CID) and electron capture dissociation (ECD).

1. Experimental

1.1. Reagents

The materials used in this work, protein standards (which were used as model samples) and consumables required for sample preparation, such as solvents and acids, are listed below.

Apo-myoglobin and cytochrome-*c* (Cat# 10K7026 and 065K7001) were purchased from Sigma–Aldrich (St. Louis, MI, USA), formic acid (Cat# 94318) was purchased from Fluka. LC–MS grade methanol, water and acetonitrile (Cat# M/4062/17, W/0112/15 and A/0638/17) were purchased from Fisher Scientific.

* Corresponding author. Tel.: +44 1315613048; fax: +44 1316506453.
E-mail address: a.a.stokes@ed.ac.uk (A.A. Stokes).

1387-3806/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijms.2009.09.004

1.2. Mass spectrometry

In order to record DESI FTICR mass spectra of the intact proteins, myoglobin and cytochrome-*c* were dissolved at a concentration of 1 mg/mL in H_2O:MeOH (1:1). 50 μL of these solutions were applied to the etched side of a glass microscope slide, which was used as the DESI target plate. The slide was then incubated at room temperature to evaporate the solvent.

The tandem mass spectra presented for cytochrome-*c* required an increased sample concentration. In this case the protein (2 mg/mL) was dissolved in H_2O:MeOH (1:1) and 200 μL of the solution was applied to target plate and the solvent allowed to evaporate.

The etched glass microscope slides were mounted on the XY stage of the DESI source using a spring loaded clamp. Further details of the DESI source are given below. The critical geometrical DESI parameters, as defined by Takats et al. [15] were determined empirically, each being co-varied until the maximum signal was obtained. A solution of methanol, water and formic acid (1:1:0.001) was used as the spray solution, 3.5 kV was applied to the spray needle and approximately 2.5 L/min N_2 was used as the nebulising gas.

The geometrical source parameters used to obtain tandem mass spectra were identical to those used to record spectra for the intact proteins. The mass resolving quadrupole was set to transmit only those ions that corresponded to the $[M+15H]^{15+}$ charge state of cytochrome-*c* (*m*/*z* 824), and the sample target plate was rastered beneath the DESI probe. In order to acquire the ECD spectrum, the electron source (Heatwave Technologies) in the FTICR cell was conditioned by applying 1.8 A overnight to the cathode filament. To record the spectrum the ECD lens was set to 30 V, and a pulse length of 6 ms and ECD bias of 1.2 V was employed. For the CID spectrum the collision voltage was set between 20 and 35 V.

Fragmentation spectra were acquired at a FID size of 512 kword and were the sum of 200 acquisitions. Data analysis was performed using Bruker Daltonics DataAnalysis 3.4 software. The SNAP 2.0 algorithm was used to generate fragment mass lists and the resultant top-down fragment mass lists were searched against Prosight PTM-2.0 [16].

2. Results and discussion

2.1. DESI source design and optimisation

Autodesk Inventor (Autodesk Inc., CA) was used to design a versatile DESI source to interface with the Bruker Daltonics Apex Qe 12 T FTICR mass spectrometer. Modelling the source design permitted rapid design iteration and fault finding. Fig. 1 shows the final design fitted to the mass spectrometer.

The stepper controlled XY stage was sourced from a Bruker Daltonics Ultraflex MALDI instrument and implemented as a structural component of the DESI frame. Software control of the XY sample stage was achieved using Enhanced Machine Controller 2 to generate step and direction inputs to the motor control driver boards.

As previously mentioned DESI has critical geometrical parameters which greatly influence the ionisation efficiency. To provide a high degree of reproducibility a solid frame was used to hold both the ESI sprayer and sample stage. The spray needle was mounted in an insulated XYZ Θ micromanipulator equipped with vernier scales for accurate measurement of angles and distances. To allow the XY sample stage to raster below the mass spectrometer source inlet a hybrid glass-brass capillary with a stainless steel extension was employed. This consisted of a conventional glass-capillary modified to include a solid brass end-cap and a 1/16 in. outer

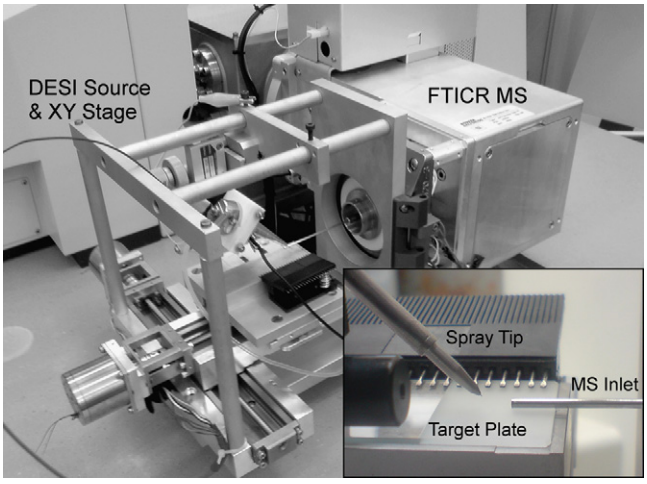

Fig. 1. DESI source mounted on Bruker Daltonics 12 T Apex Qe FTICR mass spectrometer. Insert shows a close-up view of the DESI spray needle, target plate and capillary inlet extension.

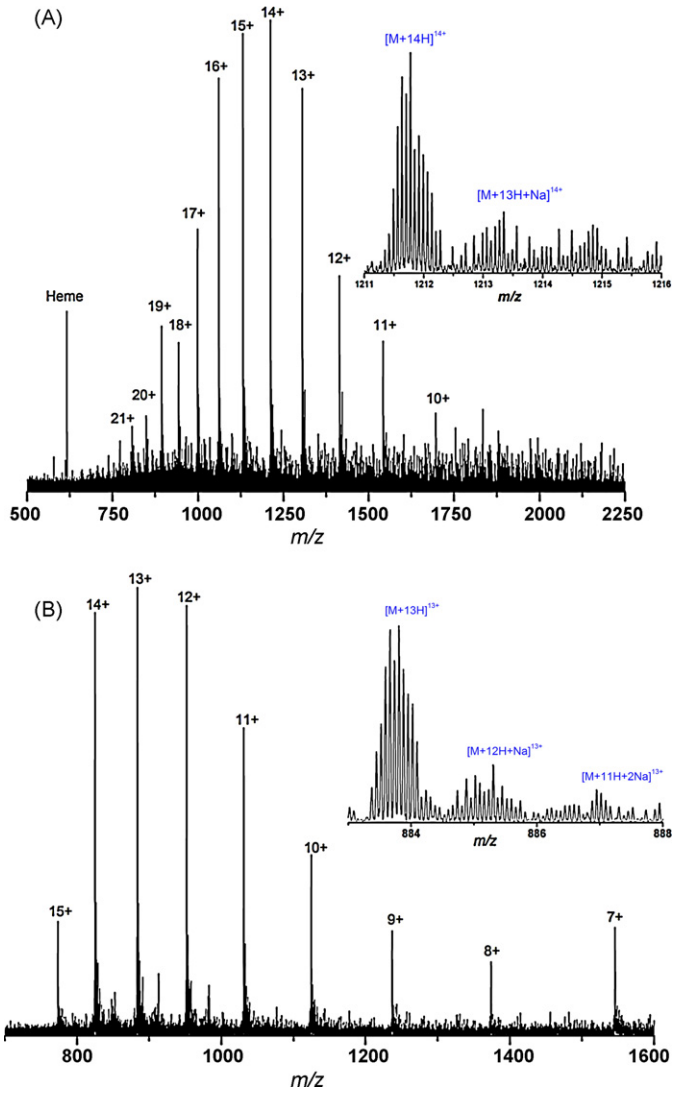

Fig. 2. Single scan DESI FTICR mass spectra for (A) myoglobin and (B) cytochrome-*c* from an etched glass substrates. Inserts show an expanded view of the most abundant charge states.

diameter–0.020 in. inner diameter stainless insert (Upchurch). The stainless insert was recessed 3 mm into the glass and extended 80 mm out through the brass end-cap.

The critical geometrical DESI source parameters, as described by Cooks et al. [1] were co-varied empirically until the largest ion-abundance was found. Nebulising gas pressure, electrospray voltage and solvent flow rate were kept constant at 3.5 kV, 100 psi and 3 μL/min respectively. The insert in Fig. 1 shows a close-up view of the DESI source, with the correct positioning of the critical geometrical parameters for the incident and collection angles, and distances. Interestingly the ion-abundance increased with the distance from the spray-tip to the mass spectrometer inlet, this may be due to the increase in time for desolvation of the nebulised droplets.

2.2. Intact protein analysis using DESI FTICR-MS

Samples were prepared and the DESI source and instrument parameters were set up as described above.

Fig. 2 shows the intact mass spectra obtained for myoglobin (16.9 kDa) and cytochrome-*c* (12.4 kDa) under mild denaturing conditions. These spectra correspond to a single-acquisition. The insert mass spectra show an expansion of the most abundant charge states, $[M+14H]^{14+}$ and $[M+13H]^{13+}$ for myoglobin and cytochrome-*c* respectively, These spectra have a FWHM resolution of 57,000 at 611 *m/z* (cytochrome-*c*) and 148,000 at 774 *m/z* (myoglobin), thus demonstrating the intrinsically high resolving power that can be achieved by DESI FTICR mass spectrometry.

Knowing the concentration and volume of sample deposited on the DESI target plates, together with the area over which the sample wets (ca. 500 mm^2) and the DESI spot size (ca. 1 mm^2) we calculate that these single-acquisition spectra correspond to approximately 5 pmol of protein sample consumed. A typical ESI experiment by comparison, using typical experimental values (10 μM protein sprayed at 3 μL/min) would require a total sample amount of 0.5 pmol, a factor of 10 less.

2.3. Top-down analysis using DESI FTICR-MS

Figs. 3 and 4 show the ECD and CID tandem mass spectra for cytochrome-*c* obtained by isolating the $[M+15H]^{15+}$ charge state in the mass resolving quadrupole of the FTICR mass spectrometer. Both the ECD and CID spectrum represent an accumulation of 200 acquisitions. Peak assignments were made using the SNAP 2.0 algorithm. In addition, the fragmentation maps obtained by searching Prosight PTM-2.0 [16–19] are shown. The software was able to match 43 (30%) of the 140 ECD peaks picked by the SNAP 2.0 algorithm with an expectation of 2.45×10^{-8}. The algorithm returned 377 peaks from the CID analysis of which 50 (13%) were matched to the theoretical fragmentation map in the database, yielding an expectation of 1.42×10^{-5}.

Once again we can estimate the amount of sample consumed, with the assumption that during the time required for 200 acquisitions the DESI probe is rastered over a maximum sample area of 200 mm^2. This would correspond to approximately 10 nmol of sample consumed. Again by comparison with ESI (10 μM protein

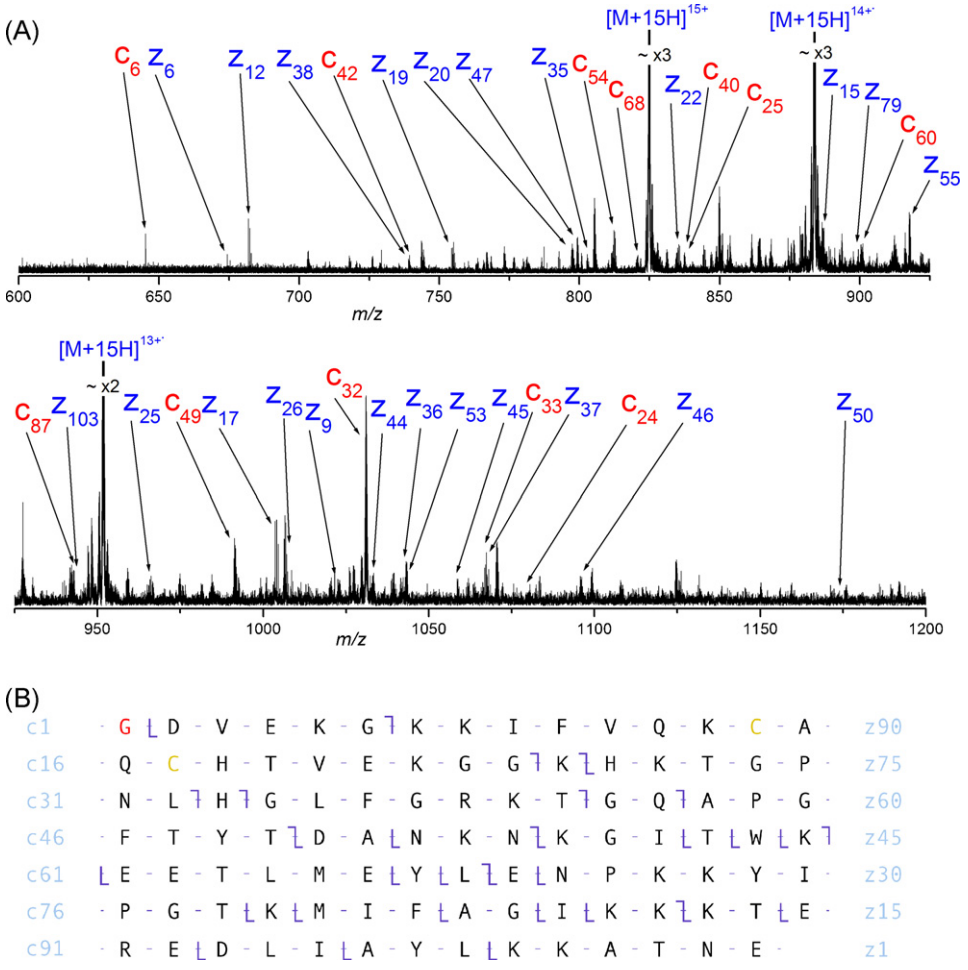

Fig. 3. (A) DESI ECD FTICR mass spectrum obtained for the isolated $[M+15H]^{15+}$ charge state of cytochrome-*c* with assigned fragments labelled. (B) Prosight PTM-2.0 fragment map.

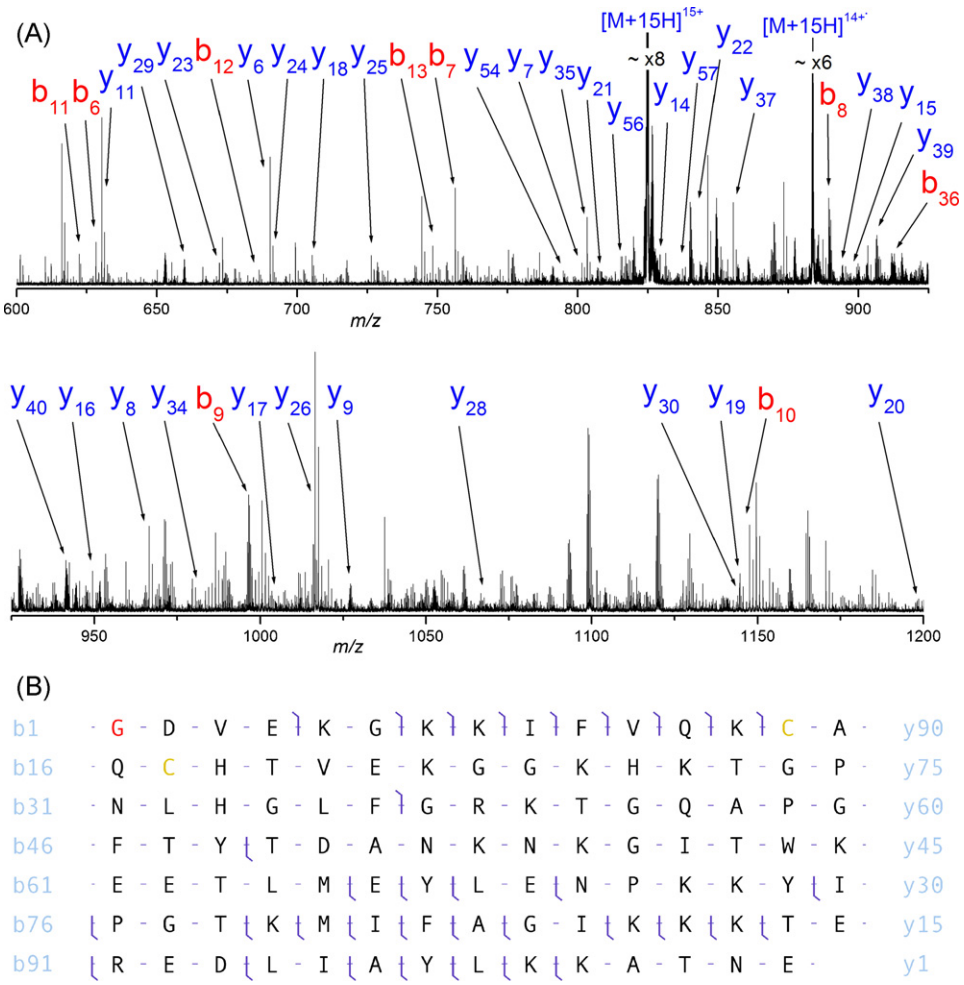

Fig. 4. (A) DESI CID FTICR mass spectrum obtained for the isolated $[M+15H]^{15+}$ charge state of cytochrome-*c* with assigned fragments labelled. (B) Prosight PTM-2.0 fragment map.

sprayed at 3 μL/min and with typically 20 acquisitions required), DESI MSMS requires 10 pmol, approximately 10^3 more sample. As others have shown [20] we find that the quality of mass spectra obtained using DESI is hugely dependent on the source parameters, particularly the respective geometrical orientation and position of the sprayer, sample stage and MS-inlet.

In this work we have shown that top-down sequencing by DESI ECD FTICR mass spectrometry of intact proteins at least a large as 12 kDa is feasible. Clearly, the addition of air amplifiers and/or flared capillaries, which have been described in the literature, to improve the ion collection efficiency from the DESI plume may well extend the mass range and sensitivity of this technique further.

Acknowledgements

The authors would like to thank Mr. Chris Galloway for his assistance with development of the DESI source. The authors gratefully acknowledge the BBSRC/EPSRC RASOR consortium for financial support.

Finally we wish to thank Prof. Neil Kelleher for the use of Prosight PTM-2.0 software.

References

[1] R.G. Cooks, Z. Ouyang, Z. Takats, J.M. Wiseman, Science 311 (2006) 1566–1570.
[2] I. Cotte-Rodriguez, R.G. Cooks, Chem. Commun. (Camb.) (2006) 2968–2970.
[3] M.S. Bereman, L. Nyadong, F.M. Fernandez, D.C. Muddiman, Rapid Commun. Mass Spectrom. 20 (2006) 3409–3411.
[4] S. Myung, J.M. Wiseman, S.J. Valentine, Z. Takats, R.G. Cooks, D.E. Clemmer, J. Phys. Chem. B 110 (2006) 5045–5051.
[5] Y. Song, N. Talaty, W.A. Tao, Z. Pan, R.G. Cooks, Chem. Commun. (Camb). (2007) 61–63.
[6] R.B. Cody, J.A. Laramee, H.D. Durst, Anal. Chem. 77 (2005) 2297–2302.
[7] C.N. McEwen, R.G. McKay, B.S. Larsen, Anal. Chem. 77 (2005) 7826–7831.
[8] J.S. Sampson, A.M. Hawkridge, D.C. Muddiman, J. Am. Soc. Mass Spectrom. 17 (2006) 1712–1716.
[9] J.S. Sampson, K.K. Murray, D.C. Muddiman, J. Am. Soc. Mass Spectrom. 20 (2009) 667–673.
[10] J.S. Sampson, A.M. Hawkridge, D.C. Muddiman, Rapid Commun. Mass Spectrom. 21 (2007) 1150–1154.
[11] P. Nemes, A. Vertes, Anal. Chem. 79 (2007) 8098–8106.
[12] Y.H. Rezenom, J. Dong, K.K. Murray, Analyst 133 (2008) 226–232.
[13] Z. Takats, V. Kobliha, K. Sevcik, P. Novak, G. Kruppa, K. Lemr, V. Havlicek, J. Mass Spectrom. 43 (2008) 196–203.
[14] Y.S. Shin, B. Drolet, R. Mayer, K. Dolence, F. Basile, Anal. Chem. 79 (2007) 3514–3518.
[15] Z. Takats, J.M. Wiseman, R.G. Cooks, J. Mass Spectrom. 40 (2005) 1261–1275.
[16] L. Zamdborg, R.D. LeDuc, K.J. Glowacz, Y.B. Kim, V. Viswanathan, I.T. Spaulding, B.P. Early, E.J. Bluhm, S. Babai, N.L. Kelleher, Nucleic Acids Res. 35 (2007) W701–W706.
[17] N.L. Kelleher, Anal. Chem. 76 (2004) 197A–203A.
[18] R.D. LeDuc, G.K. Taylor, Y.B. Kim, T.E. Januszyk, L.H. Bynum, J.V. Sola, J.S. Garavelli, N.L. Kelleher, Nucleic Acids Res. 32 (2004) W340–W345.
[19] N. Siuti, N.L. Kelleher, Nat. Methods 4 (2007) 817–821.
[20] F.M. Green, P. Stokes, C. Hopley, M.P. Seah, I.S. Gilmore, G. O'Connor, Anal. Chem. 81 (2009) 2286–2293.