Data Dependent Electron Capture Dissociation (ECD) of Phosphorylated Proteins

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Overview

- Although electron capture dissociation (ECD) is known to retain post-translational modifications on peptide backbone fragments, it has yet to be established as a data dependent proteomic method for assigning sites of phosphorylation. Here we ask:

- Can data dependent LC ECD be used to classify sites of phosphorylation in bottom up proteomics?

Introduction

- We show online data dependent liquid chromatography (LC) ECD analysis of tryptic digests of three phospho-proteins (β casein, NEK2A and PKB) analysed on a 7T Thermo Finnigan LTQ FT mass spectrometer.
- The results demonstrate that data dependent ECD is a highly useful tool in the structural elucidation of post-translational modifications (PTMs).
- NEK2A is a cell cycle-regulated kinase of the never in mitosis A family. It is thought NEK2A regulates cohesion between the mother and daughter centriole.
- PKB is a general protein kinase. It is capable of phosphorylating several proteins and is thought to be a key mediator of signal transduction processes.

Method

- β casein (Sigma Aldrich) and kinase proteins NEK2A and PKB (MRC Protein Phosphorylation Unit) were digested with trypsin and diluted to ~40 fmol/µl in 0.1% formic acid. Both PKB and NEK2A were reduced and alkylated prior to digestion with trypsin.
- 200 fmol of sample were injected onto a 75µm C18 reversed phase column (New Objective). Peptides were separated over a 60 or 120 minute gradient from 5 to 60% acetonitrile.
- Samples were infused by use of an external nanospray ionization source into a 7T Thermo Finnigan LTQ FT mass spectrometer. ECD was performed for 100 ms with a cathode potential of -12.55V. Electrons for ECD were produced by a heated dispenser cathode.
- Data were analysed with Xcalibur and Bioworks 3.2 software (Thermo Electron Corp).

Results

MKVLILACLV ALALARELEE LNVPGEIVES LSSSEESITR INKKIEKFQS
EEQQQTEDEL QDKIHPFAQT QSLVYPFPGP IPNSLPQNIP PLTQTPVVVP
PFLQPEVMGV SKVKEAMAPK HKEMPFPKYP VEPFTESQSL TLTDVENLHL
PLPLLQSWMH QPHQPLPPTV MFPPQSVLSL SQSKVLPVPQ KAVPYPQRDM
PIQAFLLYQE PVLGPVRGPF PIIV

45% sequence coverage

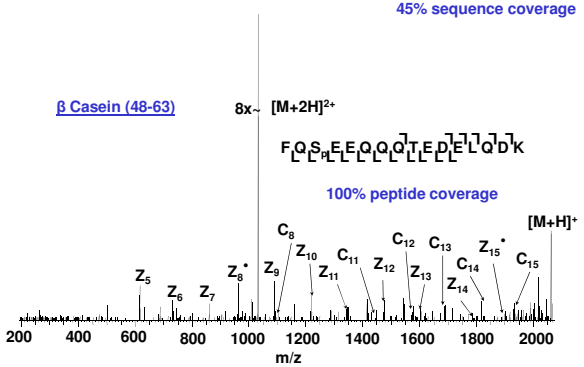

Figure 1: ECD mass spectrum of $[M+2H]^{2+}$ ions of the phosphopeptide (48-63) from β Casein.
$_P$ = Site of phosphorylation

PKB Results

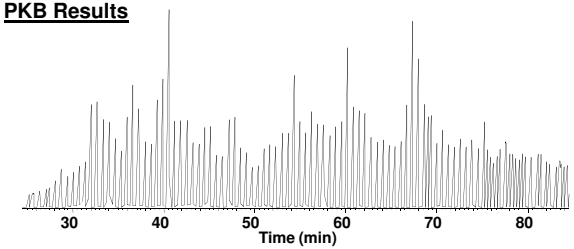

Figure 2: Total ion current (TIC) chromatogram for PKB, 120 minute gradient.

DYDIPTTENL YFQAMGSMDF RSGSPSDNSG AEEMEVSLAK PKHRVTMNEF
EYLKLLGKGT FGKVILVKEK ATGRYYAMKI LKKEVIVAKD EVAHTLTENR
VLQNSRHPFL TALKYSFQTH DRLCFVMEYA NGGELFFHLS RERVFSEDRA
RFYGAEIVSA LDYLHSEKNV VYRDLKLENL MLDKDGHIKI TDFGLCKEGI
KDGATMKTFC GTPEYLAPEV LEDNDYGRAV DWWGLGVVMY EMMCGRLPFY
NQDHEKLFEL ILMEEIRFPR TLGPEAKSLL SGLLKKDPKQ RLGGGSEDAK
EIMQHRFFAG IVWQHVYEKK LSPPFKPQVT SETDTRYFDE EFTAQMITIT
PPDQDDSMEC VDSERRPHFP QFSYSASGTA

36% Sequence coverage

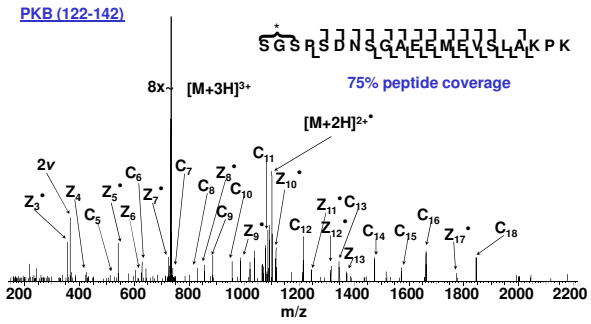

Figure 3: ECD mass spectrum of $[M+3H]^{3+}$ ions of phosphopeptide (122-142) from PKB.
* The site of phosphorylation is localised to Ser 1 or Ser 3.

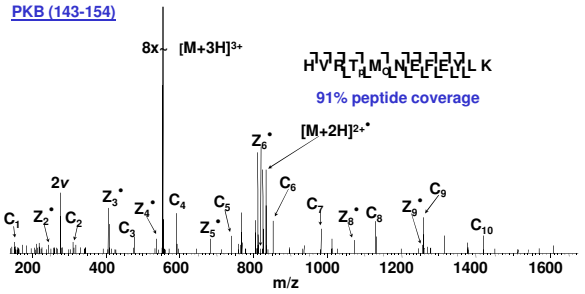

Figure 4: ECD mass spectrum of $[M+3H]^{3+}$ ions of phosphopeptide (143-154) from PKB
v = Harmonic, M_O = Methionine oxidation, $_P$ = Site of phosphorylation

NEK2A Results

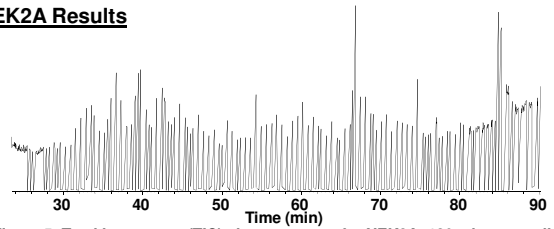

Figure 5: Total ion current (TIC) chromatogram for NEK2A, 120 minute gradient.

MPSRAEDYEV LYTIGTGSYG RCQKIRRKSD GKILVWKELD YGSMTEAEKQ
MLVSEVNLLR ELKHPNIVRY YDRIIDRTNT TLYIVMEYCE GGDLASVITK
GTKERQYLDE EFVLRVMTQL TLALKECHRR SDGGHTVLHR DLKPANVFLD
GKQNVKLGDF GLARILNHDT SFAKTFVGTP YYMSPEQMNR MSYNEKSDIW
SLGCLLYELC ALMPPFTAFS QKELAGKIRE GKFRRIPYRY SDELNEIITR
MLNLKDYHRP SVEEILENPL IADLVADEQR RNLERRGRQL GEPEKSQDSS
PVLSELKLKE IQLQERERAL KAREERLEQK EQELCVRERL AEDKLARAEN
LLKNYSLLKE RKFLSLASNP ELLNLPSSVI KKKVHFSGES KENIMRSENS
ESQLTSKSKC KDLKKRLHAA QLRAQALSDI EKNYQLKSRQ ILGMR

33% Sequence coverage

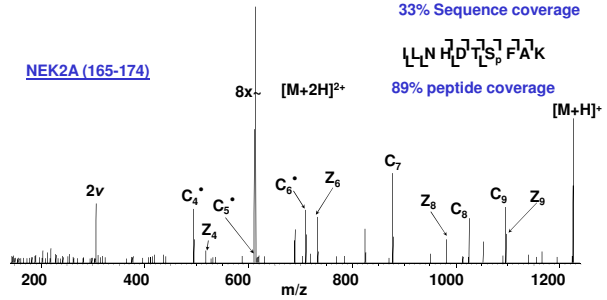

Figure 6: ECD mass spectrum of $[M+2H]^{2+}$ ions of phosphopeptide (165-174) from NEK2A .

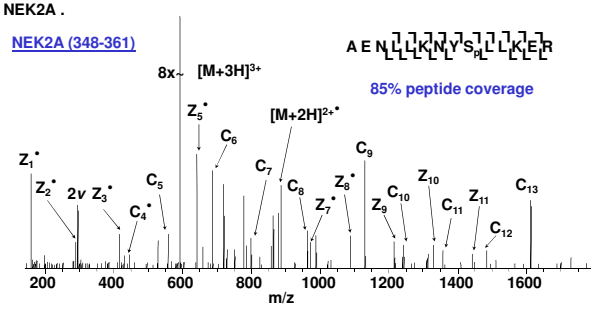

Figure 7: ECD mass spectrum of $[M+3H]^{3+}$ ions of phosphopeptide (348-361) from NEK2A .

Conclusion and Future Work

- We have demonstrated data dependent ECD of tryptic proteins with the detection of phosphorylated peptides.
- We have localised 4 out of 14 known phosphorylation sites in NEK2A and 3 out of 7 known phosphorylation sites in PKB. Three of the phosphoylation sites in PKB and eight in NEK2A were in regions of the proteins where there was no sequence coverage.
- Further work in this area will include different digestion enzymes to yield greater sequence coverage.

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