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TANDEM MASS SPECTROMETRY OF MULTIPLY CHARGED PROTEINS WITH ELECTROSPRAY IONIZATION

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Tandem Mass Spectrometry of Multiply Charged Proteins with Electrospray Ionization

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In conjunction with proteolytic digestion and tandem mass spectrometry (MS/MS), complete sequence information for proteins can be obtained. However, a potential gain in speed and sensitivity can be realized from collisionally activated dissociation (CAD) of *intact* proteins, provided improvements in CAD efficiency, instrument performance, and increased understanding in the CAD process of large molecules are obtained.

We have previously demonstrated that limited sequence information for proteins as large as ribonuclease A (13.7 kDa) [1] and serum albumins (66 kDa) [2] can be obtained from CAD of multiply charged molecules generated by electrospray ionization (ESI). Even for "known" compounds, difficulties in interpretation of *low resolution* MS/MS spectra arise because of the possibility of multiply charged product ions. However, probable assignments can be determined by searching for features common to most MS/MS spectra we have examined. For example, aids for interpretation of CAD mass spectra of multiply charged precursor ions include:

- A progression of product ion charge states (e.g., y_{25}^{2+} , y_{25}^{3+} , y_{25}^{4+}).
- Product ion series (e.g., y_{25}^{2+} , y_{26}^{2+} , y_{27}^{2+} , ...).
- MS/MS of product ions ("MS/MS/MS") to confirm assignments (and provide additional structural information). Product ions can be generated in the atmospheric pressure/vacuum interface by increasing the voltage difference between the nozzle and skimmer elements, or in the quadrupole collision cell (Q2). Fragment ions produced in the interface region can be selected by Q1 and be further dissociated in Q2.
- Tandem mass spectrometry of structurally related molecules (e.g., variants, sequence homology) help to identify the approximate location of dissociation, and thus the assignment of the product ion due to the expected shifts in m/z .

Dissociation of bovine carbonic anhydrase (29 kDa, 259 residues) produces a progression of charge states for several product ions (e.g., y_{67}^{6+} , y_{67}^{7+} , y_{67}^{8+}). Fragmentation is directed towards the COOH-terminal region of the polypeptide. Dissociation of the amide bond to a *proline* residue is favored for a number of proteins studied, especially carbonic anhydrase.

The presence of *complementary ion pairs*, which in sum (mass and charge) account for the entire molecule, alleviates some problems of interpretation of tandem mass spectra. Simple algorithms can determine whether two product ions are possibly complementary. MS/MS of the $(M+34H)^{34+}$ of carbonic anhydrase (Fig. 1) produces several sets of complementary ion pairs (e.g., $y_{67}^{7+} -- b_{192}^{27+}$, $y_{61}^{7+} -- b_{198}^{27+}$, $y_{67}^{8+} -- b_{192}^{26+}$, $y_{61}^{8+} -- b_{198}^{26+}$).

Transferrins are iron-binding glycoproteins of approximately 79 kDa, widely distributed in the physiological fluids of vertebrates. The available amino acid sequences for several species show a high degree of homology (sequence alignment). Product ions can be produced by molecular dissociation in the atmospheric pressure/vacuum interface. Because of the known sequence homology, these product ions can be compared and assigned as a series of multiply charged b_n ions from the NH_2 -terminal region near residue 60. Although the sequence for bovine transferrin is *unknown*, the fragmentation pattern is consistent with the other transferrins studied and can be assigned from the measured peak spacings.

Reduction of disulfide bonds with 1,4-dithiothreitol expectedly allows higher charged molecules to be produced, and *unexpectedly* releases a small, 6 - 8 kDa peptide from the COOH-terminus for the *mammalian transferrin proteins* studied (Fig. 2). Tandem mass spectrometry data for the released peptide from human transferrin is consistent with the expected amino acid sequence for the COOH-terminus.

Measured M_r of COOH-Terminal Transferrin Peptide

Species	M_r (exptl)	M_r (sequence)	Residues
Human	6485.1 $\pm$ 0.4	6484.4	624-679
Rabbit	7752.6 $\pm$ 0.6	7752.9	609-675
Bovine	6066.2 $\pm$ 0.6	n.a	n.a

Dissociation of large, multiply charged proteins can be induced. However, for CAD of larger proteins, higher m/z resolution (e.g., Fourier transform MS) may be necessary to assign product ion charge states.

References:

- [1] J.A. Loo, C.G. Edmonds, and R.D. Smith, *Science* **1990**, *248*, 201-204.
- [2] J.A. Loo, C.G. Edmonds, and R.D. Smith, *Anal. Chem.* **1991**, *63*, 2488-2499.

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