Ionic charge manipulation using solution- and gas-phase chemistry to facilitate analysis of highly heterogeneous proteins by ESI-MS

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Structural heterogeneity of protein therapeutics

- enzymatic PTMs (glycosylation, etc.)
- non-enzymatic PTMs (oxidation, deamidation, disulfide scrambling, etc.)
- “designer” PTMs (PEGylation, small-molecule drug conjugation, etc.)
- Conformational heterogeneity (misfolding, mis-assembly, aggregation, etc.)
Intact mass measurements of biopharmaceutical products
Enable straightforward assessment of structural heterogeneity
Can be carried out in the on-line format (LC/MS)
Can be carried out in the “native MS” format

• Higher order structure integrity (conformational heterogeneity assessment)
• Interaction with physiological partners/therapeutic targets (function assessment)

Monitoring protein interactions with therapeutic targets: why does the native ESI MS alone frequently fail?

structural heterogeneity does not allow meaningful information to be obtained

ILBP: ca. 300 kDa
(CHO > 20%)
Limited charge reduction

- extensively glycosylation of mAb gives rise to a convoluted profile

✓ select ions within narrow m/z windows
✓ brief exposure to electrons or anions to induce charge transfer
✓ charged-reduced species give rise to well-defined charge ladders in MS
✓ can be successfully applied to a range of biopharmaceuticals
  - PEGylated proteins (Analyst. 2017, 142, 336)
  - glycoproteins (Anal. Chem. 2010, 82, 18)
  - intact heparin (Anal. Chem. 2016, 88, 3)
**Limitation**
the inability of most mass spectrometers to isolate ions beyond certain threshold at high m/z values

- limited charge redaction of lower-m/z ions can be used to determine their masses (and assign the binding stoichiometry for the lower-mass complexes)

- denaturation results in dissociation of non-covalent protein assemblies or unstable proteins

- ions at higher m/z cannot be isolated, making it impossible to use limited charge reduction to determine their masses

- the mAb/Ag mixture contains ionic signals corresponding to complexes with different binding stoichiometry

- composition & binding stoichiometry cannot be assigned unambiguously
Strategy

- the utility of supercharging
- supercharging is achieved by adding m-nitrobenzyl alcohol (mNBA)
- higher charges enhance the charge reduction efficiency
- monitor the onset of denaturation

Add supercharging reagent

Isolate an ionic population within a narrow m/z window

Induce limited charge reduction in the gas phase by standard reagent
Haptoglobin (Hp)

- acute phase glycoprotein
- binds free hemoglobin (Hb) in circulation
- several isoforms
- high degree of heterogeneity (CHO ≥ 20%)

Haptoglobin human P00738

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MSALGAVIAL LLWGQLFAVD SGNDVTDIAD DGCPKPPEIA HGYHEHSVRY32
OCKNYYKLRTE GDGVYTLND KKQWINKAVGD DKLPECEADD DGCPKPPEIAH82
GYVEHHSVRYQ CKNYYKLRTE GDGVYTLNNE KWINKAVGD KLPECEAVCL32
KFKNPANPQV RILGHHLDAK GSFQWQAKMV SHM2LTTGAT LINEQWLLTT39
AKNLFLMHSNMATAKDIAPT LTLYVGGKQL VEIEKVVLPN NYSQVDGLI89
KLQKVSVNEDVMPICLPSK DYAEGVGKQG VSGWGRNANF KFTDHLYVLM139
LPVADQDQCI RHYEGSTVPE KKTPKSPVGV QPILENHTFC AGMSKYQEDT189
CYGDAGSAFA VHDEEDTYW ATGILSPDKS CAVAEGVYV KVTSIQDWHVQ239
KTIAEN245
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L*-chain (15.9 kDa) containing L-chain (9.1 kDa) sequence and an extra segment; H-chain (27.2 kDa) including four glycosylation sites.
Hp 1-1: (92 kDa)

Gaussian distributions reveals the overlapping of adjacent peaks during mass selection process.
Limited charge reduction of the most abundant ions gives rise to three well defined charge ladders corresponding to 90 kDa, 140 kDa and 190 kDa, respectively.
Expanding the scope of applications

Hp 1-1 & Hb complex

- the mixture of Hp and Hb presents an overwhelmingly challenging case
- Hp/Hb proves that this method works well with non-covalent assemblies (native MS)

Hp 1-1 & Hb + 0.7% mNBA
Hp 2-1 & Hb complex

Hp 2-1 & Hb

Hp 2-1 & Hb + 0.7% mNBA

unable to generate charge ladder for this species because its ionic signal is above the mass-selection threshold

ion isolation limit

type is well resolved now after adding mNBA to shift m/z value below the threshold
Conclusions

• a new analytical tool that manipulate ionic charge states using solution and gas phase chemistry opens up an exciting opportunity to make accurate mass determination of highly heterogeneous proteins

• each type of extensively glycosylated haptoglobin can be discerned from the convoluted MS spectrum

• this technique, for the first time, demonstrates the interpretable MS information for the Hp/Hb binding
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References