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Separation of up to 100 Conformers of Large Protein Ions by FAIMS/MS and Approach to their Structural Attribution

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Overview

In FAIMS, positively charged species with mass (m) >400 Da normally have negative compensation voltage (CV). Among protein ions with m = 8 – 66 kDa derived from ESI, three with m >29 kDa have exhibited extremely broad CV ranges, including strongly positive CV [1].

This abnormal behavior was ascribed to reversible alignment of ion macrodipoles during the periods of high electric field in FAIMS.

Here, we expand the exploration of FAIMS separation of protein ion conformers in several directions:

1. Improved delineation of the ~"30 kDa" boundary by studying more proteins with m ~30 kDa
2. Extension to yet larger proteins and non-covalent protein complexes
3. Working toward a quantitative model for dipole alignment in IMS and FAIMS, including efforts to better clarify the origin of the 30 kDa boundary

Introduction

Ion mobility spectrometry (IMS) separates and characterizes ions by some property of their transport through a gas under the influence of electric fields.

Conventional (drift-tube) IMS:
Based on absolute mobilities (K) measured at fixed electric field intensity (E). Usually employs constant (dc) moderate E; K does not depend on E.

Differential IMS (DMS) =
Field Asymmetric waveform IMS (FAIMS):
Based on variation of K as a function of E at high E
Involves time-dependent (rf) oscillatory E

Traditionally, ions in both conventional IMS and FAIMS were deemed to undergo free rotation. Hence all formulas for ion mobility [2-5] feature orientationally-averaged cross sections (Ω_{avg}).

Like neutrals, ions are electric dipoles or multipoles. At any particular E, ions with sufficient dipole moment (p) align with **p** along **E**. Statistically, p increases for larger molecules [1].

At typical E in IMS (~10 - 10^3 V/cm), alignment requires $p > 10^5$ - 10^7 D, possible only for extremely heavy species (>1 MDa). In FAIMS, E is higher by ~30 - 3000 times; alignment occurs for p > ~300 D. For proteins, those are found mostly for m > ~30 kDa.

The difference between orientationally-averaged and directional cross sections of ions could be >20%, far exceeding the usual high-field effect in FAIMS [1].

Methods

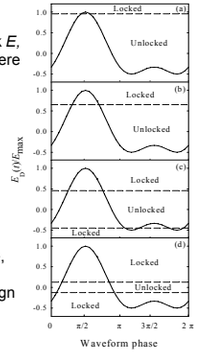
Approach: Dipole locking in FAIMS

Ion alignment will vary from null at E = 0 to max. at peak E:

Locking always stronger in high-E half-cycle

As p increases:

- Dipole locks at peak E, free rotation elsewhere
- Locking over a part of high-E half-cycle
- Locking appears in low-E half-cycle
- Locking everywhere, except near points where E changes sign



Experimental Platform

Field Asymmetric waveform IMS (FAIMS) system:

- Cylindrical FAIMS (Ionalytics Selectra) [6] coupled to LCQ Deca ion trap MS (Thermo Fisher)
- Gap 2 mm, Electrode position 2.1 mm (low resolution)
- Carrier gas N_2 at 2.5 L/min; Dispersion voltage −5 kV (P2 mode)
- Compensation voltage range: ± 70 V, scan speed 5 V/min

Sample preparation

- Proteins dissolved in water, water/methanol, or acetonitrile to 1 - 30 μM, acidified by 0 - 5% acetic, formic, or HCl acid
- To cover the broadest range of conformers, two samples prepared for each protein:
  - I. Pseudonative media. Minimum organic content, pH ~7
  - II. Denaturing media. High organic content, low pH, annealing at 80 °C for 2 h. Disulfide bonds (if any) reduced using DTT

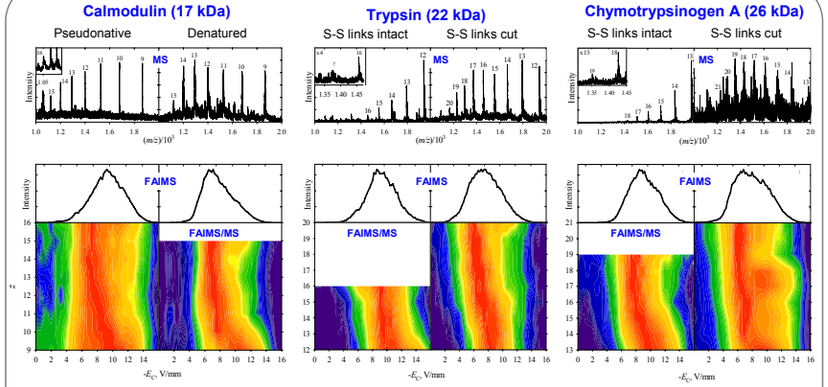
Proteins studied to date (new in red, others from [5])

Identity	# residues	m, Da	# S-S	p, D
Ubiquitin	76	8,566	No	189
Cytochrome c	104	12,361	No	253
Lysozyme	129	14,305	4	218
Hemoglobin chain A	141	15,053	No	185
Calmodulin (bovine)	149	16,790	No	427
Myoglobin	153	16,950	No	225
Trypsin (bovine pancreas)	223	23,294	6	327
β-Casein	209	23,978	No	??
Chymotrypsinogen A	245	25,660	5	376
Carbonic anhydrase II (CA II)	256	29,020	No	318
Alcohol dehydrogenase (ADH)	374	39,896	No	721
Bovine serum albumin (BSA)	578	66,430	17	1139
Transferrin (bovine)	667*	78,000	19	1098*
Serum albumin (bovine) dimer	1156	132,860	34	??

*values for human transferrin

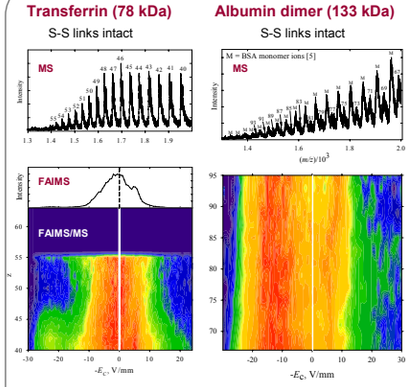
Experimental Results (2-D FAIMS/MS plots)

for m < 30 KDa



Relatively few separated conformers with negative E_c of about −(2 - 16) V/mm only, abs. values slightly decrease at higher charge states

for m >> 30 KDa



Multitude of separated conformers with E_c > 0 and E_c < 0 over the ± ~30 V/mm range, no general dependence on z

New observations match previous findings [1]

Insights from Theory

Minimum lockable dipole moment exists regardless of E !

For locking, the work to rotate dipole must exceed available thermal energy.

Textbook equation is:

$$pE > kT \quad (1)$$

This means p > 300 D at E = 25 kV/cm, T = 300 K [1].

Inequality (1) assumes ion temperature (T_{ion}) to equal gas temperature (T_{gas}).

At high E

$$T_{ion} = T_{gas} + M(KE)^2/(3k) \quad (2)$$

where K is mobility and M is gas molecule mass.

Eq (1) becomes quadratic in E:

$$(MK^2/3)E^2 - pE + kT > 0 \quad (3)$$

This has solutions when

$$p > 2K(kMT/3)^{1/2} \quad (4)$$

For typical K = 1 cm²/(V×s) in N_2 at 300 K, eq (4) yields p > 480 D.

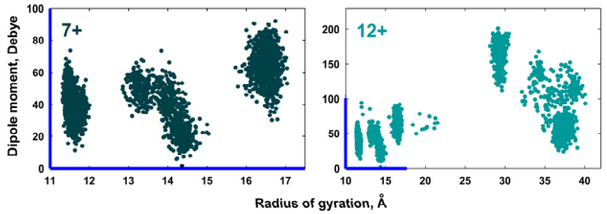
Eq (4) shows abrupt p cutoff at any E and matches observations better.

Calculations for model geometries

Full understanding of FAIMS properties of proteins requires mobility calculations for ensembles of model geometries. Presently, reliable optimization of 3-D protein structure is limited to m < ~15 - 20 kDa, i.e., below the 30 kDa boundary.

However, simulations for smaller proteins provide useful insights.

Ubiquitin is a standard system to model protein folding. Simulations indicate that p tends to increase for higher z and more unfolded conformers of same z.



Additional calculations further support the dipole alignment concept. More detailed simulations in progress.

Conclusions

- FAIMS separation capability does not appear to diminish at m ~100 kDa, showing the utility of FAIMS analyses for very large macromolecules
- FAIMS can separate conformers of non-covalent protein complexes (different docking sites?)
- Ions of transferrin (m = 77 kDa) and albumin dimer (133 kDa) exhibit same "abnormal" FAIMS separation properties (extremely wide CV range of ± ~25 V/mm) as previously studied proteins with m ~ 30 - 60 kDa. Ions of calmodulin, trypsin, and chymotrypsinogen A (17 - 26 kDa) exhibit "normal" properties, similarly to other known proteins with m < 29 kDa. These data support the existence of a "boundary" between "normal" and "abnormal" proteins (or protein complexes) at ~30 kDa.
- High-field rotational heating of ions in FAIMS raises the dipole moment needed for alignment, leading to better agreement with experiment.
  The minimum p is independent of electric field.
- Replica exchange MD simulations suggest that dipole moments of proteins tend to increase for unfolded conformers and higher z, matching observations.

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