Probing Conformations of Large Proteins by FAIMS: A New Characterization Method Based on Direction-Specific Ion Mobilities?

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Overview

Ion mobility methods

Ion mobility methods separate ions based on transport in gases under the influence of electric field. Previously, ions were assumed to undergo *free thermal rotation,* with mobility controlled by orientationally-averaged cross-section Ω_{avg}.

Many macromolecules have large dipole moments. Such dipoles *may be oriented* by strong electric fields in FAIMS and (in extreme cases) in IMS -> Mobilities depend on directional cross sections, Ω_{dir}.

We show evidence for dipole alignment in ion mobility studies of proteins. Alignment breaks a tight dependence of cross-section on mass and raises separation peak capacity by an order of magnitude.

Introduction

Ion mobility separations

- Ion mobility spectrometry (IMS)
- Field asymmetric waveform IMS (FAIMS) = Differential mobility spectrometry (DMS)

Advantages

- Often distinguish species (especially isomers) that challenge LC and other solution-based methods
- Allow very fast analyses, can be "nested" into LC/MS
- IMS provides structural information about ions
- IMS and FAIMS are becoming more available; several commercial systems introduced

However:

- Have a peak capacity less than solution methods
- Ion mobilities substantially correlated to mass → IMS is not highly orthogonal to MS; FAIMS is more orthogonal

IMS

- Based on *absolute* mobility values, *K*
- Uses a moderate (dc) electric field

FAIMS

- Exploits the dependence of *K* on field intensity, *E*
- Based on the *difference* between *K* at high and low *E*
- Uses a periodic asymmetric (rf) field

In IMS or FAIMS, *K* has been assumed to be set by Ω_{avg} – the result of uniform orientational averaging of collision integrals Ω over all collision geometries.

Methods

Physical Foundation

Dipole alignment in electric fields

Ions in gases rotate randomly.
Mean rotational energy set by gas temperature, *T*

To orient a dipole, the electric field must overcome rotation. Energy of dipole with moment *p* at angle α to *E*:

$$A = pE(1 - \cos\alpha)$$

Hence, three alignment regimes:

pE << 0.5 kT: Free rotation
pE ~ 0.5 kT: Hindered rotation, Pendular motion
pE >> 0.5 kT: Firm locking

Accurately, probability of orientation α is proportional to:

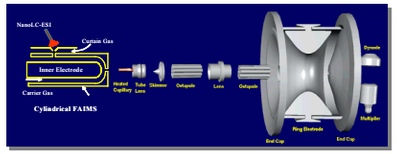
$$Pr(\alpha) = (\sin\alpha)\exp(-pE\cos\alpha/(kT))$$

For oriented dipoles, mobility is set by a *weighted* average of directional cross-sections

$$\Omega_{wei} = \int[\Omega_{dir}(\alpha)Pr(\alpha)d\alpha] / \int Pr(\alpha)d\alpha$$

Experimental Platform

Cylindrical FAIMS - LCQ Deca ion trap MS system



FAIMS parameters

- Gap 2 mm; electrode position 2.1 mm (low resolution)
- Carrier gas N_2; gas flow 2.5 L/min
- Dispersion voltage −5 kV (P2 mode)
- Compensation voltage range: ± 70V, 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 hydrochloric acids
- Two samples prepared for each protein:
  1. Pseudonative media. Minimum organic content, pH ~7
  2. Denaturing media. High organic content, low pH, some samples annealed at 80 ^{0}C for 2 h. Disulfide bonds (if present) reduced using DTT

FAIMS in dipole-oriented regime

Ion alignment will vary periodically, from null at *E* = 0 to max. at peak *E*

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

Locking always stronger in high-*E* half-cycle

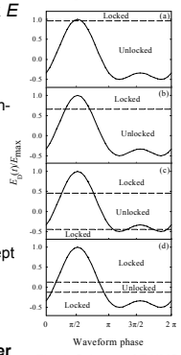


Fig. 1. Scheme of FAIMS waveform showing the dipole locking regimes

Which dipoles will orient?

At *E* = 25 kV/cm, effective dipole locking (panel b above) requires *p* ~ 400 - 600 D

Values of *p* tend to increase for larger proteins:

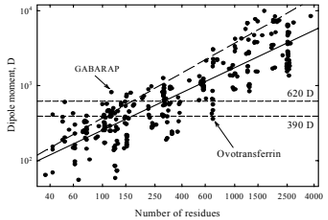


Fig. 2. Dipole moments of native proteins (~400 picked from PDB database at random)

In PDB, average *p* = 665 D,
>1/2 of proteins will align in FAIMS!

Protein dipoles depend on conformation, unfolding may create geometries with *p* either < or > than the native

Of proteins studied here, CA II, ADH, BSA should substantially align in FAIMS

Results

Effect of dipole alignment on ion mobility

Depending on the angle between **p** and large ion axis, Ω_{wei} may be > or < than Ω_{avg}.

For BSA (Fig. 3), Ω_{dir} perpendicularly to **p** is ~10% lower than Ω_{avg} (using any model), so $\Omega_{wei} < \Omega_{avg}$. Hence *K* should strongly increase at high *E*.

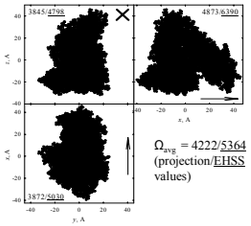


Fig. 3. Views of native BSA in the plane perpendicular to **p** and two orthogonal planes. The direction of **p** is labeled.

FAIMS behavior as a function of ion size

K(*E*) tends to increase for small ions (termed A-type) and decrease for large ions (C-type). This trend is for any gas, but the region of C- depends on the gas. With N_2, all ions >400 Da were C-type.

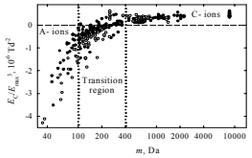


Fig. 4. FAIMS separation parameters of various ions (compiled from literature)

Conventional FAIMS behavior for proteins

All proteins studied by FAIMS previously (ubiquitin [2,3], cytochrome c [3,4], β_2-microglobulin [5], hemoglobin chain A) had *m* <16 kDa and *p* < ~250 D. Those (and lysozyme, myoglobin, casein) exhibit regular C-type behavior.

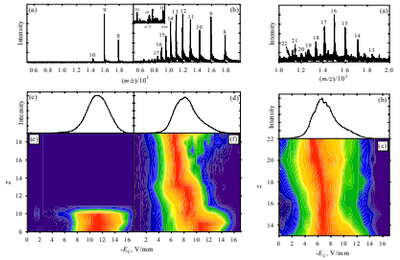


Fig. 5. MS (top) and 2-D FAIMS/MS (bottom) spectra for disulfide-intact lysozyme (M: 14,305 Da, *p*: 218 D) (left), disulfide-reduced lysozyme (middle) and β-casein (M: 23,978 Da)(right).

Abnormal FAIMS behavior for large proteins

Carbonic anhydrase II:

- M: 29,020 Da, *p*: 318 D
- In FAIMS, has a peak at positive CV, in the A-type ion range (in the rectangle): **without precedent for large ions**
- Feature is for the new high-*z* envelope
- Abundance increases for more acidic solutions

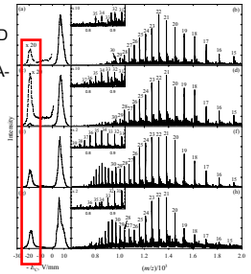


Fig. 6. Total FAIMS (left) and MS (right) spectra of CAII ions, pH decreases from (a) to (d)

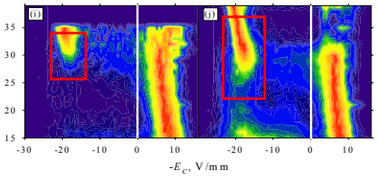


Fig. 7. 2-D FAIMS/MS spectra of CAII, for the most native-like and most denaturing solutions, (a) and (d).

"Abnormal" FAIMS features must be due to denatured conformers:

- Appear for the new envelope at high *z*
- Appear for lower *z* at lower pH
- Abundances increase at lower pH

Similar findings for ADH (M: 39,896 Da, *p*: 721 D) and BSA (M: 66,430 Da, *p*: 1139 D), "abnormal" conformers:

- Have a broad CV distribution
- More abundant than for CA II

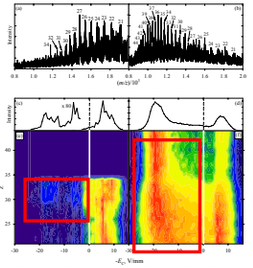


Fig. 8. MS (top) and 2-D FAIMS/MS (bottom) spectra for ADH from pseudonative (left) and denaturing (right) solutions.

Conclusions

❖ Electric field in ion mobility experiments may orient ions having a dipole moment. Mobilities of aligned and rotating ions differ, usual IMS models cease working.

❖ In FAIMS, species with *p* > 300 D should be substantially aligned. This covers most proteins and protein complexes. In IMS, alignment is possible for much higher *p*.

❖ Normally, *K*(*E*) of macroions decrease at high *E*, but may increase for aligned ions. In FAIMS, those would appear as A-type.

❖ Protein dipole moments tend to increase with size. Most proteins with *p* > 300 D have *m* > 30 kDa. Indeed, smaller proteins exhibit standard FAIMS behavior, larger proteins are "abnormal".

❖ Dipole alignment breaks the dependence of ion mobility on mass, expands FAIMS peak capacity by an order of magnitude.

❖ Alignment may be extended to smaller ions at higher *E* or lower temperature.

Acknowledgements

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