Ion Mobility Separations of Protein Conformers with Resolving Power up to 400 Using Hydrogen-Rich Gas Buffers

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Overview

- High-resolution FAIMS using H_2/N_2 gas with up to 85% H_2 applied to a protein - ubiquitin.
- Resolving power up to ~400: five times the previously highest for proteins (obtained using He/N_2 buffers).
- Fully separated >10 conformers for higher charge states.
- Peak width same or narrower than ever seen in FAIMS, scales with the charge state and filtering time properly for diffusion-limited separations.
- Hence individual protein geometries (not ensembles as previously) were apparently resolved.

Introduction

- Proteins and other biomacromolecules in solution or gas phase exhibit numerous conformers.[1]
- Major structural families (compact, partly folded, unfolded) are readily distinguished, but each comprises multiple unresolved geometries. This causes drastic peak broadening for separations in solution (by chromatography) or gas phase (by ion mobility).[2,3] Thus studies of protein chemistry and physics (e.g., fragmentation via CID or ECD, isomerization, and H/D exchange) have dealt with conformer ensembles, not individual geometries.
- Resolving power (*R*) of differential IMS (FAIMS) is improved by buffers rich in a light gas, where ion mobilities are high. Use of He/N_2 mixtures has increased *R* for protein conformers to ~80, but specific geometries were still not resolved.[4]
- Hydrogen resists electrical breakdown better than He, allowing H_2/N_2 mixtures with up to ~85% H_2. These buffers have raised *R* for smaller species by 2 - 3 times.[5] Here we explore their use for proteins.

Methods

Experiments employed a high-resolution planar FAIMS unit integrated with an ion trap MS (Thermo LTQ) via an electro-dynamic ion funnel interface.[4]

We studied a common model protein - bovine ubiquitin (Ub, 8,565 Da). The solution (~4 μM in 50:49:1 water/ methanol/ acetic acid) was infused to a single ESI emitter at 0.5 μL/min.

The H_2/N_2 gas mixtures were formulated by flow meters (MKS Instruments) and delivered to the FAIMS device at the rate of $Q = 2$ L/min (for the "standard" separation time of $t = 0.2$ s) or $Q = 1$ L/min ("extended" $t = 0.4$ s).

The amplitude of bisinusoidal FAIMS waveform ("dispersion voltage") was 5.4 kV - the maximum with existing generators. With a 1.88 mm gap width, the dispersion field is ~29 kV/cm.

Compensation voltage (CV) was supplied by high-definition electronics with exceptional reproducibility and small scanning increment, ensuring <1 mV precision.[6]

As usual, ESI of ubiquitin in denaturing solvents produces protonated ions with the charge states (*z*) of 5 – 14.

PNNL custom planar FAIMS device coupled to a mass spectrometer

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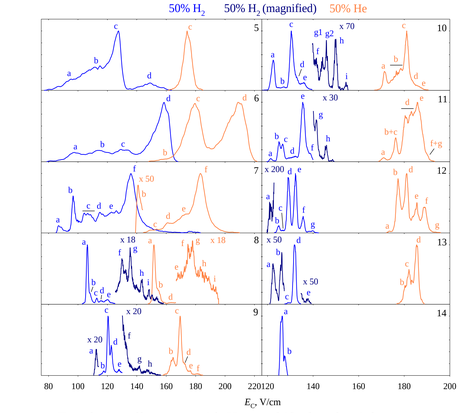
Results

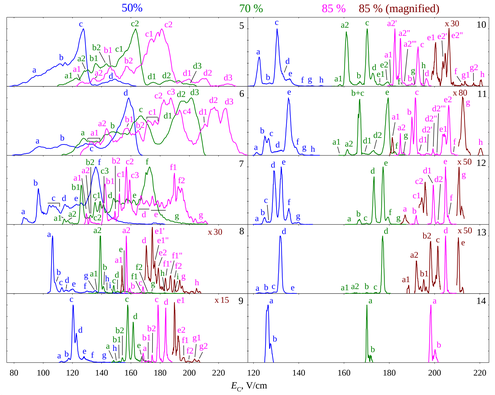
Spectra at "standard" separation time

FAIMS spectra at 50% H_2 similar to those[4] at 50% He, though the resolution is somewhat better and more features are separated.



For clarity, all spectra with He are shifted to the right.

Raising the H_2 content to 70% and then 85% shifts all spectra to higher CV and dramatically improves resolution: >10 conformers fully separated for $z = 8 – 11$.



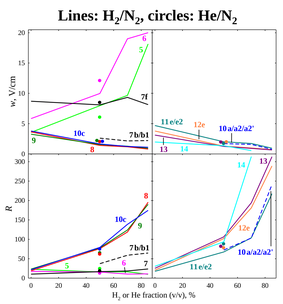
Peak width and resolving power

As H_2 fraction grows, the peak widths (*w*) increase for $z = 5$ and 6, stay constant for $z = 7$, and drop for $z = 8$ - 14.

Narrowing of peaks (because of higher ion mobility upon H_2 addition) is typical,[7,8] the opposite trend for smaller *z* reflects augmented structural diversity upon unfolding of compact conformers (induced by stronger field heating at greater He or H_2 fractions).[4]

Combination of higher CV and narrower peaks rapidly elevates the resolving power, to ~200 - 300 for $z > 7$. Made possible by combination of:

(i) strong durable field heating that unfolds Ub for $z \geq 8$ and effectively anneals the unfolded conformers, reducing their number to under 15.
(ii) high resolving power that affords full separation of an isomeric mixture of that complexity.

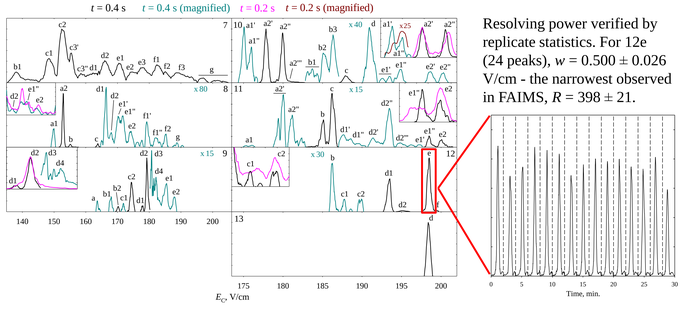


Resolving power up to 400 with extended separations

As typical for separations in media, in FAIMS *R* scales[9] as $t^{1/2}$. Extending *t* to 0.4 - 1 s has raised *R* for smaller ions by ~50 - 100%.[10] Using $t > 0.2$ s with H_2-rich gases was previously precluded by fast diffusion, but is possible for proteins that diffuse slowly because of large size.

Here, extending *t* to 0.4 s has increased the resolving power by ~50%, up to ~400.

Many new conformers distinguished.



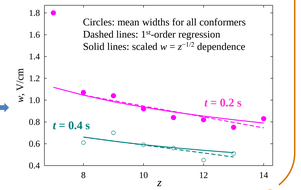
Resolving power verified by replicate statistics. For 12e (24 peaks), $w = 0.500 \pm 0.026$ V/cm - the narrowest observed in FAIMS, $R = 398 \pm 21$.

Dependence of peak widths on the charge state and separation time

Diffusion-limited peak widths in FAIMS for defined geometries are proportional to $z^{-1/2}$ and $t^{-1/2}$

Here:

1. Mean widths of features for each *z* scale as $z^{-1/2}$ at either $t = 0.2$ s or 0.4 s.
2. On average, peaks are ~1.5 times narrower at $t = 0.2$ s: close to the expected scaling ($2^{1/2} = 1.41$).



Conclusions

- H_2/N_2 buffers with up to 85% H_2 increase FAIMS resolving power for protein conformers fivefold, up to 400.
- Slower diffusion for large ions permits extended separations even at 85% H_2.
- Over 10 conformers were fully resolved for some charge states of ubiquitin.
- Peak widths equal to or narrower than those for smaller ions under same conditions, scale as a function of separation time and protein charge as prescribed for instrumental (diffusional) broadening.
- Results indicate that individual protein geometries rather than conformer ensembles were largely distinguished.
- Initial work shows that the capability extends to larger proteins.

Acknowledgements

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