ion and photon interactions with trapped biomolecular ions

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1. motivation
2. experimental
3. mass spectra and soft X-ray spectra
   • leucine enkephalin as a typical system
4. the role of protein size
   • from small peptides to larger proteins
5. secondary structure
   • soft X-ray spectroscopy and gas-phase structure
6. soft X-ray photoionization of oligonucleotides
why energetic ions & photons?

driving forces:

• molecular movies of protein dynamics with atomic resolution at X-ray free electron lasers
• mass spectrometry
  • CID, multiphoton IR dissociation: slow heating and scission of weak bonds
  • UV photoionization: dissociation via excited states
  • keV ion and soft X-ray photoionization: fs deposition of > 10 eV
• site sensitivity, electronic structure sensitivity - geometric structure sensitivity?
• biological radiation damage

key questions

• is soft X-ray or ion induced fragmentation proceeding via IC/IVR or is it fast and localized?
• how are excitations and charges migrating through the system?
• is soft X-ray absorption spectroscopy sensitive to gas-phase protein structure?
the Groningen RF ion trap

- high fluence electrospray source (>100 pA mass selected protonated peptides)
- pulsed operation (ion accumulation within RF 8-pole, transfer to 3D RF-trap)
- exposure of trap content to photon beam
- TOF mass spectrometry of trap content
C K-edge X-ray absorption

- Core excitation followed by Auger ionization → mainly single ionization
- Core ionization followed by Auger ionization → mainly double ionization

Resulting excitation energy distribution is broad around ~20 eV
C K-edge X-ray absorption

photofragmentation exhibits clear photon energy dependence

and for keV ions ...

tyrosine PES
Plekan et al.,
and for keV ions ...

- similar fragmentation pattern
- same dependence on excitation energy
peptide fragmentation

sequence ions

immonium ion
C K-edge X-ray absorption

photofragmentation exhibits clear photon energy dependence

photofragment yield can be recorded as function of photon energy

C K-edge X-ray absorption

partial ion yield spectroscopy
leu-enk (YGGFL)
m/z=120 (F)
BESSY II, Germany

O. González-Magaña et al., JPCA 116 (2012) 10745

partial ion yield spectroscopy
cytochrome C, 105 amino acids
M=11833
non-diss. single/double ionization
SOLEIL, France

A. R. Milosavljević et al., JPCL 3 (2012) 1191

high quality data for smaller biomolecules – for instance from K. Prince’s group - and for thin films
C K-edge X-ray absorption

| A: C(1s)-π* transitions in the aromatic F and Y sidechains |
| B: C(1s)-π*C=O amide group transitions – NO specificity |
| C: C(1s)-σ* transitions, Rydberg transitions, ionization |

A and B deexcite non-radiatively by Auger processes, ionization
C (ionization part) followed by Auger de-excitation, double ionization
protein size

immonium ions and small sequence ions
Protein size

Immonium ions and small sequence ions
proteins size

immonium ions and larger sequence ions
protein size

GIGAVLKVTGLPALIWSIKRKRQ

Immonium ions, larger sequence ions and non-dissociative ionization.
protein size

immonium ions, larger sequence ions and non-dissociative ionization
immonium ions, few larger sequence ions non-dissociative ionization, neutral losses
protein size

immonium ions, non-dissociative ionization, neutral losses
core ionization of gas-phase glycine @ LCLS – AMO

from the Auger spectrum, typical excitation energies can be obtained

assume deposition of 18.5 eV in the different systems

\[ T = \frac{E_{exc}}{c(T, \nu)sk} \]

degrees of freedom
temperature and frequency dependence

→ approximate T as a function of number of degrees of freedom (harmonic oscillator model)

\[ c_{peptide}(T) = 5.61 \times 10^{-4} K^{-1}T - 1.24 \times 10^{-7} K^{-2}T^2 \]
protein size

fragmentation yield as a function of T

similar trend as for direct heating, CID, SID but onset at lower T

• excitation energy dissipates in large proteins
• even from the largest molecules, immonium ions can be formed
• IVR competes with fast local dissociation channels (repulsive states?)
• radiation damage in thin films (soft X-ray microscopy) might only be an issue for small peptides!
back to melittin

(melittin+3H)$^{5+}$ $m=2.85$ kDa

288.3 eV

m/z=84:K,Q
m/z=86:I,L
m/z=101:K,Q

m/z=129:R,Q,z$^+$

m/z=130:W

m/z=143:a$^+_2$

m/z=171:b$^+_2$

m/z=228:b$^+_1$

m/z=271:a$^+_1$

m/z=370:x$^+_5$,a$^+_5$

m/z=541:z$^+_4$,a$^+_1$

m/z=129:R,Q,z$^+$

298 eV

no (melittin+3H)$^{5+}$!
linear trap geometry $\rightarrow$ much larger capacity
LHe cooling $\rightarrow$ $T \sim 10K$
mass dependent transmission $\rightarrow$ good for partial ion yield scans, bad for mass spectra
melittin – conformation effects

• resolution:
similar as for gas-phase amino acids
Plekan et al., JPCA, 111 (2007) 10998

• problem:
averaging over residues with slightly shifted peaks

• advantage:
T=10K
→ less thermal broadening
→ non-diss ionization stronger
melittin – conformation effects

gas phase structure:
(melittin +2H)$^{2+}$: helical
(melittin +3H)$^{3+}$: mainly helical
(melittin +4H)$^{4+}$: not helical (relaxation due to Coulomb repulsion)

hydrogen bonds induce out of plane distortion of the backbone

symmetries of the transition densities are broken

(too) small reduction in the transition dipoles

very sensitive to the structure
melittin – conformation effects

1s ionization energy increase and conformational relaxation at the same time
melittin – conformation effects

at variance with previous data on ubiquitin

• DFT calculations (T. Jansen): helicity reduces the main resonance, but the effect is (too?) weak
• there is no circular dichroism observed for any channel (within the accuracy of the experiment)
  • other explanations?
  • future: IMS!
exciting DNA

DNA interactions with energetic photons

• biological radiation action / radiotherapy
• charge transport in DNA
• interactions with aqueous environment

negatively charged in solution
Moller-Plesset perturbation theory:  
the telomere sequence TTAGGGG can function as a profound hole trap. 


The image shows a graph with peaks labeled with m/z values, indicating ionization events. Examples include:

- N below K-edge: \((a_2^*G)^+\), 673.5 Da
- M below K-edge: \((a_4^*G)^-\), 704.2 Da
- O below K-edge: \(b_2^*\), 633.4 Da
- O below K-edge: \(w_2^*\), 704.2 Da
- O above K-edge: \(a_4^*\), 1018.7 Da
- O above K-edge: \(a_4^*\), 1347.9 Da

The text also mentions single/double ionization of **deprotonated** telomer containing oligos induced by soft X-ray absorption.
telomers as hole traps?

all major fragmentation channels involve strand breakage somewhere in the GGG sequence!
thanks

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next step:
- large protein \((\text{ubiquitin}+10\text{H})^{10+}\)
- many photons  

\[ \Rightarrow \text{FLASH} \]
large protein—many photons

single photon conditions:
- non-dissociative ionization
- immonium ions weak

multiphoton conditions:
- immonium ions dominate
large protein—many photons

\[ I(r, z) = I_0 \frac{4 \ln 2}{\pi \Delta(z)^2} \exp \left( - \frac{4 \ln 2}{\Delta(z)^2} r^2 \right) \]
ubiquitin – multiphoton

$\sigma_{\text{total}}(90 \text{ eV}) \sim 6.7 \times 10^{-18} \text{ cm}^2$ from summation of atomic data


non-diss processes due to single/double ionization in the “halo” of the FEL beam
ubiquitin – multiphoton

Immonium ion formation upon multiphoton absorption is NOT due to initial charge state!

a) 90 eV, synchrotron
(ubi-6H)^6-

[ubi-6H]^5-
[ubi-6H]^4-
[ubi-6H]^3-

b) 90 eV, FEL

m/z
ubiquitin – multiphoton

linear increase of immonium yields

protein behaves as ensemble of free amino acids or peptides

fast local structural response