New insights in intact protein analysis with CE-TOF/MS

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Protein analysis

1. **Fundamental studies**
   - Drug-protein interactions | Protein sizing
   - Post-translational modifications

2. **Pharmaceutical analysis**
   - Biotechnology medicines | Protein aggregation
   - Drug seizure | Counterfeits

3. **Clinical analysis**
   - Biomarkers discovery | Diagnostics
   - Toxicology | Forensics | Doping control
Strategy for intact protein analysis

- High efficiency
  \[ N = \frac{\mu \cdot I \cdot U}{2 \cdot D \cdot L} \]
- High selectivity
  \[ \mu = \frac{q}{6 \cdot \pi \cdot \eta \cdot r} \]
- Green chemistry
- Ease of operation and maintaining
- No mechanical constraints
Strategy for intact protein analysis

CE modes for intact protein analysis

• Capillary zone electrophoresis (CZE)
  • Capillary isoelectric focusing (CIEF)
  • Micellar electrokinetic chromatography (MEKC)
  • Capillary gel electrophoresis (CGE)

Adsorption! MS compatibility!
Intact protein analysis by CE-TOF/MS | applications

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*Laboratoire de chimie analytique pharmaceutique*
Pharmaceutical analysis

Identification of human growth hormone in seized samples

hGH analysis with CE-TOF/MS
Human growth hormone (hGH)

- Polypeptide hormone secreted by the anterior pituitary gland
  - Protein chain of 191 aa | pI 5.1
  - Endogenous hGH presents several isoforms: 22 kDa (most abundant one), 20 kDa, 17 kDa, ...
  - Physiologic function: to stimulate growth and cell reproduction
  - Pulsating secretion: 20-30 min half-life

- hGH trafficking is relatively widespread

- Production by recombinant DNA technology
  - Recombinant hGH possesses an identical sequence to the naturally occurring 22 kDa hormone
  - Therapeutic use: to treat shortness due to numerous reasons
  - Illegal use in doping: to increase overall physical performance

hGH rhGH
CE-UV | pH optimization

Basic pH
- literature-like
- adsorption
- degradation

Staub et al., Electrophoresis (in Press)

Acidic buffer | ACN
- no adsorption
- no degradation
- high efficiency
- short analysis time

→ LOD (UV) | 5 ppm | 226 nM
1 fmol (26 pg) injected
Method transfer to ESI-TOF/MS

**TOF/MS features**

- high **resolution** (m/z = 600, 9'000 FWHM)
- high **mass accuracy** (<5 ppm)
- high **dynamic range** (4 orders of magnitude)
- **identification** based on true isotopic pattern

Staub et al., Electrophoresis (2009)
CE-ESI-TOF/MS | coupling issues

Interfaces

Sheath liquid 79%
Others 8%
Liquid junction 2%
Sheathless 11%

Sheath liquid

CE capillary

nebulizing gas

Nebulizing gas

Sheath liquid

Desolation / Ion Transfer Optics
Orthogonal Deflection
Detector

Reflectron

Drift region

Laboratoire de chimie analytique pharmaceutique
CE-ESI-TOF/MS | Interface optimization

Coaxial interface

- Sheath liquid | Composition, flow
- Nebulizing gas | Pressure
- Drying gas | Flow, temperature
- Capillary voltage
- CE capillary | Positioning

Multivariate approach
(design of experiments)

- Screening with fractionate factorial design (N=14)

- Modelization with Box-Behnken design (N=30)

• Staub et al., Electrophoresis (in Press)
hGH | CE-ESI-TOF/MS

N = 750'000

CE-ESI-TOF/MS

- unambiguous identification
- 22 kDa / 20 kDa ratio | ca. 5%

⇒ LOD (TIC/MS) | 50 ppm | 2.3 µM
36 fmol (788 pg) injected

- Staub et al., Electrophoresis (in Press)
hGH vs. rhGH | CE-ESI-TOF/MS

- 2 selectivity levels
- hGH vs. rhGH discrimination

Staub et al., Electrophoresis (in Press)
Seized samples analysis | CE-ESI-TOF/MS

Seizure #1
- 22125.68
- Dioxy-Met^{14}/Met^{125} +32 amu

Seizure #2
- 22157.28

• Staub et al., Electrophoresis (in Press)

hGH 22 kDa
- 22125.46

rhGH Humatrope
- 22125.05

Counts vs. Deconvoluted Mass (amu)
- 21000
- 22000
- 23000

Counts 100%
CZE-ESI-TOF/MS | insulin analysis

acidic pH

basic pH

Counts (%) vs. Acquisition Time (min)

Counts vs. Deconvoluted Mass (amu)

Counts vs. Mass-to-Charge (m/z)

acidic pH

basic pH

Counts vs. Deconvoluted Mass (amu)

Counts vs. Mass-to-Charge (m/z)

ESI+
hGH | discussion

No sample preparation
Intact protein analysis with high efficiency
Fast and repeatable analysis
Small sample consumption

Accurate identification

Applications
Drug seizure
Pharmaceutical quality control

Staub et al., Electrophoresis (in press)
Doping control

Identification of hemoglobin-based oxygen carriers in plasma samples

HBOC analysis with CE-TOF/MS
Hemoglobin

- Hemoprotein
  - 4 protein globular subunits
  - 1 chain + 1 heme
- Hb presents several variants
  - Hb A₀ (α+β)
  - Hb A₂ (α+δ)
  - Hb F (α+γ)
  - …
- Physiologic function
  - To transport oxygen and increase total blood oxygen capacity
- Normal level in blood > 10 g/dL

Hb and HBOC

- Hb polymerization
  - To avoid organ dysfunctions and toxicities
- Therapeutic use
  - To mimic blood oxygen transport
- Illegal use in doping
  - To increase oxygen transport
- Approved for veterinary use in US and Europe
- Polymerized bovine hemoglobin
- Phase III trials
- Polymerized human hemoglobin
HBOC doping | analytical challenge

Selectivity between HBOC and circulating Hb

Mechanical haemolysis
- HBOC and Hb can be found simultaneously in plasma
- false-positive results have to be eliminated
CE-UV/VIS | pH optimization

**Alkaline buffer**
- no adsorption
- OXY vs. Hb selectivity
- short analysis time

→ LOD (UV/VIS)
0.10 g/dL | 6.7 µM
528 fmol (8 ng) injected

UV/VIS 415 nm | detection of hemoproteins
CZE-ESI-TOF/MS | hemoglobin analysis

**Basic pH**

Intact protein

Dissociated protein

\[ \mu^- \]

\[ \alpha | 15126.65 \]

\[ \beta | 15867.39 \]
CE-ESI-TOF/MS | interface feature

**CE separation**
- migration under their **intact form**

**ESI-TOF/MS detection**
- **sheath liquid** | acid, organic solvent
- in-Taylor cone **dissociation**
- **individual globine** detection
  - accuracy < 4 ppm
CE-ESI-TOF/MS | Hb variants

ESI-TOF/MS detection
- Hb variant selectivity
CE-ESI-TOF/MS | human and bovine Hb and OXY

- ESI-TOF/MS detection
  - species selectivity
  - OXY vs. Hb selectivity
CE-UV/VIS-ESI-TOF/MS | tuning of selectivity

Sample preparation

Separation

Detection

3 selectivity levels

CE mobility

Hemoglobin vs. Oxyglobin

UV/VIS at 415 nm

Hemoproteins vs. Others

Human hemoglobin vs. Bovine Oxyglobin
HBOC | discussion

Sample preparation

Separation

Detection

4

Immuno depletion 50%

Hemoproteins vs. Highly abundant others

Immunodepletion-CE-UV/VIS-ESI-TOF/MS

- 4 selectivity levels
- unambiguous identification
- fulfills doping control requirements

→ LOD | 0.2 g/dL in plasma | 13 µM
1 pmol (16 ng) injected

Staub et al., Electrophoresis (submitted)
Acknowledgements

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Heart of Steel (Hemoglobin)
by Julian Voss-Andreae

Images show the 5’ (1.60 m) tall sculpture right after installation, after 10 days, and after several months of exposure to the elements.
Plasma samples | protein depletion

- **Dilute and shoot** | modified CE method
- **1 depletion (ca. 50%)** | original CE method
- **2 depletions (ca. 70%)** | original CE method

UV 280 nm | detection of all proteins

**Immunodepletion**
- no more CE adsorption and ESI suppression
- no more coating and harsh rinsing
- MS-compatibility | standard-like results
Immunodepletion features

- specific interactions with antibodies
- 20 highly abundant proteins simultaneously removed from plasma
- spin column format | only 40 µL of plasma
- CE compatible | direct injection, no desalting

Decrease of the dynamic range from complex biological samples