BINDING OF PFOS AND RELATED PERFLUORINATED CHEMICALS TO SERUM PROTEINS

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Introduction

• PFOS (perfluorooctane sulfonic acid) has recently been detected in a wide range of wildlife species – even in areas remote from production and use.

• PFOS accumulates in blood serum/plasma

• We need to understand to which components of blood it is bound and need to understand its possible effects.
Sulfonic Acids
- PFOS
- PFHS
- PFBS

Carboxylic Acids
- PFOA
- PFDA
- PFBA
• Blood contains proteins of many functions
  • Enzymes
  • Antibodies
  • Carrier proteins (specific & non-specific)
  • Clotting agents

• PFOS accumulates in blood serum/plasma
• To which components of blood is it bound ?
• Need to understand possible effects
Carrier Proteins

• Can be non-specific (albumin) or specific (steroid binding globulins).
• Ligand binding to carriers may be required for normal cellular uptake and function.
• Effects of PFOS (surface active) on steroid binding proteins could cause ‘endocrine disruption’.
• ‘Strategies’ vary by organism groups:
  • Some low specificity high capacity carriers
  • Some high specificity low capacity carriers
• Later of concern as these are steroid binding globulins – can PFOS displace hormones?
Protein Ligand Interactions

• Can be very labile
• Generally equilibrium processes so any ‘separation’ of ‘bound’ from ‘unbound’ disrupts equilibrium - measurement ‘unreliable’

• Multiple lines of evidence approach
  • Hormone displacement
  • Direct binding
  • Equilibrium dialysis
  • Direct observation of binding by QTOF MS
Incubate; add DCC; centrifuge

Hormone remains in solution: Measure in Scintillation counter

PFOS blocks hormone binding

Hormone Displacement Assay

- Hormone
- PFOS
- Binding protein
- DCC
Effect of perfluorinated carboxylic and sulfonic acid compounds on the binding of $^3$H-estradiol to carp serum

Percent relative to control vs. µM
Threshold Values and Relative Affinities for Compounds in Carp Serum.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Estimated $in vivo$ Carp estradiol displacement</th>
<th>Affinity Relative to E2 $EC_{10}$ values (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFBS (C4)</td>
<td>&gt;8000</td>
<td>&lt;9.1x10$^{-6}$</td>
</tr>
<tr>
<td>PFHS (C6)</td>
<td>&gt;8000</td>
<td>&lt;9.1x10$^{-6}$</td>
</tr>
<tr>
<td>PFOS (C8)</td>
<td>3576</td>
<td>2.1x10$^{-5}$</td>
</tr>
<tr>
<td>PFBA (C4)</td>
<td>4772</td>
<td>1.5x10$^{-5}$</td>
</tr>
<tr>
<td>PFOA (C8)</td>
<td>4244</td>
<td>1.7x10$^{-5}$</td>
</tr>
<tr>
<td>PFDA (C10)</td>
<td>2500</td>
<td>2.9x10$^{-5}$</td>
</tr>
</tbody>
</table>
Displacement of Cortisol from Bird Serum.

**Chicken**

**Bald Eagle**

% $^3$H-C binding (control=100)

- PFBS(C4)
- PFHS(C6)
- PFOS(C8)
- PF BUTYRIC ACID (C4)
- PF OCTANOIC ACID (C8)
- PF DECANOIC ACID (C10)
Summary

• PFCs relatively weak at displacing steroids from serum proteins

• Even at high concentrations displacement of E2 from carp serum was not complete

• Only minor species differences in cortisol displacement were observed
Q-TOF Mass Spectrometry

Analysis of high molecular weight polymers like proteins

Binding proteins only active in native tertiary structure

Using ‘soft’ ionization can observe proteins in delicate ‘native’ configuration

Protein integrity and MS performance diametrically opposed - need to do a very delicate balancing act
Q-TOF Mass Spectrometry

Soft ionization Techniques:

• Vacuum ‘poor’ – as close to atmospheric as will permit
  $10^{-4}$ Torr not $10^{-6}$
• Low temperatures – ionization usually at 200-400°C
  $< 80^\circ$C
• Low voltages – voltages to accelerate, focus, filter ions
• Best results in absence of any other ions in solution
The Q-Tof™ combines a quadrupole mass filter [MS 1], a hexapole collision cell and an oaTOF mass analyser [MS 2] to deliver high performance MS-MS.
Quadrapole used as a “mass filter” - removes unbound PFOS

Depending on the temperature and voltage in the collision cell, protein:PFC complexes can pass through TOF sector either intact or with free PFC.
Effect of Buffers on Sensitivity and Profile of 'native' Protein MS.

1 mg/ml BSA in H₂O

1 mg/ml BSA in 10 mM NH₄Ac

National Food Safety and Toxicology Center
Michigan State University
Mass determination for BSA in the absence or presence of PFOS
## Binding of PFOS to BSA

Using mathematical software to de-convolute the multiply charged peaks seen in Q-TOF MS to derive Mr for the protein (theoretical Mr for albumin approx. 66,780).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mr (n=2)</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>67,336</td>
<td></td>
</tr>
<tr>
<td>Albumin+PFOS</td>
<td>68,087</td>
<td>750</td>
</tr>
</tbody>
</table>

Mr of PFOS is 499 suggesting 1-2 PFOS per BSA molecule. Other assays suggest 1 PFOS bound per BSA molecule.

In combination this suggests 1 PFOS bound per BSA.
Gel Filtration
Separates binding proteins based on their size.

PFCs do not remain bound to proteins during the separation. Possibly due to interaction with the column or simply disruption of equilibrium.

Q-TOF Mass Spectrometry
Can use Quadrupole-Time-of-Flight (Q-TOF) instrument to separate bound and unbound PFCs allowing determination of protein fractions which bind these compounds.
Only PFOS bound to protein can reach the detector.
Mass Spectrometer Output

Protein (BSA)

PFOS Binding capacity

Mass Spectrometer Output

2: Diode Array
280 1.00Da
2.44e5

1: TOF MS ES-
499 1.00Da
1.74e3

Protein (BSA)
Binding of PFOS to BSA and carp serum
Ligand Binding Sites on Human Serum Albumin
Conclusions

PFOS binds tightly to serum albumin under physiological and non-physiological conditions.

Indications are that only one PFOS molecule binds per BSA molecule.

Would require serum PFOS concentrations of 100-150 ppm to saturate albumin before other sites (lower affinity) were affected.

Serum albumin will act to significantly ameliorate possible adverse effects of PFOS in vivo.
Acknowledgement

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