Non Specific Binding (NSB) in Antigen-Antibody Assays

Chem 395
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Instructor : Dr. James Rusling

Presenter : Bhaskara V. Chikkaveeraiah
OUTLINE

• Immunoassays Introduction

• Factors contributing to Non-specific binding (NSB)

• NSB Blocking agents

• Minimizing NSB

• NSB in Immunoassay.
Immunoassay

- **Immunoassay**
  A biochemical test-measures levels of a particular molecule in biological samples- e.g. serum – uses antibody binding to its antigen (specific binding).

- **Importance**
  - Detecting a disease at very early stage, at lowest antigen concentration.
  - Accuracy of the detection.

**Non-specific binding (NSB) affects these two factors**
In addition to binding to receptors of interest, sec. antibody may also bind to other sites. Binding to the receptor of interest is called specific binding, while binding to the other sites is called nonspecific binding (NSB).

NSB can be minimized by saturating the unoccupied binding sites with a blocking reagent (NSB agent) without taking active part in specific assay reaction.
Immunoassay without NSB blocking agent

Antigen → PG electrode → Voltage + H₂O₂ → Signal → Enzyme labelled Secondary antibody → Primary Antibody

I vs. t
Immunoassay with NSB Blocking agent

Antigen

Blocking agent

PG Electrode

Voltage + H_2O_2

Signal

Enzyme labeled Secondary antibody

Primary antibody

I

t
Properties of the blocking agent.

• Inhibit NSB (passive or covalent) of assay components to the surface,

• Inhibit non-specific protein - protein interaction.

• Exhibit no cross reactivity with subsequent assay components ( antibodies, protein )

• Not disrupt the bonds that immobilize the specific protein or biomolecule to the surface.

• Exhibit consistent, reproducible performance with every lot.
Blocking agents

- **Detergent Blockers**
  - Tween-20, Triton X-100

- **Protein Blockers**
  - Bovine serum albumin, Casein, Fish Gelatin, Whole Sera,

- **Polymer based Blockers**
  - Polyethylene glycol (PEG), Polyvinyl alcohol (PVA), Polyvinylpyrrolidone (PVP), Polyacrylic acid (PAA), Polyacrylic maleic acid (PAMA).
Detergent Blockers

- Disrupt ionic and hydrophobic biomolecule-surface bonds.
- Inhibits enzyme-substrate reactions.

- Inexpensive,
- stable, can be stored at room temperature (wash buffers)
- strips off loosely bound molecules in wash steps.
- 0.01 – 0.10% is commonly used.
Protein Blockers

- Blocks the non occupied sites on the surface.
- Space out and stabilize biomolecules bound to the surface to reduce the steric hindrance.

- Bovine serum albumin (BSA)
  - Widely used, Inexpensive, blocks non specific protein-surface binding. ~1 – 3% ic commonly used.

- Fish Gelatin
  - Mainly blocks protein-protein(Ab1-Ab2) interactions

- Whole sera
  - Blocks biomolecule-surface passive, covalent interactions, protein-protein interactions, and acts as protein stabilizer.
Factors affecting NSB

• Ab1 / Ab2 ratio ( ~ 500:1)

• Type of blocking agent used.
  – Bovine serum albumin, Casein, etc.

• Washing steps
  – Consistent and vigorous.
PSMA Immunoassay
(Prostate Specific Membrane Antigen)

- PSMA
- Blocking agent
- SWCNT
- PG Electrode

Voltage + $H_2O_2$

Signal

HRP labeled anti-PSMA1

anti-PSMA2

PSMA (Prostate Specific Membrane Antigen)
Effect of blocking agents in PSMA Immunoassay

PBS buffer + 0.05% Tween-20 + 2% BSA

<table>
<thead>
<tr>
<th>PSMA ng/mL</th>
<th>Current nA</th>
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<tbody>
<tr>
<td>10 ng/mL</td>
<td>345 ± 22</td>
</tr>
<tr>
<td>100 ng/mL</td>
<td>467 ± 83</td>
</tr>
<tr>
<td>Control</td>
<td>351 ± 16</td>
</tr>
<tr>
<td>0 ng/mL</td>
<td></td>
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</tbody>
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PBS buffer + 0.05% Tween-20 + 2% Casein

<table>
<thead>
<tr>
<th>PSMA ng/mL</th>
<th>Current nA</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 ng/mL</td>
<td>325 ± 106</td>
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<tr>
<td>100 ng/mL</td>
<td>583 ± 97</td>
</tr>
<tr>
<td>Control</td>
<td>246 ± 53</td>
</tr>
<tr>
<td>0 ng/mL</td>
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Reduction of the Nonspecific Binding of a Target Antibody and of Its Enzyme-Labeled Detection Probe Enabling Electrochemical Immunoassay of Antibody through the 7 pg/mL – 100 ng/mL (40 fM - 400 pM) Range

Yongchao Zhang and Adam Heller*
Department of Chemical Engineering and Texas Materials Institute
University of Texas at Austin.

Experimental Procedure

- Screen Printed Electrode
- Rabbit IgG
- Biotin-labeled anti-rabbit IgG
- Avidin
- H_{2}O_{2}
- H_{2}O
- PAA / PAMA
- PAA-PVP-[Os(bpy)$_2$Cl]$_2$ polymer
- HRP labeled anti-rabbit IgG

Experimental Procedure Diagram:

1. Screen Printed Electrode
2. Rabbit IgG
3. Biotin-labeled anti-rabbit IgG
4. Avidin
5. PAA / PAMA
6. PAA-PVP-[Os(bpy)$_2$Cl]$_2$ polymer
7. HRP labeled anti-rabbit IgG

Diagram:

- Oxidation of H$_2$O$_2$ to H$_2$O

Graph:

- Current (I) vs. Time (t)

Legend:

- Os$^{2+}$
- Polymeric layer

Note: The diagram illustrates the interaction of various components in a bioelectrochemical system, highlighting the use of HRP labeled anti-rabbit IgG and PAA-PVP-[Os(bpy)$_2$Cl]$_2$ polymer for enhanced detection.
Results and Discussion

Suppression of the nonspecific binding-noise current by polyanions having terminal functions forming covalent bonds with amines and with thiols.
Dependance of $\text{H}_2\text{O}_2$ electroreduction current on the antigen concentration.
Summary

• Selection of appropriate blocking system is essential for the development of a specific and sensitive assay.

• Empirical testing is required to both choose and optimize the blocking procedure and is influenced by the surface chemistry.
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