Redox Switching of Protein Function

- Flavins and electrochemical methods
- Redox Switching of Protein Function
- Proline Metabolism
  - DNA vs Membrane Binding
Discovery of flavin mononucleotide (FMN) by a biochemist at KI

Showed the biochemical basis for riboflavin as a vitamin

Axel Hugo Theodor Theorell

Nobel Prize in Physiology or Medicine 1955

Nobelprize.org
Redox Chemistry of Flavin

Flavin semiquinone is EPR active (S=1/2).

Figure 1.6. Absorption spectra of neutral and anionic forms of flavosemiquinone, as illustrated with glucose oxidase, from Massey & Hemmerich (1980).

Flavins support one-electron and two-electron transfer processes involving the N(1), C(4a) and N(5) positions of the isoalloxazine ring system.

Because flavins are involved in catalyzing oxidative and reductive mechanisms, characterizing the redox potentials of the substrates, products, and flavins are important. The redox potential of free flavin is -219 mV (pH 7). However, when bound to an enzyme, the redox potential can vary anywhere from + 100 mV to - 400 mV. Thus, the active site environment has a tremendous influence on the redox and electronic properties of the flavin coenzyme in order to optimize catalysis.
Electrochemistry Methods for Studying Redox Proteins

**Potentiometry**
An important thermodynamic parameter is the establishment of equilibrium between all redox active species. The standard redox potential ($E^\circ$) or midpoint potential ($E_m$) is determined along with the number of electrons ($n$) transferred in the reduction step. Measurement is taken at zero current flow.

$$E_{\text{meas}} = E^\circ + \frac{RT}{nF} \ln \left(\frac{\text{ox}}{\text{red}}\right)$$

**Spectroelectrochemistry**
Combines electrolysis, potentiometry, and spectroscopy (UV-visible, electron spin resonance, IR, etc.)

**Cyclic voltammetry**
"Electronic scanning” Potential is scanned while current is measured

**Coulometry**
number of electrons ($n$) transferred to a protein
molar absorptivity ($\varepsilon$)

$$Q = nFVC$$

$Q$ = total charge required (Coulombs, C)
-determined by measuring current ($I = \text{amp or C/s}$) x time (sec)

$n$ = number of electrons
$F =$ Faraday constant (96,485 Coulombs (C)/mole e-)
$V =$ volume
$C =$ concentration of redox center
Prosthetic Groups are buried in Proteins

Space-Filling Model

FAD
Electrochemical Methods

\[ E_{\text{meas}} = E^o + \frac{RT}{nF} \ln(\text{ox/red}) \]

- \( R = 8.314 \text{ J mol}^{-1} \text{ K}^{-1} \)
- \( n = \text{number of electrons} \)
- \( T = \text{K} \)
- \( F = 96485 \text{ J V}^{-1} \text{ mol}^{-1} \)
Redox potential of bound FAD in PutA

\[ E_m = -0.076 \text{ V (pH 7.5)} \]

Xanthine/Xanthine Oxidase Method


\[ E_{m(\text{protein-FAD})} = -67 \text{ mV (pH 7.5)} \]

Slow reduction!

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xanthine

-380 mV, pH 7.5

urate

Methyl viologen (ox)

Methyl viologen (red)

resorufin(red) + FAD(red)

-79 mV, pH 7.5

resorufin(ox) + FAD(ox)
Redox linked protein-ligand interactions

The binding of molecule (L) to the oxidized and reduced forms of a protein can be linked to differences in the potential of the protein in the presence ($E_m(bound)$) and absence ($E_m(free)$) of the ligand by the following thermodynamic box.

\[ \text{Protein}_{\text{ox}} + 2e^- \rightleftharpoons \text{Protein}_{\text{red}} \]

\[ K_{d(ox)} = \frac{[\text{Protein}_{\text{ox}}][L]}{[\text{Protein}_{\text{ox}}*L]} \]

\[ K_{d(red)} = \frac{[\text{Protein}_{\text{red}}][L]}{[\text{Protein}_{\text{red}}*L]} \]

There are two binding equilibria:

\[ P_{\text{ox}} + L \rightleftharpoons P_{\text{ox}}*L \]

\[ K_{d\text{ox}} = \frac{[P_{\text{ox}}][L]}{[P_{\text{ox}}*L]} \]

\[ P_{\text{red}} + L \rightleftharpoons P_{\text{red}}*L \]

\[ K_{d\text{red}} = \frac{[P_{\text{red}}][L]}{[P_{\text{red}}*L]} \]
Flavin Redox Switch

Inputs

substrates
electron donors
light

FAD_{ox}

\[ \begin{array}{c}
\text{R} \\
\text{N} \\
\text{5} \\
\text{4a} \\
\text{3NH} \\
\text{2} \\
\text{1} \\
\text{N} \\
\text{10}
\end{array} \]

\rightarrow \text{e-}

FAD_{red}

\[ \begin{array}{c}
\text{R} \\
\text{N} \\
\text{N} \\
\text{H} \\
\text{NH} \\
\text{OH}
\end{array} \]

Outputs

transcriptional regulation
membrane binding
cell signaling
**Examples of Flavin Dependent Switches**

*E. coli* Pyruvate oxidase (cytosol → membrane bound ubiquinone oxidoreductase)

\[
\text{Pyruvate} + \text{CoQ}_8 + \text{OH} = \text{acetate} + \text{CO}_2 + \text{CoQ}_8\text{H}_2
\]

Per-Arnt-Sim (PAS) domain family

Blue-light photoreceptor regulators (440 – 480 nm) (Light Oxygen Voltage)

Physiological Roles

B. abortus
- intracellular proliferation in macrophage

C. crescentus
- Cell attachment

S. elongatus
- light
- 3αLOV-HK-P

B. subtilis
- light
- stress
- light

Mechanisms of Functional Switching

For light sensing, flavin must be oxidized.

In response to light absorption by the LOV domain, a cysteinyI-C(4a) covalent adduct is formed.

Proline Metabolic Pathway

**PutA**

![Diagram of proline metabolic pathway](image)

**E. coli**

1- [D] PRODH P5CDH -1320

**B. jap.**

1- [D] PRODH P5CDH -999
PutA Proteins

Tanner JJ and Becker DF. PutA and Proline Metabolism. 2013 De Gruyter Publisher
Structure of full-length bifunctional PutA

PutA Proteins

Tanner JJ and Becker DF. PutA and Proline Metabolism. 2013 De Gruyter Publisher
Functional Switching of Proline Utilization A (PutA)

PutA-DNA Binding

A

putP

O1/2

O3/4/5

putA

B

183-210 (O1) 5’-TTTCACTACGTTGCACTCTCTCACATTT-3’
211-231 (O2) 5’-TTTGCGGTTCGACCCTTTCAAA-3’
342-365 (O3/4) 5’-CATGGTTGCAACAAAGTTGCAACAT-3’
388-412 (O5) 5’-TAAGTTGCAACCTTTCTGAACACAG-3’

C

Model of EcPutA and EcPutA-DNA complex

How does EcPutA switch functions?

Proline decreases PutA-DNA binding by 2-fold

PutA Redox Properties in the Presence of *put* Intergenic DNA

\[ E_{m(bound)} = -0.086 \text{ V (pH 7.5)} \]

\[ E_m = -76 \text{ mV} \quad \text{PutA}_{\text{ox}} \leftrightarrow 2 \text{ e-} \quad \text{PutA}_{\text{red}} \]

\[ K_d = 45 \text{ nM} \quad \text{DNA} \quad \text{DNA} \quad K_d = 98 \text{ nM} \]

\[ E_m = -86 \text{ mV} \quad \text{PutA}_{\text{ox}}-\text{DNA} \leftrightarrow 2 \text{ e-} \quad \text{PutA}_{\text{red}}-\text{DNA} \]

Only ~ 2-fold increase in \( K_d \)

Proline Dependent Binding to Lipid Bilayers
(Surface Plasmon Resonance Study)

Sensorograms of oxidized PutA (20 nM) and PutA (20 nM) with 5 mM proline binding on E. coli polar extract lipids. The arrows indicate the starting and ending of injection of protein sample. Buffer: 10 mM HEPES, 150 mM NaCl, pH 7.4

Conformational change in PutA with FAD reduction

$E_{\text{meas}}$ (mV)  OX  -31  -36  -48  -60  -66  -74  -85  RED  Re-OX

135 kDa →

111 kDa →

$E_{m(\text{Conf})} = -58$ mV
(pH 7.5)

$log ([\text{ox}]/[\text{red}])$

1-D PRODH P5CDH

PutA

$E_m$ (FAD) = -76 mV

Conformational change

How do signals from the FAD mediate PutA conformational changes?

How does FAD control PutA conformation and function?

Structural changes induced by reduction of the FAD in PutA

Yellow
Oxidized EcPutA PRODH domain
(PDB code 1TIW)

Gray
Reduced EcPutA PRODH domain
(PDB 3ITG)

Structural changes:
2’-OH group rotates 90°
FAD(N)5-R431 and R431-D370 interactions are disrupted

D370 and E372 mutations impair PutA functional switching

Cell based reporter assays

$\text{putC:}\text{lacZ}$

$\text{putC:}\text{lacZ}$ proline $\text{putC:}\text{lacZ}$

PutA (ox) PutA (red)-Membrane

Miller Units

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<th>WT</th>
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Model for Redox Dependent PutA Membrane Binding

Summary

• Flavins support one-electron and two-electron transfer processes involving the N(1), C(4a) and N(5) positions of the isoalloxazine ring system.
• Electrochemistry is a versatile tool for studying redox regulation and conformational changes.
• Proline/PRODH generates mitochondrial ROS and influences signaling pathways that determine cell survival and cell death outcomes.
• PutAs have fused PRODH and P5CDH domains that are connected by a substrate channeling cavity.
• FAD reduction induces conformational changes in EcPutA that lead to activation of membrane binding.
• Redox signals originating at the flavin N(5) are likely transmitted to the surface of EcPutA via internal hydrogen bond rearrangements.
• Alpha-domain of EcPutA is involved in conformational changes and membrane binding.