Water Biology and Medicine: from aquaporins to aquaphotomics

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Keio School of Medicine
## Water permeabilities of plasma membrane vesicles

<table>
<thead>
<tr>
<th>Membrane</th>
<th>$P_t$ (μm/s)</th>
<th>$E_A$ (kcal/mol)</th>
<th>Mercurial sensitivity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Planar lipid bilayer</td>
<td>1 - 104</td>
<td>10.8 - 14.9</td>
<td>-</td>
<td>[25]</td>
</tr>
<tr>
<td>Toad bladder</td>
<td>4</td>
<td>11.0</td>
<td>-</td>
<td>[24]</td>
</tr>
<tr>
<td><strong>apical membranes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(- ADH)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hog gastric vesicles</td>
<td>2.8</td>
<td>15.1</td>
<td>-</td>
<td>[26]</td>
</tr>
<tr>
<td>Human placenta</td>
<td>19</td>
<td>13.9</td>
<td>-</td>
<td>[27]</td>
</tr>
<tr>
<td>Brain synaptosomes</td>
<td>45</td>
<td>18</td>
<td>-</td>
<td>[22]</td>
</tr>
<tr>
<td>Rat small intestine</td>
<td>60</td>
<td>13.3</td>
<td>-</td>
<td>[21]</td>
</tr>
<tr>
<td>Rabbit proximal tubule</td>
<td>166</td>
<td>n.d.</td>
<td>+</td>
<td>[28]</td>
</tr>
<tr>
<td>Rabbit erythrocytes</td>
<td>530</td>
<td>4.6</td>
<td>+ +</td>
<td>[29]</td>
</tr>
<tr>
<td>Rat proximal tubule</td>
<td>760</td>
<td>3.1</td>
<td>+ +</td>
<td>[30]</td>
</tr>
<tr>
<td>Toad bladder</td>
<td>450</td>
<td>n.d.</td>
<td>+ +</td>
<td>[23]</td>
</tr>
<tr>
<td><strong>apical membrane</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(+ ADH)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat collecting duct endosomes</td>
<td>300</td>
<td>3.8</td>
<td>n.d.</td>
<td>[31]</td>
</tr>
<tr>
<td>Toad bladder endosomes</td>
<td>1000</td>
<td>3.9</td>
<td>+</td>
<td>[32]</td>
</tr>
</tbody>
</table>
Transmembrane water permeability

Bilayer Diffusion

All biological membranes
Low capacity
No known inhibitors
$E_a$ 10 kcal/mol

Water Channels

Renal tubules, secretory glands, red cells
High capacity for H$_2$O, not H$_3$O$^+$
Reversibly inhibited by Hg$^{++}$
$E_a$ 5 kcal/mol
Discovery of Aquaporin-1
Functional expression

Hypo-osmolar swelling
Hg\textsuperscript{++} inhibited, no currents
Preston et al., Science 1992
Human Aquaporin Repertoire

<table>
<thead>
<tr>
<th>Aquaporin (AQP)</th>
<th>Tissue or Cell Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>AQP0</td>
<td>Eye (Lens)</td>
</tr>
<tr>
<td>AQP1</td>
<td>Choroid Plexus, Erythrocytes, Eye (Cornea), Gallbladder, Kidney, Lung, Liver, Pancreas</td>
</tr>
<tr>
<td>AQP2</td>
<td>Kidney</td>
</tr>
<tr>
<td>AQP3</td>
<td>Erythrocytes, Eye ( Conjunctiva), Kidney, Skin, Upper Respiratory Tract</td>
</tr>
<tr>
<td>AQP4</td>
<td>Brain (Glial Cells), Eye (Retina), Kidney, Muscle</td>
</tr>
<tr>
<td>AQP5</td>
<td>Lacrimal Gland, Lung, Salivary Gland, Skin (Sweat Glands)</td>
</tr>
<tr>
<td>AQP6</td>
<td>Kidney</td>
</tr>
<tr>
<td>AQP7</td>
<td>Adipocytes, Kidney, Testis</td>
</tr>
<tr>
<td>AQP8</td>
<td>Colon, Kidney, Liver, Pancreas</td>
</tr>
<tr>
<td>AQP9</td>
<td>Brain, Leukocytes, Liver, Spleen</td>
</tr>
<tr>
<td>AQP10</td>
<td>Small Intestine</td>
</tr>
</tbody>
</table>

Source: Masato Yasui, MD, PhD/Johns Hopkins Medicine

(Yasui, JAMA, 2004)
Clinical relevance of aquaporins

Cell Biology:
- Secretion/absorption
- Epithelial regeneration
- Tumor Growth
- Vascular regeneration
- Cell cycle

Brain edema
Malaria infection
Obesity
Lung edema
Polycystic kidney
DI
DM

Brain edema
Cataract
Dry eye
Mood disorders
Dry mouth
Dry skin
Skin regeneration
DI
Schizophrenia
Glaucoma
Toxemia of pregnancy

Clinical relevance of aquaporins
AQP2—A regulated water channel

cDNA cloned by homology (Fushimi et al., Nature, 1993)

AQP2 localization in kidney (Nielsen et al., Proc Natl Acad Sci, 1993)
AQP2—Acute regulation by AVP

Isolated renal collecting ducts

Nielsen et al., Proc Natl Acad Sci, 1995

100 pM AVP
P$_f$ up 5-fold

Inherited defects (rare)
Nephrogenic DI (severe)

Acquired defects (very common)
Overexpression—Fluid retention
Underexpression—Enuresis
AQP0 and Cataracts

Major intrinsic protein (MIP)
- Lens fiber cells

Human AQP0 mutations
- Congenital cataracts
- Dominant inheritance
  - T138R—Multifocal opacities
  - E134G—Unilamellar cataract

Importance in neonatal lens development?

Berry et al., Nature Genetics, 2000
Francis et al., Human Mol Genetics, 2000
Coherent anti-Stokes Raman scattering (CARS)

Imaging specific molecular vibration

H\(_2\)O molecule

OH-stretching vibration
Energy diagram for OH CARS

\[ \omega_{\text{pump}} \] (793nm) \rightarrow \omega_{\text{Stokes}} \] (1064nm) \rightarrow \omega_{\text{CARS}} \] (633nm)

\[ \omega_{\text{pump}} - \omega_{\text{Stokes}} = 3200 \text{ cm}^{-1} \]

Raman shift for OH-stretch vibration = 3200 cm\(^{-1}\)
CARS signals from H\textsubscript{2}O and D\textsubscript{2}O

Detection of H\textsubscript{2}O, but not D\textsubscript{2}O (deuterium oxide)
Experimental procedure for flushing isotonic D$_2$O/HBSS

1. HeLa cells in H$_2$O/HBSS
2. Flush D$_2$O/HBSS
3. Image efflux of H$_2$O from the cell
Frame by frame pictures of $\text{H}_2\text{O}$ efflux from single cell
(Ibata et al, Biophys. J. 2011)
Structure and Function

Stopping the protons!
It is crucial for a cell's function that the pH difference between its inside and its outside is kept constant - leakage of protons through the membrane would be disastrous. For this reason the water channel rejects positively charged ions such as protons, while water molecules can worm their way through without difficulty.

Outside the channel the ions are surrounded by water molecules
The orientation and hydrogen bonding of water molecule at ar/R region

Isolation from other water molecule interrupts proton conduction.
# SIMCA (1300-1600nm, transform : MSC)

![Graph showing interclass distance]

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>M1</th>
<th>M23</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>0</td>
<td>2.7401</td>
<td>3.556</td>
</tr>
<tr>
<td>M1</td>
<td>2.7401</td>
<td>0</td>
<td>1.3314</td>
</tr>
<tr>
<td>M23</td>
<td>3.556</td>
<td>1.3314</td>
<td>0</td>
</tr>
</tbody>
</table>

*(Tsenkova & Yasui, unpublished)*
Difference spectra from Wild type

C2:1364 Water shell
C5:1410 S0
C9:1462 S2

ave (AQP M1)
ave (AQP M23)
Difference spectra WT (AQP4+) – KO (AQP4-)

AquaGram of difference spectra (WT-KO)

Difference spectra WT and KO was indicated the positive band around 1409nm and negative band around 1491nm (left figure). (right figure). AquaGram of Difference spectra WT (AQP4+) and KO (AQP4-) (right figure). These data shown the increased free water by AQP4.
Effects of AQP expression on cryopreservation

(Kato et al., PLoS One, 2014)
Anti-freezing effects of AQP

Before freezing

(-): (+) = 3:1

(-): (+) = 1:1

(-): (+) = 1:3

Ultra-quick freezing

After freezing

(-) AQP4 (+)

(-) AQP4 (+)

(-) AQP4 (+)

(Kato et al., PLoS One, 2014)
Aquaphotomics Team at Keio Univ.  
: from basic to clinical applications

Project leader: Dusan Kojic
KEIO Aquaphotomics projects: from basic to clinical application

**Electrolytes**

- **α-D-glucose**
- **β-D-glucose**

**Amino-acids**

- by Nakajima and Kojic

**Sugars**

- by Kojic

**Ab-initio or flexible MD simulation**

- by Tanaka, Iijima, et al.
- by Tomobe, Nakamura and Yasuoka
Clinical Applications


Prediction of Ovulation in Women

10 healthy women

Patient code: 503

Relation to LH

(Unpublished)

Diagnosis of Prostate Cancer

Biomarkers:
1. Plasma PSA
2. Urine PCA

200 urine samples have been collected and analyzed from patients whose PSA is between 4 and 20, and taken biopsy at Keio Hospital

By Kojic, Tanaka, Iijima, etc.