Determination of *in vivo* Rubisco kinetics in *Arabidopsis*

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• One of the first Canadian women MD
• Studied in Germany, then the US
• Took 30 years to receive full professorship
• Studied everything from cancer to bacterial toxins
• But most importantly for today…
Michaelis-Menton Kinetics Seminar Summary

- Enzymes
- Enzyme activity can be modeled
- Competitive inhibition
- Rubisco
- *In vivo* kinetics
- Models of photosynthesis
- Uses of photosynthetic models
  (Who cares?)
- PLEASE ASK QUESTIONS IF I LOSE YOU!
Enzyme

- Molecular machines
- Facilitate favorable reactions (Catalyze)
- Coordinate life

\[
\begin{align*}
\text{Pyruvate} & \quad \text{Lactate} \\
\text{CH}_3\text{C} & \quad \text{CH}_3\text{CH} \\
\text{COOH} & \quad \text{COOH} \\
\text{NADH} & \quad \text{NAD}^+ \\
\text{LDH} &
\end{align*}
\]
Enzymes

• Long chains of amino acids
• Chains fold to create shape
• Shape gives catalytic ability by stabilizing intermediates
Enzyme definitions

$$H_2O + CO_2 \xleftrightarrow{CA} H_2CO_3 \xleftrightarrow{} HCO_3^- + H^+$$

Enzyme

Substrates/Reactants

Products
Modeling of enzymes

Which enzyme is faster? (higher activity)
Modeling enzymes

- Enzyme activity will increase with substrate concentration to a certain point
- Enzyme activity will saturate
- Single-substrate enzymes can be modeled by…
\[ V_o = \frac{V_{\text{max}} [S]}{K_m + [S]} \]

\( V_o = \text{reaction rate} \)
Modeling enzymes

- $K_m$ and $v_{max}$ describe the affinity and maximum velocity.
- High $K_m$ = low affinity, low $K_m$ = high affinity.
Competitive Inhibition

• Previous equation only works with a single substrate
• What if there is a second molecule the enzyme reacts with?
Modeling of enzymes

Which enzyme is faster?
Competitive inhibition

- Need to account for the specificity ($K_i$) and concentration ($[I]$) of the inhibitor
- What happens as $[I]$ increases? Decreases?

$$V_o = \frac{v_{\text{max}} [S]}{K_m + [S]}$$

One substrate

$$V_o = \frac{v_{\text{max}} [S]}{K_m \left( 1 + \frac{[I]}{k_i} \right) + [S]}$$

Competitive inhibition
Rubisco

- Most abundant form of organic N on the planet
- Captures CO$_2$ in the first step of sugar production
- Often the limiting step to photosynthesis
- Ribulose 1-5 bisphosphate Carboxylase Oxygenase
• The schizophrenic enzyme essential for life on earth
• Modeled with competitive inhibition
**In vivo kinetics**

- Data from earlier from *in vitro*
- Enzymes are in different conditions in a living plant
- How can we determine the *in vivo* kinetic descriptions of Rubisco?
6CO₂ + 6H₂O → C₆H₁₂O₆ + 6O₂
How gas exchange works

Leaf

Boundary layer

Bulk air

CO₂

Guard cell

Stoma

Cuticle

Mesophyll cells

Epidermal cell

H₂O

Chapin et al., (2002)
How gas exchange works
How gas exchange works
In vivo Kinetics

\[ V_{\text{max}} \]

\[ K_c \]

\[ V_c = \frac{v_{max} [CO_2]}{K_m \left(1 + \frac{[O_2]}{k_o}\right) + [CO_2]} \]

(A) Graph showing the relationship between \( A \) (\( \mu \text{mol m}^{-2} \text{s}^{-1} \)) and \( p_i \) (\( \mu \text{bar} \)) with different \( O_2 \) concentrations.

- \( O_2 \) = 2%  
- \( O_2 \) = 5%  
- \( O_2 \) = 21%  
- \( O_2 \) = 35%  
- \( O_2 \) = 50%
Leaf Models of photosynthesis

\[ \text{Assimilation} = V_c - 0.5V_o - R_d \]

- With Kinetic parameters for Rubisco one can then model CO$_2$ assimilation rates under a given condition
Models of Photosynthesis

*Why should I care?*

- How fast will a plant fix carbon in a given \( \text{CO}_2 \) concentration?
- How does photosynthesis respond to changing temperature?
- What are inefficiencies in photosynthesis?
- Understand plant energy use
What this project will involve:

• Measuring leaf gas exchange of CO$_2$ under different conditions (Temp and atm)
• Fitting these measurements to models of Rubisco kinetics to determine constants
• Using these constants to predict photosynthesis in model plants
In vivo Kinetics