FTIR Difference Spectroscopy study of Y(B718)T Mutant Species from *Synechocystis* sp. PCC 6803

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What is Photosynthesis?

Process by which plants, algae and some bacteria use light energy to produce Carbohydrates, the food for all living organisms.

\[ 6\text{H}_2\text{O} + 6\text{CO}_2 \xrightarrow{\text{light, plant}} \text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2 \]
Classification of Photosynthetic Organisms

Theory of Endosymbiosis

A prokaryote ingested some aerobic bacteria. The aerobes were protected and produced energy for the prokaryote.

Over a long time, the aerobes became mitochondria, no longer able to live on their own.

Some primitive prokaryotes also ingested cyanobacteria, which contain photosynthetic pigments.

The cyanobacteria become chloroplasts, no longer able to live on their own.
Where does photosynthesis take place?

In plants and algae: in chloroplast-thylakoid membrane

In bacteria: in thylakoid membrane

http://www.daviddarling.info
Overview of Photosynthesis

Major protein-cofactor complexes in the thylakoid membrane
Subunit Organization of Photosystem I

Amino Acids

- Nonpolar (Hydrophobic)
- Polar (Hydrophilic)
- Acidic
- Basic
Protein Backbone

Amino acid 1

\[ \text{R}_1 \]
\[ \text{HC} - \text{C} - \text{O} \]
\[ \text{NH}_2 \]

Amino acid 2

\[ \text{R}_2 \]
\[ \text{HC} - \text{C} - \text{O} \]
\[ \text{NH} \]

+ \[ \text{OH} \]

\[ \text{HO}_2 \]

\[ \text{OH} \]

\[ \text{NH} \]

\[ \text{R}_2 \]

\[ \text{HC} - \text{C} - \text{O} \]

Dipeptide

\[ \text{Peptide bond} \]
Infrared Spectroscopy

- IR spectroscopy is the measurement of the wavelength and intensity of the absorption of infrared light by a sample.
- Spectral range: 4000 – 200 cm\(^{-1}\) (2.5-50\(\mu m\))
- The infrared spectra gives sharp features that are characteristic of specific types of molecular vibrations.
Amide Vibrations

- The peptide group, the structural repeat unit of proteins, gives up to 9 characteristic bands named amide A, B, I, II ... VII.

- **Amide I** and **Amide II** bands are two major bands of the protein infrared spectrum.

- The **Amide I band** (between 1600 and 1700 cm\(^{-1}\)) is mainly associated with the C=O stretching vibration (70-85%) and is directly related to the backbone conformation.

- **Amide II** results from the N-H bending vibration (40-60%) and from the C-N stretching vibration (18-40%). This band is conformationally sensitive.
Amide I is due to THOUSANDS of C=O peptide bonds 
Need Difference Spectroscopy
Only bands affected by the radical show up. Everything else is subtracted.

Negative band --- Ground state
Positive band --- Excited state
ET Chain of Photosystem I
Structure of Chlorophyll-a

FIGURE 1 Molecular structure and International Union of Pure and Applied Chemistry numbering scheme for chlorophyll a.
Structure of P700 in *Synechocystis* sp. PCC 6803
A **ketone** is a compound that contains the functional group

\[
\begin{array}{c}
\text{R}_1 \text{C} \text{R}_2 \\
\end{array}
\]

**Esters** are organic compounds in which an organic group (\( \text{R}' \)) replaces a hydrogen atom (or more than one) in an oxygen acid.

\[
\begin{array}{c}
\text{R} - \text{C} \text{O} \\
\text{O} - \text{R}' \\
\end{array}
\]
Crystal Structure of P700 at 2.5 Å

Asymmetric Environment

$P_A$ side in H-Bond network

$P_B$ side free from H-Bond network
Structure of Tyr and Thr

Tyrosine (Tyr)

Threonine (Thr)
Crystal Structure of P700 on Mutation
Light Induced P700$^+/P700$ Difference Spectra
Conclusion

- The $^{13}_1$ keto and $^{13}_3$ ester C=O mode of $P_A/P_A^+$ is unaltered upon mutation.
- The $^{13}_3$ ester C=O mode of $P_B/P_B^+$ upshifts by ~ 2 cm$^{-1}$ which could be due to a possible charge redistribution upon mutation.
- The $^{13}_1$ keto C=O of $P_B/P_B^+$ is considerably downshifted which could indicate the possibility of the introduction of a hydrogen bond to $P_B$ in the mutant.
Questions?