THE SUCCESSFUL TURNOVER OF PHOTOSYSTEM II CORE COMPLEXES USING LASER FLASH ILLUMINATION

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Abstract. Kinetics of back reaction in the S states starting with $S_2 \rightarrow S_1$, was studied. This involves electron transfer from Q_A^- back to Mn cluster. The electron transfer process start when a photon of light is absorbed by P680, which becomes excited to high energy level P680*. It acts as a primary electron donor, hence donates electron to the primary acceptor molecule Pheophytin (Pheo). Pheo then transfers electron to plastoquinone Q_A which becomes Q_A^- after accepting electrons. This is how primary photosynthesis happens. To verify if the electron is returned to the donor (cyclic electron transfer) or noncyclic electron transfer, the decay kinetics was examined assuming a very rapid back reaction. Only back reaction following S_2 to S_1 state transition was measured. For decay of the multiline (ML) intensity in flash illuminated photosystem II (PSII) core samples, a first order reaction was assumed. The time course of decay of the S_2 ML state is assumed to follow the kinetics. Results shows that the amount of energy needed for the back-reaction is very high, Ea=75 KJ/mol. This is energy needed for the electron to back react from S_2 to S_1 state. This high activation energy means back reaction will need high input of energy. PSII proceeds forward (noncyclic electron transfer).

Keywords: kinetics; back reaction; photosystem II; electron transfer; illumination; Kok cycle.

1. Introduction

There is need for alternative fuel source as oil and gas fuel resources are being depleted. The new technologies need to be developed such as solar decomposition of water into oxygen and hydrogen gas. This process releases electrons which can be used as electricity, and the hydrogen gas maybe used as a storable chemical fuel [1]. Photosynthesis is an important process for life on earth. It produces organic compounds, especially sugars from carbon dioxide with sunlight being energy source. Photosynthesis simply means “synthesis of light”. Molecular oxygen is crucial for the existence of most animal life on earth and all heterotrophs for respiration [2]. Photosynthetic organisms create their own food and are called photoautotrophs. The oxygenic phototrophs characterized to date use the same PSII

core for solar energy conversion [5]. There are about 5 distinct Chls identified in the oxygenic phototrophs light harvesting complex so far, namely: Chls *a*, *b*, *c*, *d* and *f* [6-8]. The Chl *a* is used by most of the PSII reaction centres (RC) [5]. Photosynthesis releases oxygen as a waste product while consuming carbon dioxide and water. This maintains the normal level of oxygen in the atmosphere, hence most of the life depends on it either directly or indirectly as a source of energy in their food [3]. The process of photosynthesis is initiated by PSII. Understanding the crucial process of photosynthesis can help in non-polluting electricity generation and fuel production using solar energy. The primary photosynthesis process is made up of the RC photochemistry as well as the Oxygen Evolving Complex (OEC).This work focused on oxygenic photosynthesis, with emphasis on sub unit PSII, specifically focusing on the kinetics of back reaction (reverse process) in the S states starting with $S_2 \rightarrow S_1$.

The OEC generates the evolution of oxygen in a four step cycle process known as the Kok cycle [4]. The Kok cycle comprises a progression through five redox intermediate states known as the S states, labelled S_0 to S_4 (Fig.1). This progression through the S states from S_0 to S_4 happens by successive absorption of quanta of light in the PSII turnover. The S_1 to S_4 states symbolise successive oxidation of the Mn atoms. S_1 is very stable in the dark, S_4 very short-lived, electron rearrangement, water uptake, reformation of S_0 to restart S state cycle characterise the S_4 state, with oxygen released. The manganese cluster successively donates electrons to the PSII RC; therefore the oxidation states of manganese undergo changes. This is a step-wise oxidation process. S_0 is the most reduced state. Each oxidation step advances the Mn_4Ca to the succeeding S-state. Specific electron paramagnetic resonance (EPR) signals for S- states have been used to study the S-cycle. There is no definitive establishment of the oxidation states of the four manganese in this cycle, but the following scheme has been proposed: S_0: Mn^{II} $Mn^{III}Mn^{IV}$ Mn^{IV} or $Mn^{III}Mn^{III}Mn^{III}Mn^{II}$, S_1: Mn^{III} Mn^{III} Mn^{IV} Mn^{IV} or $Mn^{III}Mn^{IV}Mn^{III}Mn^{II}$ or Mn^{III} Mn^{III} Mn^{III} Mn^{III}, S_2: Mn^{III} Mn^{IV} Mn^{IV} Mn^{IV} or Mn^{III} Mn^{IV} Mn^{III} Mn^{III}, S_3: $Mn^{I-II}Mn^{IV}Mn^{III}Mn^{IV}$, S_4: Mn^{IV} Mn^{IV} Mn^{IV} Mn^{IV} or equivalent combinations [9 – 11, 15 – 17, 25]. In the transition from S_2 to S_3 it is assumed that ligand oxidation occurs. Umena *et al*. recently revealed the atomic level detail of the S_1 state of the Mn_4Ca at 1.9 Å resolution for the PSII structure [12], also at 2.1 Å resolution for the Sr/Ca-substituted enzyme [13] recently 1.95 Å resolution of the PSII structure [14]. Two possibilities have been proposed for the redox levels of the Mn ions within the catalytic cluster, the so called 'high oxidation state (HOS)' and 'low oxidation state (LOS)' paradigms [10, 17, 25].

S_0 and S_2, have odd numbers of electrons hence ML signals can be observed (total spin, $S_T = ½$), S_1 and S_3 have even number of electrons hence no ML signals (Fig.1). PSII has the ability to carry out this decomposition, and can do it at high efficiencies. About ninety five percent of the light absorbed is transferred to photosynthetic RC in charge separation [18]. It is a very fast process, light energy and electron transfer processes occur in picoseconds at room temperature with little energy lost as heat. Therefore the primary process of photosynthesis has the po-

tential for practical applications as it produces high-energy electrons. A catalytic four-manganese complex (Mn_4Ca) can split water into oxygen and hydrogen. P680 RC can activate electrons to higher energy. Even-though the overall process of photosynthesis is known; the exact mechanisms behind key steps in photosynthesis are still not fully understood. The mechanism behind catalytic splitting of water by the manganese centre, the transfer of electrons in photosynthetic reactions as well as types of species involved, their spatial arrangement and oxidation states are very crucial processes about which much is still unknown. This is a barrier in development of photosynthetic technologies.

The focus on PSII functionality has emerged as one of cutting edge researches. This research focused on special detergent solubilised, functional sub-assemblies of PSII, called PSII core complexes. Kinetics of back reaction in the S states starting with $S_2 \rightarrow S_1$, was the aim of this project. This involves electron transfer from Q_A^- back to Mn cluster. This is a process which the organisms must avoid if primary photosynthesis is to be efficient.

The electron transfer process start when a photon of light is absorbed by P680, which becomes excited to high energy level $P680^*$. It acts as a primary electron donor, hence donates electron to the primary acceptor molecule Pheophytin (Pheo). Pheo then transfers electron to plastoquinone, Q_A which becomes Q_A^- after accepting electrons. This is how primary photosynthesis happens. Fig.2 above shows the reverse of this process which was studied. The P680 after donating electrons becomes cation $P680^+$, hence electron deficient. It then captures electron from the nearby Try_Z, which intern draws electrons from the manganese cluster.

1. Materials and methods

1. 1. PSII samples

Green English spinach (*Spinacia oleracea*) leaves were used to prepare the PSII samples used for this study. Bricker et al. [26] method with modification by Smith et al. [19] was applied to extract the PSII membranes from *Spinacia oleracea*, with the final PSII membrane samples washed twice in storage buffer (0.02 M MES pH 6.0, 0.4 M sucrose, 0.01 M MgCl2, 0.015 M NaCl and 0.006 M CaCl2) and stored at 8-15 mg/ml chlorophyll in the storage buffer at −80 °C−80 °C. Then, the PSII core complex was separated by applying a technique developed by van Leeuwen et al. [27] with modification by Smith et al. [20]. This technique separated the LCHII (which contains the PSII core complex) from the PSII membranes. Isolation of the PSII core complex (which is enclosed within the LHCII) required application of Fast protein liquid chromatography (FPLC) system. The eluted PSII core complexes concentrated using Centriprep 30 Kda cut -off concentrators to a concentration of 1.5 to 4 mg/ml Chlorophyll *a* in the eluting buffer fraction: (0.02 BisTris pH6.5, 0.4 M sucrose, 0.02 M $MgCl_2$, 006 M $CaCl_2$,), 0.03 M $MgSO_4$ and 0.3% w:v DDM), and then stored at -80 ºC using ethylene glycol (EG) as a cryoprotectant.

1. 2. Turnover and kinetic studies.

PSII core samples were diluted to 1.20 mg/ml or 2.0 mg/ml chlorophyll *a* (42 µM or 70 µM RCs respectively using the 0.85 mg/ml elution fraction buffer (without $MgSO_4$ 30 µM) and pPBQ (i50:50 ethanol: DMSO) was added at varying quinone: P680 RC ratios to monitor the effect of excess acceptor. The quinone: RC ratios were 0 (no quinone added), 15:1, 25:1, 35:1 and 90:1. Sample aliquots of 250 µl were carefully loaded into 4 mm O.D quartz EPR tubes (Wilmad quartz) and freeze thaw cycled twice to 196 K under argon flush atmosphere twice to deplete the solutions of O_2.

1. 3. Illumination

The PSII samples (cores) prepared as above were thawed on ice/water mix (0 ºC/273 K) for 1 hour before use. Aliquots of approximately 250 µl were carefully loaded into 4 mm O.D. quartz EPR tubes (Wilmad quartz), and subsequently frozen in liquid nitrogen. The sample was annealed for approximately 10 to 30 minutes (this ensured a full relaxation to the S_1 state) and subsequently freeze trapped in the S_1 state using liquid nitrogen. To generate the S_2 state thorough $S_1 \rightarrow S_2$ turnover using PSII cores, firstly the temperature of the sample was monitored and controlled by a nitrogen gas flow system, then samples were subjected to 12 seconds illumination at 235 K using a narrow window filter set transmitting 515 nm to 620 nm envelop (peak 540 nm) light only to generate full turnover of the PSII core complex OEC to the S_2 state. EPR measurements of S_2 ML state and the stably oxidisable tyrosine signal, signal II(slow),Y_D^+ were recorded as reference spectra (effectively 100% turnover into the S_2 ML state). Samples were subsequently annealed at 273 K (ice water mix) for 10 minutes, to allow full return to the S_1 state, then set in a water bath for 1 minute at reference temperatures between 9°C°C and 18 °C °C (282 K to 291 K), then transferred to an N_2 gas flow cryostat set at the reference temperature for 1.5 minutes. Flash turnover illumination used a Continuum Powerlite 7000 YAG pulse laser operated at 532 nm set at a 200 mJ per pulse (7 ns pulse width at half height), with the laser output broadened using a cylindrical lens to approximate dimensions of 2 cm by 0.5 cm ellipsoid. This generates turnover within the sample for the ellipsoid dimension of the flash. However, the sample dimension was typically 2.5 cm to 3 cm in length. This was taken into account in the ML Decay curves by subjecting the zero time (y-intercept) to be not forced to the 100% turnover (continuous illumination at 235 K) ML intensity.

A mirror surface was attached to the rear of the N_2 flow system to enhance illumination probability. Single laser illumination flashes were applied and the samples were either frozen to liquid N_2 within 1 second or allowed to incubate in the N_2 flow for set of times between 2 and 15 seconds before freezing to liquid N_2. In the time course analyses, 100% ML intensity was taken as the value generated by continuous illumination (time zero).Then samples were flashed and immediately frozen sample at time=1 second (transfer to liquid N_2) or the incubation time plus 1 second for transfer to liquid N_2. Each figure shows the accumulated time (delay plus transfer to liquid N_2).

1. 4. ML analysis

The S_2 state ML signals across full width (<200mT) and the component upfield of signal II(slow) were measured. The S_2 state ML intensity was measured as an average of the peak and "anti-peak" surrounding the ML Hyperfine peak upfield Peak 5 (see Supplementary material Fig.S2). That is, the height and integrated weight of upfield hyperfine peak 5 and the spacing between upfield peaks 4 and 5. These presented the best signal to noise component isolated from other EPR signals co-present in the S_2 state (FeQ_A^- and Mn^{2+}) near this region.

1. 5. Decay kinetics analysis

The turnover of PSII core samples using flash illumination, were previously studied by two groups [21-22]. Here the decay kinetics was examined assuming a very rapid back reaction. As the PSII core sample evolve O_2 at a high rate, it is assumed the Kok S-state cycle functions normally in these samples, with some driving force causing very rapid decay of ML on a short time scale for each S-state transition. Only back reaction following S_2 to S_1 state transition was measured. For decay of the ML intensity in flash illuminated PSII core samples, a first order reaction was assumed. The time course of decay of the S_2 ML state is assumed to follow the kinetics.

$$ML(t) = ML(t = 0) \times e^{-kt} \quad (1)$$

Where *k* is the reaction rate, ML (*t*=0) is the initial turnover rate for the S_2 ML formation, ML (t) is the remnant ML intensity at a delay time *t* after the flash illumination.

In PSII membrane samples, the half decay time for the ML signal at 290 K is of the order of a few minutes [23], [24]. A graph of $\ln(percentage\ ML\ turnover) \times (\frac{ML(t)}{ML(t=0)})$ versus time results, with slope being *-k* for the various temperatures for which the measurements were recorded. It is hypothesised here that the lack of membrane surrounding the PSII core samples potentially accelerates the back reaction process, so delay times of orders of seconds were measured. The decay rate *k*, is assumed to be temperature dependent, so the flash turnover and ML decay was measured for temperatures between 9℃ and 18℃ (Figs.3 &4). The slopes of the lines were the rate constant, k. The graphs are a plot of ln(relative ML) by delay time in seconds. The lines of best fit were taken for all the samples, that is, at each there were three lines of best fit for each temperature. An indication of the driving energy for the back reaction may be determined from the temperature dependence of the rate constant, *k*, for S_2 ML decay:

$$k = Ce^{-\frac{Ea}{RT}} \quad \text{(In effectively mol unit terms)} \quad (2)$$

Where *R* is the gas constant 8.31447J/mol/K, *T* is the temperature in Kelvin and *C* is a constant associated with the maximal rate.

Graphing ln(k) versus 1/T (Fig.5) should show a straight line with slope -Ea/R and intercept lnC. The intercept lnC is not discussed, as only the driving energy was considered important for the development of protocols for measurement of flash turnover of PSII core samples from higher plants.

2. Results and discussions

Values greater than 100% can occur because samples are referenced to the ML level generated by continuous illumination. Five samples with different concentrations were used for the analysis, sample 1 (1.2 mix per ml chlorophyll, 2.8 mmol, 65 quinones per RC, sample 2(1.2 mix per ml chlorophyll, 0.6 mmol, 15 quinones per RC). Sample 3 (2 mix per ml chlrophyll, 1.2 mmol), sample 4 (0.85 mix per ml chlorophyll, 2 mmol) and sample 5 (0.85 mix per ml chlorophyll, 0.7 mmol).

When rearranging the Arrhenius equation:

$$\ln k = \ln C - \frac{Ea}{RT} \tag{3}$$

Where:

ln$k$ (see Tables 1 & 2) is the natural log of the rate constant, C is the Arrhenius parameter, Ea is the activation energy, R is the ideal gas law constant, and T is the absolute temperature.

When making use of this equation for a straight line $(y = mx + c)(y = mx + c)$ it is noted that Ea/R is the slope, T is a variable and C is the y-intercept. From Fig.6, the linear equation is $y = -8676.3x + 28.075$ $y = -8676.3x + 28.075$. Only the slope of the graph which is -8676.3 is required.

Ea/R=8676.3 (R=8.31447 $JK^{-1}mol^{-1}$)

Ea=8676.3×8.31447 $JK^{-1}mol^{-1}$=72 138.8361 $JK^{-1}mol^{-1}$

Ea=72 138.8361 $JK^{-1}mol^{-1}$/96485.3 $Cmol^{-1}$

Ea =0.75eV≈75 $KJmol^{-1}$

75 $KJmol^{-1}$ is the amount of energy needed for the back reaction (Fig.2). This energy is very high and it occurs in seconds which is good for the primary photosynthesis process.

3. Conclusion

The amount of energy needed for the back-reaction is very high, Ea=75 KJ/mol. This is energy needed for the electron to back react from S_2 to S_1 state. This high activation energy means back reaction will need high input of energy. PSII proceeds forward. The back reaction requires very high activation energy and occurs in seconds, which is very slow. Errors may have occurred during the analysis, but this was minimised by taking more results.

Declarations

Not applicable

Acknowledgements

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Conflict of interest

No conflict of interest

REFERENCES

Pace, R.J. (2006). An Integrated Artificial Photosynthesis Model. *In: Artificial Photosynthesis: From Basic Biology to Industrial Application*

Blankenship, R.E. (2008). *Molecular Mechanisms of Photosynthesis*

Bryan, D.A., Frigaard, N.U. (2006). Prokaryotic photosynthesis and phototrophy illuminated. *Trends Microbiol*.

Kok B, Forbush B, McGloin M (1970) Cooperation of Charges in Photosynthetic O2 Evolution – I. A Linear Four Step Mechanism *Photochem Photobiol.* https://doi.org/10.1111/j.1751-1097.1970.tb06017.x

Vinyard, D.J., Ananyev, G.M., Dismukes, C. (2013) Photosystem II: the reaction center of oxygenic photosynthesis. *Annu Rev Biochem 82:*577 – 606. https://doi.org/10.1146/annurev-biochem-070511-100425

Chen, M., Schliep, M., Willows, R.D., et al (2010). A red-shifted chlorophyll. *Science* (80-). https://doi.org/10.1126/science.1191127

Manning, W.M., Strait, H.H. (1943.) Chlorophyll D, a Green Pigment of Red Algae. *J Biol Chem*

Björn, L.O., Papageorgiou, G.C., Blankenship, R.E., Govindjee (2009). A viewpoint: Why chlorophyll a? *Photosynth. Res.*

Bergmann, U., Grush, M.M., Horne, C.R., et al (2002) Characterization of the Mn Oxidation States in Photosystem II by Kβ X-ray Fluorescence Spectroscopy. *J Phys Chem B.* https://doi.org/10.1021/jp982038s

Baituti, B. (2017). What the multiline signal (MLS) simulation data with average of weighted computations reveal about the Mn hyperfine interactions and oxidation states of the manganese cluster in OEC? *Hyperfine Interact* 238:. https://doi.org/10.1007/s10751-017-1440-8

Baituti, B. (2018). Computational studies of the Mn4/Ca cluster in photosystem II. J *Theor Comput Chem 17:.* https://doi.org/10.1142/S0219633618500074

Umena, Y., Kawakami, K., Shen, JR., Kamiya, N. (2011). Crystal structure of oxygen-evolving photosystem II at a resolution of 1.9Å. *Nature*. https://doi.org/10.1038/nature09913

Koua, F.H.M., Umena, Y., Kawakami, K., Shen, J-R. (2013). Structure of Sr-substituted photosystem II at 2.1 A resolution and its implications in the mechanism of water oxidation. *Proc Natl Acad Sci 110:*3889–3894. https://doi.org/10.1073/pnas.1219922110

Suga, M., Akita, F., Hirata, K., et al (2015) Native structure of photosystem II at 1.95A° resolution viewed by femtosecond X-ray pulses. *Nature*. https://doi.org/10.1038/nature13991

Gatt, P., Petrie, S., Stranger, R., Pace, R.J. (2012). Rationalizing the 1.9 Å crystal structure of photosystem II-A remarkable Jahn-Teller balancing act induced by a single proton transfer. *Angew Chemie – Int Ed.* https://doi.org/10.1002/anie.201206316

Pace, R.J., Stranger, R., Petrie, S. (2012). Why nature chose Mn for the water oxidase in Photosystem II. *Dalt Trans 41:*7179 – 7189. https://doi.org/10.1039/c2dt30185g

Gatt, P., Stranger, R., Pace, R.J (2011). Application of computational chemistry to understanding the structure and mechanism of the Mn catalytic site in photosystem II â€“ A review. *J Photochem Photobiol B Biol 104:*80 – 93. https://doi.org/10.1016/j.jphotobiol.2011.02.008

Kruse, M.L., Arlt, A., Sieke, A., et al (2005). Immediate early gene X1 (IEX-1) is organized in subnuclear structures and partially co-localizes with promyelocytic leukemia protein in HeLa cells. *J Biol Chem.* https://doi.org/10.1074/jbc.M501571200

Smith, P.J., Åhrling, K.A., Pace, R.J. (1993). Nature of the S2 state electron paramagnetic resonance signals from the oxygen-evolving complex of photosystem II: Q-band and oriented X-band studies. *J Chem Soc Faraday Trans.* https://doi.org/10.1039/FT9938902863

Smith, P.J., Peterson, S., Masters, V.M., et al (2002). *Magneto-Optical Measurements of the Pigments in Fully Active Photosystem II Core Complexes from Plants* 2:1981 – 1989

Ho, F.M., Styring, S. (2008). Access channels and methanol binding site to the CaMn4 cluster in Photosystem II based on solvent accessibility simulations, with implications for substrate water access. *Biochim Biophys Acta – Bioenerg.* https://doi.org/10.1016/j.bbabio.2007.08.009

Kolling, D.R.J., Brown, T.S., Ananyev, G., Dismukes, G.C. (2009) Photosynthetic oxygen evolution is not reversed at high oxygen pressures: Mechanistic consequences for the water-oxidizing complex. *Biochemistry*. https://doi.org/10.1021/bi801774f

Geijer, P., Peterson, S., Åhrling, K.A., et al (2001). Comparative studies of the S0 and S2 multiline electron paramagnetic resonance signals from the manganese cluster in Photosystem II. *Biochim. Biophys. Acta – Bioenerg. 1503:*83 – 95

Styring, S., Rutherford, A.W. (1988). Deactivation kinetics and temperature dependence of the S-state transitions in the oxygen-evolving system of Photosystem II measured by EPR spectroscopy. *BBA – Bioenerg*. https://doi.org/10.1016/0005-2728(88)90046-1

Baituti, B., Akofang, L. (2020). The Two Alternative Oxidation State Assignments of Manganese Ions: What S2 CW-EPR Multiline (ML) Signal Simulations Reveal? *Appl Magn Reson.* https://doi.org/10.1007/s00723-020-01190-5

Bricker, T.M., Pakrasi, H.B., Sherman, L.A. (1985). Characterization of a spinach photosystem II core preparation isolated by a simplified method. *Arch Biochem Biophys 237:*170 – 176. https://doi.org/10.1016/0003-9861(85)90266-8

Van Leeuwen, P.J., Nieveen, M.C., Van de Meent, E.J., et al (1991). Rapid and simple isolation of pure photosystem II core and reaction center particles from spinach. *Photosynth Res.* https://doi.org/10.1007/BF00054128

Figures and Tables

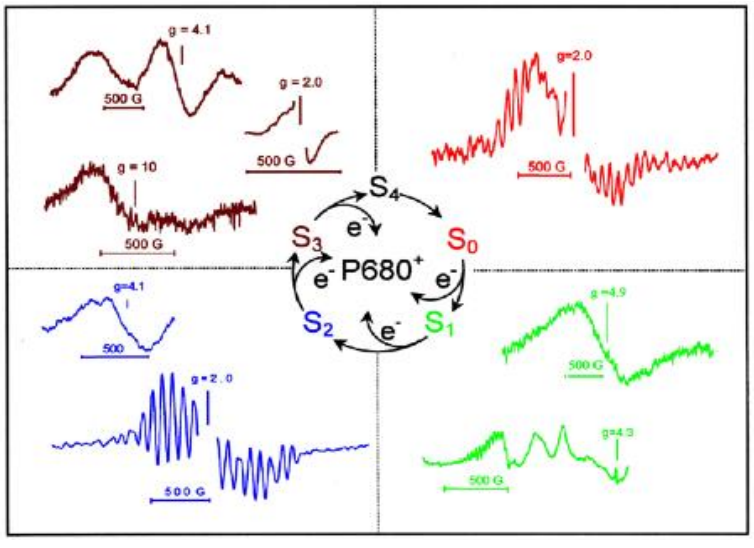

Figure 1. EPR Signals from S states [4]

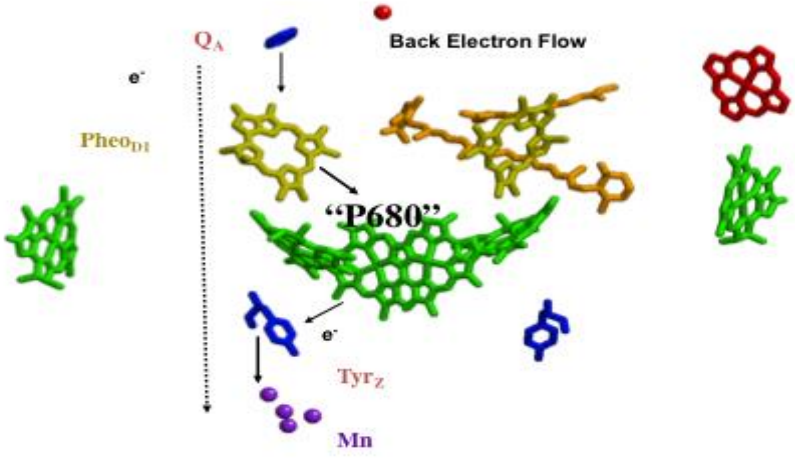

Figure 2. Shows the back reaction process under investigation

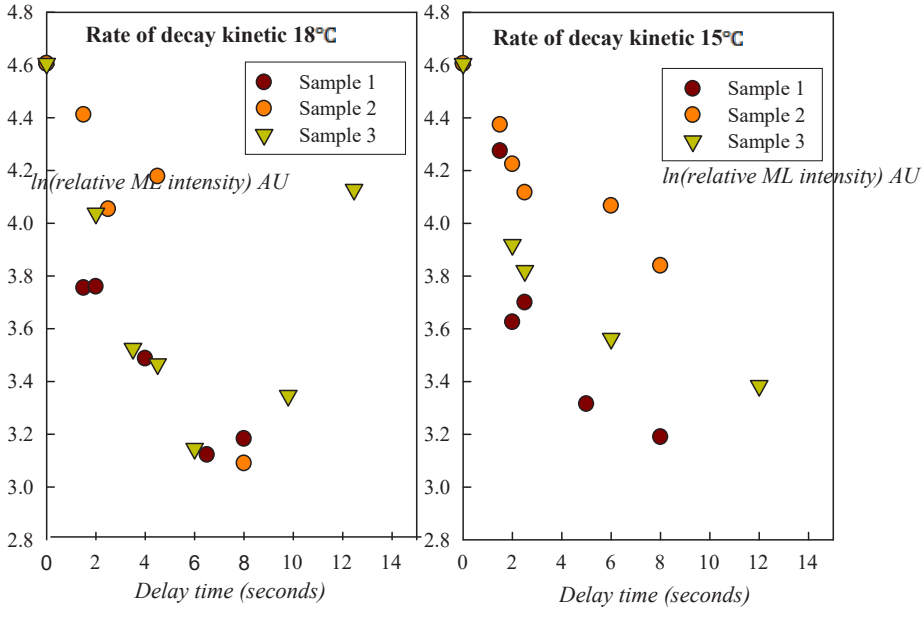

Figure 3. At 18°C and 15°C the above graphs were obtained for sample 1, 2, 3

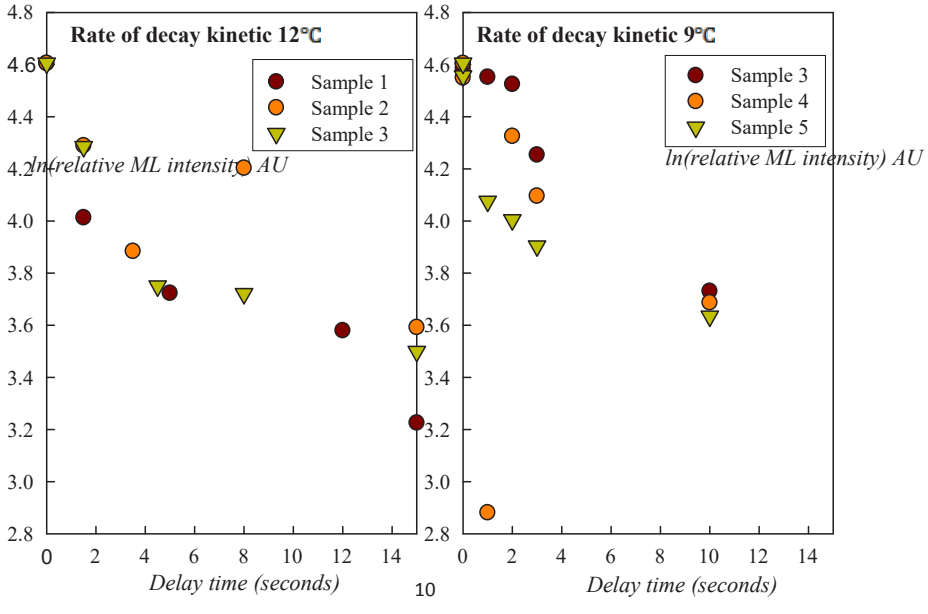

Fig.4. At 12°C and 9°C the above graphs were obtained for sample 1, 2, 3, 4 and 5

The activation energy and Arrhenius parameter can be found using the values below:

Table 1. Showing ln*k* for each temperature

Temperature(K)	1/T(K^{-1})	ln*k*
291	0.003436426	-1.35, -1.70, -2.10
288	0.003472222	-2.10, -1.90, -2.40
285	0.003508772	-2.05, -2.25, -2.55
281	0.003558719	-2.45,-2.50, -3.00

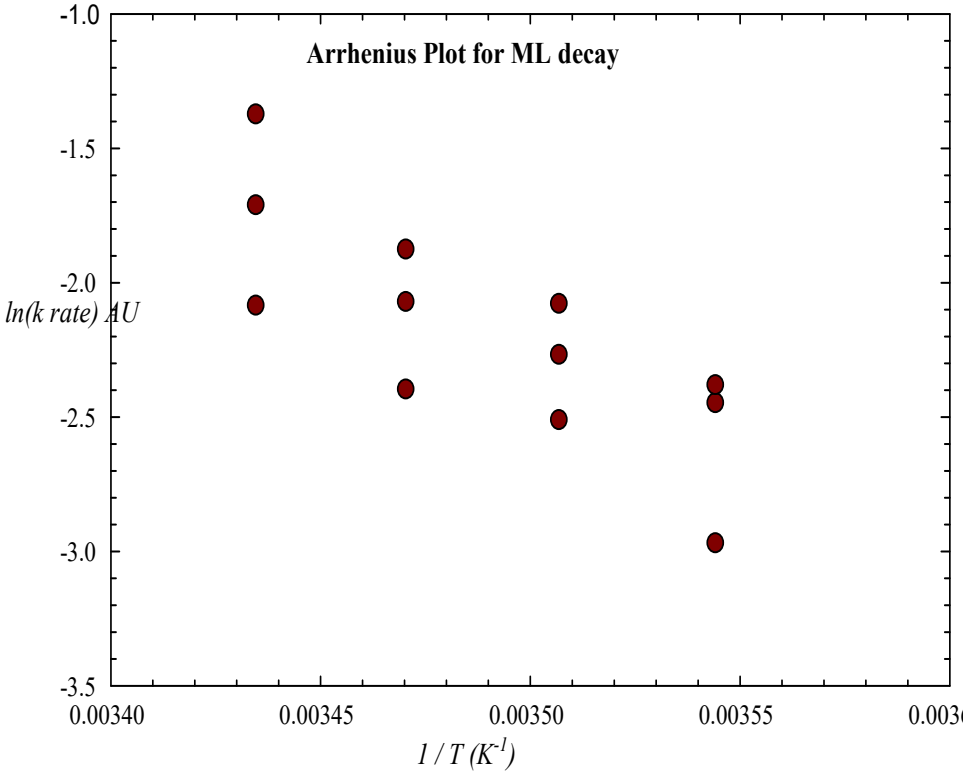

Figure 5. Shows the Arrhenius plot for ML decay (lnk versus 1/T)

From the values above, the average ln*k* for each temperature was taken and the following values were obtained and used to draw a linear graph with 4 points:

Table 2. Showing average ln*k* for each temperature

Temperature(K)	1/T(K^{-1})	lnk (average)
291	0.003436426	-1.72
288	0.003472222	-2.13
285	0.003508772	-2.28
281	0.003558719	-2.81

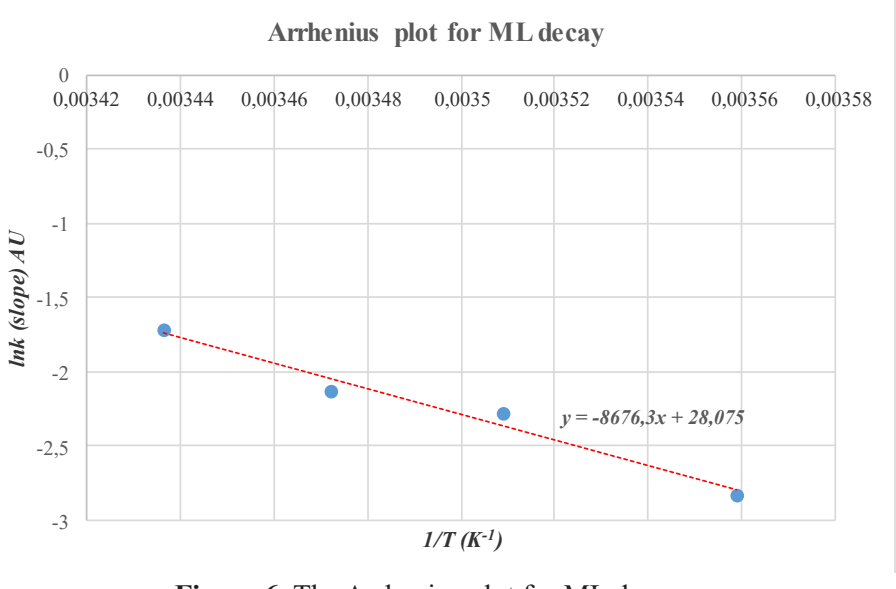

Figure 6. The Arrhenius plot for ML decay

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