The time course of acclimation to the stress of triose phosphate use limitation:

Authored by: Alan M. McClain, Jeffrey A. Cruz, David M. Kramer, and Thomas D. Sharkey

DOI: https://doi.org/10.22541/au.165572650.04132192/v1

Correspondence: Thomas D. Sharkey, tsharkey@msu.edu +1 (517) 353 3257

Preamble

The data that was used to compile this paper is presented here. Data is provided in a tabular "tidy data" format and in our use was analyzed in the R programming language. The data is divided into data chunks largely associated with one figure each in the paper. These chunks are addressed one at a time with an acknowledgement of the associated figure, a general description of the data and its organization, and a definition of variables. We also include brief suggestions for re-analysis in R using the "tidyverse" package.

A/C$_i$ curve fit outputs

This folder contains the data required to reconstitute figure 1. Plants are adapted to one of three treatment conditions for 30 hours: elevated CO2 (1500 ppm), ambient CO2 (420 ppm), or low CO2 (150 ppm). During treatment, A/Ci curves are taken approximately every 2.5 hours. The parameters "Vcmax", "J", and "TPU" are the three primary fitting parameters of the Farquhar, von Caemmerer, Berry, and Sharkey A/Ci fit model in umol m^{-2} s^{-1}. The treatment is registered in the "Type" column, the replicate is "Count", and the order of the aci curves is listed as "aci".

In the publication figure, the data was normalized to the first curve. Here, data is presented in three ways. First, the "raw" folder contains the fitting data from each individual replicate before normalization. The "normalized" folder contains the fitting data normalized for each replicate. Finally, in the root folder, "combined aci data.csv" is the compiled data in tidy data format which can be used to replicate the publication figure. This file also contains an "Hours" variable that represents how long after the beginning of experimentation the A/Ci curve was taken in hours.

This code will produce a low-formatting copy of the original figure 1.

```
read_csv("combined aci data.csv") %>%
  pivot_longer(cols=c("VcMax","J","TPU")) %>%
  ggplot(mapping=aes(x=Hours,y=value,color=Type)) +
  geom_smooth()+
  facet_wrap(~name, nrow=1)
```

Elevated CO2 ACi ECS+PSI data

This folder contains the data required to reconstitute figure 2. This data compares the effect of a step change in CO2 concentration on the light reactions before and after acclimation te elevated CO2. Data is taken along A/Ci curves from before acclimation and at the end of 30 hrs of acclimation. The variables here are "A", photosynthetic assimilation in umol m^{-2} s^{-1}; "Ci", the interior leaf CO2 concentration in ppm; "gsw", the stomatal conductance to

water in mol m^{-2} s^{-1}; "Phi2", the ratio of absorbed light energy committed to photochemistry; "Time", the length of time after beginning logging in seconds; "NPQt", theoretical non-photochemical quenching of chlorophyll fluorescence; "Conductivity", also known as gH+, is the conductivity of protons across the thylakoid membrane; "Velocity", also known as vH+, is the initial decay of the proton gradient during a dark interval and is proportional to the rate of protons crossing the thylakoid membrane; "PMF", measured here as total electrochromic shift (ECSt), is proportional to the total proton-motive force across the thylakoid membrane; "num", the replicate; "ID", whether the data was taken before or after acclimation.

Data for every A/Ci for every replicate is provided in the folders labeled "Plant 1-5", representing the 5 replicates. For the figure, only the first and last A/Ci for each replicate was used. These two curves for each replicate are compiled in the table "combined adaptation data.csv".

This code produces a minimally formatted copy of the original figure 2.

```
read_csv("combined adaptation data.csv") %>%
  filter(Conductivity > 0) %>%
  pivot_longer(cols = c(A, Phi2, NPQt, Conductivity, PMF)) %>%
  ggplot(mapping = aes(x=Ci, y=value, color=ID))+
  geom_smooth()+
  facet_wrap(ncol=1, ~name, scales="free_y")
```

Pre vs post-adaptation qL response

Data in this folder was used to produce figure 6. This data compares the effect of a step change in CO2 concentration on qL depending on whether the leaf has acclimated to elevated CO2 or not. Data is presented in two files, "Pre adaptation.csv" and "Post adaptation.csv" depending on whether data was taken before or after acclimation to elevated CO2. Variables in these files are "NPQt", theoretical non-photochemical quenching; "Phi2", the yield of photochemistry; "PhiNPQt", the yield of non-photochemical quenching; and "qL", the oxidation state of Qa (higher qL represents more oxidized Qa). "Time_post" is the duration between the start of the addition of a step change in CO2 from 420 ppm to 1500 ppm CO2, and "ID" is the replicate.

This code will produce a minimally formatted copy of the original figure 6.

```
bind_rows(
  read_csv("Post adaptation.csv") %>% add_column("Type" = "Post"),
  read_csv("Pre adaptation.csv") %>% add_column("Type" = "Pre")
) %>%
  pivot_longer(cols=c("Phi2", "NPQt", "PhiNPQt", "qL")) %>%
  ggplot(mapping=aes(x=Time_post, y= value, color = Type))+
  geom_smooth()+
  facet_wrap(ncol=1, ~name, scales="free_y")
```

PSI kinetics during saturating flashes

Data in this folder was used to produce figure 5. This folder contains raw traces of PSI absorbance during a saturating flash protocol a period of time after addition of a step change in CO2. The oxidation state of PSI is measured as the change in absorbance at 820 nm, called "DeltaA". DeltaA is calculated from the Raw_Voltage. "Time" is the duration since the beginning of the experiment, not the length of time after addition of the step change in CO2. The length of time after addition of CO2 is listed in a separate file, "Trace order and time post-CO2.csv" contained in a folder for each replicate. This file reveals the scrambling pattern used in creation of the overall protocol.

Here is the protocol for each trace. First, the absorbance is measured with the actinic light on. Then, the leaf is given a period of darkness during which PSI becomes reduced (DeltaA goes down). Next, the leaf is given a 1-s

period of very bright light, where PSI should become oxidized; however, in many cases, it becomes re-reduced during the flash, indicating an acceptor side limitation of PSI.

Each trace is plotted in the same scrambled order for each replicate in "traces.pdf". The original figure 5 was made by combining and formatting a choice of 3 of these trace graphs.

Rubisco activation state

Data in this folder was used to produce figure 3. This folder contains worked-up data with rubisco activation state data before, during, or after acclimation to elevated CO2. Data in the "Hours adaptation" folder was used to produce figures 3a and 3b. "Type" is the duration at elevated CO2 before collecting the sample; "Total" is total rubisco activity of the activated sample; "Activation State" is the activation state of rubisco at that time.

Data in the "Minutes timecourse" folder was used to produce figures 3c and 3d. "Before" refers to whether rubisco is deactivating after exposure to elevated CO2 (Before = 0) or reactivating after the end of exposure to elevated CO2 (Before = 2.5); "Time" is the duration after beginning the treatment in minutes; "Count" is the replicate; "Act State" is the activation state; "Activity" is the activity of the non-activated sample, and "Tot activity" is the activity of the activated sample.

Scrambled PSII+PSI+ECS

Data in this folder was used to produce figure 4. Leaves were exposed to a step change in CO2, then electrochromic shift was measured by DIRK after a randomized period of time. After 20 more seconds, PSII and PSI were measured by saturating flash. Then, the leaf was reverted to ambient CO2 for 10 minutes to reset before sampling at another randomized period of time. Data is compiled into a single table, "Combined data table.csv". Many measurements are combined here.

Many variables are in this table from several measurements. "Conductivity" is the conductivity of protons across the thylakoid membrane; "Velocity" is the rate of proton transfer across the thylakoid membrane; "PMF" is an electrochromic shift-derived measurement of proton-motive force. "Phi2" is the yield of photochemistry; "Fm", "Fs", and "Fo" are raw measurements of fluorescence used to calculate other parameters (all of these measurements are light-adapted); "NPQt" is the theoretical non-photochemical quenching of fluorescence; "qL" is the redox state of Qa; "e- conductivity" is ket, calculated from the decay of the ΔA_{820} signal, and represents the rate constant of electron transfer from cytochrome b6f to PSI. "e- velocity" is a kinetic parameter calculated from the decay of the ΔA_{820} signal, but is not used in the publication; "dA820t" is the total difference in the ΔA_{820} signal from the steady-state to full oxidation; "ps1ratio" is the oxidation state of PSI, where higher means more oxidized; "TimePost" is the time after the addition of elevated CO2 before the electrochromic shift measurement begins; "PhiNPQt" is the yield of non-photochemical quenching; and "TimeFOP" is the time after the addition of elevated CO2 before the measurement of PSI and PSII.

An additional file is provided, "sample CO2 trace.csv". This is a sample trace of the flow of CO2 into the sample cuvette after the imposition of a step change in CO2.

This code will produce a minimally-formatted copy of figure 4.

```
satflash <- read_csv("Combined data table.csv") %>%
  select(qL, Phi2, PhiNPQt,NPQt,ps1ratio,TimeFOP,`e- conductivity`) %>%
  rename("TimePost" = "TimeFOP") %>%
  pivot_longer(cols = -TimePost)
ecs <- read_csv("Combined data table.csv") %>%
  select(Conductivity, PMF, TimePost) %>%
  pivot_longer(cols = -TimePost)
co2trace <- read_csv("sample CO2 trace.csv")
bind_rows(satflash,ecs,co2trace) %>%
```

```
ggplot(mapping=aes(x=TimePost,y=value))+
stat_summary()+
facet_wrap(~name,ncol=1,scales="free_y")
```

Sharing/Access Information

This data has not been made available on any other public source. This data was not derived from another source.