Flash photometric studies of proton release inside thylakoids in dark-adapted chloroplasts with the pH indicator dye neutral red

Verena Förster and Wolfgang Junge

Universität Osnabrück, Schwerpunkt Biophysik, Postfach 4469
D-4500 Osnabrück, Germany (F.R.G.)

Photosynthetic water oxidation at the expense of even red quanta of light is a unique performance of green plants. Unfortunately the water-oxidizing enzyme complex is highly resistive to biochemical and biophysical characterization. We report on the liberation of protons during stepwise water oxidation under excitation of photosystem II by a group of flashes. So far protons are the only kinetically resolvable indicator of the events in the enzyme complex.

There are at least two sites of proton release inside thylakoids: the water-splitting enzyme and the site of plastohydroquinone reoxidation.

Proton release inside thylakoids can be monitored via absorption changes of neutral red under conditions when the outer phase is strongly buffered with bovine serum albumin (1). Proton release which is assumed to be due to the water-splitting enzyme is measured under conditions when electron transport between the photosystems is blocked by DBMIB (2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone).

According to the model of Kok (2) the water-splitting enzyme accumulates four oxidizing equivalents cycling through the states S_0, S_1, S_2, S_3 and S_4 before one molecule of oxygen is liberated. Proton release does not follow the pattern of oxygen evolution. Since S_1 is most stable in the dark excitation of chloroplasts by a series of laser flashes reflects proton release during the transitions $S_i \rightarrow S_{i+1}$. Although S_2 and S_3 are known to be almost totally relaxed to S_1 after 7 minutes in the dark we obtained two different patterns of proton release depending on the mode of dark adaptation. Assuming an equilibrium of 75% S and 25% S in the dark, 10% double hits ($S_i \rightarrow S_{i+1} \rightarrow S_{i+2}$) and 10% misses (no transition) in each turnover these patterns were fitted by a stoichiometry of $0\ H^+(S_0 \rightarrow S_1) : 1\ H^+(S_1 \rightarrow S_2) : 1\ H^+(S_2 \rightarrow S_3) : 2\ H^+(S_3 \rightarrow S_4, S_0)$ and 1:0:1:2, respectively. The former pattern was found after 7-20 minutes of dark adaptation. The latter one was found when the chloroplasts (which had been stored under liquid N_2) were not exposed to light while thawing and preparing the sample. The kinetics of the respective proton releases during $S_0 \rightarrow S_1$, $S_2 \rightarrow S_3$ and $S_3 \rightarrow S_4$ could be correlated with the kinetics of the reduction of the assumed intermediate Z between the water-splitting enzyme and P680 as detected by EPR measurements (3). Protons were released with half rise times of $\leq$250µs ($S_0 \rightarrow S_1$), 500µs ($S_2 \rightarrow S_3$) and 1ms ($S_3 \rightarrow S_4$). However, one 100µs phase in the multiphasic rise after the second flash cannot be attributed to a distinct transition, if H^+ are assumed to be released with single exponential kinetics during each transition. In the presence of DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) we found even more rapid proton release ($\leq$100µs) suggesting a protolytic site at the donor side of PSII other than the water-splitting enzyme. The 100µs phase in the absence of DCMU may be caused by proton release from this site.

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(2) Kok,B., Forbush,B. and McGloin (1970), Photochem. Photobiol.11, 457

(3) Babcock,G.T., Blankenship,R.E. and Sauer,K.(1976),FEBS Lett61, 286