IS3.4 - Resolving the *in vivo* mechanisms governing the inhibition of algal hydrogenase

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Photoproduction of hydrogen by green algae is considered a transitory release valve of excess reducing power. It is also considered as a potential carbon-free source of sustainable energy given a method allowing for a continuous production. It is generally accepted that the transitory production of hydrogen is mainly due to the intrinsic sensitivity of [FeFe]-hydrogenase to oxygen. In contrast, we propose an alternative hypothesis, suggesting that photosynthetic electron loss to competing processes, mainly carbon fixation, precedes the inevitable inactivation by oxygen. Here we show that when transitioning from dark anaerobiosis to light, hydrogen production ceases within 2 minutes, regardless of oxygen levels. By simultaneously monitoring the size of the active hydrogenase pool, we show that it remains entirely intact up to 4 minutes of illumination. Thus, our data provides a safety window of 4 minutes in which the hydrogenase pool is not being inactivated by oxygen. Furthermore, we show that electron loss to carbon fixation outcompetes hydrogen production and leads to hydrogen uptake. Indeed, supplying additional reducing power to hydrogenase at the cessation point regenerates the accumulation of hydrogen. In conclusion, our results imply that in contrast with the common dogma, the fast cessation of hydrogen production is governed by electron loss rather than oxygen inactivation, which takes place minutes later.