S3-030

Is Non-Photochemical Quenching Protective?

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Keywords: Non-Photochemical Quenching, Photoinhibition, D1 Degradation.

Introduction

Photosynthetic organism are often exposed in their natural environment to photon flux densities which can lead to the saturation of electron transport chain in thylakoids membranes. Under these circumstances oxidative stress at the level of photosystem II (PSII) may occur which determines the loss of photochemical activity and, in a successive stage, the degradation of the reaction centre binding D1 protein. It was initially demonstrated by Jones and Kok (1966) and confirmed by others (e.g. Tyystjarvi et al. 1994, Park et al. 1995) that photoinhibition is linear with the number of absorbed photons (reciprocity rule). Therefore it has been proposed that, although the molecular nature of the mechanism is still largely unclear, non photochemical quenching of chlorophyll fluorescence (qNP) plays a key role in photo-protective strategies by lowering the excited state population in the antenna matrix. On the other hand a number of recent reports regarding the photoinhibition sensitivity to the modulation of the excited state population have shown that only a moderate protection is achieved compared to what would be expected from the reciprocity rule. To explain this contradiction it was postulated that a small population of Chls which are uncoupled, or only weakly coupled to PSII antenna and hence less sensitive to the antenna excited state levels, are involved in photo-oxidative damage in thylakoids (Sinclair et al. 1996, Santabarbara et al. 1999**,** Tyystjarvi et al. 1999). The recently published action spectrum of the F_V/F_M lowering in spinach thylakoids is significantly blue-shifted (about 3-4 nm) compared to PSII photon absorption spectrum in the membrane, and has therefore been interpreted in terms of a small population of damaged, or incompletely assembled Chl-protein complexes (Santabarbara et al. 2001). In the present study we present a comparison between *in vitro* experiments on thylakoids isolated from a number of different species (Spinach, Barley and *Arabidopsis*) and *in vivo* experiments on the unicellular alga *Chlamydomonas*. The data are consistent with previous results and underline the question of the importance of qNP as a useful protective strategy against photoinactivation.

Materials and Methods

Thylakoids from spinach and barley were prepared from freshly harvested leaves as previously described, (Jennings et al. 1981) and those from *Arabidopsis* following the modification described by Casazza et al.(2001). *Chlamydomonas reinhardtii* were grown in a carbon supplemented medium and harvested in the exponential growth phase. DCMU, DNB

(m-dinitrobenezene) and DBMIB were added in ethanol and the final v/v ratio was less than 1%. The photoinhibitory treatment was with a 900 W Xenon lamp with appropriate heat filters (fluence 0.35 W/cm^2, unless otherwise indicated), with samples in a 1cm optical cuvette at a Chl concentration equivalent to 4 μg/ml. Samples were illuminated in the presence of 10 μM DCMU at 4 °C. Photoinhibition was estimated by the initial slope of the relative decrease of the F_V/F_M ratio by a least-square regression or by the light induced F_M quenching rate parameter (k_I) as previously described (Santabarbara et al. 1999) Fluorescence induction was measured as previously described (Jennings et al. 1981) after 15 min of dark incubation at 4 °C to allow complete Q_A oxidation.

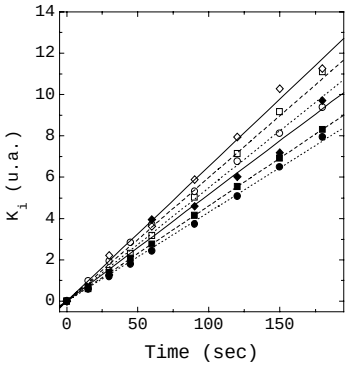

Fig. 1. Kinetics of photoinhibition-induced quenching (k_I) in spinach (diamonds, solid lines), *Arabidopsis* (squares, dashed lines)and barley (circles, dotted lines) thylakoids in the presence (closed) and absence (open) of singlet quenchers.

Results

In table 1 are presented the photoinhibition rates, as judged by F_V/F_M changes, for thylakoids extracted from several species and for intact cells of Chlamydomonas, in the presence and absence of antenna singlet quenchers. It can be seen that both in the *in vivo* and *in vitro* systems there is only a limited protection induced by excited state quenching of 60%-70% during the light treatment.

Table 1. The effect of excited state quenching on photoinhibition (F_V/F_M). In all cases quenching was about 65%.

	Photoinhibition Unquenched	Photoinhibition Quenched
Spinach thylakoids	28.9%	25.7%
Barley thylakoids	20.9%	17.8%
Arabidopsis thylakoids	27.8%	26.5%
Chlamydomonas cells	34.8%	33.3%

As the F_V/F_M decline is largely due to a quenching of F_M the dynamics of this F_M quenching were analysed in terms of a light-induced quenching rate constant (k_I) in thylakoids from different species in the presence and absence of singlet quenchers (figure 1). The kinetics and the extent of this irreversible light induced quenching are comparable in all the *in vitro* systems and furthermore only a limited protection is induced by the lowering of the excited state population, about 30% that expected from the reciprocity rule.

In figure 2 the F_M quenching in *Chlamydomonas* is analysed with respect to light intensity and light treatment time. It is clear that the k_I parameter varies linearly with

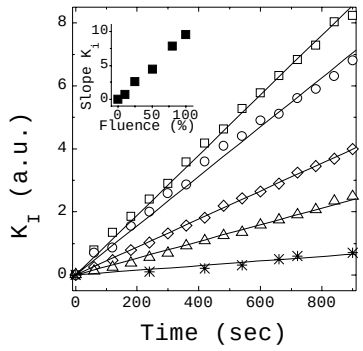

Fig. 2. Photoinhibition-induced quenching as a function of time and light fluence in *Chlamydomonas* . Squares (100%, 60 mW/cm^2)**,** Circles (80%), Diamonds (51.5%), Triangles (25%), Stars (10%). Insert: rate of k_I development versus fluence.

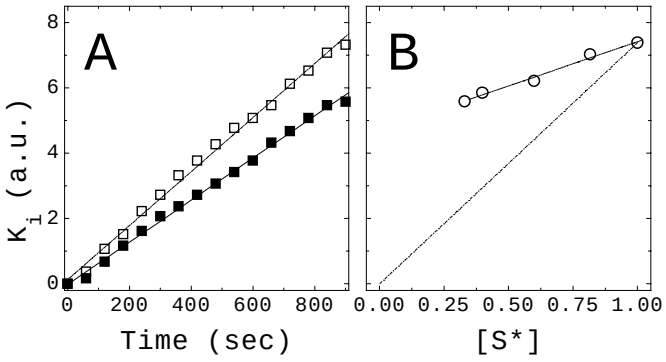

Fig. 3. Effect of singlet quenching on photoinhibition in *Chlamydomonas* cells. A: Kinetics of k_i in the presence (closed) and absence (open) of the singlet quencher DNB (666μM, 70% quenching). B: Relation between the excited state population [S*] and light-induced F_M quenching (k_I).

treatment time and light fluence and hence obeys the reciprocity rule, as previously described for thylakoids (Santabarbara et al 1999, 2001). When the fluorescence was quenched by 70% with DNB the kinetics of the light-induced k_I were reduced by about 23% (Fig. 3A). When k_I is plotted against the integrated excited state population during the treatment time a protection of about 35% with respect to the reciprocity rule was found (Fig. 3B).

In figure 4 are presented the immunoblots which describe the kinetics of D1 protein degradation in spinach thylakoids illuminated in the presence or absence of the singlet quencher DNB. Within the measurement errors no protection was apparent for an excited state lowering of 40% integrated over the treatment time.

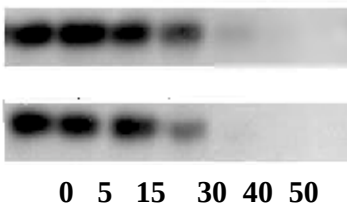

Fig. 4. Kinetics of D1 protein degradation in spinch Thylakoids illuminated in the presence and absence of DNB.

Discussion

The present results confirm and extend previous data on the photo-protective role of qNP, experimentally simulated by addition of singlet quenchers to thylakoids from a number of species and an intact algal system (*Chlamydomonas*), and also on the triggering of photoinhibition. In all cases the protective effect of excited state quenching was estimated to be about 30% that expected on the basis of the reciprocity rule. The data suggest that the often proposed mechanism involving Chl triplet formation on P_{680} (recombination triplet) (Durrant

et al. 1990**,** Vass and Styring 1993) does not play a major role in triggering photoinhibition, as the levels of the recombination triplet should be linear with the antenna excited states under our experimental conditions (traps closed). This interpretation is supported by our recent observation that the photoinhibition action spectra is blue shifted 3-4nm with respect to PSII absorption (Santabarbara et al, 2001). The data are easily explained by the presence of uncoupled chlorophylls, with a high triplet yield, which may be present on damaged or incompletely assembled complexes and which will be less sensitive to singlet quenchers than the coupled antenna matrix due to their smaller cross section. We would furthermore point out that the figure of about 30% for photoprotection with respect to excited state quenching represents an upper limit for an *antenna based* process as it is expected that some singlet quenching of the putative damaged or incompletely assembled complexes will occur, leading to reduction of triplet formation on them, and which will have been included in our k_I estimates. In this scenario the role of qNP as an *antenna based* photo-protective strategy would seem to have been somewhat overestimated. This point is further emphasised by the data on the light induced D1 protein degradation, widely considered to by an initial step in the repair of photo-damaged complexes. This phenomenon seems to be insensitive to the levels of antenna excited states. The possibility that light-induced qNP is photoprotective at the level of damaged or incorrectly assembled should be considered.

Acknowledgements

This study has been in part supported by the MURST sub-project “Fotosintesi: stress da luce e protezione” and the CNR Target Project on Biotechnology.

References

Casazza A.P., Tarantino D. and Soave C. (2001) *Photosynthesis Research,* in press.

Durrant, J. R., Giorgi, L. B., Barber, J., Klug, D. R. and Porter, G. (1990) *Biochimica et Biophysica Acta,* **1017**, 167-175.

Jennings, R.C., Garlaschi, F. M., Gerola, P. D., Etzion-Katz, R. and Forti, G. (1981) *Biochimica et Biophysica Acta,* **638**, 100-107.

Jones L.W and Kok. B. (1966) *Plant Physiology*, **41**, 1037-1044

Park, Y.I., Chow, W.S. and Anderson J.M (1995) *Planta,* **196**, 401-411

Santabarbara, S., Garlaschi, F. M., Zucchelli, G. and Jennings, R. C. (1999) *Biochimica et Biophysica Acta,* **1409**, 165-170.

Santabarbara, S., Neverov, K., Garlaschi, F. M., Zucchelli, G. and Jennings, R. C. (2001) *FEBS Letters*, **491** 109-113.

Sinclair, J., Park, Y. I., Chow, W. S. and Anderson, J. M. (1996) *Photosynthesis Research*, **50**, 33-40.

Tyystjarvi, E., Kettunen R.. and Aro, E. M. (1994) *Biochimica et Biophysica Acta*, **1186**, 177-185.

Tyystjarvi, E., King, N., Hakala, M. and Aro, E. M. (1999) *Journal of Photochemistry and Photobiology. B:Biology*. **48**, 142-147

Vass, I. and Styring, S. (1993) *Biochemistry,* **32**, 3334-3341.