热带亚热带植物学报 1997, 5(1):59－64
Journal of Tropical and Subtropical Botany

蛋白质修饰剂、变性剂和活性氧对菠菜叶片光系统Ⅱ光失活的影响

林植芳 李晓萍 林桂珠 彭长连
(中国科学院华南植物研究所，广州 510650)

摘要 菠菜叶圆片以活性氧、蛋白质修饰剂和变性剂结合强光处理半小时，检测叶绿素荧光动力学并与单照强光的对照作比较。加入H_2O_2、$\cdot O_2^-$、OH·和$^1O_2$等活性氧加剧了PSⅡ的光抑制作用，Fv/Fm, q_P和ΦPSⅡ降低，q_N和$1-q_P$相应上升。4种蛋白质修饰剂NBS、DEPC、BDE和ρCMB的处理，使叶片的Chl荧光参数发生了与活性氧作用相类似的变化，尤以精氨酸残基修饰剂BDE的影响较为显著。SDS明显地影响Fv和q_P但未改变q_N值。Gu-HCl导致q_P完全丧失，q_N达最大值。低浓度的脲素和DMSO略为降低PSⅡ活性和增大q_N。结果认为对叶绿体蛋白构象或结构的任何改变皆引起光下PSⅡ光失活作用的增强。

关键词 活性氧；蛋白质修饰剂；蛋白质变性剂；PSⅡ光失活；菠菜叶片

EFFECTS OF PROTEIN MODIFIERS, DENATURANTS AND ACTIVE OXYGEN ON PHOTOSYSTEM Ⅱ PHOTOINACTIVATION OF SPINACH LEAVES

Lin Zhifang Li Xiaoping Lin Guizhu Peng Changlian
(South China Institute of Botany, Academia Sinica, Guangzhou 510650)

Abstract Leaf discs of spinach were exposed to high light in the presence of active oxygen, protein modifiers and denaturants, and chlorophyll fluorescence kinetics was detected and compared with high light treatment alone.

The addition of H_2O_2, $\cdot O_2^-$, OH· and $^1O_2$ enhanced photoinhibition, showing the decrease of Fv/Fm, q_P and ΦPSⅡ, which were accompanied by the increase of q_N and $1-q_P$. Treatment of four protein modifiers, NBS, DEPC, BDE and ρCMB, also exhibited the similar changes of fluorescence parameters. More evident change was caused by BDE, an Arg-modifier of protein. SDS affected significantly on Fm, Fv and q_P, but didn't change q_N. A complete lose of q_P associated with maximum q_N were induced by Gu-HCl. Urea and DMSO at low concentration reduced the PSⅡ activity and increased q_N in less extent. The

The project was funded by the Natural Science Fundation of China.

Abbreviations: NBS, N-bromosuccinimide; DEPC, diethylpyrocarbonate; BDE, butanedione; ρCMB, ρ-chloromercuribenzoic acid; Gu-HCl, guanidinium chloride; SDS, sodium dodecylsulfate; DMSO, dimethyl sulfoxide

1996－06－07 收稿； 1996－09－25 修回

results suggest that any change in conformation or structure of chloroplast protein all led to the enhancement of PSII photoinactivation.

Key words Active oxygen; Protein modifier; Protein denaturant; PSII photoinactivation; Spinach leaf

1 Introduction

Photoinhibition has become an important area of intensive study during recent years. It has been pointed out that two different types of photoinhibition occurred either at the acceptor-side or donor-side of PSII reaction centre[1]. The photodamage and photoinactivation were considered to be in relation to the changes of conformation and structure of PSII complex. The formation of active oxygen species was promoted in chloroplasts under high light intensity. Active oxygen may involved in the initiation, acceleration and sequence of events leading to impairment of PSII [2,3]. Some specific chemical modifiers and denaturants of protein are useful probes for exploring the relationship between function and change of conformation or structure of membrane, protein/enzyme and their regulation mechanism. However, the direct effects and their differences among these chemicals and exogenous active oxygen on inactivation of PSII are not clear.

The present paper reports data on PSII inactivation of spinach leaf discs treated by four kinds of active oxygen, protein modifiers and denaturants, via the detection of chlorophyll fluorescence modulation kinetics.

2 Materials and methods

Leaf discs from mature spinach plants were infiltrated with different kinds of active oxygen ($\cdot O_2^-$, OH·, $^1O_2$, H_2O_2), protein modifiers (N-bromosuccinimide, diethylpyrocarbonate, butanedione, *p*-chloromercuribenzoic acid) and protein denaturants (urea, guanidinium chloride, sodium dodecyl sulfate, dimethyl sulfoxide) for 30 min, then exposured to 25 ℃ and high light (1 000 μmol $m^{-2}s^{-1}$) for another 30 min with dark treatment as control. Chlorophyll fluorescence was measured with a PAM pulse modulation fluorometer and the fluorescence parameters were calculated according to Schreiber *et al* [4]. The intensity of weak modulated measuring beam was less than 0.01 μmol photon $m^{-2}s^{-1}$, actinic light was 250 μmol $m^{-2}s^{-1}$ and saturating light pulse was up to 6 600 μmol $m^{-2}s^{-1}$.

Singlet oxygen ($^1O_2$) was generated by hematoporphyrin photosensitization[5]. Superoxide anion (O_2^-) was produced by the illuminated methylviologen[6], while hydroxyl radical (OH·) was obtained through the Fenton reaction[7].

3 Results and discussion

3.1 Effect of active oxygen

In comparison with dark control, high light induced the photoinhibition of spinach leaf showing decrease on Fm, Fv, Fv/Fm, q_P and increase of q_N (Table 1). In treatments with four kinds of active oxygen under high light, the changes of chlorophyll fluorescence parameters were enhanced. Addition of H_2O_2 (150 mmol/L) and the chemicals which produced $\cdot O_2^-$, OH · radicals and $^1O_2$ in the dark resulted in increase of q_P and decrease of q_N, but a slight effect on Fv/Fm. When illuminating leaf discs in the presence of the above active oxygen species, Fv/Fm and Fv fell down to 5−16% and 9−50% of light control, respectively. The change of fluorescence quenchings (q_P and q_N) showed different behavior. Treatments with H_2O_2, Fo and q_N rose with low q_P, indicating that the dissipation of excessive light energy was altered in favour of heat dissipation. However, under conditions treated with other active oxygen species, both q_P and q_N dropped. The decrease of q_N may imply that the energization of chloroplast was also affected by the strong species of active oxygen and led to the reduction of photoprotective ability.

Table 1 Effect of active oxygen on chlorophyll fluorescence

Active oxygen		Fm	Fv	Fo	Fv/Fm	q_P	q_N
H_2O	D	89.8	74.9	18.2	0.834	0.722	0.658
	L	76.0	54.3	21.7	0.714	0.588	0.712
	L/D	0.85	0.73	1.19	0.86	0.81	1.08
H_2O_2	D	93.2	74.3	17.0	0.797	0.696	0.705
	L	75.5	49.8	25.7	0.659	0.400	0.787
	L/D	0.81	0.67	1.51	0.83	0.58	1.11
OH ·	D	93.9	74.0	19.8	0.788	0.571	0.803
	L	77.2	53.2	24.0	0.689	0.577	0.689
	L/D	0.82	0.72	1.21	0.87	1.01	0.87
$^1O_2$	D	77.8	61.2	16.6	0.786	0.658	0.689
	L	52.3	34.7	18.0	0.663	0.500	0.641
	L/D	0.67	0.57	1.08	0.84	0.76	0.93
O_2^-	D	84.2	64.0	20.2	0.760	0.304	0.854
	L	50.8	27.3	23.7	0.537	0.222	0.565
	L/D	0.60	0.43	1.17	0.70	0.73	0.66

D— in the dark; L— under the light (1000 μmol $m^{-2}s^{-1}$)

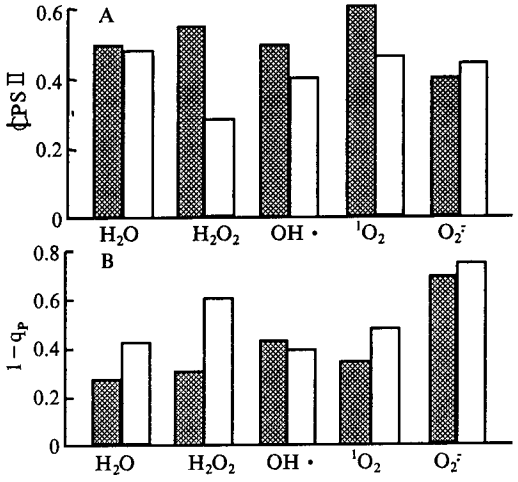

Fig. 1 Changes of ΦPS Ⅱ (A) and $1-q_P$ (B) in the presence of active oxygen

Fig. 1 showed the calculated value of Φ PSⅡ (photochemical quantum efficiency of open PSⅡ reaction centre) and $1-q_P$ (approximate measure of the reduction state of PSⅡ centre) under light or dark condition with and without active oxygen. Involvement of exogenous active oxygen with high light all showed a decreasing ΦPSⅡ (7−49%) and an increasing $1-q_P$ value (3−89%), which suggests that a high Q reduction state reflecting the potentially harmful overexcitation of PS Ⅱ centres occurred in these cases. The results

formed that active oxygen mediated photooxidative damage of PSII. It has been reported that excessive oxidative conditions can seriously perturb normal cell metabolism, cause pigment bleaching and protein cross-linking of PSII complex[8], and involve in the targeting and breakdown process of protein[3]. Some special amino acid residues in protein, such as histidine, cystein, tryptophan are very susceptible to oxidative damage[9], and the oxidized proteins showed more proteolytic hydrolysis than natural protein[10]. Thus, the possible explanation for the enhancement of photoinhibition by active oxygen might be mainly due to the oxidative damage of PSII complex in the acceptor side (by $^1O_2$, $\cdot O_2^-$, H_2O_2) or in the donor side (by OH· and H_2O_2). On the other hand, oxidative modification of proteins in chloroplast may result in enhancing their susceptibility to proteolysis.

3.2 Effect of protein modifiers

Table 2 summarizes the effects of four kinds of protein modifiers on fluorescence parameters of spinach leaf. The tryptophan residue modifier NBS, cysteine residue modifier ρCMB, arginine residue modifier BDE and histidine residue modifier DEPC all caused the significant decrease of Fm, Fv, Fv/Fm and q_P. The quenching of Fm was almost paralleled to a decrease in Fo, and the decrease in q_P was correlated with high q_N. Additionally, the high reduction state of PSII (1.3−1.9 fold of control) and low photochemical activity of PSII (ΦPSII, 86−95% of control) were observed by these covalence modification of chloroplast protein (Fig. 2). Among the test modifiers in the same concentration, ρCMB induced an obvious decrease in Fm and Fo, while the evident changes of PSII, $1-q_P$ and q_P, q_N were found by the treatments with BDE and DEPC.

Table 2 Effects of protein modifiers and denaturants on chlorophyll fluorescence

Additions	Fm	Fv	Fo	Fv/Fm	q_P	q_N
Protein modifiers (μmol/L)						
No addition	96.2	72.2	24.0	0.751	0.559	0.771
ρCMB(100)	65.0	46.3	18.7	0.712	0.429	0.790
NBS(100)	70.1	50.3	19.8	0.717	0.340	0.825
Butanedione(100)	73.2	52.7	20.5	0.720	0.158	0.817
DEPC(100)	80.5	57.7	22.8	0.716	0.318	0.807
Protein denaturants (mmol/L)						
Gu-HCl (60)	95.7	62.2	35.8	0.650	0	0.921
Urea (80)	90.0	66.8	23.2	0.742	0.150	0.809
SDS (1.7)	69.0	43.3	26.7	0.627	0.250	0.770
DMSO (60)	85.9	64.0	21.9	0.745	0.417	0.810

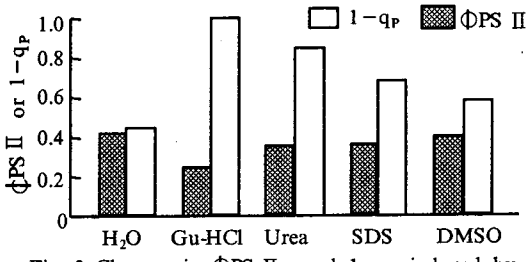

Fig. 2 Changes in ΦPS II and $1-q_P$ induced by protein modifiers

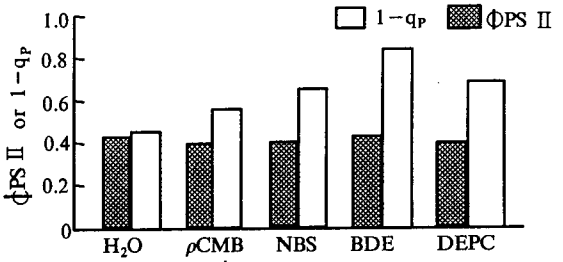

Fig. 3 Changes in ΦPS II and $1-q_P$ induced by protein denaturants

It is known that histidine mediating the quenching of $^1O_2$[3], and a serine-type endopeptidase with -SH catalytic mechanism in reaction centre complex of PSII might

involve in the turnover decomposition of D_1 protein[11]. The present data indicated that changes of protein conformation of chloroplast led to the enhancement of photoinhibition, the different effect on fluorescence parameters depened on the modifiers used, but the induced increasing photoinhibition might only express a response of photoprotection in heat dissipation in present experimental conditions.

3.3 Effect of protein denaturants

Guanidinium chloride and urea disturbed the hydrophobic group and/or hydrophilic group of protein which resulted in the unfolding of protein. The guanidinium chloride treated leaf showed very high level of q_N (up to 0.921) and Fo (149%), and a complete loss of q_P (Table 2). The lowest ΦPSⅡ (59%) and low Fv/Fm ratio (86%) were also observed in Fig. 3. Urea at 80 mmol/L did not changes Fo and Fv/Fm, but reduced PSⅡ activity by decreasing Fm and q_P. Its effect on PSⅡ was less than that of guanidinium chloride.

Treatment with low concentration of detergent sodium dodecye sulphate (SDS) significantly decreased Fm(28%), Fv(40%), Fv/Fm(17%) and q_P(55%), but the same q_N value remained as compared with control. The result showed that SDS-treatment inhibited the function of PSⅡ, which was in agreement with the inhibition of PSⅡ electron transport (H_2O-DCPIP) and the redistribution of excitation energy between PSⅡ and PSⅠ in SDS-treated pea chloroplasts as reported by Apostolova et al[12].

Dimethyl sulfoxide (DMSO) showed less effect on PSⅡ fluorescence parameters (Table 2 and Fig. 3). DMSO is a cleavager of hydrogen bond in protein and also a scavenger of hydroxyl radical[13]. This two different function may be as part of the reason for explaining its slight effect under high light.

In conclusion, we proposed that the conformation and structure of protein in chloroplast were altered either by protein modifiers and denaturants, or by the oxidative conformation of protein and pigments under oxygen stress condition, they all led to the photoinactivation of PSⅡ. The pattern and extent of changes might be dependent upon the property, concentration of the used compounds, and the importance of modified component in PSⅡ centre.

References

1 Aro E M, Virgin I, Andersson B. Photoinhibition of photosystem Ⅱ. Inactivation, protein damage and turnover. Biochim Biophys Acta, 1993, 1143:113－134

2 Gong H, Nilsen S, Allen J F. Photoinhibition of photosynthesis *in vivo*: Involement of multiple sites in a photodamage process under CO_2 and O_2 free conditions. Biochim Biophys Acta, 1993, 1142:115－122

3 Hidey E, Spetea C, Vass I. Singlet oxygen and free radical production during acceptor and donor-side-induced photoinhibition. Studies with spin trapping EPR spectoscopy. Biochim Biophys Acta, 1994, 1186:143－152

4 Schreiber U, Schliwa U, Bilger W. Continuous recording of photochemical and non-photochemical chlorophyll

fluorescence quenching with a new type of modulation fluorometer. Photosynth Res, 1986, 10:51－62

5 Bodannes R S, Chan P C. Ascorbic acid as a scavenger of singlet oxygen. FEBS Lett, 1979, 105:195－196

6 Chia L S, McRae D G. Thompson J E. Light dependence of paraquat-initiated menbrane deterioration in bean plants, evidence for the involvement of superoxide. Physiol Plant, 1982, 56:492－499

7 Cohen G. The Fenton reaction, In: Greenwald R A ed. Handbook of Methods for Oxygen Radical Research, CRS Press, Boca Raton, Florida, 1985, 55－64

8 Mishra N P et al. Destructive role of singlet oxygen during aerolic illumination of the photosystem II core complex. Biochim Biophys Acta, 1994, 1186:81－90

9 Kawakishi S et al. The oxidative degradation of some special amino acid residues in protein by active oxygen species. In: Asada K, Yoshikawa T eds. Frontiers of Reactive Oxygen Species in Biology and Medicine. Elsevier Science BV. Amsterdam, 1994, 157－160

10 Agarwal S, Sohal R S. Aging and proteolysis of oxidized proteins. Arch Biochim Biophys, 1994, 309:24－28

11 Polgar L, Halasz P. Current problems in mechanistic studies of serine and cysteine proteinase. Biochem J, 1982, 207(1):1－10

12 Apostolova E L, Ivanov A G, Petkova R. Photochemical activity of pea chloroplasts after sodium dodecyl sulphate-treatment. Photosynthetica, 1990, 24:173－175

13 Fuijimoto Y et al. Effect of reactive oxygen species on cyclooxygenase and lipoxygenase metabolism of arachidonic acid in platelets. In: Asada K, Yoshikawa T eds. Frontiers of Reactive Oxygen Species in Biology and Medicine. Elsevier Science BV. Amsterdam, 1994, 187－188