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Lipid peroxidation in thylakoid membranes under nitrogen stress in Spirulina platensis

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Abstract
Proteins in the thylakoid membranes undergo stress and affect the photosynthetic electron transport chain. The changes in the lipid-protein interaction show effects on electron transport activity and photo system II activity is much effected and lipid peroxidation takes place in thylakoid membrane. The alteration in the thylakoid membrane is responsible for improper functioning of photosystem II under stress. The depletion of nitrogen causes an enhancement in the lipid peroxidation.

Keywords: Nitrogen, Stress,Protiens,Lipids,Spirulina

Introduction

A series of reactions of photosynthesis takes place in thylakoid membranes. These membranes consist of two main compartments, the grana and the stroma lamellae. Four membrane bound pigment protein complexes are embedded in the thylakoid membranes to perform the primary process of photosynthesis. They are photosystem (PS) II, cytochrome (Cyt) b_6f complex, PS I and ATP synthase complex (Fig. 2). PS I and ATP synthase excluded from the grana membranes and PS II are abundantly present in the stacked parts of the thylakoid membranes (Andersson and Andersson, 1980; Barber, 1982), and the Cyt b_6f complex is found in both grana and stroma regions (Albertsson, 1995). Lipid protein interaction in the thylakoid membrane is disturbed due to depletion of nitrogen.

Materials and methods

Lipid peroxidation (LPO) has been measured according to the method of Heath and Packer (1968). The cells/ thylakoids were homogenized in 0.1 % TCA. The homogenized suspension was centrifuged at 10000 x g for 5 min. 4 ml of 0.5 mM of TBA- TCA mixture was added and boiled for 30 min at 95° C. Then the samples were kept in ice for 5 min and centrifuged at 10000 x g for 15 min. The absorbance of supernatant was measured at 532 nm (specific absorbance) and 600 nm (non specific absorbance). For the exact specific absorption value of the LPO, non specific absorption OD value of 600 nm was substracted from the OD value of 532 nm. The

malondialdehyde (MDA) calculations were made by using the extinction coefficient 155 mM^{-1} cm^{-1}. The amount of MDA was expressed as n M of MDA per mg protein.

Results

In algae and higher plants thylakoid membranes are rich in the above lipids, particularly monogalactosyl diacyl glycerol (MGDG) and digalactosyl diacyl glycerol (DGDG) for about 60-70 % of the membrane lipids (Nishihara *et al.,* 1980; Douce and Joyard, 1980). Studies on the mixtures of MGDG and DGDG suggest that the increase in the temperature favours the formation of non-lamellar structures where as low temperature causes the mixture to exist in the lamellar phase. The non-bilayer lipids are required for the package of light harvesting units of PSI and PS II into thylakoid membranes (WIlliams *et al.,* 1982). In the case of the thylakoid membranes, this tendency causes the destabilization of chlorophyll protein complexes of photosystems and alters the non-bilayer lipid and protein interaction.

Any treatment of stress tends to destabilize lipid protein interaction and can also induce phase transition in membranes. Keeping the above studies in mind, an attempt is made to study the peroxidation of thylakoid membrane under nitrogen stress.

Thylakoid membranes contain proteins in addition to lipid bilayer (Quinn and Williams, 1985). The major proteins present in thylakoid membranes are polypeptides related to WOC and LHC, polypeptides related to intersystem electron carriers (Cyt b_6f), and polypeptides related to PS I and PS II.

The changes under stress in the membrane organization can also affect the functional aspects of photosynthetic electron transport chain besides PS II.

Therefore an attempt was made to study the effect of nitrogen depletion on thylakoid membrane organization in this cyanobacterium by depleting the nitrogen.

Changes in the lipid protein interaction are responsible for the alteration of electron transport activities. To analyse this, an attempt is made to study the effect of nitrogen depletion on the lipid peroxidation thylakoid membranes.

For this purpose the cells were grown in media containing nitrogen between 80 μM to 40 μM and lipid peroxidation was measured in terms of MDA formed.

In control cells 39 n mole MDA/mg protein was observed (Table 1).

Table 1

Concentration $NaNo_3$ (μM)	Lipid peroxidation (nmol MDA mg^{-1} protein)	Percent enhancement
Control	39± 3.1	0
80	50 ± 5.0	28
60	57± 4.1	46
40	52± 4.9	33

The depletion in the concentration of nitrogen from 80 μM to 40 μM gradually caused enhancement in the lipid peroxidation up to 60 μM concentration. At 40 μM nitrogen concentration in the medium only 33% enhancement was noticed.

An attempt was made to correlate PS II catalyzed electron transport activity with lipid

peroxidation of thylakoid membranes. For this study, the cells were grown under nitrogen depleted medium containing concentration nitrogen from 80 to 40 μM and in the same samples both PS II electron transport activity as well as lipid peroxidation was measured (Table 2).

Table 2

Concentration $NaNo_3$ (μM)	PS II activity μmol O_2 evolved mg Chl^{-1} h^{-1}	Lipid peroxidation nmol(MDA) mg^{-1} (protein)
control	374±24	35 ± 3.1
0	302±14	44 ± 5.0
60	247±15	53 ± 4.1
40	176±10	49 ± 4.9

The decrease in nitrogen concentration gradually caused inhibition in the PS II activity. The inhibition of PS II activity is very much related with the increase of lipid peroxidation upon decrease in nitrogen concentration. In this way there was an inverse relationship between electron transport activity and lipid peroxidation in treated samples.

After incubation of cells at 60 μM nitrogen concentration they showed 54% enhancement of lipid peroxidation.

The same cells were kept at 80 μM nitrogen containg media after incubation showed only 26% of lipid peroxidation. But when the 80 μM nitrogen containing media incubated cells were transferred to 220 μM concentration of nitrogen containing medium and allowed it for 24 h incubation, there was the reversal of nitrogen stress induced alterations and the lipid peroxidation of thylokoids was equal to that of control cells (Table 3).

Table 3

Concentration $NaNO_3$ (μM)	Lipid peroxidation (nmol MDA mg^{-1} protein)	Percentage change
Control	35± 3.1	0
60	54± 5.1	54
80	44± 4.1	26
220	37± 3.5	5

Thus, nitrogen starved cells have ability to reverse the alterations in thylakoid membrane by modulating the protein and lipid biosynthesis when they were transferred to nitrogen containing medium.

Discussion

In photosystem thylakoid membrane plays a key role in cyanobactria as well as in higher plants. Generally biological membranes contain high proportions of non bilayer forming lipids in association with proteins to perform photosynthesis.

Photosynthetic membranes contain MGDG and DGDG in high proportions for proper packing of pigments and proteins related to light reactions of photosynthesis. Therefore the alterations in thylakoid lipid organization in the membrane play a key role and it will be responsible for the improper functioning of PS II under stress conditions.

To characterize the alterations in thylakoid membrane, lipid peroxidation has been measured in

both control and treated samples. To achieve this, the lipid peroxidation in control and 80-40 µM nitrate stressed samples by estimating the MDA formations were measured. The depletion of nitrogen caused enhancement in the lipid peroxidation up to 40 µM nitrogen source. Almost 43% enhancement in lipid peroxidation was noticed in nitrogen stressed samples.

The enhancement in lipid peroxidation was brought to the normal level as in control cells due to the shifting the cells to the optimum nitrate containing medium.

To correlate the lipid peroxidation with PS II catalyzed electron transport an attempt has been made to compared the above two parameters. These studies showed an inverse relationship between PS II photochemistry and lipid peroxidation. Thus both alterations of energy transfer and changes in the lipid organization of thylakoid membranes are responsible for the loss of PS II photochemistry.

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