nonviability of spermatozoa[20]. Thus the present findings on cisplatin effect on germinal cells of tumour bearing mouse may constitute helpful guide for better monitoring of cisplatin-mediated chemotherapy, particularly to male cancer patients who are in reproductive age.

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Regulation of Hill reaction coupled to photoreduction of nitrate and nitrite in *Anabaena doliolum*

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The photoreduction of nitrate and nitrite with water as the electron donor was observed in *Anabaena doliolum*. Dicyclohexyl carbodiimide, a specific inhibitor of ATPase, inhibited O_2 evolution as this process involved ATP hydrolysis. Cyanide-*m*-chlorophenyl hydrazone, an uncoupler, abolished O_2 evolution by breakdown of the active energy dependent transport of nitrate and nitrite and of the proton gradient. NH_4Cl, methylamine and hydroxylamine inhibited O_2 evolution by acting as bases and cleaving high energy bonds. EDTA combined with the metal ions that were required for oxygen evolution, synthesis of photosynthetic units and photophosphorylation. This resulted in the blockage of the nitrate and nitrite photoreduction coupled to O_2 evolution by preventing transfer of electrons from water and reductant for nitrate and nitrite reduction.

A significant feature of all nitrate reductases is their tight association to membranes with photosynthetic activity[1–5]. The coupling of nitrate reduction with the photolysis of water in cyanobacteria has been observed and use reduced ferrodoxin as the physiological electron donor[1–6]. It is in the thylakoid membrane that the light energy is absorbed by the highly organized assemblies of photosynthetic pigments and electron carriers. The expulsion of high energy electrons from the chlorophyll molecules in the reaction centres and their flow down a redox potential gradient results in the formation of strongly electronegative electron carriers like ferredoxin and NADPH. Part of the released energy is incorporated during this electron transport into ATP in the process of photophosphorylation[1–9].

Uncouplers dissociate the electron transport from ATP formation while the electron transport inhibitors act on one or more of the intermediate electron carriers[10–13].

This paper reports that in *Anabaena doliolum*, there is a breakdown of the nitrate-dependent oxygen evolution due to the effect of inhibitors and uncouplers of the photosynthetic electron transport chain.

A. doliolum was grown axenically in Allen and Arnon medium[14] diluted eight times at $26 \pm 2^{\circ}$C in light at a photon flux density of 40 μmol m^{-2} s^{-1} incident at the surface of the culture vessel. The experiments were carried out with log phase cultures. The measurement of oxygen evolution coupled to nitrate reduction was carried out in a Clark-type oxygen electrode fitted with a circulating water jacket. The temperature was adjusted to 25°C. The light intensity on the surface of the cuvette was 10 Wm^{-2} (20,000 lux). The reaction

mixture contained in a final volume of 3 ml; 150 μmol tricine–NaOH buffer, pH 7.7; 60 μmol KNO_3 or $NaNO_2$ (as the case may be); 30 μmol $MgCl_2$; 0.3 mg ferredoxin; *A. doliolum* particles (0.4 mg chlorophyll). Dicyclohexyl carbodiimide (DCCD, 50 μM), carboxyl cyanide-*m*-chlorophenyl hydrazone (CCCP, 10 μM), ammonium chloride (NH_4Cl, 1 mM), hydroxylamine–HCl (20 μM), methylamine–HCl (20 μM), ethylene diamine tetraacetic acid (EDTA, 2 mM), atrazine (10^{-5} M) and 3-(3,4-dichlorophenyl)-1, 1-dimethylurea (DCMU, 10^{-5} M) were added to the reaction mixture and incubated for 15 min before the oxygen evolution measurements. The chlorophyll content was estimated[15].

The uptake and assimilation of inorganic nitrogen by cyanobacteria is governed by various factors in the immediate environment of which the principal one is light[1-9]. The photoreduction of nitrate, nitrite and ammonia with water as electron donor is observed in cyanobacteria[1-5,8,9,16].

Results on the effect of photosynthetic inhibitors and uncouplers are shown in Table 1. DCCD, a specific inhibitor of ATPase[10,12], inhibited O_2 evolution. This indicated that the process of nitrate and nitrite-dependent O_2 evolution involved ATP hydrolysis, which is also a requirement for the uptake of nitrate and nitrite as seen in *Nostoc muscorum*[5,8,12]. Thus, DCCD acts as an energy-transfer inhibitor preventing electron flow and ATP formation.

CCCP, NH_4Cl, hydroxylamine and methylamine, all nonherbicidal uncouplers of photophosphorylation inhibited the O_2 evolution (Table 1). The sensitivity of the nitrate and nitrite-dependent O_2 evolution to these uncouplers could reflect a breakdown of the transport and of the ability of the cells to maintain their pH levels[7,9,11]. Cyanobacteria synthesize ATP in light by the same mechanism used by higher plants. A proton gradient is formed as a result of the light-driven electron transport and the proton gradient then drives the synthesis of ATP. These gradients are formed at two or three sites in the electron transfer sequence[9].

Table 1. Effect of inhibitors and uncouplers of photosynthetic electron transport and photophosphorylation on the nitrate and nitrite-dependent O_2 evolution by *Anabaena doliolum* cells

Incubation conditions in which the cyanobacterial cells were suspended for 15 min prior to O_2 measurements	O_2 evolution μmol mg^{-1} Chl *a* min^{-1}
Control (None)	48
DCCD	0
CCCP	0
NH_4Cl	0.3
Hydroxylamine-HCl	0
Methylamine-HCl	0
EDTA	2.1
Atrazine	2.7
DCMU	3.1

All data are the mean of three experiments.

CCCP abolished both the light-induced acidification and photosynthetic oxygen evolution completely, suggesting that the proton gradient depends in some way on electron transport and/or on photophosphorylation[7-12].

The amines act as uncouplers by possibly acting as bases which can penetrate to sites not available to highly polar bases, thereby causing hydroxyl ion catalysed cleavage of high energy bonds, which would otherwise transfer their energy to the pyrophosphate bond of ATP[13]. Ammonia inhibited indirectly by uncoupling photophosphorylation which increased the production of reductants from water through the photosystems[9,10].

Mn^{2+}, Ca^{2+}, Mg^{2+} and Cl^- are all required for the turnover of the reaction centres, for activation of the photosynthetic units in photosystem II (PS II), for photophosphorylation and oxygen evolution[9,18]. EDTA, a chelating agent, probably binds one of these metal ions and thereby, affecting the O_2 evolution capacity (Table 1).

As shown in Table 1, both the herbicides DCMU and atrazine, blocked O_2 evolution as they inhibit the electron transfer on the reducing side of PS II[17-19]. Hence, the supply of electrons from water and of reductant for nitrate reduction is prevented. The inhibitory effect of DCMU on nitrate reductase enzyme has already been reported in *A. doliolum*[4].

Thus, it is concluded that the photoreduction of nitrate and nitrite coupled to O_2 evolution is subject to control by factors governing both O_2 evolution and the assimilation of inorganic nitrogen.

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MEETINGS/SYMPOSIA/SEMINARS

15th Scientific Meeting of the International Society of Hypertension

Date: 19-26 March 1994
Place: Melbourne, Australia,
Contact: Dr Shailendra Vajpeyee
President, Indian Society of Hypertension
Govt. Medical College, Surat 395 001
Phone: (261) 46130 (Off.), (261) 41371 (Res.)
Fax: 91–261–652338.

Hypertension and Heart Research in Developing Countries (A Satellite Symposium of ISH 1994 Melbourne Meeting)

Date: 14–15 March 1994
Place: Bombay
Contact: Dr K. G. Nair
President, Organizing Committee
206 Doctor House, Pedder Road
Bombay 400 026
Phone: (22) 3865008
Fax: 91–22–467780

TROPMET-94 — A National Symposium of Climate Variability

Date: February 1994
Place: IITM, Pune
Topics: Climate and monsoon variability: intraseasonal, interannual, decadal, century and palaeo time-scales; Climate modelling: atmospheric, ocean and coupled models; Anthropogenic effects on climate: role of greenhouse gases, measurements and modelling; Chaos and predictability; Role of oceans in climate variability; Role of land surface processes; Satellite applications in climate studies; Impacts of climate change.
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