Stoichiometric Photophosphorylation in Thylakoids from the Blue-Green Alga, *Anabaena variabilis*

Erika Wax and Wolfgang Lockau

Institut für Botanik, Universität Regensburg, Universitätsstr. 31, D-8400 Regensburg

Z. Naturforsch. **35 c**, 98–105 (1980); received October 4, 1979

Cyanobacterium, Thylakoids, Photophosphorylation, P/e_2 Ratio, pH Gradient

A method is described for the preparation of thylakoids from the blue-green alga *Anabaena variabilis* which exhibit photosynthetic control. The thylakoids can be stored in liquid N_2 with little loss in activity. P/e_2 ratios (number of ATP molecules formed per pair of electrons transported) have been determined for partial reactions of the photosynthetic electron transport chain. Electron transport from water to an electron acceptor of photosystem I is coupled to phosphorylation with a P/e_2 ratio of 0.9–1.2, from water to electron acceptors of photosystem II with ratios of 0.21–0.3, and oxidations of hydrogen carriers by photosystem I with ratios of 0.3–0.35. Electron transport in these assay systems generates a proton gradient across the thylakoid membrane (acid inside), which is decreased by the substrates of phosphorylation and by uncouplers.

Introduction

Blue-green algae are prokaryotes possessing a photosynthetic machinery similar to that of chloroplasts from higher plants (see ref. [1]). Their thylakoids, located within the cytoplasm, contain both photosystem I and photosystem II. Photophosphorylation coupled to linear electron transport in extracts from a blue-green alga was first demonstrated by Gerhardt and Trebst [2]. Subsequently, stoichiometries (P/e_2 ratios) of this phosphorylation comparable to those usually found with thylakoids from higher plants were reported for cell-free preparations from several species [3–5]. Recently, the phenomenon of photosynthetic control, *i. e.* stimulation of electron flow by the substrates of phosphorylation, was also observed [6, 7]. In a study of cyclic phosphorylation mediated by an artificial redox compound, Padan and Schuldiner [8] have demonstrated that a pH gradient across the thylakoid membrane is primarily involved in energy conservation.

In this report, we describe a method for the isolation of very stable photosynthetic lamellae from *Anabaena variabilis*, a species which is frequently used for studies of cyanobacterial photosynthesis and from which photosynthetic mutants can be easily propagated [9]. Phosphorylation and formation of a pH gradient across the thylakoid membrane (acid inside) are shown to be associated with photoreductions by photosystem II, photooxidations by photosystem I, and overall electron transport. The results are discussed with reference to current concepts of energy conservation in electron transport of photosynthesis.

Materials and Methods

Culture conditions

Anabaena variabilis Kütz. (American Type Culture Collection 29413) was grown in a 4-fold dilution of the medium of Allen and Arnon [10]. Batches of 300 ml were inoculated with algae containing approximately 0.5 µg Chl/ml (final concentration) and kept in an illuminated thermostat at 30 °C. The cultures were continuously bubbled with filtered air enriched with 2% CO_2 and illuminated by two 40 W cool-white fluorescent lamps providing a light intensity of about 2300 lux. The minimal doubling time was 17 h, as measured by apparent absorption at 540 nm.

Abbreviations: AQS, anthraquinone-2-sulfonic acid; Chl, chlorophyll a; DAD, 2,3,5,6-tetramethyl-*p*-phenylenediamine; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DCIP, 2,6-dichloroindophenol; DCMU, 3(3′,4′-dichlorophenyl)-dimethylurea; FCCP, carbonylcyanide-*p*-trifluoromethoxy-phenylhydrazone; FeCy, potassium ferricyanide; HEPES, N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid; P_i, inorganic phosphate; TMPD, N,N,N′N′-tetramethyl-*p*-phenylenediamine.

Reprint requests to W. Lockau.

0341-0382/80/0100-0098 $ 01.00/0

Isolation of thylakoids

Algal filaments were harvested by centrifugation for 20 min at 2000×*g*, washed in 30 mM NaH_2PO_4-KOH, pH 6.8 (10 min centrifugation at 4000×*g*), washed again in 0.6 M sucrose, 10 mM NaCl, 30 mM NaH_2PO_4-KOH, pH 6.8, and resuspended in this buffer containing, in addition, 10 mM ethylenediaminetetraacetic acid, trisodium salt, and 1 mg egg white lysozyme/ml, at a concentration of 360 µg Chl/ml. This suspension was incubated in the dark at 30 °C for 1 h, with slow shaking. All subsequent steps were performed at 0–4 °C. The cells were collected by a 10 min centrifugation at 4000×*g*, resuspended in 0.6 M sucrose, 5 mM NaH_2PO_4, 50 mM NaCl, 10 mM $MgCl_2$, 30 mM HEPES, adjusted to pH 7.5 with KOH, at a concentration of 0.5–0.7 mg Chl/ml, and cavitated for 0.25 s/ml with a Sonifier B-12 equipped with a 0.5 inch tip (Branson Sonic Power Co., Danbury, Connecticut) at 70 W output. After removal of large particles by two centrifugations (5 min at 1000×*g*), the thylakoids were sedimented by a 1 h centrifugation at 48000×*g* and resuspended in the buffer used during cell breakage at a concentration of about 1 mg Chl/ml. The thylakoids were stored in 0.5 ml aliquots in liquid N_2. Before use, they were thawed under running tap water.

Assays

Chlorophyll was determined in methanolic extracts according to Mackinney [11].

Electron transport and coupled phosphorylation were assayed at 25 °C in the oxygen electrode set-up described before [12] except that the intensity of the red (610–750 nm) actinic light was 730 kerg · cm^{-2} · s^{-1}. Since buffers of high osmolarity were used, the oxygen electrode was calibrated by the method of Robinson and Cooper [13].

The concentration of thylakoids used in the individual experiments is given in the legends and Tables. Electron transport and coupled phosphorylation comprising the activities of both photosystem I and photosystem II were assayed in a reaction mixture consisting of 0.6 M sucrose, 10 mM NaCl, 10 mM $MgCl_2$, 2 mM NaH_2PO_4, 2 mM ADP, 0.1 mM anthraquinone-2-sulfonate (AQS), 30 mM HEPES, adjusted to pH 7.5 with KOH. The oxygen uptake resulting from the oxidation of the reduced electron acceptor by O_2 was followed. Addition of a catalase inhibitor was not necessary, since the thylakoid preparations were free of catalase activity. For assays of the photosynthetic reduction of $NADP^+$, AQS was omitted from the reaction mixture, and 12 µM ferredoxin, a saturating amount of ferredoxin-$NADP^+$-oxidoreductase, and 0.3 mM $NADP^+$ were added. Actinic illumination was as in the oxygen electrode experiments, $T = 22$ °C. Formation of NADPH was measured by the absorption increase at 366 nm. For assays of photosystem I activity, the reaction mixture containing AQS was supplemented with 1 µM DCMU (a photosystem II inhibitor), 5 mM sodium ascorbate plus either 0.5 mM N,N,N′,N′-tetramethyl-*p*-phenylenediamine (TMPD), 0.5 mM 2,3,5,6-tetramethyl-*p*-phenylenediamine (DAD), or 0.2 mM 2,6-dichloroindophenol (DCIP) as artificial electron donor couples to photosystem I, and with 233 µg superoxide dismutase/ml. Ascorbate alone did not support photosystem I activity. The concentration of superoxide dismutase used inhibited light-induced O_2 uptake maximally (37% inhibition). The rationale for the addition of superoxide dismutase has been described by *e. g.* Ort and Izawa [14]. Photosystem II activity was determined in three assay systems. Firstly, the ferricyanide Hill reaction was assayed in the presence of 10 µM DBMIB, a plastoquinone antagonist [15]. In this case, AQS in the reaction mixture described above was replaced by 2 mM potassium ferricyanide. Secondly, DBMIB was used as an autoxidizable electron acceptor at an alkaline pH [16]. The reaction mixture consisted of 0.6 M sucrose, 10 mM NaCl, 10 mM $MgCl_2$, 2 mM NaH_2PO_4, 2 mM ADP, 20 µM DBMIB, 30 mM N-[tris(hydroxymethyl)-methyl]-glycine, adjusted to pH 8.35 with KOH. Thirdly, silicomolybdic acid was used as an electron acceptor in the presence of DCMU. The reaction mixture, composed similarly to the one described by Berg and Izawa [17], consisted of 0.6 M sucrose, 20 mM NaCl, 3 mM $MgCl_2$, 2 mM NaH_2PO_4, 2 mM ADP, 5% (v/v) glycerol, 0.5% dimethyl sulfoxide, 0.2 mg bovine serum albumin/ml, 1 µM DCMU, 127 µM silicomolybdic acid, 30 mM HEPES, and was adjusted to pH 7.5 with KOH.

Phosphorylation was assayed by the esterification of [^{32}P]-P_i (500000–900000 cpm/µmol). Unreacted [^{32}P]-P_i was removed by a modification of a published procedure [18]: After addition of 2% (w/v, final concentration) trichloroacetic acid, the sample was centrifuged, 0.5 ml of the supernatant added to 5 ml 0.8 N $HClO_4$ containing 4% ammonium heptamolybdate. This solution was extracted consecutively

with 5 ml each of benzene/*iso*-butylalcohol (1/1), H_2O-saturated *iso*-butyl alcohol, and finally diethyl ether. Radioactivity was determined with a gas-flow counter (Berthold, Wildbad, FRG). Appropriate dark controls were always substracted.

Light-induced intrathylakoid pH changes were estimated by the fluorescent amine method of Schuldiner *et al.* [19]. The extent of the fluorescence quench of 9-amino acridine upon actinic illumination was measured in a model ZFM4 fluorometer (Zeiss, Oberkochen, FRG), modified for side illumination using fiber glass optics. Fluorescence of 9-amino acridine was excited with the emission lines of a mercury lamp around 405 nm, isolated with the appropriate Zeiss glass filter and attenuated to 10% intensity by a neutral density filter. Fluorescence emission was measured at 456 nm through the Zeiss monochromator M4 QIII at 1.5 mm slit width. The temperature was 25 °C, the red actinic light (610 to 750 nm) had an intensity of 72 kerg · cm^{-2} · s^{-1} at the surface of the cuvette.

[^{3}H]H_2O and [^{14}C]sucrose were used to determine the sucrose-impermeable [^{3}H]H_2O space of the thylakoids. To 0.2 ml of a thylakoid suspension in the buffer used for isolation with 240 – 280 µg Chl, either 0.04 µCi [^{14}C]sucrose or 0.1 µCi [^{3}H]H_2O were added. After mixing and a 15 min centrifugation at 27000×*g* in the dark, the supernatant was removed and the surface of the pellet quickly washed with the buffer. The pellet was dissolved in 0.5 ml of a 0.1% solution of Triton X-100, 50 µl of 40% trichloroacetic acid added, and the precipitate removed by centrifugation. 50 µl aliquots of the supernatants, which served as the standard, were treated in the same way. Radioactivity was determined by liquid scintillation counting. Quenches were estimated by internal standardization.

Ferredoxin, ferredoxin-NADP$^+$-oxidoreductase (both from spinach), and superoxide dismutase (from bovine erythrocytes) were isolated by published procedures [20 – 22]. Silicomolybdic acid was prepared according to ref. [23]. DBMIB and DCMU were gifts from Dr. A. Trebst (Bochum, FRG).

Results

General properties of the isolated thylakoids

The logarithmic phase of growth of the alga in batch culture lasts for about 4 days. When thylakoids are isolated from logarithmically growing cultures, electron transport from H_2O to AQS/O_2 decreases after the onset of illumination (Fig. 1 b). Similar results are obtained for the Hill-reaction with ferricyanide as the electron acceptor (AQS replaced by 2 mM ferricyanide). More stable and active thylakoid preparations can be isolated from cultures in the early stationary phase of growth (Fig. 1 a). Preparations obtained from such cultures (usually harvested after 7 days of growth) were used throughout this study. These thylakoids retain their photosynthetic activities after storage in liquid N_2 (Table I). After 20 h on ice, rates of electron trans-

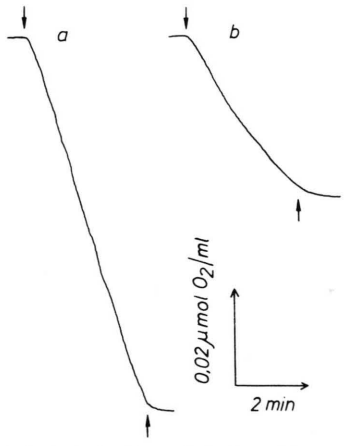

Fig. 1. Kinetics of electron transport in thylakoids isolated from cultures of different age. Electron transport from water to AQS/O_2 was assayed in the oxygen electrode. Trace a, thylakoids isolated from a batch culture after 7 days of growth. Trace b, thylakoids isolated from a batch culture after 3.5 days of growth. The assay mixtures, described under Methods, contained thylakoids with 20 µg Chl/ml. Downward arrows, light on; upward arrows, light off.

Table I. Stability of photosynthetic activities upon storage of thylakoids in liquid N_2.

Assays performed	Rate of		P/e_2 ratio
	electron flow	phosphorylation	
after isolation	416	196	0.94
after 8 days in liquid N_2	372	165	0.89

Rates of electron flow are given in µequivalents · mg^{-1} Chl · h^{-1}, those of phosphorylation in µmol P_i esterified · mg^{-1} Chl · h^{-1}. The Hill reaction with AQS/O_2 as the electron acceptor system was assayed with thylakoids containing 20 µg Chl/ml, the illumination time was 2 min.

port and phosphorylation decreased by about 10%. As judged from absorption spectra (not shown), the thylakoid preparations are depleted of phycobiliproteins.

Photosynthetic control and uncoupling of phosphorylation

Photosynthetic electron transport from H_2O to AQS/O_2 is coupled to phosphorylation. For different preparations, the number of ATP molecules formed per pair of electrons transported (P/e_2 ratio) varies from 0.9–1.2. Addition of ADP to the reaction mixture already containing Mg^{2+} and P_i stimulates the rate of electron transport about twofold (Table II). Electron transport is also stimulated by the uncouplers NH_4Cl, gramicidin D, and FCCP. However, only partial uncoupling is obtained with NH_4Cl (25–50% inhibition even at a concentration of 20 mM) and with 4 µg/ml gramicidin D. As recorded in Table II, the addition of ADP to reaction mixtures containing either NH_4Cl or gramicidin D further

Table II. Effects of ADP and of uncouplers on electron flow, and of uncouplers on phosphorylation.

Addition	No ADP Rate of electron flow	+2 mM ADP		P/e_2 ratio
		Rate of electron flow	Rate of phosphorylation	
–	159	308	159	1.03
4 µg gramicidin/ml	317	333	53	0.32
2 µM FCCP	224	227	5	0.04
2.5 mM NH_4Cl	282	329	134	0.81
1.5 µM valinomycin	113	242	123	1.02
2.5 mM NH_4Cl plus 1.5 µM valinomycin	344	343	10	0.06

Rates of electron flow are given in electron µequivalents · mg^{-1} Chl · h^{-1}, those of phosphorylation in µmol P_i esterified · mg^{-1} Chl · h^{-1}. The Hill reaction with AQS/O_2 as the electron acceptor system was assayed with thylakoids containing 17.1 µg Chl/ml, the illumination time was 4 min. The reaction mixture contained Mg^{2+}, P_i and about 15 mM K^+ (see Methods).

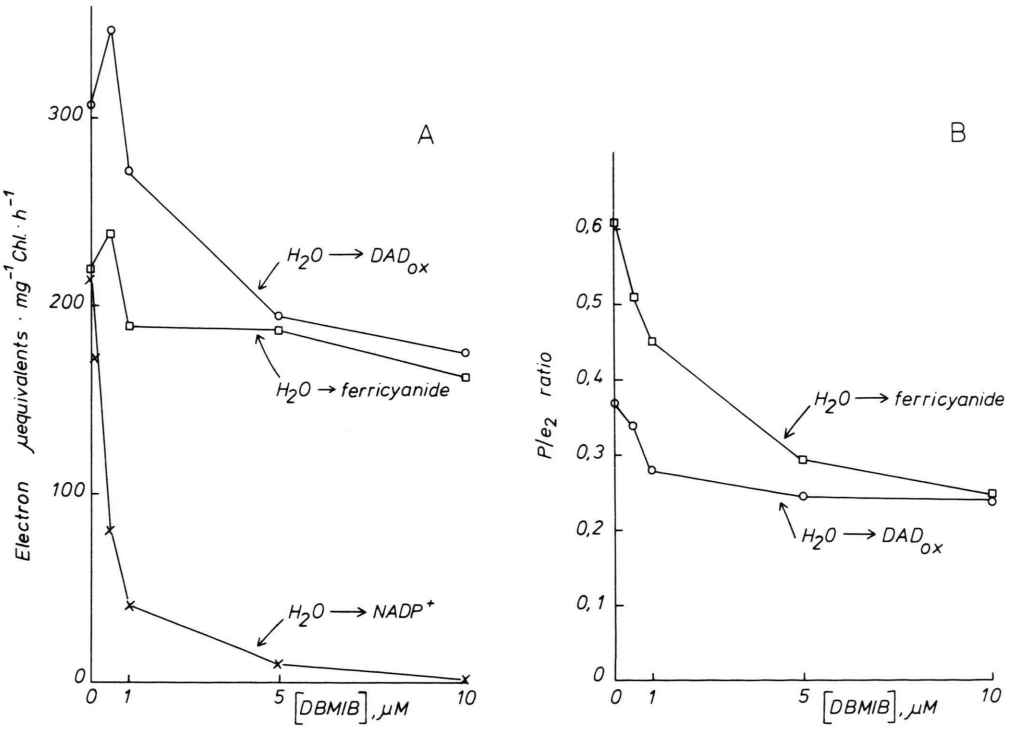

Fig. 2. Effect of DBMIB on electron transport and coupled phosphorylation with ferricyanide or oxidized DAD as electron acceptors. For comparison, the effect on photoreduction of $NADP^+$ is also shown. The assay mixtures, described under Methods, contained thylakoids with 20 µg Chl/ml. The illumination time was 2 min for the assay of $NADP^+$ reduction, and 4 min in the other cases.

stimulates the rate of electron transport. 1.5 μM valinomycin, while acting as an inhibitor of coupled and basal electron transport [24], has no effect on the coupling efficiency. However, the combination of NH_4Cl and valinomycin stimulates electron transport strongly and inhibits photophosphorylation almost completely. Strong uncoupling is also observed with 2 μM FCCP (Table II). An inhibitory effect of this compound on electron transport is noted (compare ref. [25]), which is not relieved by ADP.

Phosphorylation in partial reactions of the photosynthetic electron transport chain

In Fig. 2 and Table III, rates of electron transport and of coupled phosphorylation in partial reactions of photosynthesis are shown. For the isolation of a photoreduction driven by photosystem II alone, the plastoquinone antagonist DBMIB [15] is used. 5 to 10 μM DBMIB inhibit photoreduction of $NADP^+$ almost completely, whereas the Hill-reactions with either ferricyanide or the oxidized form of DAD

Table III. Electron transport and coupled phosphorylation in partial reactions of the photosynthetic electron transport chain.

Photosystem (s) involved	Electron donor	Electron acceptor	Inhibitor	Rate of		P/e_2 ratio
				electron flow	phosphorylation	
2+1	H_2O	AQS/O_2	–	276	152	1.10
2	H_2O	ferricyanide	DBMIB	156	24	0.31
2	H_2O	$DBMIB/O_2$	DBMIB	128	14	0.22
2	H_2O	silico-molybdate	DCMU	116	13	0.22
1	ascorbate/DAD	AQS/O_2	DCMU	3744	568	0.30
1	ascorbate/DCIP	AQS/O_2	DCMU	840	130	0.31
1	ascorbate/TMPD	AQS/O_2	DCMU	1364	34	0.05

Rates of electron flow are given in electron μequivalents · mg^{-1} Chl · h^{-1}, those of phosphorylation in μmol P_i esterified · mg^{-1} Chl · h^{-1}. The illumination time was 2 to 4 min, depending on the rate of electron transport. The chlorophyll concentration in the photosystem 1 assays was 2 μg/ml, and 19.6 μg/ml in all other cases. Assay conditions are described in Methods.

Table IV. Light-induced pH gradients across the thylakoid membrane in partial reactions of the photosynthetic electron transport chain.

Experiment	Photosystem (s) involved	Electron donor	Electron acceptor	Addition	ΔpH
A	2+1	H_2O	AQS/O_2	–	2.8
				5 mM NH_4Cl +0.7 μM valinomycin	not detected
	2	H_2O	DBMIB	–	2.4
	1	ascorbate/DAD	AQS/O_2	–	3.2
B	2+1	H_2O	AQS/O_2	–	2.75
				2 mM NaH_2PO_4	2.8
				0.2 mM ADP	2.6
				2 mM NaH_2PO_4 +0.2 mM ADP	2.25
				1 μM FCCP	not detected

The reaction mixture used for the assay of photosystems II plus I is described in Methods. In experiment B, NaH_2PO_4 was omitted from the basic reaction mixture. For assay of photosystem II, AQS was replaced by 20 μM DBMIB. These reaction mixtures contained thylakoids with 7.6 μg Chl/ml. The photosystem I assay, performed as in Methods, contained 5.3 μg Chl/ml. The concentration of 9-amino acridine was 10 μM. See text for further details.

(kept oxidized by ferricyanide) as electron acceptors still procede at a considerable rate (Fig. 2A). From this observation, and from the relatively low P/e_2 ratio in the absence of DBMIB (Fig. 2B), it is concluded that ferricyanide can be reduced by both photosystem II and photosystem I. The phosphorylation efficiencies of these photoreductions decrease with increasing concentrations of DBMIB, yielding P/e_2 ratios of 0.25 – 0.3 at 5 – 10 μM DBMIB (Fig. 2B, Table III). As shown in Table III, the photoreductions of DBMIB (kept oxidized by O_2 at an alkaline pH, see Methods) and of silicomolybdic acid (in the presence of DCMU) are also coupled to phosphorylation, although at a somewhat lower efficiency.

Photooxidation of the hydrogen carriers DAD and reduced DCIP by photosysem I (photosystem II blocked by DCMU) is coupled with P/e_2 ratios of 0.3 – 0.35, in different experiments. The electron donor TMPD, however, supports phosphorylation only at a very low rate (Table III). In contrast to DAD and reduced DCIP, TMPD does not liberate protons upon oxidation (see [26, 27]).

Determination of light-induced pH gradients

The internal aqueous space of the thylakoids estimated as described under Methods is 15 ± 6 μl/mg Chl (mean of 4 experiments ± standard deviation). Using this value, the extent of the light-induced pH gradient across the thylakoid membrane was calculated from the quench in fluorescence of 9-amino acridine according to [19]. Electron transport comprising the activities of both photosystems, of photosystem II alone or of photosystem I alone (with DAD as reductant) induces reversible quenches of the fluorescence of 9-amino acridine, indicating an acidification of the intrathylakoid space. The calculated Δ pH values (Table IV) should be considered as crude because it is assumed that the internal volume of the thylakoids does not change upon illumination, and because it is uncertain for other systems and for thylakoids from blue-green algae [8, 28, 29] whether 9-amino acridine reports internal pH values correct. As shown for electron transport from water to AQS/O_2, the presence of the substrates of phosphorylation decreases the extent of Δ pH (Table IV). In the presence of NH_4Cl plus valinomycin, and of FCCP (which inhibit photophosphorylation almost completely, Table II), no light-induced transmembrane pH gradient is observed by the 9-amino acridine technique.

Discussion

The isolation procedure described is a convenient method for the preparation of stable thylakoid preparations active in electron transport and coupled phosphorylation. We do not have a firm explanation for the observation that thylakoids isolated from cultures in their early stationary phase are the most stable ones obtained. It seems likely that variations in the composition of the membranes in different stages of growth influence the stability of the isolated thylakoids. Such variations have been observed for photosynthetic catalysts of blue-green algae [30].

An outstanding feature of our thylakoid preparation is the photosynthetic control. The addition of either uncouplers or of the substrates of phosphorylation at least doubles the rate of electron flow of overall electron transport (Table II). Photosynthetic control has not been observed in thylakoids from *Anabaena variabilis* prepared by a long lysozyme digestion followed by osmotic shock [4]. By comparison with thylakoids from higher plants, Lee *et al.* [4] therefore suggested that photophosphorylation in these preparations may only be losely coupled to electron transport. However, the phosphorylation efficiencies (P/e_2 ratios) in our preparations exhibiting photosynthetic control do not exceed those reported by Lee *et al.* The range of P/e_2 ratios for phosphorylation coupled to electron transport from water to a photosystem I electron acceptor (0.9 – 1.2) is that usually found with thylakoids from chloroplasts of higher plants considered to be well coupled (compare *e. g.* ref. [27]).

The pattern of energy conservation is, in general, identical to that observed with higher plant thylakoids. Photoreductions by photosystem II and photooxidations of hydrogen carriers (which liberate protons upon oxidation) by photosystem I are coupled to phosphorylation and generate a proton gradient (acid inside) across the thylakoid membrane (Fig. 2, Tables III and IV), which is decreased by phosphorylating conditions [31] and by uncouplers. These findings indicate a vectorial arrangement of the components of photosynthetic electron transport in the membrane, the oxidizing sides of photosystem I and of photosystem II (or the water splitting reaction) being oriented towards the inside of the thylakoid vesicle (see [26]). It should be noted in this context that no electrochromic absorption changes, indicative of a transmembrane orientation

of photosystems [32], have been reported so far for blue-green algae. The sensitivity of steady-state phosphorylation to uncouplers and its insensitivity to valinomycin/K^+ is similar to chloroplasts, as has been noted before for cyclic phosphorylation in osmotically shocked spheroplasts from *Plectonema boryanum* [8]. There are, however, some differences in details.

Firstly, phosphorylation is relatively insensitive to uncoupling by gramicidin D or NH_4Cl (Table III), reminiscent of the situation in subchloroplast vesicles prepared by sonication [33, 34]. It is not possible to decide whether this uncoupler resistance is an intrinsic feature of the thylakoids of *Anabaena variabilis* or induced by the isolation procedure, which includes a short time of sonication. Partial insensitivity of ATP synthesis to uncoupling by amines may indicate a contribution of the membrane potential to phosphorylation [33], at least in the presence of amines. This problem, however, has not been solved beyond doubt [34, 35]. Valinomycin may increase the uncoupling effect of NH_4Cl (Table II) by increasing the permeability of the membranes to NH_4^+ [33].

Secondly, the P/e_2 ratios determined for the partial reactions of the photosynthetic electron transport chain are relatively low (Table III). In photosystem II phosphorylation, where the rates of ATP synthesis and, presumably, the electrochemical potential are rather low, the stoichiometry may be suboptimal because a greater proportion of the electrochemical potential may be dissipated unspecifically (*i. e.* not coupled to ATP synthesis) than in systems giving higher rates of phosphorylation [36]. This may also explain the observation [3, 4] that non-cyclic phosphorylation is more sensitive to various treatments of thylakoids from blue-green algae than is cyclic phosphorylation with artificial redox cofactors. In the latter case, the rate of proton pumping is expected to be high, thus overriding an increased leakiness of the membranes to protons. The same reasoning, however, does not explain why the P/e_2 ratios of phosphorylation coupled to photosystem I electron transport (0.3 – 0.35, in the presence of a saturating amount of superoxide dismutase) are lower than half the overall stoichiometry (Table III), which is found with higher plant chloroplasts (see [27]). Also, a limitation of the rate of phosphorylation by the turnover of the ATPase can be excluded, because the stoichiometry is invariant when the rate of phosphorylation is decreased to the rate associated with overall electron transport by decreasing the light intensity (not shown under Results).

In conclusion, the concept of at least two energy conserving sites, each associated with one of the photosystems, seems applicable to thylakoids from *Anabaena variabilis*. The formation of pH gradients indicates a transmembrane orientation of both photosystems, their oxidizing sites being located towards the inside of the thylakoids. The results further stress the close relationship between the photosynthetic machineries of blue-green algae and higher plants.

Acknowledgement

We are thankful to Dr. G. Hauska, Universität Regensburg, for constant support and reading of the manuscript.

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