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Oxidative Phosphorylation and Energy Buffering in Cyanobacteria

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The onset of respiration in the cyanobacteria *Anacystis nidulans* and *Nostoc* sp. strain Mac upon a shift from dark anaerobic to aerobic conditions was accompanied by rapid energization of the adenylate pool (owing to the combined action of ATP synthase and adenylate kinase) and also the guanylate, uridylate, and cytidylate pools (owing to nucleoside diphosphate and nuclesoide monophosphate kinases). Rates of the various transphosphorylation reactions were comparable to the rate of oxidative phosphorylation, thus explaining, in part, low ~P/O ratios which incorporate adenylates only. The increase of ATP, GTP, UTP, and CTP levels (nanomoles per minute per milligram [dry weight]) in oxygen-pulsed cells of *A. nidulans* and *Nostoc* species was calculated to be, on average, 2.3, 1.05, 0.8, and 0.57, respectively. Together with aerobic steady-state pool sizes of 1.35, 0.57, 0.5, and 0.4 nmol/mg (dry weight) for these nucleotides, a fairly uniform turnover of 1.3 to 1.5 min^{-1} was derived. All types of nucleotides, therefore, may be conceived of as being in equilibrium with each other, reflecting the energetic homeostasis or energy buffering of the (respiring) cyanobacterial cell. For the calculation of net efficiencies of oxidative phosphorylation in terms of ~P/O ratios, this energy buffering was taken into account. Moreover, in *A. nidulans* an additional 30% of the energy initially conserved in ATP by oxidative phosphorylation was immediately used up by a plasma membrane-bound reversible H^+-ATPase for H^+ extrusion. Consequently, by allowing for energy buffering and ATPase-linked H^+ extrusion, maximum P/O ratios of 2.6 to 3.3 were calculated. By contrast, in *Nostoc* sp. all the H^+ extrusion appeared to be linked to a plasma membrane-bound respiratory chain, thus bypassing any ATP formation and leading to P/O ratios of only 1.3 to 1.5 despite the correction for energy buffering.

The efficiency of oxidative phosphorylation is commonly expressed in terms of P/O ratios calculated, e.g., from the oxygen-induced increase of the adenosine phosphate free energy content of intact cells during transition from anaerobic to aerobic conditions. Cyanobacteria and other bacteria have mostly been reported to give P/O ratios around 1 (19, 26, 29; W. H. Nitschmann, Ph.D. thesis, University of Vienna, Austria, 1982), which is considerably less than the well-known mitochondrial coupling ratio of 3.0 with NAD-linked substrates. Reasons for this discrepancy may be: (i) inherently lower coupling efficiencies as discussed for, e.g., alcalophilic bacteria, with H^+/ATP ratios of up to 8 (8); (ii) direct coupling of energy-dependent transmembrane transport processes to respiratory electron flow via the proton motive force, thus bypassing the synthesis of ATP as such (6, 19, 21); or (iii) ATP-utilizing reactions in the cytosol proceeding at a rate comparable to that of oxidative phosphorylation. An example of the last possibility was demonstrated in *Escherichia coli* by the operation of adenylate kinase (EC 2.7.4.3), which catalyzes the fast and freely reversible reaction ATP + AMP = 2ADP (*K* near 1) (11, 15), thereby recycling ADP as the phosphate acceptor in oxidative phosphorylation without wasting energy (10). Thus, the onset of respiratory electron transport resulted in a rapid increase of the ATP level paralleled by a corresponding decrease of AMP, with only little effect on ADP (10). Similar findings were reported for other bacteria (29) and cyanobacteria (19). Consequently, the initial rate of formation of "energy-rich" adenosine phosphate bonds (~P) is not simply accounted for by ΔATP but must be calculated from Δ~P = 2ΔATP + ΔADP (10). Note that in the noncompartmentalized cell of a procaryote the ATP newly synthesized by electron transport phosphorylation is equally available to any of the ATP-consuming reactions in the cytosol.

Under physiological conditions the action of nucleoside diphosphate (NDP) kinase (NDPK; NDP + ATP = nucleoside triphosphate [NTP] + ADP; EC 2.7.4.6) and nucleoside monophosphate (NMP) kinase (NMPK; NMP + ATP = NDP + ADP; EC 2.7.4.4) might well necessitate similar corrections (as with adenylate kinase) with respect to the other NTPs and NDPs newly, and rapidly, synthesized at the expense of ATP from oxidatiave phosphorylation. These enzymes catalyze a roughly isoenergetic phosphate transfer between different nucleoside phosphates, as is reflected by equilibrium constants *K* near 1 (28), analogous to, and as fast as, adenylate kinase (11). As an overall result, therefore, in steady-state conditions all the nucleoside phosphates within a noncompartmentalized cell may be conceived of as being in equilibrium with each other, meaning that the γ-phosphate bond of each NTP and the β-phosphate bonds of two NDPs are energetically equivalent to the γ-phosphate bond of ATP (energy buffering). In contrast to the rapid transphosphorylation reactions, turnover of nucleoside phosphates owing to biosynthesis and RNA metabolism is negligible in resting cells (5).

Another energy-requiring process that might interfere with experimentally determined P/O ratios is proton extrusion from oxygen-pulsed (respiring) cells (21). This process can be powered either by a H^+-translocating respiratory chain in the plasma membrane (23, 24; V. Molitor, M. Trnka, and G. A. Peschek, Curr. Microbiol., in press), thereby accelerating oxygen uptake without concomitant ATP production (21), or by a reversible (25) or unidirectional (26) H^+-translocating ATPase in the plasma membrane utilizing the ATP as soon as it is formed by oxidative phosphorylation at the (intracellular) thylakoid membrane (see Fig. 4). Clearly, in either case the apparent P/O ratios would not reflect the true efficiency of respiratory energy coupling.

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In the present study, the impact of the combined action of NMPK and NDPK in the cytosol and of H^+ extrusion across the plasma membrane on the overall efficiency of oxidative phosphorylation in the cyanobacteria *Anacystis nidulans* and *Nostoc* sp. strain Mac is discussed. For comparison, relevant data from other bacteria are also mentioned.

MATERIALS AND METHODS

Culture of organisms. Axenic cultures of *A. nidulans* (*Synechococcus* sp. strain 1402-1, Gottingen, Federal Republic of Germany) and *Nostoc* sp. strain Mac (PCC 8009) were grown and harvested as described previously (20). Unless otherwise mentioned, harvested cells were washed twice with 30 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES)-Tris buffer (pH 7.5) and resuspended in the same medium (10 to 20 mg [dry weight]/ml; 1 mg [dry weight] is equivalent to 0.02 mg of chlorophyll).

Assay of NDPK and NMPK. Harvested cells were washed twice with distilled H_2O and suspended to a concentration of 12 to 40 mg (dry weight)/ml in 50 mM Tris hydrochloride (pH 8.0). After cooling to 0°C and addition of 0.25 mM dithiothreitol, cells were broken with a Branson sonifier, model B 15 (output control 7, 40% duty cycle, 45 min, 0°C). About 60 to 70% of the cells were broken as concluded from packed cell mass determination before and after the sonication. Unbroken cells and cell debris were removed by centrifugation (10 min, 3,000 × *g*, 4°C). Protein was determined as described in reference 4.

Enzyme assays were performed at room temperature in the direction of ATP synthesis (ADP + NTP → ATP + NDP for NDPK; ADP + NDP → ATP + NMP for NMPK; and 2ADP → ATP + AMP for adenylate kinase). Reaction mixtures contained: 1 ml of 50 mM Tris hydrochloride buffer, pH 8.0; 10 μl of 400 mM $MgCl_2$; 10 μl of 2.5 mM dithiothreitol; 10 μl of 100 mM ADP; and 10 μl each of 100 mM GTP, UTP, and CTP (NDPK assay [12, 32]); or 1 ml of 50 mM Tris hydrochloride buffer, pH 8.0; 10 μl of 400 mM $MgCl_2$; 10 μl of 2.5 mM dithiothreitol; 10 μl of 20 mM ADP; and 10 μl each of 100 mM GDP, UDP, and CDP (NMPK assay [28]). Activities of adenylate kinase causing ATP production with ADP alone were measured on the same

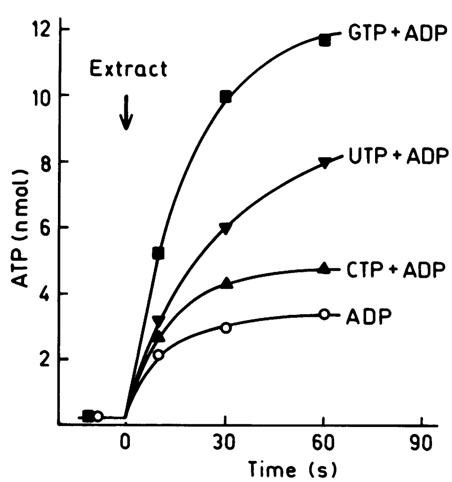

FIG. 1. Activities of NDPK in cell extracts of *A. nidulans* as apparent from the increase in ATP after addition of the extracts (arrow) to assay mixtures containing 1 mM ADP or 1 mM ADP plus 1 mM NTP. Samples contained 9.4 mg of protein per ml. Qualitatively similar results were obtained with extracts from *Nostoc* sp. strain Mac (data not shown; see Table 1, footnote[a]).

TABLE 1. Activities of NDPK and NMPK in cell extracts of *A. nidulans*[a]

Enzyme	Substrate(s)	Activity (%)
NDPK	1 mM ADP	100
	1 mM ADP + 1 mM GTP	237
	1 mM ADP + 1 mM UTP	144
	1 mM ADP + 1 mM CTP	121
NMPK	0.2 mM ADP	100
	0.2 mM ADP + 1 mM GDP	217
	0.2 mM ADP + 1 mM UDP	189
	0.2 mM ADP + 1 mM CDP	119

[a] Initial rates of ATP formation were determined on two different preparations and expressed as the percentage of adenylate kinase activity (2ADP → ATP + AMP) representing the background activity of the extracts after the addition of ADP alone. For NDPK, 100% = 1.42 nmol/min per mg of protein; for NMPK, 100% = 0.05 nmol/min per mg of protein. Data for NDPK activity were calculated from Fig. 1. Qualitatively similar results were obtained with extracts from *Nostoc* sp. strain Mac, 100% activity in this case amounting to 2.88 nmol/min per mg of protein for NDPK and 0.15 nmol/min per mg of protein for NMPK. All data given are mean values from five independently prepared extracts, deviations from corresponding means ranging within ±20%. Samples contained between 6.9 and 9.4 mg of protein per ml.

reaction mixtures as for NDPK and NMPK but with additional nucleotides omitted. Reactions were started by the addition of 100 μl of cell extract. ATP formation was measured at various time intervals in 30-μl samples of the reaction mixture transferred to 1 ml of 20 mM Tris hydrochloride buffer (pH 7.8) containing 2 mM EDTA, which stopped the reaction through Mg^{2+} binding. No ATP formation was observed without the addition of cell extract or of ADP.

Determination of nucleotide concentrations. ATP was assayed by a luciferin-luciferase assay with a specially designed photometer (Biolumat LB 9500; Berthold, Wildbad, Federal Republic of Germany). Other nucleotides were measured after treatment of the samples with suitable enzymes: for ADP and AMP (16), 3 U of pyruvate kinase and 3 U of adenylate kinase per ml; for GTP, GDP, and GMP (13), 3 U of pyruvate kinase and 0.1 U of guanylate kinase per ml; for CTP, as described in reference 18. Intracellular nucleotides were extracted from steady-state cells after flushing of the suspensions with air or nitrogen for at least 45 min (see Table 2).

Nucleotides and enzymes necessary for nucleotide assays were obtained from Sigma Chemical Co., St. Louis, Mo. Lyophilized enzymes (pyruvate kinase [EC 2.7.1.40] from rabbit muscle; hexokinase [EC 2.7.1.1]–glucose-6-phosphate dehydrogenase [EC 1.1.1.49] [mixed enzymes] and 3-phosphoglycerate kinase [EC 2.7.2.3], each from bakers' yeast) were dissolved in 20 mM Tris hydrochloride (pH 7.8)–1% bovine serum albumin. Adenylate kinase (EC 2.7.4.3) from rabbit muscle was obtained in $(NH_4)_2SO_4$ solution and used as described previously (16). Guanylate kinase (EC 2.7.4.8) was obtained in 50% glycerol solution. Firefly extracts were prepared as described previously (18) except that 0.25 mM dithiothreitol was included.

P/O ratios. P/O ratios were determined in a translucent vessel of 20-ml volume which contained a Clark-type oxygen electrode (model 53; Yellow Springs Instrument Co., Yellow Springs, Ohio) and could be sealed with a screw cap equipped with a capillary inlet. The vessel was filled with 20 ml of cell suspension. The suspension was stirred in the dark while being bubbled with N_2 for 20 min to induce anaerobiosis, while the decrease in oxygen concentration

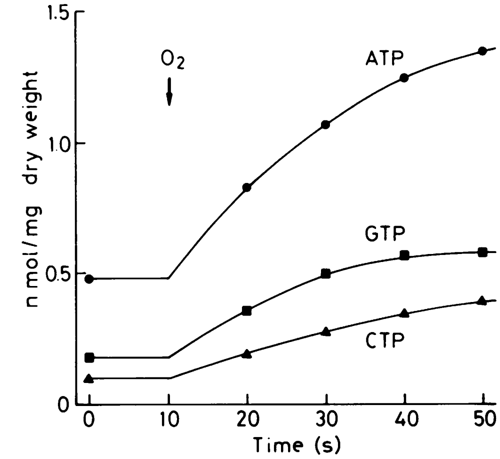

FIG. 2. Increase of intracellular NTP concentrations after aeration (arrow) of dark anaerobic *A. nidulans*. Qualitatively similar results were obtained with *Nostoc* sp. strain Mac (Tables 2 and 3) (19) and also with illuminated samples (dark-light transitions [data not shown; see Materials and Methods]).

was monitored with the oxygen electrode. For the assay of intracellular nucleotides, samples of the anaerobic suspension were withdrawn into a syringe containing the extraction medium (18; Nitschmann, Ph.D. thesis). If dark-light transitions were performed, 10 μM 3-(3′,4′-dichlorophenyl)-1,1′-dimethylurea (DCMU) was added before nitrogen flushing was started, and the cells were illuminated with 800-W/m^2 incandescent light as measured with a radiometer (model 65; Yellow Springs Instrument Co.), illumination replacing the oxygen pulse. Oxygen uptake by the cells and intracellular nucleotides (in the extracts) was measured after injection of 1 ml of O_2-saturated 30 mM HEPES-Tris buffer (pH 7.5) into the anaerobic cell suspension (pulse experiments; see Tables 3 and 4 and Fig. 2 and 3). All measurements were performed at 35°C.

Proton fluxes. Measurements of oxygen-induced proton extrusion from the cells were performed as described previously (6, 20).

RESULTS

In cell extracts of *A. nidulans*, ATP formation was observed after the addition of ADP (Fig. 1; Table 1), obviously caused by the endogenous adenylate kinase. However, the stimulation of ATP production from ADP in the presence of GTP, UTP, and CTP or GDP, UDP, and CDP (Fig. 1; Table 1) indicates the presence of NDPK and NMPK. The data show that the activities of both enzymes are comparable to that of adenylate kinase. Consequently, the onset of oxidative or photophosphorylation should qualitatively cause the same relative concentration changes in mono-, di-, and triphosphates of all types of nucleosides, namely, an increase of NTP and a decrease of NMP together with a constant NDP level. This was confirmed with intact cells of *A. nidulans* and *Nostoc* sp. upon transition from dark anaerobic to aerobic conditions (Fig. 2 and 3; Table 2). Dark aerobic and anaerobic pool sizes of all nucleotides currently accessible to the firefly assay by enzymatic treatment of biological samples were determined, i.e., ATP, ADP, AMP, GTP, GDP, GMP, and CTP. The results are shown in Table 2, which for comparison also presents some of the scant data previously reported for other bacteria.

Modest changes of the mass action ratios of NDPK, adenylate kinase, and guanylate kinase reactions during the transition demonstrate that the activities of these enzymes are high enough to maintain the equilibrium (*K* near 1) between different nucleotides; the following extreme values were calculated from Fig. 2: [ATP] · [GDP]/[ADP] · [GTP] = 0.95 to 1.89; $[ATP] \cdot [AMP]/[ADP]^2$ = 0.30 to 1.35; [ATP] · [GMP]/[ADP] · [GDP] = 0.36 to 1.31. NTP turnover (rate of NTP formation divided by the aerobic steady-state concentration) was calculated to be 1.50 min^{-1} for ATP, 1.85 min^{-1} for GTP, and 1.32 min^{-1} for CTP, thus being identical within the limits of error. The ATP turnover in several cyanobacteria and, for comparison, other bacteria is given in Table 3; rates of dicyclohexylcarbodiimide (DCCD)-

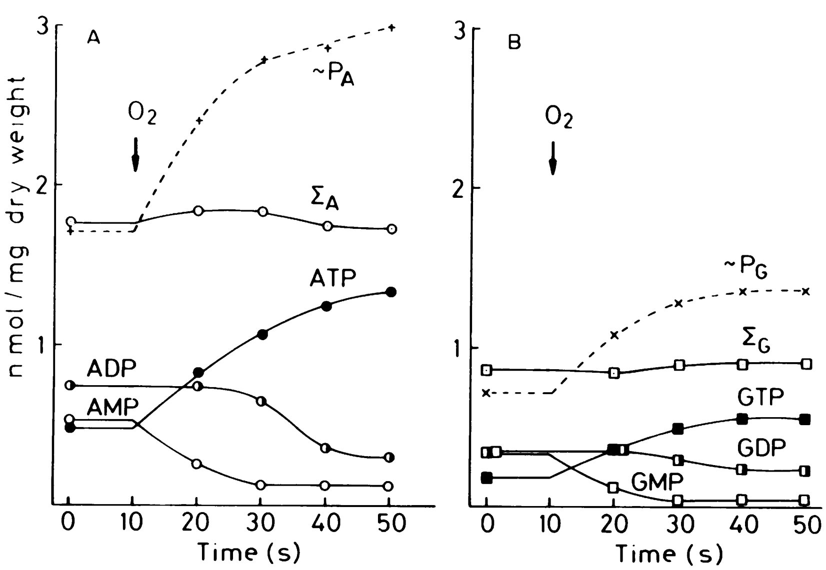

FIG. 3. Changes in adenine (A) and guanine (B) nucleotide concentrations in aerated *A. nidulans* in the dark. Σ_A = [ATP] + [ADP] + [AMP]; Σ_G = [GTP] + [GDP] + [GMP]; $\sim P_A$ = 2[ATP] + [ADP]; $\sim P_G$ = 2[GTP] + [GDP]. Qualitatively similar changes were seen with *Nostoc* sp. strain Mac (Table 2) and also with illuminated samples (dark-light transitions; data not shown).

TABLE 2. Intracellular concentrations of nucleotide phosphates in cyanobacteria and other bacteria[a]

Organism	ATP	ADP	AMP	GTP	GDP	GMP	CTP	CDP	UTP	UDP	References
Anacystis nidulans	1.35 (0.48)	0.30 (0.75)	0.09 (0.53)	0.57 (0.18)	0.24 (0.23)	0.07 (0.34)	0.40 (0.10)	ND[e]	ND	ND	This paper (Fig. 2 and 3)
Anacystis nidulans[b]	2.87 (2.11)	2.44 (2.11)	1.06 (1.19)	ND	ND	ND	ND	ND	ND	ND	3
Anacystis nidulans	1.0	0.61	0.11	ND	ND	ND	ND	ND	ND	ND	14
Nostoc sp. strain Mac	1.19 (0.34)	0.75 (0.91)	0.40 (0.93)	0.44 (0.20)	0.30 (0.33)	0.07 (0.23)	0.23 (0.05)	ND	ND	ND	This paper (Fig. 2 and 3)
Escherichia coli	3.7 (0.4)	1.2 (1.9)	0.9 (2.6)	ND	ND	ND	ND	ND	ND	ND	10
Escherichia coli[c,d]	6.9–10.7	1.0–1.6	ND	3.4–5.1	0.7	ND	1.9–2.3	ND	2.4–3.0	ND	1
Escherichia coli[c]	6.3	2.4	ND	3.3	1.9	ND	1.7	1.8	2.9	1.1	17
Klebsiella pneumoniae[c]	6.5 (3.7)	2.2 (3.9)	0.4 (1.1)	ND	ND	ND	ND	ND	ND	ND	9

[a] Values (nanomoles per milligram [dry weight]) refer to dark aerobic conditions. If available, dark anaerobic values are given in brackets. Assays were performed on resting cells under steady-state conditions unless otherwise stated.
[b] Values recalculated assuming a chlorophyll content of 2.6% of the dry weight and a cellular volume of 90 μl/ml of chlorophyll.
[c] Assays performed on growing cells.
[d] Values recalculated assuming a protein content of 50% of the dry weight.
[e] ND, Not determined.

sensitive (hence, ATPase-mediated [6, 20]) H^+ extrusion from oxygen-pulsed cells as necessary for additional correction of P/O ratios are also shown.

Table 4 permits a comparison among progressively corrected P/O ratios displayed by oxygen-pulsed *A. nidulans* and *Nostoc* sp. as calculated from changes of (i) adenine nucleotide concentrations alone (column 1), (ii) adenine and guanine nucleotide pools together (column 2), (iii) the pool

TABLE 3. Rate of ATP formation, ATP turnover, and proton extrusion in dark aerated (respiring) suspensions of cyanobacteria and other bacteria[a]

Organism	Rate of ATP formation (nmol of ATP/min per mg [dry wt])	Aerobic ATP pool size (nmol of ATP/mg [dry wt])	ATP turnover (min^{-1})	Proton ejection (nmol of H^+/min per mg [dry wt])[b]		References
				− DCCD	+ DCCD	
Anacystis sp. strain SAUG 1402-1 (*Synechococcus* sp. strain ATCC 27144)[c]	5.1	2.5	2.0	6.6	3.1	This paper[d]
Nostoc sp. strain PCC 8009[e]	2.1	2.2	1.0	4.1	4.2	This paper[d]
Anabaena variabilis ATCC 29413	8.3	5.3	1.6	11.6	3.6	26
	4.5	2.2	2.0	ND[f]	ND	Nitschmann, Ph.D. thesis
	ND	ND	ND	1.5	0.3	20
Anabaena variabilis ATCC 27892	ND	ND	ND	1.8	ND	27
Paracoccus denitrificans	ND	ND	ND	45–50	—	30
Escherichia coli	50	2.5	20	—	—	29
Proteus mirabilis	100	3.0	33	—	—	29
Pseudomonas aeruginosa	30	2.0	15	—	—	29

[a] Values were derived from oxygen pulse experiments (see Materials and Methods).
[b] DCCD, an inhibitor of the reversible H^+-ATPase, was added to the suspensions 20 min before the assay of proton extrusion at a concentration of 15 to 20 nmol/mg (dry weight) which had been shown to completely eliminate all oxidative phosphorylation (6, 20).
[c] SAUG, Sammlung von Algenkulturen der Universität Göttingen, Gottingen, Federal Republic of Germany.
[d] Mean values of five experiments. Standard deviations were within ±20%.
[e] PCC, Pasteur Culture Collection, Paris, France.
[f] ND, Not determined.

TABLE 4. Coupling efficiencies of oxidative phosphorylation in *A. nidulans* and *Nostoc* sp. as expressed in terms of ~P/O ratios and corrected for different ATP-consuming processes[a]

Organism	$\sim P_A/O$	$\sim P_{A+G}/O$	$\sim P_{\Sigma N}/O$	$(\sim P_{\Sigma N} + \sim P_{H^+\text{-ATPase}})/O$
Anacystis nidulans	0.5–1.0	1.0–1.6	1.5–2.1	2.6–3.3
Nostoc sp.	0.8–1.0	1.0–1.2	1.3–1.5	1.3–1.5

[a] ~P/O ratios were calculated from initial rates of ~P formation and oxygen consumption immediately after the oxygen pulse. Oxygen uptake rates were between 6 and 12 nmol of O per min per mg (dry weight). Proton extrusion was between 5.7 and 10.5 (*A. nidulans*) and 9.8 and 12.5 (*Nostoc* sp.) nmol of H^+/min per mg (dry weight). Extremes from five determinations are shown. Explanations (also see Discussion): $\sim P_A = 2[ATP] + [ADP]$; $\sim P_{A+G} = 2[ATP] + [ADP] + 2[GTP] + [GDP]$; $\sim P_{\Sigma N} = 2\Sigma[NTP] + \Sigma[NDP]$; $\sim P_{H^+\text{-ATPase}} = n\Delta H^+_{ATP}$, with $n = ATP/H^+$ (assumed to be 0.5) and ΔH^+_{ATP} = rate of DCCD-sensitive (i.e., ATP-dependent) H^+ extrusion.

sizes of all nucleoside phosphates NXP (column 3), and eventually (iv) by allowing also for the ATP utilized by the plasma membrane-bound ATPase for H^+ extrusion from the oxygen-pulsed cells (column 4). It is seen that, formally, P/O ratios rise, on an average, from 0.75 to 1.3, 1.8, and 2.95 for *A. nidulans* and from 0.9 to 1.1, 1.4, and 1.4 for *Nostoc* sp. by stepwise taking into account the immediate oxygen-induced increase in the energy content ($\Delta\sim P$) of AXP, AXP plus GXP, and all the nucleoside phosphates and finally also the ATP requirement of the H^+-pumping ATPase (if any), respectively (see Discussion).

DISCUSSION

The experiments described in the previous section clearly show that the induction of respiratory electron transport and, hence, oxidative phosphorylation in the cyanobacteria *A. nidulans* and *Nostoc* sp. strain Mac resulted in an immediate increase of intracellular concentrations of not only ATP but also GTP and CTP. Lower rates of increase in NTP concentrations are paralleled by correspondingly lower steady-state pool sizes, thus eventually yielding the same turnover ($minute^{-1}$) for each type of NTP. Even if a minor contribution from direct phosphorylation of GDP by the membrane-bound ATP synthase might occur (2, 7), the increase of CTP clearly demonstrates the action of NDPK.

Upon aeration or illumination of anaerobic cell suspensions, complementary changes of NTP and NMP levels but little variation of the NDP levels were found for the adenylate and guanylate pools, reflecting the combined action of oxidative phosphorylation (ADP + P_i = ATP) and the freely reversible transphosphorylation between ATP and other NDPs and NMPs as catalyzed by NDPK (NDP + ATP = NTP + ADP) and NMPK (NMP + ATP = NDP + ADP) or adenylate kinase (AMP + ATP = 2ADP). The activities of NDPK with UDP and CDP and of NMPK with UMP and CMP (Table 1) and the nearly identical turnover of ATP, GTP, and CTP (see Results) may be taken as evidence that in energized cells also the uridylate and cytidylate pools show kinetic patterns similar to those of the adenylate and guanylate pools. In other organisms NDPK was shown to be nonspecific, utilizing any deoxyribonucleoside triphosphate or diphosphate (12), whereas NMPK comprises distinct enzymes which are rather specific for ATP and the respective monophosphate (11, 31). Nothing is known about the specificity of these enzymes in cyanobacteria.

Taking into account the increase of ~P in both adenylate and guanylate pools raises the calculated P/O ratios in *A. nidulans* and *Nostoc* sp. by about 73 and 22%, respectively. A minimal estimate for the increase of ~P in the pyrimidine nucleotide pool (uridine and cytidine nucleotides) was obtained as follows. The ratios of steady-state levels of ATP, GTP, and CTP in cyanobacteria are obviously very similar to that in, e.g., *E. coli* and *Klebsiella pneumoniae* (Table 2), with UTP levels intermediate between GTP and CTP. Assuming a parallel increase of UTP and CTP, together with constant CDP and UDP concentrations during the transition

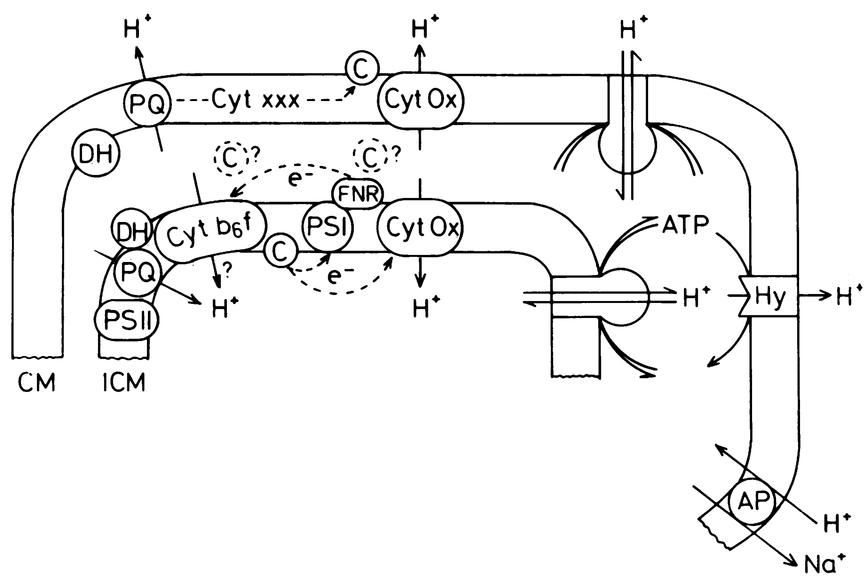

FIG. 4. Polarity and sidedness of electron transport, proton translocation, and ATP synthesis and hydrolysis in the energy-transducing membranes of cyanobacteria. CM, Cytoplasmic membrane; ICM, thylakoid membrane; DH, dehydrogenases; PSI and PSII, photosystems I and II; PQ, plastoquinone; Cyt, cytochrome; CytOx, cytochrome oxidase; c, cytochrome *c*; Cyt ×××, functionally uncharacterized cytochromes in the plasma membrane; FNR, ferredoxin:$NADP^+$ oxidoreductase; Hy, unidirectional ATP hydrolase; AP, antiporter; broken lines indicate the path of electrons (e^-).

from dark anaerobic to aerobic conditions, the increase of ~P was calculated according to Δ~P = 2ΔNTP + ΔNDP. Adding this estimated value to the Δ~P that incorporates only adenylates and guanylates, "true" P/O ratios corrected for the increase of ~P in the total nucleotide pool were obtained (Table 4, column 3). The foregoing discussion may apply also to other bacteria whenever the activities of NDPK and NMPK are as high as, or higher than, the rate of oxidative phosphorylation, thereby leading to underestimated P/O ratios (29).

Yet, in our cyanobacteria, although allowance was made for oxygen pulse-induced changes in the concentration of all nucleotides, the calculated coupling ratios remained lower than 3. Therefore, unless an inherently lower efficiency of oxidative phosphorylation is invoked for cyanobacteria (however, see reference 22), there must be still another energy-consuming process that affects the final P/O balance. The energy gap, with respect to P/O ratios, was identified in the form of active H^+ extrusion across the plasma membrane (19, 20) which thus adds a further lowering of the experimentally determined P/O ratios to the previously discussed cytosolic processes of NDPK and NMPK reactions (Table 4).

Under physiological conditions around pH 7 the transmembrane H^+ gradient at the plasma membrane of *A. nidulans* is built up, in part, by a DCCD-sensitive, reversible, H^+-translocating ATPase in the membrane (Fig. 4). From quantitative measurements of the net synthesis of ATP in dark anaerobic cells exposed to artificial gradients of the transplasma membrane proton electrochemical potential, a minimum requirement of $2H^+$/(ATP = ~P) was derived for the ATPase (25). Together with the known rate of DCCD-sensitive H^+ extrusion from oxygen-pulsed cells (Table 3) (6, 20), the rate of ATP hydrolyzed for active H^+ extrusion could be calculated and added to the total ~P increase in the nucleotide pools, resulting in a maximum P/O ratio of 2.6 to 3.3 for *A. nidulans* (Table 4, column 4).

Another part of respiratory proton extrusion, which is insensitive to ATPase inhibition (6, 20), can be accounted for directly by respiratory electron transport in the plasma membrane (23; Molitor et al., in press; also see reference 24 for a review). While in *A. nidulans* this mechanism is most pronounced below pH 5 only (6), it seems to prevail over the total external pH range tested in *Nostoc* sp. (Table 3, columns 4 and 5) (20).

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