Biochimica et Biophysica Acta, 682 (1982) 293–296
Elsevier Biomedical Press

BBA Report

BBA 40013

ACTIVE SODIUM EXTRUSION REDUCES NET EFFICIENCIES OF OXIDATIVE PHOSPHORYLATION IN THE STRICTLY PHOTOAUTOTROPHIC CYANOBACTERIUM *ANACYSTIS NIDULANS*

W.H. NITSCHMANN, G. SCHMETTERER, R. MUCHL and G.A. PESCHEK *

Biophysical Chemistry Group, Institute of Physical Chemistry, University of Vienna, Währingerstrasse 42, A-1090 Vienna (Austria)

(Received March 16th, 1982)
(Revised manuscript received July 2nd, 1982)

Key words: Oxygen evolution; Oxidative phosphorylation; Photosynthesis; Phototrophy; Na^+ efflux; (A. nidulans)

Efficiencies of oxidative phosphorylation (P/O ratios), intracellular high-energy phosphate pools (ATP and ADP) under aerobic and anaerobic dark conditions, and photosynthetic oxygen evolution measured with intact cells of *Anacystis nidulans* were found to be specifically depressed by NaCl, but not by KCl. A scheme is proposed which explains the deleterious effect of sodium on the energy metabolism of *A. nidulans* by competition for protons between ATP synthesis and active sodium extrusion.

Cyanobacteria are a major group of phototrophic prokaryotes uniquely capable of plant-type, oxygenic photosynthesis [1–3]. Along with photosynthetic oxygen production, cyanobacteria presumably were the first organisms to develop some type of aerobic respiration as well [3]. Unfortunately, very little is known about respiratory electron transport and oxidative phosphorylation in cyanobacteria [1,2]. Yet it would be particularly desirable for reasons of both evolutionary considerations and comparative biochemistry to have a better understanding of the mechanisms of aerobic energy conversion in the very organisms whose direct ancestors are believed to mark the turning point from a basically anaerobic to an aerobic biosphere. Nowadays, the growth of more than 50% of the cyanobacterial species investigated strictly depends on supply of light as the sole energy source, i.e., they are obligate phototrophs, unable to grow on organic substances in the dark [4]. Among a couple of hypotheses attempting to explain the reasons for obligate photo(auto)trophy [4–7] the possibility of inherently insufficient ATP generation by oxidative phosphorylation has received least attention.

Here we want to show that apparent efficiencies of oxidative phosphorylation, i.e., P/O ratios [8,9], in whole cells of the obligately phototrophic cyanobacterium *Anacystis nidulans* (SAUG 1402/1) are severely curtailed by elevated sodium levels present in the environment. Implications of the energy costs of active sodium extrusion for the phenomenon of obligate phototrophy will be discussed.

Fig. 1 shows formation rates of ~P and oxygen consumption during a transition from anaerobiosis to aerobiosis with intact *A. nidulans* cells containing different amounts of intracellular sodium. The formation rates of ~P and oxygen consumption rates varied by a factor of 2–3 with different batches of cells. However, the calculated P/O values were always as shown in Fig. 2, depending only on the intracellular Na^+ concentration. Furthermore, using the same batch of cells (Fig. 1b

* To whom correspondence should be addressed.
Abbreviation: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

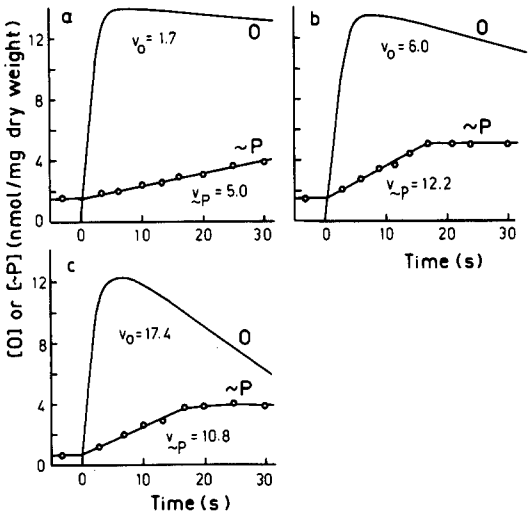

Fig. 1. Formation of ~P and oxygen uptake in whole cells of *Anacystic nidulans* on transition from anaerobiosis to aerobiosis. v_O and $v_{\sim P}$ are the rates expressed as nmol O or ~P/min per mg dry weight. (a) Na^+-depleted cells grown photoautotrophically in modified Medium D [28] as previously described [20,29] but with all Na^+ salts replaced by corresponding K^+ salts except for Na_2EDTA and Na_2MoO_4 (total Na^+ concentration 0.3 mM). Assay medium: 30 mM Hepes-KOH buffer, pH 7.5. Intracellular sodium concentration $[Na^+]_i < 1$ mM. (b) Cells grown in modified Medium D [29] (containing about 30 mM Na^+). Assay medium: 30 mM Hepes-KOH buffer, pH 7.5. $[Na^+]_i = 10-15$ mM. (c) Cells grown in modified Medium D as for b. Assay medium: 30 mM Hepes-NaOH buffer, pH 7.5. $[Na^+]_i = 25-30$ mM. Intracellular sodium was measured by flame photometry [10,12], assuming cell water to account for 70% of packed cell volume [30]. All experiments were performed at 35°C in strict darkness, suspensions containing 10–15 mg dry weight of cell/ml. Anaerobiosis was achieved by sparging cell suspensions with oxygen-free nitrogen and maintained for 20 min before adding oxygen-saturated assay medium (time zero). Oxygen concentration was measured polarographically [24,25]. For determination of ~P (=2[ATP]+[ADP] [31]) cells were extracted with trichloroacetic acid/EDTA [32]. ATP and ADP were assayed by the luciferin-luciferase method [33]. ATP synthesis was completely abolished by 50 μm of the uncoupler carbonyl cyanide *m*-chlorophenylhydrazone (not shown), thus excluding major contribution from substrate-level phosphorylation.

and c) the formation rates of ~P were essentially independent of the presence of Na^+, while Na^+ enhanced oxygen uptake drastically, suggesting a role for oxygen in active Na^+ extrusion. It is clearly seen (Fig. 2) that the highest efficiencies (P/O = 2.9) were displayed only by cells depleted of Na^+ and assayed in K^+ buffer. The low P/O

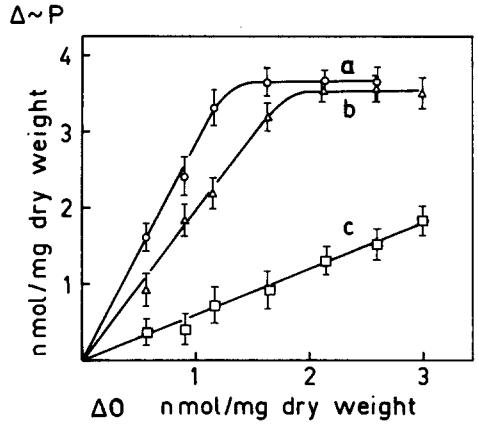

Fig. 2. Formation of ~P as function of oxygen formation. Curves a, b and c were calculated from Fig. 1a, b and c, respectively [9,26,34]. The slopes indicate the P/O ratios: (a) 2.9, (b) 2.0, (c) 0.6. ΔO: Amount of oxygen taken up by the cells after different periods of time following aeration. As the O_2 electrode had response time of 5–10 s, oxygen consumption during the first seconds was calculated from v_O by assuming respiratory O_2 uptake to be constant during the whole experiment (see Fig. 1a–c). ΔP: Increase of ~P from anaerobic level within the same time as corresponding ΔO.

ratios of Na^+-rich cells were thus caused by the striking postanaerobic enhancement of oxygen uptake rather than reduced rates of synthesis of energy-rich adenylates.

The specific effect of Na^+ on energy metabolism is also demonstrated by the results shown in Figs. 3 and 4. Addition of NaCl, but not KCl,

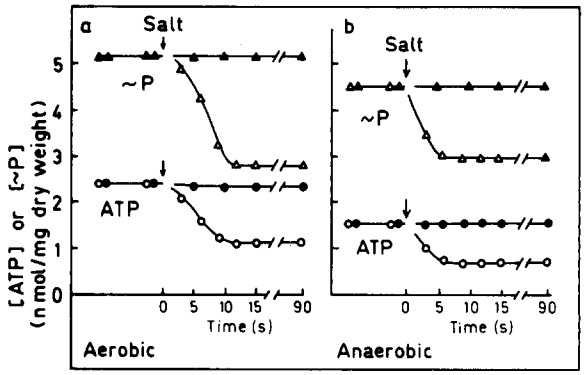

Fig. 3. Intracellular levels of ATP and ~P (=2[ATP]+[ADP]) in the dark as influenced by addition of 50 mM NaCl (open symbols) or KCl (filled symbols) at zero time to aerobic or anaerobic cells grown as described in Fig. 1a and suspended in 30 mM Hepes-Tris buffer, pH 7.5.

lowered the levels of ~P and ATP in both aerobic and anaerobic cells in the dark (Fig. 3). The effect is somewhat higher aerobically, presumably because more energy is available when respiration is possible. Indeed, more Na^+ is transported out of the cells aerobically than anaerobically as shown by the cells containing higher Na^+ concentrations in the latter case [10]. Also, illuminated cells under energy-limited conditions obviously suffered from the energetic burden imposed on them by Na^+ as seen from the lowering of oxygen evolution (Fig. 4).

Stimulation of respiratory oxygen uptake following addition of NaCl but not KCl [11] had previously been explained by energy-dependent extrusion of sodium through an Na^+/H^+-antiporting system in the cytoplasmic membrane of *A. nidulans* ultimately powered by ATP ('salt respiration', cf. Ref. 12). Another explanation is suggested, however, by our own observations. In cells passing from anaerobiosis to aerobiosis in the dark (Fig. 1b and c) oxygen uptake is enhanced by Na^+ while formation of ~P remains unaffected. Moreover, Na^+ causes a depression of O_2 evolution in the light. While it may be assumed that photosynthetic oxygen evolution is the same under all conditions in Fig. 4, aerobically part of the evolved O_2 and anaerobically all of the O_2 is used in respiration. According to our hypothesis, therefore, a cytoplasmic membrane-bound proton-translocating terminal oxidase participates directly

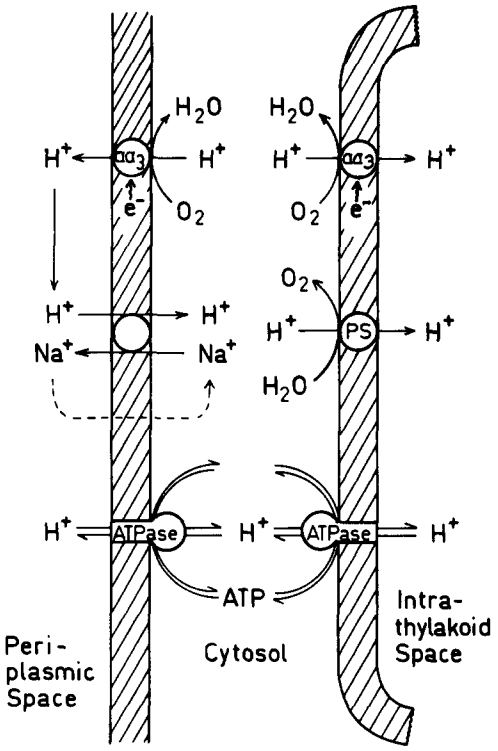

Fig. 5. Proposed scheme of membrane functions in *A. nidulans*. PS, photosynthetic electron transport; aa_3, proton-pumping cytochrome oxidase; e^- ↿, electron donation to cytochrome oxidase through hitherto unknown intermediates; ----→, passive influx of sodium. The separated representation of respiratory and photosynthetic electron transport on the thylakoid membrane was chosen for clarity and does not necessarily imply that they share no common components.

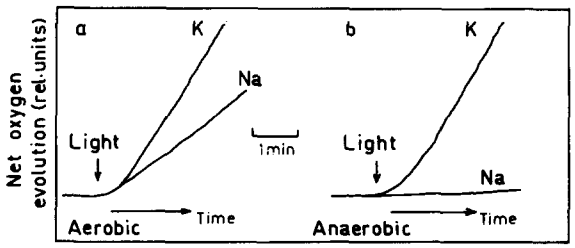

Fig. 4. Photosynthetic oxygen evolution (CO_2 as substrate) by cells suspended in aerobic (0.12 mM O_2) or anaerobic K^+- or Na^+-Hepes buffer (30 mM, pH 7.5). CO_2 fixation as measured radiochemically with $NaH^{14}CO_3$ [29] was found to parallel exactly O_2 evolution under the respective conditions (not shown). Arrows indicate onset of illumination with 5 $W \cdot m^{-2}$ white light as measured with a YSI radiometer model 65. The inhibiting effect of sodium was not seen if saturating light of 100 $W \cdot m^{-2}$ was used (not shown).

in the buildup of a proton gradient across the cytoplasmic membrane, the gradient being used primarily for active sodium extrusion (Fig. 5). With *A. nidulans*, cytochrome *c* oxidase (cytochrome aa_3) characterized in this and other cyanobacterial species [13–17] could recently be shown to be located not only in the thylakoid but also in the cytoplasmic membrane [18–21], and to be capable of proton translocation [22] similar to mammalian cytochrome oxidase [23].

Thus, under conditions when much energy must be expended for Na^+ extrusion (e.g., in the common media used for laboratory growth of the organisms), and under the premise of constitutively low rates of respiratory electron transport

(and, hence, of oxidative phosphorylation) as compared to photosynthetic electron transport (photophosphorylation), the situation discussed above might necessarily result in 'obligate' phototrophy. Be it noted that just the condition of inherently low respiration rates is well proven for cyanobacteria [7,24,25]. Preliminary experiments of the type described here for *A. nidulans* also were conducted with *Anabaena variabilis* ATCC 29413 and *Nostoc* sp. strain MAC [26]. The results were qualitatively similar to those shown for *A. nidulans* though, interestingly, the deleterious effect of sodium was far less pronounced in these facultatively chemoheterotrophic species. This might best be explained by intrinsically different permeabilities for Na^+ of the cytoplasmic membrane in the different species. However, in this context it is tempting to mention that typically halophilic (or at least halotolerant) cyanobacteria such as *Oscillatoria limnetica* and *Aphanocapsa halophytica* are obligate phototrophs [27].

This work was supported by financial aid from the Fonds zur Förderung der wissenschaftlichen Forschung in Österreich. One of us (G.A.P.) thanks the Kulturamt der Stadt Wien for a grant. The interest of Professor E. Broda and the skilful technical assistance of Mr. O. Kuntner are gratefully acknowledged.

References

1 Carr, N.G. and Whitton, B.A. (1982) The Biology of the Cyanobacteria, 2nd edn., Blackwell Scientific Publications, Oxford, in the press
2 Broda, E. (1973) The Evolution of the Bioenergetic Processes, Pergamon Press, Oxford
3 Broda, E. and Peschek, G.A. 91979) J. Theor. Biol. 81, 201–212
4 Stanier, R.Y. and Cohen-Bazire, G. (1977) Annu. Rev. Microbiol. 31, 225–274
5 Carr, N.G. (1973) in The Biology of the Blue-Green Algae (Carr, N.G. and Whitton, B.A., eds.), pp. 39–65, Blackwell Scientific Publications, Oxford
6 Singer, R.A. and Doolittle, W.F. (1975) Nature 253, 650–651
7 Smith, A.J., London, J. and Stanier, R.Y. (1967) J. Bacteriol. 94, 972–983
8 Stouthamer, A.H. (1978) in The Bacteria (Gunsalus, I.C., ed.), Vol. 6, pp. 389–462, Academic Press, New York
9 Van der Beek, E.G. and Stouthamer, A.H. (1973) Arch. Mikrobiol. 89, 327–339
10 Nitschmann, W.H. and Peschek, G.A. (1982) FEBS Lett. 139, 77–80
11 Paschinger, H. (1977) Z. Allg. Mikrobiol. 17, 373–379
12 Paschinger, H. (1977) Arch. Microbiol. 113, 285–291
13 Peschek, G.A. (1981) Biochim. Biophys. Acta 635, 470–475
14 Peschek, G.A. (1981) Biochem. Biophys. Res. Commun. 98, 72–79
15 Peschek, G.A., Kienzl, P.F. and Schmetterer, G. (1981) FEBS Lett. 131, 11–16
16 Kienzl, P.F. and Peschek, G.A. (1982) Plant Physiol. 69, 580–584
17 Peschek, G.A., Schmetterer, G., Lauritsch, G., Nitschmann, W.H., Kienzl, P.F. and Muchl, R. (1982) Arch. Microbiol. 131, 261–265
18 Peschek, G.A. and Schmetterer, G. (1978) FEMS Microbiol. Lett. 3, 295–297
19 Peschek, G.A., Schmetterer, G. and Sleytr, U.B. (1981) FEMS Microbiol. Lett. 11, 121–124
20 Peschek, G.A., Muchl, R. and Schmetterer, G. (1981) Curr. Microbiol. 6, 233–237
21 Peschek, G.A., Kienzl, P.F., Muchl, R. and Schmetterer, G. (1982) Biochim. Biophys. Acta 679, 35–43
22 Peschek, G.A., Schmetterer, G., Lauritsch, G., Much, R., Kienzl, P.F. and Nitschmann, W.H. (1982) in Photosynthetic Prokaryotes: Cell Differentiation and Function (Papageorgiou, G.C. and Packer, L., eds.), Elsevier Science Publishers Co. Inc., New York, in the press
23 Wikström, M. and Krab, K. (1979) Biochim. Biophys. Acta 549, 177–222
24 Peschek, G.A. (1980) Arch. Microbiol. 125, 123–131
25 Peschek, G.A. (1979) Biochim. Biophys. Acta 548, 203–215
26 Nitschmann, W.H. (1982) Thesis, University of Vienna
27 Garlick, S., Oren, A. and Padan, E. (1977) J. Bacteriol. 129, 623–629
28 Kratz, W.A. and Myers, J. (1955) Am. J. Bot. 42, 282–287
29 Peschek, G.A. (1978) Arch. Microbiol. 119, 313–322
30 Dewar, M.A. and Barber, J. (1973) Planta 113, 143–155
31 Hempfling, W.P. (1970) Biochim. Biophys. Acta 205, 169–182
32 Larsson, C.-M. and Olsson, T. (1979) Plant Cell Physiol. 20, 145–155
33 Lundin, A. and Thore, A. (1975) Appl. Microbiol. 30, 713–721
34 Baak, J.M. and Postma, P.W. (1971) FEBS Lett. 19, 189–192