The cytochrome *f*–*b* electron-transport complex

A common link between photosynthesis and respiration in the cyanobacterium *Anacystis nidulans*

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Reduced-minus-oxidized difference spectra were recorded on particle preparations of the cyanobacterium *Anacystis nidulans*. Physiological oxidation of anaerobic membranes was effected either by O₂ or by light. In both cases the spectral changes observed in the 550–570 nm region were essentially the same. The results were confirmed by dual-wavelength spectrophotometry. It is concluded that a membrane-bound cytochrome *f*–*b* complex participates in both respiratory and photosynthetic electron transport.

The intimate relationship between photosynthesis and respiration in phototrophic prokaryotes has long been recognized (cf. Stanier & Cohen-Bazire, 1977; Clayton & Sistrom, 1978). In certain anoxygenic photosynthetic bacteria (Rhodospirillales) the ubiquinol–cytochrome *c*₂ electron-transport sequence, possibly including a membrane-bound cytochrome *b*–*c*₁ complex (Wood, 1980*a,b*; Gabelini *et al.*, 1982), serves both photosynthesis and respiration (cf. Baccarini-Melandri & Zannoni, 1978). Much less is known about the other large group of phototrophic prokaryotes, the cyanobacteria, whose outstanding property is plant-type oxygenic photosynthesis (Broda & Peschek, 1979). In particular, the respiratory electron transport of cyanobacteria still is far from being adequately resolved. Location of both respiratory and photosynthetic electron-transport assemblies on the intracytoplasmic (thylakoid) membranes (Bisalputra *et al.*, 1969; Peschek *et al.*, 1981) and the existence of common intermediates (Hoch *et al.*, 1963; Jones & Myers, 1963; Eibrenner & Bothe, 1979; Hirano *et al.*, 1980; Lockau, 1981) have been suggested, mostly on the grounds of indirect evidence. In the present paper it will be shown that the oxidation of anaerobic membrane particles from *Anacystis nidulans* gave similar spectral changes in the α -region of cytochromes *f* and *b* no matter whether the reaction was triggered by light or by O₂.

Materials and methods

Growth of *Anacystis nidulans* and isolation of

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membranes were described previously (Peschek, 1979). The isolated membranes were devoid of water-splitting activity, but did catalyse high rates of light-dependent electron transport through both photosystems using, e.g., diphenylcarbazide as an electron donor to Photosystem II (Peschek *et al.*, 1982*b*); also the respiratory activities of the membranes were unimpaired (Peschek *et al.*, 1982*b*). For measurements, the particles were suspended in a medium containing 0.6 M-mannitol, 10 mM-KCl, 3 mM-MgCl₂ and 30 mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid]/phosphate buffer, pH 7.5. Difference spectroscopy and dual-wavelength measurements were performed at 35°C with an Aminco–Chance UV-Vis dual-wavelength spectrophotometer equipped for cross-illumination. Actinic light was provided from a Leitz slide projector and passed through a 707 nm interference filter of 12 nm half-bandwidth (Schott AG, Mainz, Germany). Light intensity was 18 W/m² at the surface of the cuvettes as measured with a YSI radiometer, model 65. All samples contained 10 μ M-dichlorophenyldimethylurea to exclude any residual photosynthetic O₂ evolution, and 3 mM-NADPH (or NADH, which gave the same effect) as a physiological reductant (Peschek, 1981). Dual-wavelength measurements were done with (initially) anaerobic membrane suspensions; the optical band-pass of the spectrophotometer was 1 nm throughout. Anaerobiosis was achieved by sparging with O₂-free N₂, finally layering 0.5 ml of paraffin oil on top of the suspensions. Aerobiosis was induced by injecting 0.1 ml of O₂-saturated assay buffer, giving 20–25% air saturation in the cuvettes, which always con-

tained 2.0 ml (final volume). Chlorophyll was determined as described by Mackinney (1941).

Results and discussion

Difference spectra obtained by selective chemical reduction and oxidation of the membranes revealed the α -bands of reduced cytochromes *c*-556 (cytochrome *f*), *b*-563 and *b*-559 (Fig. 1; cf. Kienzl & Peschek, 1982). Addition of 2% (v/v) Triton X-100 to the membranes did not significantly change the appearance of the spectra (not shown); thus in contrast with the situation with membranes from the thermophilic cyanobacterium *Phormidium laminosum* (Stewart & Bendall, 1980) the contribution of high-potential cytochrome *b*-559 to the spectra displayed by the *Anacystis* membranes appeared to be negligible. Physiological difference spectra using NAD(P)H and O₂ (darkness) or light as reductants and oxidants respectively are shown in Fig. 2. It is seen that both cytochrome *f* and *b* gave a remarkably similar response, regardless whether the oxidation was brought about by light (photosynthesis; Fig. 2a) or by O₂ in the dark (respiration; Fig. 2b), though discrimination between cytochromes *b*-563 and *b*-559 was no longer possible owing to the rather broad band. However, by analogy with chloroplasts (Böhme, 1977, 1979; Wood, 1977), it can be assumed that cytochrome *b*-563, together with cytochrome *f*, is the physiologically active species. Thus it is derived from Fig. 2 that cytochromes *f* and *b*-563, which may be present as a cytochrome *f*-*b* complex in the *Anacystis* membranes (cf. Hurt & Hauska, 1981; Krinner *et al.*, 1982), could be physiologically oxidized and reduced under conditions of both respiration and photosynthesis.

Dual-wavelength measurements at 563–577 nm (Fig. 3) and 556–577 nm (Fig. 4) during transition of the membranes from anaerobic dark to light conditions (Figs. 3a, 3b and 4a) or from dark anaerobic to aerobic conditions (Figs. 3c, 3d and 4b) were consistent with this interpretation. Photo-oxidation of the cytochromes apparently reflected endogenous cyclic electron flow with a rate-limiting step on the low-potential side of the cytochromes. The plastoquinone antagonist dibromothymoquinone shifted the system to a more oxidized level by interrupting electron supply from the reduced plastoquinone pool (Fig. 3b). Methyl Viologen, a dye known to withdraw electrons from the donor side of Photosystem I, elicited a still more pronounced photo-oxidation of both cytochromes *b*-563 (Fig. 3a and 3b) and *f* (Fig. 4a). Switching off the light in both cases permitted the system to return to its original reduced state, showing that the spectral changes observed were fully reversible.

Under conditions of dark respiration a similar, though slower, oxidation of cytochromes *b*-563 and *f*

Fig. 1. Reduced-minus-oxidized difference spectra of *Anacystis nidulans* particle preparations as effected by chemical reductants and oxidants

(a) Hydroquinone-reduced minus ferricyanide-oxidized; (cytochrome *f*); (b) ascorbate-reduced minus hydroquinone-reduced (cytochrome *b*-559); (c) dithionite-reduced minus ascorbate-reduced (cytochrome *b*-563). Samples contained 50 μ g of chlorophyll/ml.

Fig. 2. Reduced-minus-oxidized difference spectra of *Anacystis nidulans* particle preparations as effected by physiological reductants or oxidants

(a) Dark anaerobic minus light (707 nm); (b) dark anaerobic minus dark aerated. For details, see the Materials and methods section. Samples contained 50 μ g of chlorophyll/ml.

was measured (Figs. 3c, 3d and 4b). This oxidation was mediated by the terminal oxidase (probably an *aa*₃-type cytochrome; cf. Peschek, 1981; Peschek *et al.*, 1982a), since addition of KCN permitted immediate re-reduction of cytochromes *b*-563 and *f* through electrons from NAD(P)H. When the oxi-

dation of reduced plastoquinone was prevented by dibromothymoquinone, the dark aerobic oxidation of cytochrome *b*-563 proceeded to an even higher level, with KCN thereafter provoking only little re-reduction (Fig. 3d). Note that the effect of dibromothymoquinone thus was similar in photosynthesizing and respiring samples (cf. Eisbrenner & Bothe, 1979; Hirano *et al.*, 1980; Peschek, 1980).

The spectrophotometric data shown in Figs. 2–4

Fig. 3. Redox activities of cytochrome *b*-563 in *Anacystis nidulans* particle preparations recorded by dual-wave-length spectrophotometry at 563–577 nm (◇) 707 nm light on; (◆) light off. The addition of oxidants and inhibitors is indicated by smaller arrows (†). Abbreviations used: MV, Methyl Viologen (5 mM); DBMIB, dibromothymoquinone (5 μM). Samples contained 75 μg of chlorophyll/ml.

Fig. 4. Redox activities of cytochrome *f* in *Anacystis nidulans* particle preparations recorded by dual-wave-length spectrophotometry at 556–577 nm (◇) 707 nm light on; (◆) light off. The addition of oxidants is indicated by smaller arrows (†). Abbreviation used: MV, Methyl Viologen (5 mM). The effect of dibromothymoquinone (not shown) was similar to that in Fig. 3. Samples contained 90 μg of chlorophyll/ml.

Scheme 1. Joint respiratory and photosynthetic electron flow in thylakoid membranes of cyanobacteria. Abbreviations used: PS, photosystem; DCMU, dichlorophenyldimethylurea; PQ, plastoquinone; cyt, cytochrome; MV, Methyl Viologen; DBMIB, dibromothymoquinone; fd, ferredoxin; P700, pigment P700.

can be accommodated for by Scheme 1, which shows joint respiratory and photosynthetic electron flow in thylakoid membranes of cyanobacteria.

Recently a cytochrome *f*-*b* complex was isolated from the cyanobacterium *Anabaena variabilis* (Krinner *et al.*, 1982), and the present Scheme 1 may be checked in reconstitution experiments using the purified complex. It is to be noted, however, that these findings neither exclude nor imply the cytoplasmic membrane as an additional site of electron transport, as was previously envisaged (Raboy & Padan, 1978; Reed *et al.*, 1981; Scherer *et al.*, 1981; Peschek *et al.*, 1981, 1982b).

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