BBA 3707

STUDIES ON THE ELECTRON-TRANSFER SYSTEMS IN PHOTOSYNTHETIC BACTERIA

II. THE EFFECT OF HEPTYLHYDROXYQUINOLINE-*N*-OXIDE AND ANTIMYCIN A ON THE PHOTOSYNTHETIC AND RESPIRATORY ELECTRON-TRANSFER SYSTEMS

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SUMMARY

Heptylhydroxyquinoline-*N*-oxide and antimycin A were used to study the sequence of oxidation–reduction catalysts in the photosynthetic and respiratory electron-transfer systems of photosynthetic purple bacteria, *Rhodopseudomonas spheroides* and *Rhodospirillum rubrum*. These inhibitors caused steady-state changes of cytochromes in aerobic-cell suspensions: more reduced cytochrome *b* and more oxidized cytochrome *c*. The reduction of cytochrome *b* (by anaerobiosis) was not inhibited by these reagents but that of cytochrome *c* was blocked. It was found, too, that these reagents affected the steady-state level of carotenoids in *R. spheroides* in an anaerobic suspension.

Illumination of aerobic cells caused the reduction of cytochrome *b* and oxidation of cytochrome *c* in the presence of heptylhydroxyquinoline-*N*-oxide. It was concluded that these inhibitors block the oxidation–reduction reaction between cytochromes *b* and *c*, and that *b*-type cytochrome occurs nearer to the photochemical reducing site and to dehydrogenases, while *c*-type is closer to the photochemical and respiratory oxidizing sites.

INTRODUCTION

Antimycin A and HOQNO have been used as inhibitors of electron transfer systems in mitochondria isolated from animals, plants and bacteria[1–4]. It is well known that in the photosynthetic phosphorylation of the chromatophores isolated from purple bacteria, HOQNO and antimycin A are powerful inhibitors[5–7]. Photophosphorylation studies with flashing illumination have shown that these inhibitors block the site in the electron transport chain which limits the over-all rate of phosphorylation when the light intensity is at the saturating level[8].

Abbreviation: HOQNO, 2-*n*-heptyl-4-hydroxyquinoline-*N*-oxide.

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Our present paper deals with the steady-state changes of cytochromes induced by these inhibitors in purple bacteria. The effect of these inhibitors on carotenoid pigments was studied, too. The light-induced absorption change in the presence of these inhibitors showed an interesting blocking phenomenon between cytochromes of *c*- and *b*-types.

METHODS

Culture of bacteria

Photosynthetic non-sulfur purple bacteria, *Rhodopseudomonas spheroides* and *Rhodospirillum rubrum*, were cultured anaerobically under illumination as described before.

Spectrophotometric observations

A split-beam spectrophotometer designed in our laboratory[10,11], which has been adapted for cross-illumination of samples, was used for spectrophotometric measurements. Difference spectra at liquid nitrogen temperature were measured with a low-temperature adaptor for the split-beam spectrophotometer[12]. Other experimental procedures are described in a preceding paper[9].

RESULTS

Effect of HOQNO and antimycin A on the absorption spectrum of bacterial suspension in dark

R. spheroides: If HOQNO was added to the aerobic cells, there occurred a shift in the steady state of cytochromes (Fig. 1). This low-temperature difference spectrum

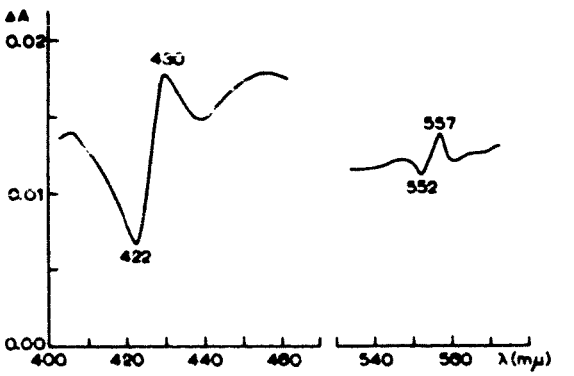

Fig. 1. Difference spectrum of *R. spheroides*, aerobic HOQNO-treated cells minus aerobic cells. HOQNO, $1 \cdot 10^{-4}$ *M*; optical path length, 1 mm; 2.70 mg dry weight/ml; temperature, 77° K.

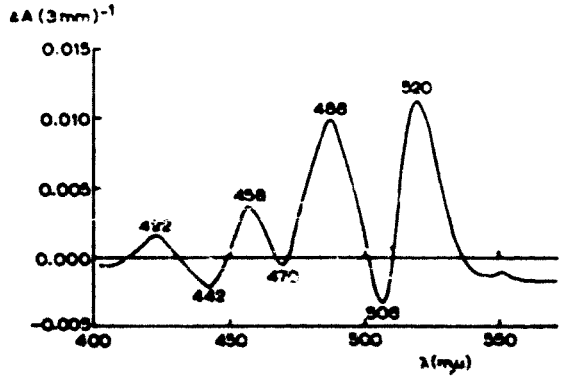

Fig. 2. Absorption spectrum change induced by antimycin A in *R. spheroides* anaerobic cells. Difference spectrum of anaerobic cells minus anaerobic antimycin A-treated cells. [Antimycin A, $3 \cdot 10^{-5}$ *M*; 2.11 mg dry weight/ml; optical path length, 3 mm; temperature, 297° K.

of two "frozen" steady states shows absorbancy increases at 430 mμ and 557 mμ and absorption decreases at 422 mμ and 552 mμ, indicating an increased reduction of a cytochrome of *b*-type and an increased oxidation of a cytochrome of *c*-type. A similar change was also observed in *R. rubrum* aerobic cells when HOQNO was added. It is to be noted that this steady-state change is similar to the response of HOQNO-treated *R. rubrum* aerobic cells to illumination.

After addition of antimycin A ($3 \cdot 10^{-5}$ *M*) or HOQNO ($1 \cdot 10^{-4}$ *M*) to the anaerobic *R. spheroides* cells, carotenoids showed a striking change (Fig. 2). It is like a reversal of the change observed in anaerobic *R. spheroides* cells caused by illumination or oxygenation, suggesting an increase of shorter-wavelength carotenoids[9, 13–15]. At the same time the reduction of *c*-type cytochrome was inhibited and this component remained in a more oxidized state than in untreated cells.

R. rubrum: Reduction of *c*-type cytochrome by anaerobiosis was inhibited by HOQNO as revealed by difference spectra, HOQNO-treated minus anaerobic cells at room and liquid-nitrogen temperatures (Fig. 3). At room temperature, absorption decreases were observed at 422 mμ and 551 mμ; at the lower temperature, the troughs were shifted towards shorter wavelengths and were located at 420 and 548 mμ, with appreciable sharpening of the bands. The band shifts to shorter wavelengths

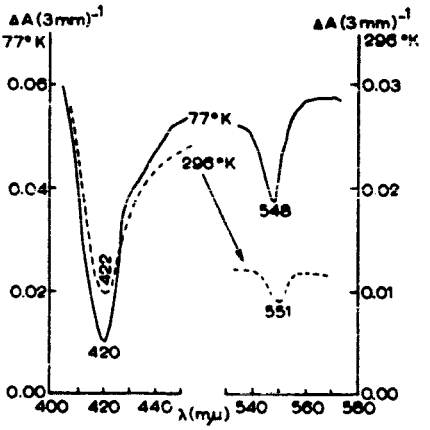

Fig. 3. Steady state change of *c*-type cytochrome in the presence of HOQNO in anaerobic *R. rubrum* cells. [HOQNO], $1 \cdot 10^{-4}$ *M*; optical path length, 3 mm; temperature, 77° K and 296° K.

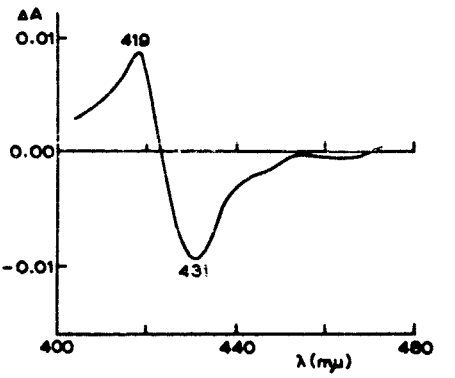

Fig. 4

Fig. 4. Difference spectrum of *R. rubrum*, aerobic minus aerobic HOQNO-treated cells. 2.31 mg dry weight/ml; [HOQNO], $6 \cdot 10^{-5}$ *M*; optical path length, 10 mm; temperature, 297° K.

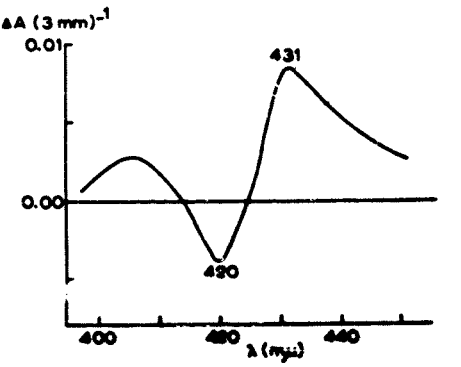

Fig. 5

Fig. 5. Absorption spectrum change by illumination in HOQNO-treated aerobic *R. rubrum* cells (illuminated minus dark difference spectrum). [HOQNO], $1 \cdot 10^{-4}$ *M*; optical path length, 3 mm; temperature, 296° K.

and the sharpening of absorption bands at low temperatures have been observed in heme proteins[13, 16–18]. This result indicates that the reduction of *c*-type cytochrome by anaerobiosis is inhibited by HOQNO.

In the aerobic *R. rubrum* cells, the steady-state changes of cytochromes following the addition of HOQNO were similar to the changes observed in the aerobic *R. spheroides* cells (Fig. 4). This difference spectrum shows that, in the presence of HOQNO, absorption decreased at 419 mμ and increased at 431 mμ, indicating that the steady state of *b*-type cytochrome changed to a more reduced level and cytochrome *c* showed a shift to a more oxidized level, as is the case in *R. spheroides*.

Effect of HOQNO on the light-induced absorption spectrum change

When HOQNO was added to the aerobic suspension of *R. rubrum*, illumination caused an increased absorption at 431 mμ and a decrease at 420 mμ (Fig. 5). This can be interpreted as a reduction of *b*-type cytochrome and an oxidation of *c*-type cytochrome by illumination when HOQNO is blocking the reaction of these two heme components. Cytochrome of *c*-type is largely reduced and *b*-type cytochrome is mainly oxidized in aerobic *R. rubrum* or *R. spheroides* cells in the dark[18,19]. In the presence of HOQNO (in the aerobic cells), *c*-type cytochrome becomes more oxidized and *b*-type more reduced. But there is still enough reduced cytochrome *c* and oxidized cytochrome *b* to permit observation of the light-induced change shown in Fig. 5 in aerobic HOQNO-treated cells.

DISCUSSION

It has been found that HOQNO and antimycin A are effective inhibitors of light-induced phosphorylation of the chromatophores isolated from photosynthetic bacteria[5–7]. Their effects upon the absorption spectrum of cell suspensions were studied in this paper. The inhibition of cytochrome *c* reduction (by anaerobiosis) by the additon of HOQNO or antimycin A to anaerobic suspensions indicates that the electron transfer system is blocked at the dehydrogenase side of cytochrome *c*. It was also shown that cytochrome *c* becomes more oxidized and cytochrome *b* more reduced when HOQNO is added to aerobic suspensions. It was noted that the illumination of an aerobic suspension in the presence of HOQNO induces reduction of *b*-type cytochrome and oxidation of *c*-type cytochrome. This is an interesting phenomenon since it would represent one of the crossover points for the light-induced response observed in intact cells, usually the light response affects the components similarly: they are all oxidized, as in the case of the typical anaerobic response[19]; or if the cells are treated with phenylmercuric acetate, one component, cytochrome *b*, is reduced[9]. Our present results suggest that the cytochromes *c* and *b* are located on the photosynthetic and respiratory electron transport chain and that the *c*-type cytochrome is closer to the photochemical oxidizing site and to the terminal oxidase, while the *b*-type cytochrome is nearer to the photochemical reducing site and to the dehydrogenases. It is indicated, too, that the site of action of these inhibitors is at, or close to, the site of the oxidation–reduction reaction of these two heme protein components.

Our recent data on photophosphorylation under flashing and continuous illumination suggest that electron transport at the site of action of these inhibitors is rate-limiting in the dark process of photosynthetic phosphorylation[8].

To observe the effect of HOQNO or antimycin A on the absorption spectrum change in the dense suspensions of intact cells, higher concentrations of these reagents were necessary than for the inhibition of photosynthetic phosphorylation of the isolated chromatophores. It was, however, noticed in the study of the effects of these reagents on photophosphorylation, that the molar ratio of the reagents to bacteriochlorophyll (or protein) was more important in determining the degree of inhibition than the apparent concentration of these inhibitors[18]. It is not unlikely that in the intact-cell experiments, the local concentrations of these inhibitors in the electron transport system are in a comparable range to those in the phosphorylation experiments with isolated chromatophores, though much larger amounts of the inhibitors are used in the former case.

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REFERENCES

1 V. R. POTTER AND A. E. REIF, *J. Biol. Chem.*, 194 (1952) 287.
2 H. BALTSCHEFFSKY AND M. BALTSCHEFFSKY, *Biochim. Biophys. Acta*, 29 (1958) 220.
3 J. W. LIGHTBOWN AND F. L. JACKSON, *Biochem. J.*, 63 (1956) 130.
4 W. D. BONNER, in J. E. FALK, R. LEMBERG AND R. K. MORTON, *Haematin Enzymes*, Pergamon Press, London, 1961, p. 479.
5 H. BALTSCHEFFSKY AND M. BALTSCHEFFSKY, *Acta Chem. Scand.*, 12 (1958) 1333.
6 L. SMITH AND M. BALTSCHEFFSKY, *J. Biol. Chem.*, 234 (1959) 1575.
7 D. M. GELLER AND F. LIPMANN, *J. Biol. Chem.*, 235 (1960) 2478.
8 M. NISHIMURA, *Biochim. Biophys. Acta*, 57 (1962) 88, 96.
9 M. NISHIMURA AND B. CHANCE, *Biochim. Biophys. Acta*, 66 (1963) 1.
10 C.-C. YANG AND V. LEGALLAIS, *Rev. Sci. Instr.*, 25 (1954) 801.
11 C.-C. YANG, *Rev. Sci. Instr.*, 25 (1954) 807.
12 R. W. ESTABROOK, *J. Biol. Chem.*, 223 (1956) 781.
13 B. CHANCE, *Brookhaven Symposia in Biology No. 11*, 1959, p. 74.
14 L. SMITH AND J. RAMÍREZ, *Arch. Biochem. Biophys.*, 79 (1959) 233.
15 L. SMITH A. D J. RAMÍREZ, *J. Biol. Chem.*, 235 (1960) 219.
16 R. W. ESTABROOK AND B. MACKLER, *J. Biol. Chem.*, 224 (1957) 637.
17 R. W. ESTABROOK AND B. SACKTOR, *Arch. Biochem. Biophys.*, 76 (1958) 532.
18 M. NISHIMURA, in preparation.
19 B. CHANCE AND L. SMITH, *Nature*, 175 (1955) 803.