[S8-5]

Oxidative Stress Generation Associated with the Bacteriochlorophyll Biosynthesis of *Rhodobacter sphaeroides*

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Rhodobacter sphaeroides, a facultative photosynthetic bacterium, contains two SODs; CuZnSOD is detected only under the conditions where photosynthetic complexes are formed (1), whereas FeSOD is constitutively expressed although its activity of the aerobically grown cell nearly doubled as compared with that of the anaerobically grown cell. The role of CuZnSOD in protecting the photoheterotrophic cells from periplasmic superoxide upon exposure to O_2 was proposed (1). Oxidative stress defense in *R. sphaeroides* is also mediated by catalases as well as by thioredoxin system which acts as thiol-disulfide redox buffer to reduce the protein thiols that were oxidized (2). Mutations in thioredoxin system lowered the formation of photosynthetic complex (2).

The formation of photosynthetic complexes of *R. sphaeroides* is redox dependent. Lowering oxygen tension induces the formation of intracytoplasmic membrane housing the photosynthetic complexes of *R. sphaeroides*. Light captured by B800-850 and B875 light-harvesting complexes is transferred to reaction center complex, where the redox reactions are initiated to convert light energy into ATP and reducing power. The oxygen-regulated expression of apoproteins of LH and RC complexes, which are encoded by *puc*, *puf*, and *puh* operons, was elucidated.

The expression of several enzymes for bacteriochlorophyll (Bchl) *a* synthesis is also subject to anaerobic induction. Protoporphyrin IX (Proto), which is synthesized from 5-aminolevulinic acid (ALA), is a common intermediate for heme and Bchl *a*. It can be easily chelated with Mg ion, giving Mg-Proto. Mg-chelatase has much lower K_m for Proto than ferrochelatse (3, 4). Mg-Proto is metabolized to chlorophyllide (Chlide) *a*, which is either used as a direct precursor of chlorophyll (Chl) *a* or further converted to bacteriochlorophyllide (Bchlide) *a*, a precursor of Bchl *a*. The first step of Chlide *a* metabolism is the reduction of its ring B by a nitrogenase-like enzyme, chlorophyllide *a* reductase (COR), which is composed of BchX, BchY, and BchZ.

COR reduces ring B of Chlide *a* under anaerobic conditions. Although the enzyme is labile in the presence of O_2, it generates superoxide at low O_2. Consistently, suppressor mutations rescuing the inability of *R. sphaeroides* FeSOD mutant to grow in succinate-based minimal medium were predominantly mapped to BchZ subunit of COR. BchX, a flavo-iron sulfur protein, acts as an ATP-dependent NADH:FMN oxidoreductase, whereas BchY is an iron-sulfur protein that can be co-purified with BchZ containing b-type heme. Neither Chlide *a* reduction nor superoxide generation was observed with the enzyme reconstituted with the wild-type subunits of BchX and BchY, and the apo-subunit of BchZ that had been refolded without heme, in which FMN of BchX was fully reduced. Thus, superoxide radical is generated not from FMN of BchX but from heme of BchZ. The enzymes reconstituted with BchX, BchY, and the mutein subunit of BchZ from suppressor mutants showed less activity not only for Chlide *a* reduction but also for superoxide generation compared with the enzyme reconstituted with the wild-type subunits. It was further found that the heme of BchZ muteins was half reduced in its redox state compared with that of wild-type BchZ (5).

The expression of COR is highly induced upon lowering O_2 tension. Especially, the induction of BchZ synthesis under the conditions results in the titration of heme in cells. The resulting effect is to lower heme availability, which leads to the activation of ALA synthase expression in addition to its O_2-dependent expression. Thus, the induction of BchZ expression upon semi-aerobiosis results in the enhanced metabolic flow to protoporphyrin IX, which readily can be used for Bchl *a* synthesis of *R. sphaeroides*.

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