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EVIDENCE FOR INVOLVEMENT OF THE ELECTRON TRANSPORT SYSTEM AT A LATE STEP OF ANAEROBIC MICROBIAL HEME SYNTHESIS

N.J. JACOBS and J.M. JACOBS

Department of Microbiology, Dartmouth Medical School, Hanover, N.H. 03755 (U.S.A.)

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Summary

The penultimate step in heme biosynthesis, the oxidation of protoporphyrinogen to protoporphyrin, can be anaerobically coupled to the reduction of fumarate in extracts of anaerobically-grown *Escherichia coli*. This coupling is approximately 90% inhibited by 2-heptyl-4-hydroxy quinoline-*N*-oxide (HQNO), a known inhibitor of the electron transport chain. This observation suggests that the mechanism of the anaerobic oxidation of protoporphyrinogen in *E. coli* involves a coupling into the anaerobic electron transport system. In contrast, the aerobic oxidation of protoporphyrinogen, which occurs in mammalian and yeast mitochondria, is known to be linked directly to oxygen without the mediation of an electron transport system.

In mammalian and yeast mitochondria, a late step in heme synthesis, the oxidation of protoporphyrinogen to protoporphyrin, is linked directly to molecular oxygen, without the mediation of the electron transport system [1—3]. Since many facultative and anaerobic bacteria are capable of synthesizing cytochromes during anaerobic growth, oxygen-independent mechanisms for this step must exist [4, 5]. We have previously reported the presence of an anaerobic system for protoporphyrinogen oxidation involving fumarate as anaerobic electron acceptor in anaerobically-grown *E. coli*. Subsequently, we reported that nitrate could also serve as anaerobic electron acceptor at this step, and we confirmed the physiological significance of these findings for the process of anaerobic heme synthesis [7]. However, the mechanism of the coupling between the protoporphyrinogen oxidizing system and the fumarate reductase enzyme was not elucidated. In the present communication, we present evidence suggesting that this coupling involves the anaerobic electron transport system.

All methods used have been previously described in detail [7]. Sonic

extracts containing both particulate and supernatant fractions were prepared from *E. coli* grown anaerobically on a complex medium containing glucose and fumarate [7]. The oxidation of protoporphyrinogen to protoporphyrin was determined spectrophotometrically by following the appearance of the four-banded spectrum of neutral protoporphyrin directly in extracts containing EDTA [7]. The anaerobic conversion of either protoporphyrinogen or protoporphyrin to protoheme was determined by extraction of protoheme with acidified acetone and spectrophotometric measurement as the pyridine hemochromogen [7]. The inhibitor, HQNO (Sigma Chemical Company, St. Louis, Mo.) was either dissolved directly in extracts, or added in an ethanolic solution.

Our results (Fig. 1) indicate that the anaerobic conversion of protoporphyrinogen to protoporphyrin is markedly stimulated by the addition of fumarate to the reaction mixture. This reaction is inhibited in the presence of the electron transport inhibitor, HQNO. A similar level of inhibition was also observed if the HQNO level was lowered to $1 \cdot 10^{-8}$ mol per mg extract protein. This inhibition was confirmed by following protoheme formation (Table I). Since protoporphyrinogen must first be converted to protoporphyrin to become a substrate for iron insertion by the ferrochelatase enzyme, following protoheme formation from protoporphyrinogen is a valid indirect measurement of protoporphyrinogen oxidation. As indicated (Table I), the anaerobic conversion of protoporphyrinogen to protoheme is dependent upon the addition of fumarate, and this reaction is also inhibited by HQNO. Although data for a 60-min incubation period are shown in Table I, fumarate-dependent heme synthesis from protoporphyrinogen was linear over a 30—100 min incubation period, and the inhibitory effect of HQNO was equivalent at each time interval. In contrast, the conversion of protoporphyrin to protoheme is predictably not dependent on fumarate addition, and is not inhibited by HQNO.

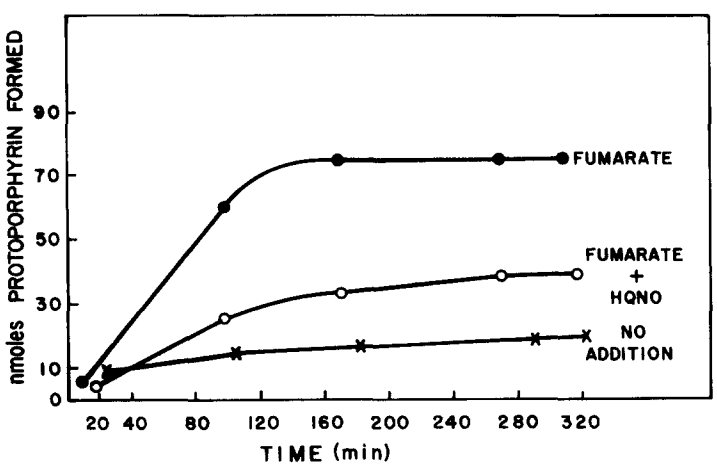

Fig. 1. HQNO inhibition of fumarate-dependent anaerobic oxidation of protoporphyrinogen to protoporphyrin. All cuvettes contained 60 mg protein of crude extract, and were incubated anaerobically under assay conditions described previously [7]. Where indicated, 123 nmol of protoporphyrinogen, 260 μmol of potassium fumarate or 1 mg of HQNO were added.

TABLE I

EFFECT OF HQNO AND FUMARATE ON ANAEROBIC PROTOHEME FORMATION FROM EITHER PROTOPORPHYRINOGEN OR PROTOPORPHYRIN

All cuvettes were incubated anaerobically under assay conditions previously described [7] for 60 min. Where indicated, the following additions were made to the 3-ml reaction mixture: protoporphyrinogen (176 nmol), protoporphyrin (176 nmol), potassium fumarate (146 μmol), HQNO (1 mg).

Substrate	Additions to reaction mixture	Total protoheme (nmol)	Increase in protoheme (nmol)
Protoporphyrinogen	none	7	2
Protoporphyrinogen	fumarate	37	31
Protoporphyrinogen	fumarate, HQNO	9	3
Protoporphyrinogen	HQNO	8	3
None	none	5	0
Protoporphyrin	none	34	29
Protoporphyrin	fumarate	48	43
Protoporphyrin	fumarate, HQNO	47	42
Protoporphyrin	HQNO	35	30

We have previously demonstrated [7] that protoheme biosynthesis in these experiments is not stimulated by the addition of ferrous salts to the reaction mixture, doubtlessly because sufficient iron is present in the crude extracts utilized.

These data clearly demonstrate the inhibitory effect of low levels of HQNO on electron transport between the protoporphyrinogen oxidizing system and the fumarate reductase enzyme. Although the exact site of HQNO inhibition is not known, it has been characterized as an inhibitor of aerobic electron transport near the level of cytochrome *b* in mitochondria and *E. coli* [8]. In anaerobic or facultative bacteria, anaerobic electron transport between dehydrogenases and the fumarate reductase is inhibited by HQNO at the level of menaquinones or cytochrome *b* rather than at the dehydrogenase or fumarate reductase level [9—15].

Thus, our data suggest the conclusion that in *E. coli*, the electrons removed from protoporphyrinogen during its anaerobic conversion to protoporphyrin are coupled into the anaerobic electron transport chain. This is the first report of a linkage between this step in heme synthesis and an electron transport system. This proposed mechanism has important implications for the process of anaerobic heme synthesis. For instance, it suggests that an anaerobic electron transport system must be present in all anaerobic bacteria which make heme. In contrast, aerobic protoporphyrinogen oxidation, which occurs in mammalian and yeast mitochondria, does not require an electron transport system. A second interesting implication is that in anaerobic heme synthesis, the six electrons removed during the oxidation of a molecule of protoporphyrinogen are available for the generation of energy through the mediation of the anaerobic electron transport system. This energy would not be available to cells oxidizing protoporphyrinogen directly with oxygen. Such energy would be useful for anaerobic bacteria synthesizing large amounts of cytochromes [5]. Other studies have shown that energy can be generated by the oxidation of substrates using fumarate as anaerobic electron acceptor in *E. coli* [16, 17] and other bacteria [18].

It is also of interest to note that the previous step in heme synthesis, the

oxidation of coproporphyrinogen, can occur under anaerobic conditions in photosynthetic bacteria and yeast, and has been reported to require ATP, methionine, pyridine nucleotides, and possibly a non-heme iron component [19, 20].

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