THE LATE STEPS OF ANAEROBIC HEME BIOSYNTHESIS IN E. COLI: ROLE FOR QUINONES IN PROTOPORPHYRINOGEN OXIDATION

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SUMMARY

The anaerobic oxidation of protoporphyrinogen with fumarate as electron acceptor in cell-free extracts of E. coli is inhibited by ultra-violet irradiation. The activity of irradiated extracts is restored by addition of menadione and the restored activity is blocked by the electron-transport inhibitor 2-heptyl-4-hydroxy quinoline-N-oxide. These observations suggest that quinones are required as electron transport carriers at this late step in the pathway of anaerobic heme biosynthesis. These findings have important implications both for the mechanism of anaerobic heme synthesis and for the physiology of cytochrome biosynthesis in anaerobic microorganisms.

INTRODUCTION

In mammalian and yeast mitochondria, the penultimate step in heme biosynthesis, the oxidation of protoporphyrinogen to protoporphyrin, is linked directly to molecular oxygen without mediation of the electron transport system (1-4). Since many facultative and anaerobic bacteria synthesize cytochromes during anaerobic growth, oxygen independent mechanisms for this step must exist (5, 6). Our previous studies have shown that fumarate is an important anaerobic electron acceptor for this oxidation in E. coli (7, 8). We also found that the coupling between the protoporphyrinogen oxidizing system and the fumarate reductase enzyme was blocked by the electron transport inhibitor 2-heptyl-4-hydroxy quinoline-N-oxide (HQNO), suggesting the involvement of an unidentified carrier in the anaerobic electron transport chain (9). In this communication, we present evidence suggesting that this unidentified carrier is a quinone.

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MATERIALS AND METHODS

Sonic extracts containing both particulate and soluble fractions were prepared from E. coli grown anaerobically on a complex medium containing 0.2% glucose and 0.6% fumarate as previously described (8). The oxidation of protoporphyrinogen directly to protoporphyrin in anaerobic cuvettes was followed spectrophotometrically in a reaction mixture containing Tris buffer (pH 7.6), EDTA and glutathione as described (8). Extracts were treated with U.V. light for 60 min. with a Blak-Ray long wave lamp (3666 λ) (Ultra-Violet Products, San Gabriel, Calif.) at a distance of 6 cm. During irradiation, a thin layer of extract in a petri dish was cooled in an ice bath and gently stirred (10). Solutions of menadione (Sigma Chemical Company, St. Louis, Missouri) and HQNO were dissolved in alcohol, and 0.05 ml amounts were added to extracts along with other reagents of the reaction mixture (8). Control experiments showed no effect of this amount of alcohol alone. Other methods and chemicals have been described previously in detail (8, 9).

RESULTS AND DISCUSSION

Irradiation of cell-free extracts with U.V. light causes a significant inhibition of anaerobic protoporphyrinogen oxidation with fumarate as electron acceptor (Fig. 1). These extracts exhibit little activity for protoporphyrinogen oxidation in the absence of added fumarate. If menadione is added to the reaction mixture, the activity of irradiated extracts with fumarate as electron acceptor is completely restored (Fig. 2). In the absence of fumarate, addition of menadione alone does not cause the oxidation of protoporphyrinogen (Fig. 2).

To determine whether the activity restored to the irradiated extract by menadione is similar to the activity of the untreated extract, we tested the effect of HQNO. We previously found that HQNO blocked electron transport between the protoporphyrinogen oxidizing system and fumarate reductase in untreated extracts (9). As shown in Fig. 2, HQNO also markedly inhibits the activity restored to irradiated extracts by the addition of menadione.

These results clearly suggest an obligatory role for quinones as electron carrier between the protoporphyrinogen oxidizing system and the fumarate reductase enzyme. Treatment of extracts with U.V. light

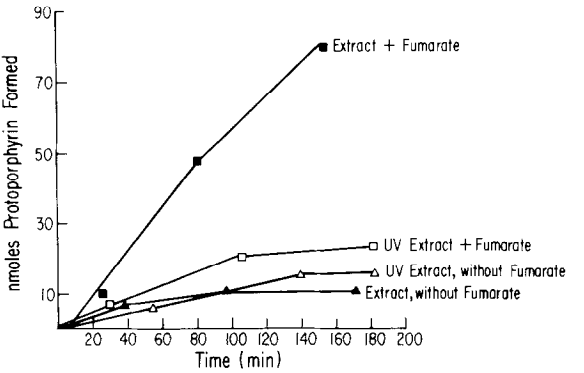

Figure 1. Effect of U.V. irradiation on the anaerobic oxidation of protoporphyrinogen to protoporphyrin. The assay procedure and contents of the reaction mixture are described in the Methods section. All extracts were present at a concentration of 60 mg per 3 ml. reaction mixture. Protoporphyrinogen (123 nmoles) was added to each cuvette, and potassium fumarate (260 μmoles) was added where indicated. Extracts were treated with U.V. light as indicated in the Methods section.

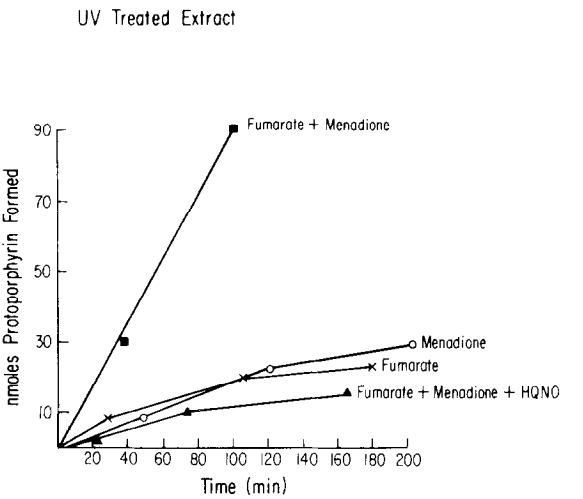

Figure 2. Effect of menadione on the anaerobic oxidation of protoporphyrinogen with fumarate as electron acceptor in extracts treated with U.V. light. Assay conditions as shown in Fig. 1. Where indicated, the following were added to the reaction mixture: menadione (1.25 μmoles); fumarate (260 μmoles); HQNO (0.15 mg). All extracts were treated with U.V. light.

is known to destroy quinones (10, 11). Restoration of activity by adding menadione is a clear indication that the treatment with U.V. light specifically destroys an intermediate electron transport carrier

rather than the protoporphyrinogen oxidizing system or the fumarate reductase. The effect of HQNO suggests that the activity reconstituted by menadione is not artificial, since HQNO inhibits as it does in untreated extracts. Investigations in similar microorganisms have correlated this effect of U.V. and the subsequent restoration by menadione with a requirement for menaquinone as obligatory electron carrier (10, 11). In our experiments menadione can presumably substitute for the natural menaquinone found in E. coli.

This probable menaquinone involvement as a carrier between protoporphyrinogen and fumarate is similar to the involvement of menaquinone as a carrier between the dihydroorotic acid dehydrogenase and the fumarate reductase in E. coli (12). Dihydroorotic acid is an intermediate in the biosynthesis of uracil. Therefore, our results indicate an interesting parallel between anaerobic heme biosynthesis and anaerobic uracil biosynthesis, where both processes probably involve menaquinone as carrier and fumarate as electron acceptor.

Although our findings clearly show the importance of menaquinones and fumarate in anaerobically oxidizing protoporphyrinogen, other electron acceptors, such as nitrate (8), may also serve this function under different growth conditions. Carriers other than menaquinones may be operative with electron acceptors other than fumarate.

Further questions remain about the mechanism of anaerobic protoporphyrinogen oxidation. For instance, the nature of the reaction between protoporphyrinogen and quinones in the E. coli membrane requires elucidation. In experiments similar to those shown in Fig. 1, we have tested the effect of NAD and NADP and have found no evidence for involvement of pyridine nucleotides as electron acceptors capable of replacing fumarate in anaerobic protoporphyrinogen oxidation (unpublished observations). Possibly the entire reaction between protoporphyrinogen and fumarate takes place within the cytoplasmic membrane, without the involvement of soluble components.

From a physiological viewpoint, it is interesting that this step in anaerobic heme synthesis contrasts sharply with the mechansim for aerobic protoporphyrinogen oxidation which occurs in mammalian and yeast mitochrondria. In these aerobic systems, this enzyme reacts directly with oxygen, without the involvement of carriers in the electron transport chain (1-4).

These observations also have implications for the physiology of obligatory anaerobic bacteria. Those anaerobes that contain cytochromes often exhibit a growth requirement for vitamin K. It seems likely that a partial explanation for this requirement can be found in our observation that vitamin K is apparently required for the anaerobic biosynthesis of heme.

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