Phytotoxic Compounds Cochlioquinones Are Inhibitors of Mitochondrial NADH-Ubiquinone Reductase

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INTRODUCTION

Previously we reported the isolation and structural elucidation of a series of cochlioquinone derivatives from a plant pathogenic fungus *Bipolaris bicolor*. These compounds are phytotoxic to the host plants of the pathogen and thus considered to play a significant role for the disease development, although the mechanism of the expression of toxicity remains unknown.

In view of the quinone (or quinol) moiety commonly contained in cochlioquinones, it was predicted that they would elicit inhibition of redox reactions in electron-transfer system in which quinone plays a crucial role like such inhibitors as piericidin A, UHNQ (undecylhydroxy-naphthoquinone) and HQNO (heptylhydroxyquinoline-N-oxide). To see this possibility, we examined the effect of cochlioquinones on the mitochondrial electron-transfer system using bovine heart and potato tuber mitochondrial enzymes.

MATERIALS AND METHODS

Four cochlioquinone analogues (Fig. 1) were obtained from a culture of *Bipolaris bicolor* El-I. Antimycin A, rotenone and cytochrome c were purchased from Sigma.

The preparation of bovine heart submitochondrial particles and the measurement of NADH oxidase and NADH-PB reductase activity were carried out as described. The complex IV activity (TMPD oxidase activity) of submitochondrial particles was determined as reported. Succinate-cytochrome c reductase activity was measured oxygraphically using intact mitochondria.

RESULTS

1. Inhibition of Bovine Heart Mitochondrial Electron-transfer by Cochlioquinones

The inhibition by cochlioquinone B of NADH oxidase (complexes I+III+IV), NADH-PB reductase (complex I), succinate-cytochrome c reductase (complexes II+III), PBH2-cytochrome c reductase (complex III) and TMPD oxidase (complex IV) activities of submitochondrial particles and isolated enzyme prepared from bovine heart mitochondria were examined (Table 1). As the index of inhibitory potency, IC50, which is the concentration to halve the control enzyme activity was used.

Fig. 1 Structures of cochlioquinone derivatives used in this study.
cochlioquinone B against NADH. In contrast, the pattern of inhibition for cochlioquinone B against PB was complicated. As shown in Fig. 2B, the reciprocal plots were curvilinear in the presence of fixed concentrations of cochlioquinone B. That is, the pattern of inhibition varied from noncompetitive to competitive as the concentration of PB increased.

The inhibitory potencies of four cochlioquinone analogues were compared for NADH oxidase activity (Table 2). Although the variation of the potencies was less than 3-folds, isocochlioquinone A which carries a reduced quinone ring was the most potent inhibitor. However, compared to a typical potent inhibitor rotenone, the inhibitory potencies of these analogues were not so large.

### DISCUSSION

The quinone-related inhibitors known to day generally possess a quinone ring (or quinone-related structure) at an edge of the molecule carrying a hydrophobic side chain, such as piericidin A, HQNO and stigmatellin. However, the quinone ring moiety of cochlioquinones is located in the center of the molecule; in other words, the quinone ring is buried in the molecule. The fact that even such sterically congested quinone-related compounds acted as the inhibitor of quinone catalytic site of complex I suggests that the quinone catalytic site of complex I, in particular for bovine enzyme, is spacious enough to accommodate "bulky" quinone-related compounds. This notion seems to be supported by other studies of the inhibitory mechanism of various potent inhibitors of complex I, where the quinone catalytic site of this enzyme was supposed to be a rather large pocket which can accommodate a wide variety of inhibitors in a dissimilar manner.

The pattern of inhibition by cochlioquinone was compli-
cated. It varied depending upon the concentration of exogenous quinone. A similar complicated inhibition regarding with complex I was reported for capsaicin,\(^9\) pungent principle of red pepper species, and for synthetic rotenone stereoisomers.\(^2\) Such kinetic data might be interpreted as that complex I bears two quinone binding sites with a similar level of affinity for exogenous quinone (PB), and the activity of this enzyme is inhibited by the binding of an inhibitor molecule to one of those quinone binding sites.\(^5,8\)

Mitochondrion is the major site of reactive oxygen production.\(^9\) For instance, the inhibition of complex I activity causes superoxide generation and lipid peroxidation.\(^10\) Since cochlioquinones turned out to be inhibitor of plant mitochondrial complex I, it is of interest to elucidate the relationship between the inhibition of energy-transducing system and the expression of disease symptoms. The study to verify this issue is currently in progress.

REFERENCES

9) A. Boveris & B. Chance: Biochem. J. 134, 707 (1973)