Note

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*.¹⁾ 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 (undecylhydroxynaphthoquinone) 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-1.¹⁾ 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.²⁾ The complex IV activity (TMPD oxidase activity) of submitochondrial particles was determined as reported.³⁾ Succinate-cytochrome *c* reductase was isolated from bovine heart mito-

Fig. 1 Structures of cochlioquinone derivatives used in this study.

chondria according to the method of Yu & Yu.⁴⁾ Using the isolated enzyme, succinate-DCIP, PBH₂-cytochrome *c* and succinate-cytochrome *c* reductase activities were determined.⁴⁾

The preparation of potato tuber submitochondrial particles and the measurement of deamino-NADH-PB reductase activity were done as described.²⁾ Succinate oxidase 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. Cochlioquinone B elicited inhibition of NADH oxidase and NADH-PB reductase activities, but no other enzyme activities. This indicates that cochlioquinone B is a specific inhibitor of complex I. The inhibitory potency was larger for NADH oxidase activity than that for NADH-PB reductase activity, being consistent with the case for other complex I inhibitors such as piericidin A and rotenone.^{2, 5)}

We next examined the pattern of inhibition of NADH-PB reductase activity by cochlioquinone B. Figure 2A is double-reciprocal plots showing the effect of fixed concentrations of cochlioquinone B on the rate of NADH-PB reductase activity. An uncompetitive pattern of inhibition is seen for

Abbreviations: complex I, NADH-ubiquinone reductase; complex II, succinate-ubiquinone reductase; complex III, ubiquinol-cytochrome *c* reductase; complex IV, cytochrome *c* reductase; deamino-NADH, reduced nicotinamide hypoxanthine dinucleotide; DCIP, dichlorophenolindophenol; PB, 2,3-dimethoxy-5-*n*-pentyl-1,4-benzoquinone; PBH₂, 2,3-dimethoxy-5-*n*-pentyl-1,4-benzoquinol; TMPD, *N,N,N',N'*-tetramethylphenylenediamine.

Table 1 Inhibition by cochlioquinone B of bovine heart mitochondrial complexes I, II, III and IV activities.

Enzyme activity	IC ₅₀ (nmol/mg of protein)
NADH oxidase (Complexes I+III+IV)	83
NADH-PB reductase (Complex I)	370
Succinate-cyt. <i>c</i> ^{a)} reductase (Complexes II+III)	> 3600
Succinate-DCIP reductase (Complex II)	> 3600
PBH ₂ -cyt. <i>c</i> ^{a)} reductase (Complex III)	> 3600
TMPD oxidase (Complex IV)	> 3600

^{a)} cyt. *c*: cytochrome *c*.

Fig. 2 Double-reciprocal plots of kinetic data of NADH-PB reductase activity of bovine heart submitochondrial particles.

The concentrations of PB in (A) and NADH in (B) were kept at 50 mM respectively. The reaction medium, in final volume of 2.5 ml, contained 50 mM potassium phosphate (pH 7.4) 0.25 M sucrose, 1 mM MgCl₂, 2 mM KCN and 0.4 mM antimycin A. In each assay, the final mitochondrial protein concentration was 30 mg/ml.

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 increase.

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,

Table 2 The inhibitory potency of cochlioquinone analogues and rotenone for NADH oxidase activity of bovine heart submitochondrial particles.^{a)}

Compound	IC ₅₀ (nmol/mg of protein)
Cochlioquinone A	115
Cochlioquinone B	83
Isocochlioquinone A	56
Stemphone	160
Rotenone	0.17

^{a)} The IC₅₀ values were measured at least twice and the results were averaged.

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.

2. Inhibition of Potato Tuber Mitochondrial Electron-transfer by Cochlioquinones

The IC₅₀ of cochlioquinone B for NADH-PB reductase activity of proton-pumping NADH dehydrogenase in potato tuber mitochondria, which is the counterpart of the mammal mitochondrial complex I, was *ca.* 3000 nmol/mg of protein. The sensitivity of this enzyme to the inhibition by cochlioquinone B was very low comparing to that of mammalian enzyme, as has been observed for other complex I inhibitors such as rotenone and piericidin A.^{2, 6)}

On the other hand, the succinate oxidase activity of potato tuber mitochondria was not inhibited by cochlioquinone B at the concentration of 3600 nmol/mg of protein. Since this concentration was nearly at the solubility limit of this compound, the effect at higher concentrations was not examined. This result indicates that cochlioquinone B has no inhibitory effect on complexes II, III and IV activities.

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^{2, 5, 7)} 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,⁸⁾ 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.^{2,8)}

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 cochlinoquinones 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.

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要 約

植物毒素コクリオキノン類によるミトコンドリア NADH-ユビキノ還元酵素の阻害

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植物病原菌 *Bipolaris bicolor* の生産する植物毒素コクリオキノン類のミトコンドリア電子伝達系に対する作用を、ウシ心筋およびジャガイモ塊茎より調整した酵素系を用いて検討した。コクリオキノン類は電子伝達複合体 I~IV のうち I の活性を阻害し、速度論的解析から阻害部位はキノン還元部位であることが明らかとなった。他の複合体に対しては実質的な阻害作用は認められなかった。複合体 I~IV がいずれもキノン還元部位をもつことを考慮すると、コクリオキノン類は複合体 I のキノン還元部位との相互作用に適した立体構造を有するものと考えられた。