J. Pestic. Sci., **30**(2), 120–121 (2005)

Society Awards 2005

(on prominent achievement)

Inhibitors of Mitochondrial Respiratory Enzymes*

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(Accepted February 23, 2005)

The active conformation of antimycin A, a specific inhibitor of mitochondrial complex-III, expected from structure–activity studies is consistent with that come from X-ray crystallography of the enzyme. The structure–activity studies of acetogenins, potent inhibitors of complex-I, indicate that these inhibitors elicit potent activities only when the γ-lactone ring and hydroxylated THF ring moieties are directly linked by an alkyl spacer. Δlac-Acetogenins that are acetogenin mimics possessing two alkyl tails without a γ-lactone ring appeared to be a novel type of complex-I inhibitor, the binding site of which differs from that of ordinary complex-I inhibitors.

Keywords: respiratory inhibitors, respiratory enzymes, acetogenins, bioenergetics, structure–activity relationship.

INTRODUCTION

Mitochondrial respiratory enzymes are important targets of recent agrochemicals. Recently structural information at the atomic resolution of several respiratory enzymes such as succinate–ubiquinone oxidoreductase (complex-II) and cytochrome bc_1 complex (complex-III) were derived from crystallographic studies. Elucidation of the inhibitory mechanism based on the atomic structure is helpful to design new agrochemicals targeting respiratory enzymes. However, it should be realized that crystallographic studies have some limitation since these studies do not cover all forms of the enzyme during a reaction cycle, and some enzyme crystals lack functionally important bound ubiquinone. On the other hand, the X-ray structure of NADH–ubiquinone oxidoreductase (complex-I), another important target enzyme, remains unavailable due to its high complexity. Therefore, various experimental approaches other than X-ray crystallography are necessary to clarify the inhibitory mechanism of respiratory inhibitors. This paper briefly describes the results of studies that we carried out to explore the mode of action of typical respiratory inhibitors.

ANTIMYCIN

Antimycin is the most potent inhibitor of the ubiquinone reduction site (Q_i site) of complex-III. This inhibitor consists of salicylic acid and dilactone ring moieties. Our structure–activity studies showed that solely the salicylic acid moiety is strictly recognized by the enzyme. Both the phenolic OH and 3-formylamino groups are essential for potent inhibition. However, the dilactone ring moiety plays a supporting role in inhibitor binding to the Q_i site by increasing the hydrophobicity of the molecule. Based on the structure–activity studies and MO calculation of the 3-formylamino group, we proposed a binding model of antimycin in the Q_i site in 1995. This model is almost supported by recent X-ray structures of antimycin bound to complex-III. The effects of UK-2A, which is a natural antibiotic and very close to antimycin A structurally, on reduced heme b_H differ from those of antimycin A since UK-2A has a methoxy group in place of a formylamino in the 3-position of the salicylic acid ring. This observation also supports the crucial function of the 3-formylamino group.

STIGMATELLIN

Stigmatellin is one of the most potent inhibitors of the ubiquinol oxidation site (Q_o site) of complex-III. The X-ray structure of stigmatellin bound to the Q_o site suggested that the 4-carbonyl group of the benzopyran ring forms a hydrogen bond to Nε-His181 of the Rieske protein, a ligand of the 2Fe–2S cluster. To determine the binding of stigmatellin directly, we tried to monitor the hydrogen bond as a change in vibration of the carbonyl group by a redox reaction induced FT–IR spectroscopy using ^{13}C-labeled stigmatellin in collaboration with Drs. Hellwig and Hunte. We observed a clear shift in the wave number (about 10 cm^{-1}) of the carbonyl

* See Part II for the full Japanese article.

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group upon binding to the enzyme as well as a change in vibration of an acidic residue (Asp255 or Glu272 of cytochrome *b*) located in the Q_o cavity.

OVERVIEW OF COMPLEX-I INHIBITORS

A large number of inhibitors of complex-I are known. With the exception of rhein and diphenyleneiodonium, which inhibit electron input into complex-I, all inhibitors act at the terminal electron transfer step of this enzyme. Radio- and fluorescent-ligand binding and photoaffinity labeling studies indicated that a certain marker ligand is displaced by numerous competitors (*i.e*. other complex-I inhibitors). These findings suggest that a wide variety of inhibitors share a common large binding domain with partially overlapping sites.

It should, however, be realized that in all these experiments, the authors demonstrated that the binding of a certain marker ligand to the enzyme is completely suppressed in the presence of an excess amount of competitors. Under these experimental conditions, one cannot rule out the possibility that even though the binding sites of the ligand and competitors are quite different, the binding of an excess amount of competitors induced structural change of the ligand binding site, which resulted in suppression of the ligand binding. Actually, several studies suggested that complex-I undergoes dynamic conformational change. From competition tests using a fluorescent inhibitor with a high quantum yield, we showed that the apparent competitive behavior among various complex-I inhibitors cannot be interpreted solely by the scenario that they share a common large binding domain with partially overlapping sites. It remains, therefore, unclear how binding sites of complex-I inhibitors relate to each other.

ACETOGENINS

More than 400 annonaceous acetogenins have been isolated from the plant family *Uvaria accuminata* (Annonaceae) during the past two decades. Acetogenins have very potent and diverse biological effects such as antitumor, antimalarial, pesticidal and antifeedant activities. The inhibitory effects of acetogenins on mitochondrial complex-I are of particular importance as the diverse biological activities are thought to be attributable to this effect. Actually some acetogenins, like bullatacin, are the most potent inhibitors of this enzyme identified to date. Although the acetogenins are thought to act at the terminal electron transfer step of complex-I, there is still no hard experimental evidence to verify whether the inhibitors in fact bind to the ubiquinone reduction site. Additionally, there are few structural similarities between acetogenins and ordinary complex-I inhibitors such as piericidin A and rotenone. Thus, considering the unusual structural characteristics as well as the very strong inhibitory effect of acetogenins, a detailed analysis of the inhibitory actions of these inhibitors is important to elucidate the structural and functional features of the terminal electron transfer step of complex-I. As the first step toward this end, identification of the crucial structural factors of acetogenins responsible for potent inhibition would be useful.

From structure–activity studies using a series of natural and synthetic acetogenins with bovine complex-I, we have shown that: i) the presence of polar functional group(s) like an OH group in the spacer, the number of THF rings and the stereochemistry around the hydroxylated THF ring(s) are not essential structural factors for potent activity; ii) the natural γ-lactone ring itself is not crucial for activity, and can be substituted with an ubiquinone ring; and iii) acetogenin acts as a strong inhibitor only when the γ-lactone and THF ring moieties are directly linked by an alkyl spacer, the optimal length of which is about 13 carbon atoms. Thus, except for the important role of the alkyl spacer, crucial structural factors including the active conformation of acetogenins remain to be elucidated. Nevertheless, based on these results, we propose that the γ-lactone and THF ring moieties act in a cooperative manner on the enzyme with the support of some specific conformation of the spacer.

*Δ*lac-ACETOGENINS

We synthesized *Δ*lac-acetogenins, which are new acetogenin mimics possessing two *n*-alkyl tails without a α,β-unsaturated γ-lactone ring. To elucidate the inhibition mechanism of *Δ*lac-acetogenins, we carried out wide structural modifications of *Δ*lac-acetogenins and characterized the inhibitory action using bovine heart mitochondrial complex-I. In contrast to common acetogenins, both the presence of adjacent bis-THF rings and stereochemistry around the hydroxylated bis-THF rings are important structural factors required for potent inhibition. The inhibitory potency of some *Δ*lac-acetogenins appeared to be equivalent to that of bullatacin, one of the most potent natural acetogenins. Double-inhibitor titration of steady state complex-I activity showed that the extent of inhibition of *Δ*lac-acetogenins and bullatacin are not additive, suggesting that the binding sites of the two inhibitors are not identical. Competition tests using a fluorescent ligand indicated that the binding site of *Δ*lac-acetogenins does not overlap with that of other complex-I inhibitors. The effects of *Δ*lac-acetogenins on superoxide production from complex-I also differ from those of other complex-I inhibitors. Our studies demonstrate that *Δ*lac-acetogenins are a novel type of inhibitor acting at the terminal electron transfer step of bovine complex-I.

CONCLUDING REMARKS

Mitochondrial respiratory enzymes have become important targets of agrochemicals over the past two decades. Some excellent agrochemicals targeting respiratory enzymes have been developed, whereas selective toxicity remains the most important theme in the development of respiratory inhibitors. To move beyond this theme, further progress in basic as well as comprehensive research on structural and functional features of the respiratory enzymes is necessary.