ELSEVIER

Biochimica et Biophysica Acta 1364 (1998) 236–244

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

Structure–activity relationships of some complex I inhibitors

Hideto Miyoshi *

Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-01, Japan

Received 17 October 1997; revised 30 December 1997; accepted 5 January 1998

Abstract

A wide variety of complex I inhibitors act at or close to the ubiquinone reduction site. Identification of the structural factors required for exhibiting inhibitory actions on the basis of structure–activity relationships is useful to elucidate the manner in which inhibitors interact with the enzyme. This review summarizes studies on the structure–activity relationship of rotenoids, piericidins, capsaicins, pyridinium-type inhibitors and modern synthetic agrochemicals acting at mitochondrial complex I. © 1998 Elsevier Science B.V.

Keywords: Complex I; Mitochondrion; Respiratory inhibitor; Structure–activity relationship

1. Introduction

There are a wide variety of complex I inhibitors acting at or close to the ubiquinone reduction site [1,2]. Structure–activity studies of complex I inhibitors are important not only to elucidate the structural factors required for their inhibitory action, but also to determine the structural properties of the ubiquinone reduction site in the enzyme. In addition, detailed comparisons of inhibitory actions of structurally different inhibitors will provide clues to the mechanism of redox-driven proton pumping of the enzyme. In general, to identify important structural factors from the study of structure–activity relationships, a series of derivatives in which the chemical structures have been systematically modified are needed. Without sufficient structural variation, important structural factors and their complementary effects may be overlooked. In this respect, however, the number of complex I inhibitors for which detailed structure–activity studies have been conducted using a wide variety of derivatives is small. In this article, structure–activity studies of rotenoids, piericidins, capsaicins and pyridinium-type inhibitors will be reviewed. Although similar studies on other types of complex I inhibitors have been published, these four series of inhibitors were chosen because of their historical importance to research on complex I and usefulness as probes for exploring complex I function. The detailed mechanisms of action of these inhibitors will not be discussed since this was reviewed in the article by Degli Esposti [2]. Furthermore, since novel types of complex I inhibitors are believed to hold important positions in most modern synthetic insecticides and acaricides [3], the chemical features of agrochemicals which have been recently marketed in many countries will also be reviewed.

* Corresponding author. Fax: +81-75-753-6408; E-mail: miyoshi@kais.kyoto-u.ac.jp

PII S0005-2728(98)00030-9

2. Structure–activity studies of rotenoids

Natural rotenone is the most widely used inhibitor of complex I because of its high inhibitory potency and commercial availability. Rotenone consists of a five-ring structure (A- to E-rings) and has three chiral centers (6a-C, 12a-C and 5′-C, Fig. 1). The stereochemistry of rotenone has been assigned as 6a*S*, 12a*S*, 5′*R* configuration [4]. All known natural rotenoids have been isolated in the thermodynamically stable *cis*-B/C ring fusion [5,6]. The three-dimensional structure of rotenone determined by X-ray crystallographic analysis [7] is also shown in Fig. 1 (the bottom). Rotenone is bent at the face of contact between the B- and C-rings.

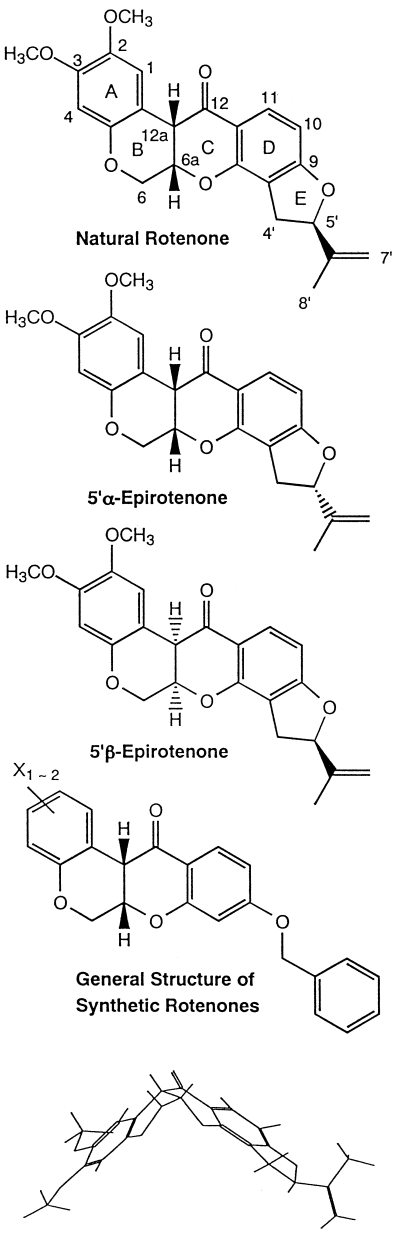

Fig. 1. Structures of natural rotenone, 5′α-epirotenone, 5′β-epirotenone and synthetic rotenones. For synthetic rotenones, X = alkyls or alkoxys [10]. The three dimensional structure of rotenone obtained by X-ray crystallographic analysis [7] is shown at the bottom.

Using synthetic rotenone stereoisomers (5′α-epirotenone and 5′β-epirotenone, Fig. 1), it has been shown that the inhibitory potency of 5′β-epirotenone, in which B- and C-ring planes are almost coplanar, is about 100-fold less active than natural rotenone with mammalian mitochondrial complex I [8,9], indicating that the bent form is essential for the activity. This conclusion is supported by the observation that the inhibitory potency of rotenol, in which the whole molecular conformation is not fixed due to opening of the C-ring is about 200-fold less active than natural rotenone [8,10]. Levett et al. [11] have also shown an important role for the B/C ring system by synthesizing rotenone-related inhibitors which lack the core B/C ring. The inhibitory potency as well as the manner of inhibition of 5′α-epirotenone differ considerably from those of natural rotenone irrespective of slight differences in the E-ring moiety [9]. That is, the potency of this isomer is about 10-fold less than that of rotenone and the inhibition pattern varies from non-competitive to competitive as the concentration of exogenous quinone increases. This indicates that the configuration of the isopropenyl group attached to the E-ring is also important for the activity. These observations obtained with rotenone stereoisomers demonstrate that the whole molecular structure (or shape) of rotenone is strictly recognized by mammalian complex I.

A similar structure–activity relationship of rotenone stereoisomers has been reported for the proton-pumping NADH-Q oxidoreductase in potato tuber (*Solanum tuberosum* L.) mitochondria [9]. In contrast, with respect to the proton-pumping NADH-Q oxidoreductase of *Escherichia coli*, the sensitivity of the enzyme to rotenone and its stereoisomers is markedly decreased and the differences in the inhibitory potencies of the three inhibitors are ambiguous [9]. This suggests that only part of the rotenone molecule is recognized by this enzyme. It is notewor-

thy that the sensitivity of non-proton-pumping NADH-Q oxidoreductase in potato tuber mitochondria and *E. coli* to inhibition by rotenone stereoisomers is much lower than that of the proton-pumping enzyme [9], as is also the case for natural rotenone [12,13]. This is consistent with the notion that the proton-pumping machinery is close to the ubiquinone reduction site [14–16].

Besides the stereochemical properties of rotenone, Ueno et al. [10] investigated the effects of the substituents in the A-ring on the activity using synthetic rotenoids in which the E-ring moiety of rotenone was substituted by a benzyloxy group (Fig. 1). The native chemical structure (2,3-dimethoxy substitution) is the most favorable for the activity with bovine heart mitochondrial complex I. This may be because a specific spatial organization of the hydrogen-bond acceptable methoxy oxygens is required for tight fitting into the binding site. It is, however, noteworthy that derivatives possessing other substitution patterns such as 1,3-dimethoxy and 2,3-dimethyl groups do not necessarily lose all activity. Replacing one of the two methoxy groups with an ethoxy group results in almost complete retention of activity, indicating that the binding environment of the A-ring moiety is sufficiently spacious to accommodate substituents larger than the methoxy group. The sensitivity of proton-pumping NADH-Q oxidoreductase of potato tuber and *E. coli* to inhibition by the rotenone analogues is much lower than that of the bovine enzyme. The 2,3-dimethoxy substituted analogue showed the best activity with the potato enzyme, although the extent of the differences in potency among the derivatives possessing various substitution patterns is rather small, being within one order of magnitude. For the *E. coli* enzyme, however, the 2,3-dimethoxy substituted derivative did not show the best activity; for instance, 2-monomethoxy and 3-monomethoxy derivatives were several times more potent than the 2,3-dimethoxy derivative. These findings indicated that the local binding environment of the A-ring moiety of rotenone in bovine complex I is specific and differs considerably from those in potato and *E. coli* enzymes.

Furthermore, Ueno et al. [10] showed that the apparent inhibitory potency of the derivative lacking the 12-C=O group in the C-ring is completely retained with bovine complex I, whereas the manner of inhibition of this inhibitor differs somewhat from that of natural rotenone. This derivative is approximately 7- and 3-fold more potent than natural rotenone for potato and *E. coli* enzymes, respectively. These findings indicate that the 12-C=O group is not essential for exhibiting apparent inhibitory action.

3. Structure–activity studies of piericidins

Many kinds of *Streptomyces* strains produce piericidin homologues. Piericidin A has been used as a very potent inhibitor of complex I from various biological sources. The structure of piericidin A originally proposed by Takahashi et al. [17] in 1965 was revised later by the same group in 1977 [18], as shown in Fig. 2. It should be appreciated that an incorrect structure of piericidin A has been often cited even in recent literature.

Detailed structure–activity studies on piericidin analogues including both natural and synthetic products have been performed by Takahashi et al. [17] and Yoshida et al. [18–21] with mammalian and insect mitochondria. The natural side chain structure of piericidin A is not essential for the activity since piericidin B, C and D analogues, in which the side chain structure in the region from C-5 to C-13 differs from that of piericidin A, exhibit activity as high as or only slightly less than that of piericidin A [21]; for instance, the relative potencies of piericidins A, B, C and D are 1.0, 0.84, 1.1, and 0.80, respectively. Based on structure–activity studies of a number of synthetic piericidin analogues possessing different side chains, it was concluded that a branched methyl group at C-3 and unsaturation between C-2 and C-3 are important for potent activity [21]. Actually, this structural unit is completely conserved for another

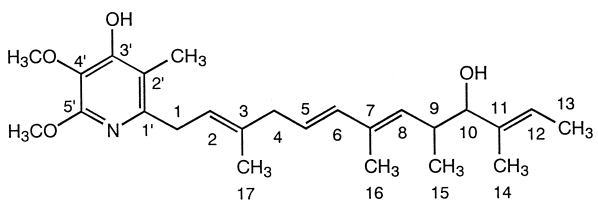

Fig. 2. Structure of piericidin A.

class of potent synthetic inhibitors, ubicidines [22]. However, as derivatives possessing a simple long alkyl chain retain fairly potent activity [23], the side chain might serve primarily to enhance the hydrophobicity of the whole molecule. In evidence of this, the location of the hydrophobic side chain at either the 1′- or 2′-position of the pyridine ring does not affect the inhibitory potency [21,23].

A free pyridinol hydroxy group in the pyridine ring moiety, which resembles the quinone ring of ubiquinone, is essential for activity [24,25]. To elucidate the functional contributions of the other substituents on the pyridine ring (namely, 2′-Me, 4′-MeO and 5′-MeO groups), Yoshida et al. [20,21] synthesized a variety of piericidin analogues in which substitution patterns on the pyridine ring as well as side chain structure were modified simultaneously. Their assay results were, however, confusing. For instance, lack of the 2′-Me group results in marked loss of inhibitory potency, whereas loss of both the 2′-Me and 5′-MeO groups restores the inhibitory potency irrespective of the side chain structure.

4. Structure–activity studies of capsaicins

Capsaicin (Fig. 3), the pungent principle of red pepper species, acts as a competitive inhibitor for ubiquinone with bovine heart mitochondrial complex I [26]. Using submitochondrial particles prepared from bovine heart mitochondria and several bacterial membranes, Yagi [27] demonstrated that the inhibition of NADH-Q oxidoreductase activities of several organisms by capsaicin correlated well with the presence of an energy coupling site in this segment of the respiratory chain. That is, the sensitivity to capsaicin inhibition of NADH-Q oxidoreductase that bears an energy coupling site (NDH-1) is much higher than that of the enzyme lacking such a site (NDH-2). Yagi [27] consequently concluded that capsaicin inhibition correlates better with the presence of a coupling site than inhibition by rotenone, piericidin A or DCCD (*N*, *N*′-dicyclohexylcarbodiimide), which had been thought to discriminate fairly well between NDH-1 and NDH-2 [14]. Thus, capsaicin is a useful reagent for investigating the mechanism of energy coupling associated with complex I.

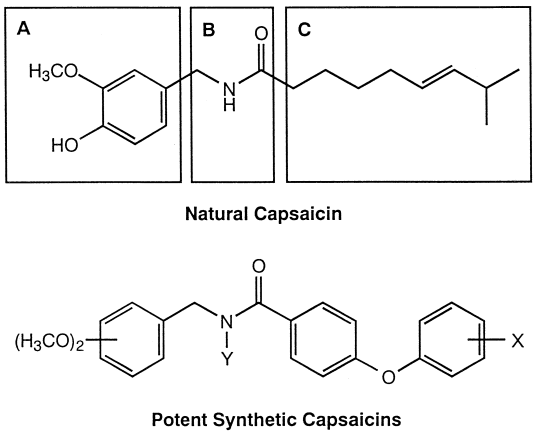

Fig. 3. Structures of natural capsaicin and potent synthetic capsaicins. For synthetic capsaicins, X = alkyls or alkoxys; Y = H or Me [31].

The structural requirements of capsaicin analogues for other pharmacological effects such as analgesic activity have been examined closely (e.g., Refs. [28,29]), whereas structure–activity studies regarding electron-transfer inhibition are limited. Chudapongse and Janthasoot [30] found that methyl capsaicin is several times more potent than natural capsaicin with mammal mitochondrial complex I, indicating that the phenolic OH group is not essential for the activity. On the other hand, Shimomura et al. [26] have shown that capsaicin analogues with an acyl group of 10 to 12 carbons are much more potent than natural capsaicin, indicating that the hydrophobicity of the acyl moiety favors activity. Based on these studies, Satoh et al. [31] performed a detailed structure–activity study using a variety of synthetic capsaicins which were prepared by modifying each region of the three sections (A to C, Fig. 3) of the prototype capsaicin, as follows.

Modification both of the substitution pattern and of the number of methoxy groups on the benzene ring, which may be superimposable on the quinone ring of ubiquinone due to a structural similarity, did not markedly affect the inhibitory potency with bovine heart mitochondrial complex I. Concerning the dipolar amide bond unit which can serve as a hydrogen-bond donor and acceptor, alteration of the position of this functional group in the molecule and chemical modifications of this unit such as *N*-methylation do not affect the inhibitory potency. Regarding the moi-

ety corresponding to the alkyl side chain, a rigid diphenylether structure (Fig. 3) is more inhibitory than a flexible alkyl chain. Thus, the rule regarding the variation of the inhibitory potency depending upon structural modification is rather ambiguous. This may be not only because that the high rotational freedom of A- and B-sections of capsaicin make these moieties fit flexibly into the binding pocket in a dissimilar way, but also that capsaicins interact at proposed multiple sites in complex I [27,31].

Satoh et al. [31] also examined the inhibition by the synthetic capsaicins of NDH-1 and NDH-2 activities in potato tuber mitochondria and *E. coli* plasma membranes. The sensitivity to inhibition by various synthetic capsaicins differs markedly between NDH-1 and NDH-2, supporting the notion that the sensitivity against capsaicin inhibition correlates well with the presence of an energy coupling site in the enzyme [27]. It is noteworthy that several potent synthetic capsaicins discriminated between NDH-1 and NDH-2 much better than natural capsaicin [31].

5. Structure–activity studies of *N*-methylphenylpyridiniums

N-Methyl-4-phenylpyridinium (MPP^+) and its alkyl derivatives (Fig. 4) have recently attracted our attention not only because their neurotoxicity is thought to be related to Parkinsonian syndrome [32], but also because mode of action studies of these inhibitors have suggested that they are bound at two distinct sites on bovine heart mitochondrial complex I [33,34]. [1] As MPP^+ binds to the so-called 'rotenone-site' [37,38], this proposal would mean that there are also two distinct ubiquinone binding sites in complex I. This might be very helpful in elucidating the terminal electron transfer step in the enzyme. Thus, mode of action as well as structure–activity studies of MPP^+ analogues should provide clues as to the mechanism of energy-coupled electron transfer in the enzyme.

The inhibitory potency of MPP^+ itself (mM order) is much poorer than that of classical potent inhibitors (nM order) such as piericidin A and rotenone for mammalian mitochondrial complex I. The development of inhibition of this hydrophillic inhibitor is a very slow process which continues for hours [33]. For simple alkyl analogues of MPP^+ (Fig. 4), the inhibitory potency increases and the inhibition is attained in a shorter time with increases in hydrophobicity of the alkyl substituent [33]. Besides the effect of the hydrophobicity, structure–activity profiles of the pyridinium-type inhibitors are complicated, and consequently important structural features for the inhibition are still ambiguous.

It is not clear whether a positive charge on the pyridinium ring is functionally essential for the activity since the neutral (i.e., pyridine) form is more potent than the pyridinium form for relatively weak inhibitors [33,39,40], although an important role for a positive charge has been claimed for relatively potent pyridiniums [33,41]. The methyl group attached to a pyridine nitrogen atom might not be essential because an ethyl or even a bulky benzyl derivative retains the activity [34,41]. For substituents on the pyridinium ring, the substitution position as well as its steric shape are not restricted for eliciting potent inhibition

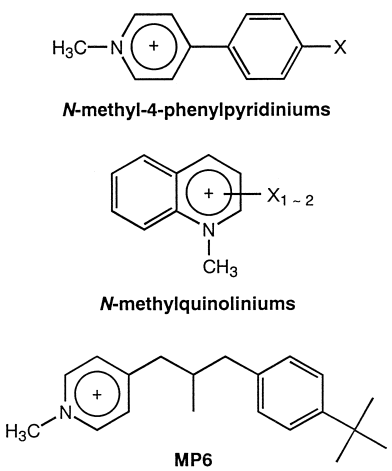

Fig. 4. Structures of *N*-methyl-4-phenylpyridiniums ($MPPs^+$), *N*-methylquinoliniums and MP6 (*N*-methyl-4-[2-(*p-tert*-butylbenzyl)propyl] pyridinium). X = alkyls or alkoxys [33,41].

[1] Although two sites of interaction have been suggested for rotenone and piericidin A using ^{14}C-labeled inhibitors [35,36], these earlier studies were challenged by the recent radioligand assay using [^{3}H]dihydrorotenone [37], wherein the signal-to-noise ratio was markedly improved due to high specific activity of the ligand.

[34,40,41]. Furthermore, the *N*-methylpyridinium ring itself is not essential for the activity since other aromatic rings such as bulky *N*-methylquinolinium (Fig. 4) can functionally substitute for it [41].

Thus, the physicochemical structural factors, except for hydrophobicity, of the pyridinium-type inhibitors required for inhibition have yet to be defined. It is, however, likely that only two factors, a positive charge (i.e., electrophilic property), which may interact with the proposed anionic residue(s) in the binding site [33], and marked hydrophobicity, which facilitates the passage of the cationic inhibitor to the binding site through the hydrophobic environment in the membrane, are required for potent inhibition.

The effects of the hydrophobic anion tetraphenylboron (TPB^-) on the inhibitory action of MPP^+ analogues is noteworthy. The presence of TPB^- potentiates the inhibition of less hydrophobic MPP^+ analogues and facilitates the development of inhibition [33], although the potentiation effects have been confirmed even for hydrophobic MPP^+ analogues [41]. These effects of TPB^- can be accounted for first by an increase in MPP^+ concentration in the membrane lipid phase due to ion pair formation and second by facilitating MPP^+ passage through the hydrophobic barrier to the binding site. Interestingly, when TPB^- present in molar excess over MPP^+ analogues, the former partially reverses the inhibition by the latter. This complicated effect of TPB^- along with pH dependence of MPP^+ inhibition prompted Gluck et al. [33] to conclude that MPP^+ analogues are bound at two sites, one accessible to relatively hydrophillic inhibitors (termed the 'hydrophillic site') and one shielded by a hydrophobic barrier on the enzyme (the 'hydrophobic site'), and that occupation of both sites is required for complete inhibition. Using novel potent pyridinium and quinolinium inhibitors, Miyoshi et al. [41] provided strong evidence to support this idea, and further demonstrated that both the MPP^+ (inevitably ubiquinone) binding sites contribute to redox-driven proton pumping. In addition, they succeeded in synthesis of a fairly selective inhibitor of the hydrophillic site, MP6 (*N*-methyl-4-[2-(*p-tert*-butylbenzyl)propyl] pyridinium, Fig. 4). The availability of this inhibitor and further modified derivatives will stimulate interesting additional studies aimed at resolving the mechanism of the terminal electron transfer step in complex I.

6. Structural feature of complex I inhibitors as pesticides

Pesticides must be safe, both to humans and to non-target organisms, and leave no harmful residues, either in the crops or in the environment. In this respect, respiratory inhibitors have been thought difficult to develop as pesticides since a highly selective toxicity between target and non-target organisms cannot be obtained due to similarities in electron-transfer

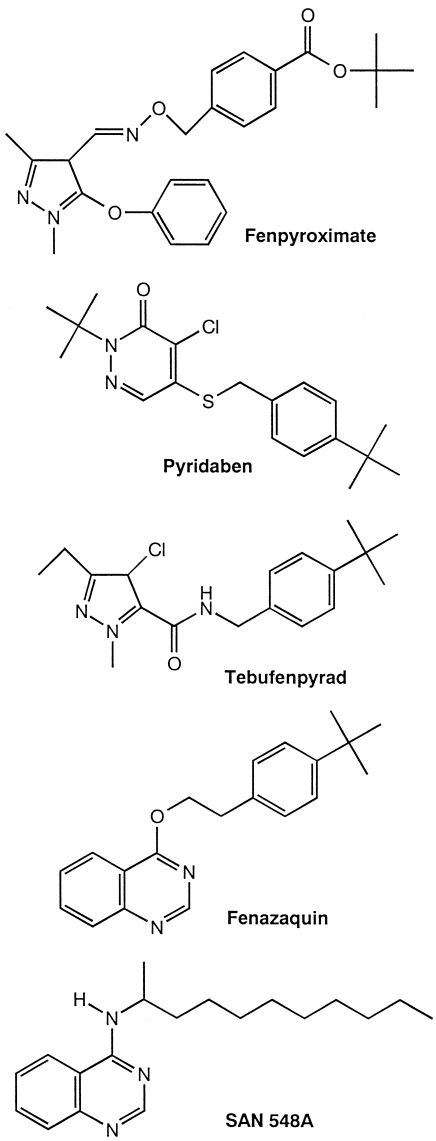

Fig. 5. Structures of Fenpyroximate (Nihon Noyaku), Pyridaben (Nissan Chemical Industries), Tebufenpyrad (Mitsubishi Kagaku), Fenazaquin (Dow Elanco) and SAN 548A (Sandoz).

machinery in the respiration chain [42]. However, a number of chemical companies have recently announced new excellent respiratory inhibitors which satisfy the above demands. These respiratory inhibitors are expected to hold important positions in most modern synthetic insecticides and acaricides [3]. Among these, compounds whose target site is mitochondrial complex I will be briefly reviewed.

Novel structural types of insecticides/acaricides are listed in Fig. 5. The properties of these compounds as insecticides/acaricides have been reviewed [43,44]. These compounds inhibit mitochondrial complex I activity by disturbing the terminal electron-transfer step between Fe–S cluster N-2 and Q (the so called 'rotenone site' [1,45–47]). Since these compounds are potent inhibitors of both mammalian and insect mitochondrial complex I, their low toxicity to mammals should be due to specific metabolic decomposition rather than target insensitivity, as investigated closely with Fenpyroximate [48,49]. Although structure–activity studies of these compounds as pesticide have been reported at international conferences on pesticide chemistry, no studies of in vitro activity have been published. Since in vitro activity is not necessarily correlated to in vivo activity, definition and comparison of the structural factors required for the inhibition of complex I are impractical at present.

It is, however, likely that they have broad structural features in common, notably heterocyclic compounds containing two nitrogen atoms and hydrophobic tail structure. The lone-pair containing group of the heterocyclic moiety might play an important role in binding to the enzyme through specific interactions with some residues. However, it seems to be meaningless to superimpose their heterocyclic ring moieties in any particular way since the steric hindrance surrounding the nitrogen atoms and electron density on the atoms differ markedly. The manner of binding of the heterocyclic ring might vary depending upon both the bulkiness of the substituents on the ring and the tail structure. On the other hand, the primary role of the tail moiety in the inhibition should be enhancement of hydrophobicity of the whole molecule. The high degree of hydrophobicity of the inhibitor might be energetically favorable to partitioning into and passage through the hydrophobic environment of the ubiquinone catalytic site which is believed to be part of the membrane-embedded segment of complex I [14–16]. This feature of the functional division of the inhibitor molecule is important for other inhibitors such as piericidins and capsaicins, as described above. On the basis of COMPASS (COMmon geometrical PAttern Search System) and 'molecular field fitting' analyses, Akagi et al. [50] suggested that the active conformations of the agrochemicals are non-planar; that is, the heterocyclic ring and the tail moieties are perpendicular to each other. It is interesting to note that a non-planar structure is also important for rotenoids and capsaicins [9,31]. Although bulky *tert*-butyl (or *tert*-butylphenyl) groups are common structural factors of the novel agrochemicals, it is unclear whether this structural unit is based on some biorational design.

7. Conclusions

Through structure–activity studies of the above complex I inhibitors, particularly on mammalian mitochondria, we conclude that the important structural factors which drastically affect the inhibitory potency (by several orders of magnitude) are not necessarily obvious for each inhibitor. For instance, only the pyridinol hydroxy group is undoubtedly essential for the action of piericidin. Other functional groups on the pyridinol ring are indeed important to maintain high potency, but do not determine the exhibition of inhibitory effects. The bent form of the rotenone molecule is a very important structural factor, whereas the two methoxy groups in the A-ring, 12-C=O group in the C-ring and presence of the E-ring itself are not essential for exhibiting apparent inhibition. Particularly for capsaicins and pyridinium-type inhibitors, it is not easy to define the structural properties important for inhibition, except hydrophobicity of the molecule. These findings along with the observation that a wide variety of structurally different inhibitors act at the ubiquinone catalytic site in common [1,2] suggest that this site in mammalian mitochondrial complex I is spacious enough to accommodate various inhibitors in a dissimilar manner depending upon structural specificity. The manner of binding of complex I inhibitors might not follow the so-called 'key and keyhole' relation in which multi-

ple functional groups of the inhibitors are thought to interact tightly with their binding environment in a similar manner. It should also be mentioned that the complexity of the explanation of the inhibitory mechanism as well as the structure–activity relationship of complex I inhibitors would be due to the presence of more than one ubiquinone reduction site in the enzyme [2,33,41,51]. The number of ubiquinone reduction sites, and the spatial and functional relationship of the sites must be elucidated to fully understand the mechanism of inhibition of complex I inhibitors.

Acknowledgements

The author thanks Drs. M. Degli Esposti (Monash Univ., Victoria, Australia), K. Motoba (Nihon Nohyaku, Osaka, Japan) and T. Obata (Ube Industories, Ube, Japan) for their helpful and stimulating discussion.

References

[1] T. Friedrich, P. Van Heck, H. Leif, T. Ohnishi, E. Forche, B. Kunze, R. Jansen, W. Trowitzsch-Kienat, G. Höfle, H. Reichenbach, H. Weiss, Eur. J. Biochem. 219 (1993) 691–698.
[2] M. Degli Esposti, Biochim. Biophys. Acta, 1998, this special issue.
[3] T.R. Perrior, Chem. Ind., London, 1993, pp. 883–887.
[4] G. Büchi, L. Crombie, P.J. Godin, J.S. Kaltenbronn, K. Siddalingaiah, D.A. Whiting, J. Chem. Soc., 1961, pp. 2843–2860.
[5] T. Unai, I. Yamamoto, Agric. Biol. Chem. 37 (1973) 897–901.
[6] M.J. Begley, L. Crombie, A.H.A. Hadi, J.L. Josephs, J. Chem. Soc. Perkin Trans. I, 1989, pp. 204–205.
[7] S.K. Arora, R.B. Bates, R.A. Grady, N.E. Delfel, J. Am. Chem. Soc. 97 (1975) 5752–5755.
[8] J. Burgos, E.R. Redfearn, Biochim. Biophys. Acta 110 (1965) 475–483.
[9] H. Ueno, H. Miyoshi, K. Ebisui, H. Iwamura, Eur. J. Biochem. 225 (1994) 411–417.
[10] H. Ueno, H. Miyoshi, M. Inoue, Y. Niidome, H. Iwamura, Biochim. Biophys. Acta 1276 (1996) 195–202.
[11] P.C. Levett, D.A. Whiting, J. Cayley, G.S. Cockerill, J.B. Weston, Bioorg. Med. Chem. Lett. 4 (1994) 505–508.
[12] I.M. Møller, W. Lin, Annu. Rev. Plant Physiol. 37 (1986) 309–334.
[13] K. Matsushita, T. Ohnishi, H.R. Kaback, Biochemistry 26 (1987) 7732–7737.
[14] T. Yagi, Biochemistry 26 (1987) 2822–2828.
[15] T. Yagi, Y. Hatefi, J. Biol. Chem. 263 (1988) 16150–16155.
[16] G. Hofhaus, H. Weiss, K. Leonard, J. Mol. Biol., 1991, 1027–1043.
[17] N. Takahashi, A. Suzuki, S. Tamura, J. Am. Chem. Soc. 87 (1965) 2066–2074.
[18] S. Yoshida, S. Shiraishi, N. Takahashi, Agric. Biol. Chem. 41 (1977) 587–591.
[19] S. Yoshida, K. Yoneyama, S. Shiraishi, A. Watanabe, N. Takahashi, Agric. Biol. Chem. 41 (1977) 855–862.
[20] S. Yoshida, Y. Nagao, N. Takahashi, Agric. Biol. Chem. 44 (1980) 2913–2920.
[21] S. Yoshida, Y. Nagao, A. Watanabe, N. Takahashi, Agric. Biol. Chem. 44 (1980) 2921–2924.
[22] M. Gutman, S. Kliatchko, FEBS Lett. 67 (1976) 348–353.
[23] K.H. Chung, K.Y. Cho, Y. Asami, N. Takahashi, S. Yoshida, Z. Naturforsch. 44C (1989) 609–616.
[24] T. Mitsui, T. Sagama, J. Fukami, K. Fukunaga, N. Takahashi, S. Tamura, Botyu-Kagaku 34 (1969) 135–142, (in Japanese).
[25] D.J. Horgan, H. Ohno, T.P. Singer, J.E. Casida, J. Biol. Chem. 243 (1968) 5967–5976.
[26] Y. Shimomura, T. Kawada, M. Suzuki, Arch. Biochem. Biophys. 270 (1989) 573–577.
[27] T. Yagi, Arch. Biochem. Biophys. 281 (1990) 305–311.
[28] J.M. Janusz, B.L. Buckwalter, P.A. Young, T.R. LaHann, R.W. Farmer, G.B. Kasting, M.E. Loomans, G.A. Kerckaert, C.S. Maddin, E.F. Berman, R.L. Bohne, T.L. Cupps, J.R. Milstein, J. Med. Chem. 36 (1993) 2595–2604.
[29] C.S.J. Walpole, S. Bevan, G. Bovermann, J.J. Boelsterli, R. Breckenridge, J.W. Davies, G.A. Hughes, I. James, L. Oberer, J. Winter, R. Wrigglesworth, J. Med. Chem. 37 (1994) 1942–1954.
[30] P. Chudapongse, W. Janthasoot, Biochem. Pharmacol. 30 (1981) 735–740.
[31] T. Satoh, H. Miyoshi, K. Sakamoto, H. Iwamura, Biochim. Biophys. Acta 1273 (1996) 21–30.
[32] I.J. Kopin, S.P. Markey, Annu. Rev. Neurosci. 11 (1988) 81–96.
[33] M.R. Gluck, M.J. Krueger, R.R. Ramsay, S.O. Sablin, T.P. Singer, W.J. Nicklas, J. Biol. Chem. 269 (1994) 3167–3174.
[34] M.P. Murphy, M.J. Krueger, S.O. Sablin, R.R. Ramsay, T.P. Singer, Biochem. J. 306 (1995) 359–365.
[35] D.J. Horgan, T.P. Singer, J.E. Casida, J. Biol. Chem. 243 (1968) 834–843.
[36] M. Gutman, T.P. Singer, J.E. Casida, J. Biol. Chem. 245 (1970) 1992–1997.
[37] D.S. Higgins, J.T. Greenamyre, J. Neurosci. 16 (1996) 3807–3816.
[38] R.R. Ramsay, M.J. Krueger, S.K. Youngster, T.P. Singer, Biochem. J. 273 (1991) 481–484.
[39] C.L. Hoppel, D. Greenblatt, H.C. Kwok, P.K. Arora, M.P. Singh, L.M. Sayre, Biochem. Biophys. Res. Commun. 148 (1987) 684–693.
[40] L.M. Sayre, M.P. Singh, P.K. Arora, F. Wang, R.J. McPeak, C.L. Hoppel, Arch. Biochem. Biophys. 280 (1990) 274–283.

[41] H. Miyoshi, M. Inoue, S. Okamoto, M. Ohshima, K. Sakamoto, H. Iwamura, J. Biol. Chem. 272 (1997) 16176–16183.
[42] J.R. Corbett, K. Wright, A.C. Baillie, The Biochemical Mode of Action of Pesticides, Academic Press, London, 1984, pp. 1–49.
[43] R.M. Hollingworth, K.I. Ahammadsahib, Rev. Pestic. Toxicol. 3 (1995) 277–302.
[44] P. Leroux, Pestic. Sci. 47 (1996) 191–197.
[45] K. Motoba, T. Suzuki, M. Uchida, Pestic. Biochem. Physiol. 43 (1992) 37–44.
[46] E. Wood, B. Latli, J.E. Casida, Pestic. Biochem. Physiol. 54 (1996) 135–145.
[47] R.M. Hollingworth, K.I. Ahammadsahib, G. Gadelhak, J.L. McLaughlin, Biochem. Soc. Trans. 22 (1994) 230–233.
[48] K. Motoba, H. Nishizawa, T. Suzuki, H. Hamaguchi, M. Uchida, Biosci. Biotechnol. Biochem. 56 (1992) 366–372.
[49] K. Motoba, T. Suzuki, M. Uchida, Pestic. Biochem. Physiol. 43 (1992) 37–44.
[50] T. Akagi, Y. Takahashi, S. Sasaki, Quant. Struct.-Act. Relatsh. 15 (1996) 290–295.
[51] U. Brandt, Biochim. Biophys. Acta 1318 (1997) 79–91.