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Suicide Substrates as Irreversible Inhibitors of Flavoenzymes

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SUMMARY

An increasing number of flavin dependent enzymes have recently been found to be inhibited by substrate analogs which fulfill the requirements set forth for suicide inhibitors. In most cases inactivation results from covalent modification of the flavin coenzyme; in other cases protein alkylation at the enzyme active center is involved. Examples of the first are the inactivation of general acyl-CoA dehydrogenase from pig kidney, and butyryl-CoA dehydrogenase from Megasphera elsdenii by a metabolite of the hypoglycaemic agent hypoglycin, and by 3,4-pentadienoyl-CoA; these are described in some detail. A survey of the suicide inhibitors investigated so far indicates that either an acetylenic, an allenic or the methylenecyclopropane moieties flanking the function to be oxidized, are required for suicide inactivation of flavin enzymes.

INTRODUCTION

The terms "suicide substrates" or "suicide inactivators" have entered the biochemists' vocabulary to describe a class of highly specific irreversible enzyme inhibitors [1-3]. The prototype of this category of inhibitors comes from the studies of Bloch and coworkers on the inactivation of ß-hydroxy-decanoyl thioester dehydrase by the actylenic substrate analog, 3-decynoyl-N-acetyl-cysteamine [4]. This enzyme catalyzes the isomerization of the analog to the corresponding 2,3-decadienoyl thioester which is subject to rapid nucleophilic attack from an active site histidine residue. Although the term "suicide substrate" has received widespread use, Miesowicz and Bloch prefer the designation "Trojan horse inhibitor": arguing that "the enzyme does not commit suicide - a deliberate act - but is the victim of deceit" [5]. Massey and co-workers [6], however, were the first to use the term "suicide", in connection with the inhibition of xanthine oxidase by allopurinol, when they described this effect as due to "a sort of suicide reaction". Walsh has termed these compounds "mechanism-based inactivators" since their mode of action is a direct consequence of chemical events occurring during catylsis [7]. Suicide substrates generally have chemical and structural features resembling those of the normal substrate, but contain, in addition, a function which is converted to a highly reactive species within the active site. This reactive moiety may attack an essential protein residue or prosthetic group before dissociation occurs causing irreversible inhibition of enzyme activity. Suicide substrates can thus be highly specific in their action and can often inactivate their target enzymes rapidly at equimolar concentrations.

Over the past 7-8 years a variety of enzymes have been demonstrated to be irreversibly inactivated by compounds which meet the criteria for suicide substrates (see [7,8] for recent reviews). The design and application of suicide substrates has proved extremely useful in the elucidation of the reaction mechanism of a number of flavoenzymes. In particular, these studies support the concept that an early catalytic event in flavin catalyzed dehydrogenation is removal of a proton from the substrate, generating a transient carbanion [7]. During normal turnover this substrate derived carbanion serves as reductant for enzyme bound flavin, whereas analogous activation of the suicide substrate leads to covalent modification of the active center.

MECHANISTIC PREMISES

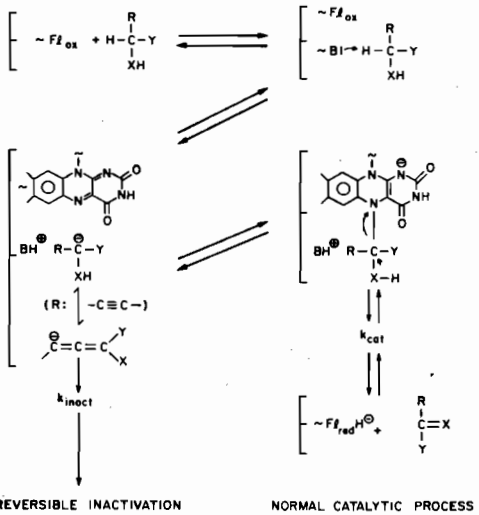
Flavin dependent enzymes catalyze a wide variety of reactions involving a diverse range of substrates, so that it is safe to assume the occurrence of several distinct general catalytic mechanisms. This subject has been reviewed recently [7,9,10,11]. A classification of flavin enzymes based on the type of reaction catalyzed has been proposed by Massey and Hemmerich [12]. In the case of the "electron transferases" the nature of the reaction catalyzed (electron transfer) seems to preclude application of the suicide substrate approach. With those flavoproteins which interact with pyridine nucleotides, the mode by which reducing equivalents are transferred from nucleotide to flavin (e.g. via hydride transfer, covalent intermediates, or radical mechanism) is still in dispute [9].

In contrast, with those flavoproteins oxidizing "activated C-H substrates" (i.e. molecules in which a C-H group is rendered weakly acidic by flanking electron withdrawing groups such as X, Y (Scheme 1) a consensus has emerged that catalysis is initiated by proton abstraction with formation of a transient carbanion [7,11,13, 14,15]. The mode of subsequent transfer of reducing equivalents from carbanion to oxidized flavin may differ from enzyme to enzyme. For example, with L-lactate oxidase [15,16] and D-amino acid oxidase [14] good evidence exists for the involvement of covalent intermediates. In other cases, this mechanism has been questioned in favor of a free radical process involving flavin-substrate radical pair intermediates [17].

Scheme 1 depicts a minimal mechanism for the oxidation of an activated "C-H substrate" and irreversible inactivation by a suicide substrate. Inactivation is represented as occurring after isomerization to yield an allenic carbanion. When inactivation involves formation of a covalently modified flavin species, e.g. with D-lactate dehydrogenase and L-lactate oxidase (Table 1), the adduct probably derives from direct attack of the allenic carbanion on the oxidized isoalloxazine ring. In other cases, inactivation may be a consequence of subsequent protonation or rearrangement of this allene carbanion on the enzyme surface.

As emphasized by Walsh [18] the ratio k_{cat}/k_{inact} (Scheme 1) i.e. the ratio of turnover of the inhibitor as a substrate to the rate of inactivation is a useful measure representing the effectiveness and specificity of the inhibitor.

Scheme 1



RESULTS AND DISCUSSION

α-Hydroxy Acid Oxidases (Dehydrogenases)

The interaction of suicide substrates with flavoenzymes using α-hydroxy acid substrates has received considerable attention, and 8 examples are given in Table 1. Among these, L-lactate oxidase and Megasphera elsdenii D-lactate dehydrogenase have been studied in detail, and the kinetic aspects of their suicide inactivation and the structures of the modified flavins have been established [19,20, 21,22,23].

The characteristic partioning ratio k_{cat}/k_{inact} for 2-Hydroxy-3-butynoate varies widely between enzymes, and may also depend on the conditions of inactivation. For example with L-lactate oxidase, the ratio is strongly dependent on the oxygen concentration; anaerobically k_{cat}/k_{inact} is $\ll 1$, while in air saturated solution a value of ~ 110 is obtained [20]. In contrast, the D-lactate dehydrogenase isolated from the strict anaerobe M. elsdenii exhibits a partioning ratio of ~ 3 which is not significantly influenced by oxygen [22]. These results were interpreted as a reflection of the close interrelation between catalytic and inactivation mechanisms. In two further cases ratio values of 15-20 and 30 were reported for E.coli D-lactate dehydrogenase and rat kidney α-hydroxy acid oxidase respectively [24,25].

TABLE 1: SURVEY OF FLAVIN ENZYMES, WHICH ARE INHIBITED BY SUICIDE SUBSTRATES

ENZYME	INHIBITOR	COVALENT MODIFICATION OF (COENZYME OR PROTEIN RESIDUE)	LIT.REF.
L-Lactate oxidase (M.smegmatis)	(L) H-C≡C-CH(OH)-COOH	FMN (cyclic, flavin C(4)-N(5) adduct with inhibitor)	19,20,64
D-α and L-α-Hydroxy acid oxidase (Rabbit kidney and rat kidney)	(D,L) H-C≡C-CH(OH)-COOH	FMN (structures n.d.)	24
Glycollate oxidase (Pisum sativum)	(L) H-C≡C-CH(OH)-COOH	FMN (analog to Lactate oxidase)	65,66
L-Lactate dehydrogenase (E.coli)	(L) H-C≡C-CH(OH)-COOH	FMN (?)	25
L-Lactate dehydrogenase (Cytochrome b_2) (Baker's yeast)	(L) H-C≡C-CH(OH)-COOH	FMN (analog to Lactate oxidase ?)	67
D-Lactate dehydrogenase (M.elsdenii)	(D) H-C≡C-CH(OH)-COOH	FAD (Cyclic, flavin N(5)-C(6) adduct with inhibitor)	21,22,23
Monoamine oxidase (Bovine liver mitochondria)	$-C\equiv C-CH_2-N$ $-C\equiv C=CH-CH_2-N$ cyclopropylamine $C_6H_5-N_2H_3$	1. FAD (flavin N(5)-adduct) 2. FAD (cyclic flavin C(4a)-N(5)-adduct) 3. Protein SH 4. FAD (flavin 4a-phenyl-adduct)	31,33,34, 35,36,68 31,37 38,39,40 42,43
Butyryl-CoA dehydrogenase (M.elsdenii) General acyl-CoA dehydrogenase (Pig kidney)	1. methylenecyclopropyl-CH_2-COSCoA (MCPA-CoA) 2. $CH_2=C=CH-CH_2-COSCoA$	1. FAD (and probably active site amino acid(s)) 2. FAD (reversible ?)	44,69,68, this work this work
L-Aminoacid oxidase (Crotalus adamanteus)	(L) HC=CH-CH(NH_2)-COOH	Amino acid residue	2,56
D-Aminoacid oxidase (Hog kidney)	1. HC≡C-CH_2-CH(NH_2)-COOH 2. $(CH_3)_2$CH-CH(NHCl)-COOH	1. His_{171}, Tyr_{259}, (Tyr_{263}?) 2. Chlorination of Tyr_{259}	1,3,5, 56,58 59
Sarcosine oxidase (Rat liver)	1. $H_2C=CH-CH_2-NH-CH_2COOH$ 2. $HC\equiv C-CH_2NH-CH_2COOH$	Amino acid residue ?	69
Succinate dehydrogenase (Beef heart mitochondria)	$O_2N-CH_2-CH_2-COOH$	Amino acid residue (SH group)	62,63

Table 1 indicates that in all the cases studied to date involving α-hydroxy acid oxidases or dehydrogenases, modification of the flavin is the principal cause of inactivation. However, alkylation of protein functional groups may represent a minor side reaction, as observed with D-lactate dehydrogenase from M. elsdenii [22].

The structures of the modified coenzymes deserves comment. Covalent modification always involves the N(5) position of the isoalloxazine ring, a locus believed to function in the transfer of reducing equivalents [19,26]. Adduct formation solely at this position (of the type $\geq N-\overset{|}{C}-XH$ (X=O,N) see Fig. 1) is probably readily reversible [26]. Irreversible attachment of the suicide substrate to the flavin results from the formation of additional stable covalent bonds at C(4a) or C(6) positions (see Fig. 1).

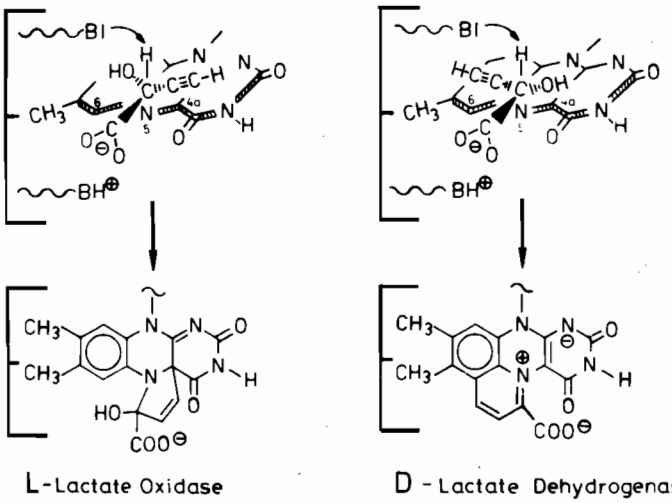


Fig. 1
Stereochemical mode of adduct formation in L-lactate oxidase and D-lactate dehydrogenase with α-hydroxy-3-butynoate.

The covalent adducts formed with D-lactate dehydrogenase and L-lactate oxidase appear to derive from the stereospecific attack of the activated butynoate moiety with the orientations shown in Fig. 1. It is reasonable to assume that the active sites of these two enzymes have evolved to accomodate an analogous orientation of their enantiomeric substrates. Further, in both enzymes it would be expected that the substrate α-carbon would be positioned above the plane, close to the N(5) position allowing interaction of a transient carbanion with the flavin π-orbitals. The structure of both adducts and other mechanistic studies suggest that both enzymes activate their substrates by initial proton abstraction, and a corresponding base residue placed along the extension of the α-C-H-bond axis would be expected. As described

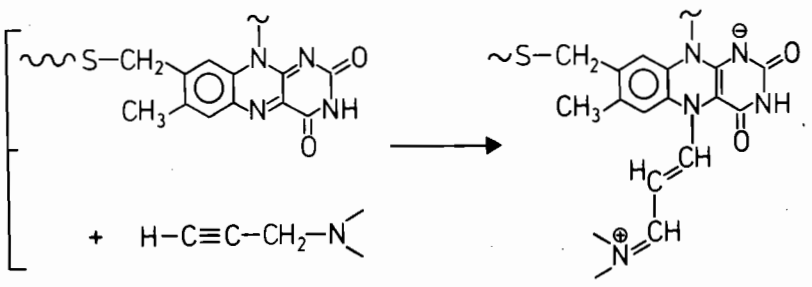
elsewhere, the carboxylate group probably participates in substrate binding and is oriented pointing away from the flavin [27]. These studies emphasize the potential of suicide substrates in the elucidation of flavoenzyme reaction mechanisms.

Monoamine Oxidase

This enzyme plays a central role in the metabolism of biogenic amines relevant to the central nervous systems. Mainly for this reason, an impressive effort has been undertaken in the past twenty years aimed at the elucidation of its reaction mechanism, and at the finding of modes for controlling its activity. The progress arising from these investigations has been presented and discussed in a series of recent monographs, and reviews to which the reader is referred for detailed information [28,29,30,31,32]. In the present paper, therefore, only a short summary is given of the inactivation reactions which are likely to proceed via suicide mechanism. From a chemical point of view, suicide substrates of monoamine oxidase can be differentiated into four categories:

Propargylamines (e.g. chlorgyline, deprenyl, pargyline) carry as activatable function the propargyl group $-CH_2-C\equiv CH$. With these inhibitors, inactivation occurs by addition of the suicide substrate to the flavin to form a cyanine-type adduct as shown in Fig. 2 below. The structural features of this adduct were elucidated by a combination of enzymatic [33,34], and chemical model studies [35,36]; its mechanism of formation is still not understood, but from chemical considerations it might proceed via a radical process [31]:

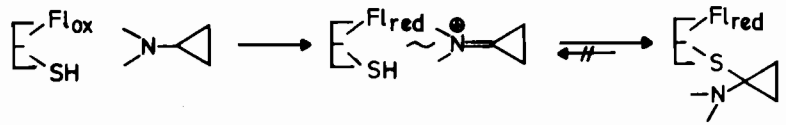
Fig. 2



Allenic amines may be related, in their mechanism of inactivation to their tautomeric propargylamines. In fact transient conjugated allenes have been proposed to be formed by the enzyme suicide reaction per se from a precursor propargylamine, and to be the actual species responsible for covalent enzyme modification [31]. From such reasoning these two classes of inhibitors would be expected to yield the same type of covalent adduct within the same enzyme class [37]. It was surprising to find that N-2,3-butadienyl-N-benzylmethylamine apparently yields a C(4a)-N(5) cyclic flavin adduct [37] similar to that found with L-lactate oxidase (see above); and different from the flavocyanine obtained with propargylamines [37]. A similarly divergent behaviour was also found for the photochemical reaction in model systems, where allenic- and propargylamines apparently yield adducts with structures opposite to those obtained in the enzymatic reaction [37].

Cyclopropylamines, The third class of monoamine oxidase suicide inhibitors, were initially supposed to inactivate the enzyme, as found with propargylamines and allenic amines, i.e. by covalent modification of the flavin coenzyme [29,38]. Recent results however suggest that inactivation results from alkylation of an active center amino acid residue [39] by a mechanism similar to that proposed for the inactivation of succinate dehydrogenase [40]. Thus, according to Peach et al. [39], the inhibitor is first oxidized to the imine, which then forms a thioaminal with an essential -SH group at the active site:

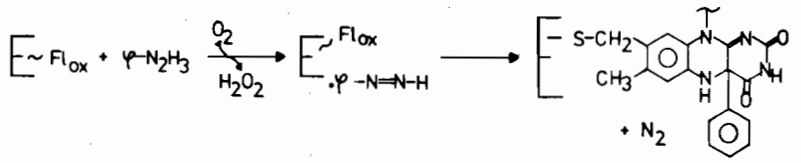
Scheme 2



Phenylhydrazines do not possess, at first sight, a chemical function which one might suspect to be activated by an enzymatic reaction related to the catalytic process of monoamine oxidase. They are known, however, to inhibit the metabolism of biogenic amines [41], and to be potent irreversible inactivators of this enzyme [42]. An indication as to the possible mechanism, and to the solution of the puzzle resulted from experiments with a related enzyme, trimethyl-

amine dehydrogenase [43]. Thus the 2-e$^-$ oxidation product of phenylhydrazine, phenyldiazene inhibits this enzyme faster than phenylhydrazine itself, and reacts also with flavin models such as lumiflavin to yield covalent adducts. The structure of these adducts is most probably that of a 4a-phenyl,4a,5-dihydroflavin [43], and resulting from a mechanistically very interesting reaction involving oxidation of the diazene with concomitant liberation of molecular nitrogen:

Scheme 3



General Acyl-CoA Dehydrogenase (GADH) from Pig Kidney, and Megasphera elsdenii Butyryl-CoA Dehydrogenase (BDH)

The second known example (cf. also succinate dehydrogenase below) of a naturally occurring suicide substrate for flavoenzymes is derived from hypoglycin A. This unusual amino acid is present in the unripe arillus of the ackee fruit (Blighia sapida) which, when ripe, serves as a dietary staple in Jamaica. Ingestion of hypoglycin A leads to the often fatal Jamaican vomiting sickness (see Tanaka [44] for a recent review). Hypoglycin was first isolated from unripe ackee in 1954 by Hassall and co-workers [45] and its structure was subsequently established in several laboratories (see references in [44]). A considerable body of evidence points to inhibition of various short chain acyl-CoA dehydrogenases,e.g. butyryl-CoA dehydrogenase, isovaleryl-CoA dehydrogenase, and glutaryl-CoA dehydrogenase by methylenecyclopropylacetyl-CoA (MCPA-CoA) as the major consequence of hypoglycin poisoning [44,46,47,48,49].

Since cyclopropane derivatives and alkynes share some similar chemical properties, we reasoned that MCPA-CoA may serve as a suicide substrate (Table 1). In view of the considerable pharmacological interest in compounds of this type, we are studying the interaction of MCPA-CoA with pure GADH and BDH. In particular we wish to establish whether inactivation occurs by irreversible formation of flavin adducts, or via covalent modification of the apoprotein.

GADH is a mammalian acyl-CoA dehydrogenase which exhibits optimal activity with medium chain (C_{10}) acyl substrates [50,51]. MCPA-CoA, whose acyl chain is 5-C long, was generated in situ from MCPA (Scheme 2, step ⓑ) using acyl-CoA synthetase [52]. MCPA was obtained either by enzymatic degradation of hypoglycin A with L-amino acid oxidase (Scheme 2, step ⓐ) or by chemical synthesis [53,54]. Incubation of GADH aerobically with MCPA-CoA as outlined in the legend to Fig. 3 results in a gradual bleaching of the typical oxidized flavin absorbance (Fig. 3) yielding a spectrum reminiscent of modified reduced flavins [55]. This formal reduction is not reversed by O_2 although GADH reduced by short chain acyl substrates is reoxidized by O_2 at an appreciable rate [50,51]. These results suggest that the active center of GADH has undergone drastic modification on MCPA-CoA treatment. As might be expected, incubation of the short chain specific enzyme, BDH, with the inhibitor under the same conditions leads to a much more rapid irreversible bleaching of the flavin spectrum.

Fig. 3

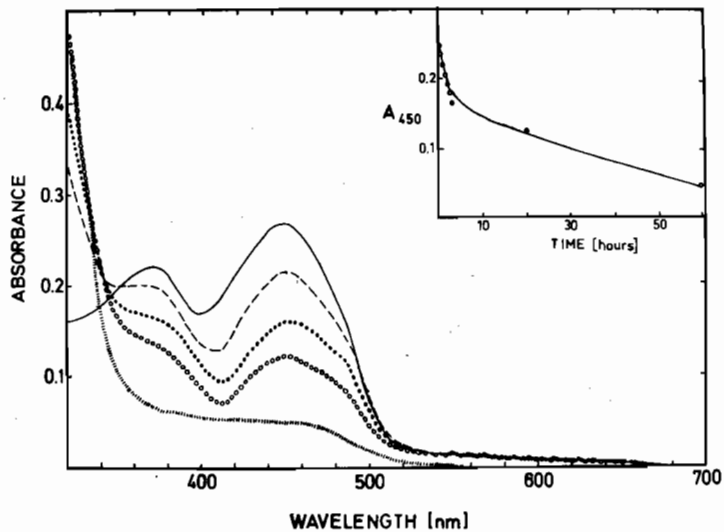


Fig. 3. Spectral course of the aerobic reaction of MCPA-CoA with general acyl-CoA dehydrogenase from pig kidney.
The enzyme, 2.2 nmol in 0.9 ml of 50 mM phosphate buffer pH 7.6 was incubated in a system generating MCPA-CoA in situ, containing 30 µM methylenecyclopropylacetate, Li-salt, 0.3 µM CoA, 60 µM ATP, 60 µM $MgCl_2$ and acyl-CoA synthetase (0.2 mg/ml, 1.7 units) in 0.1 ml of the same buffer. Curve (——) native enzyme. Curves (---), (...), (ooo) and (,,,,) were recorded after 3,5,20 and 60 min of incubation, the last curve after centrifugation in order to remove a slight turbidity. The insert shows the biphasic time dependence of the bleaching occurring at 450 nm.

With both enzymes, modification of the flavin chromophore is accompanied by an irreversible decrease in enzyme activity (shown for BDH in Fig. 4). The competitive inhibitor acetyl-CoA protects BDH against inactivation by MCPA-CoA (Fig. 4) again suggesting that inactivation occurs within the active center. It should be noted that the spectral changes observed in Fig. 3 are distinctly biphasic, a phenomenon also observed during reduction of GADH by short chain substrates [50,51]. Biphasic behaviour in the presence of MCPA-CoA might also indicate additional alkylation of the apoprotein as was observed with D-lactate dehydrogenase [22]. With BDH, the rate of inactivation is significantly faster than the rate of bleaching (Fig. 4), pointing to the same conclusion. In preliminary experiments, similar results to these described above have been obtained using purified MCPA-CoA.

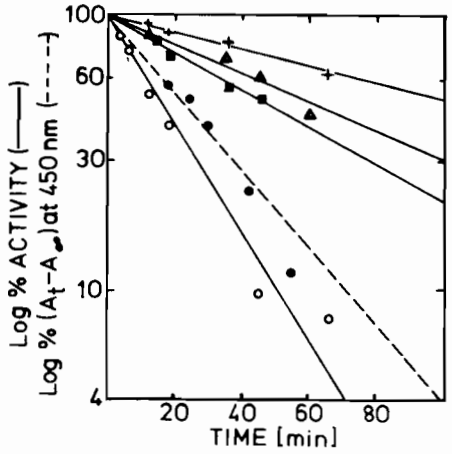


Fig. 4

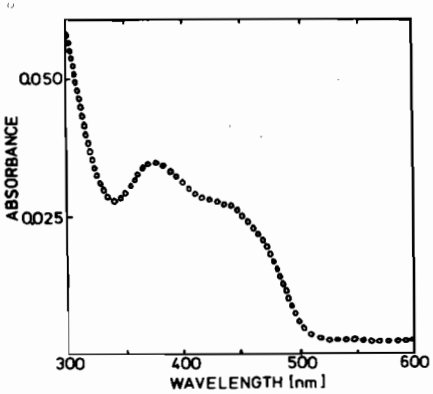


Fig. 5

Fig. 4. Course of inactivation of butyryl-CoA dehydrogenase (M. elsdenii) by MCPA-CoA and protective effect of acetyl-CoA as a competitive inhibitor.
The dehydrogenase, 48 nMol in 1.0 ml of 50 mM phosphate buffer pH 7.6 was incubated with 1.2 µM methylenecyclopropylacetate, Li-salt, 0.26 µM CoA, 6 µM ATP, 6 µM $MgCl_2$ and acyl-CoA synthetase (0.2 mg/ml, 1.7 units) and the enzyme activity was measured at the intervals shown by the method of [51]. Incubation in the absence (o-o) and in the presence of 0.6 (□-□), 1.2 (Δ-Δ) and 2.4 µMol (+-+) of acetyl-CoA. Curve (●-●) represents the decrease in absorbance at 450 nm, which occurs during the inactivation process and which was recorded in experiments similar to that of Fig. 3.

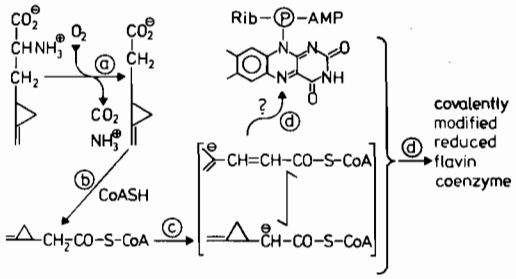
Fig. 5. Absorption spectrum of the chromophore obtained after inactivation of general acyl-CoA dehydrogenase from pig kidney and release from the protein.
The inactivation with MCPA-CoA was carried out as described in the legend of Fig. 3. The protein was dialyzed against 50 mM phosphate buffer, pH 7.6 and then denaturated with 5 % TCA. The spectrum shown was obtained after centrifugation of the protein precipitate, ether extraction of excess TCA and neutralization to pH 7.0

Neither the spectrum of the oxidized enzyme nor enzymatic activity are recovered after prolonged dialysis or gel filtration. The spectrum of the chromophore released from the enzyme on trichloroacetic acid treatment is shown in Figure 5. Thin layer chromatography of the supernatant reveals a blue fluorescent material in addition to some unmodified FAD. Similarly the fluorescence emission spectrum of the supernatant is composed of the emission of FAD (λ_{max} emiss. $\sim$ 530 nm), and of that of a blue fluorescing species with a λ_{max} at 460 nm.

These results suggest that MCPA-CoA fulfills the requirements for a suicide substrate for GADH and BDH. They also strongly suggest that irreversible inactivation occurs by covalent modification of the flavin. The alternative possibility that a modification of the active center occurs after reduction of the flavin cannot be ruled out rigorously, but appears very improbable as in such a case the normal chromophore of oxidized flavin should be recovered at least after protein denaturation. Although little can be said about the structure of the presumably modified flavin, it probably constitutes a type of modification similar to that previously encountered with L-lactate oxidase [19]. Experiments are currently in progress to determine the stoichiometry of inactivation and the structure of the modified flavin using (^{14}C)-labeled MCPA-CoA.

A plausible mechanism of inactivation of those acyl-CoA dehydrogenases susceptible to MCPA-CoA could involve deprotonation at the position of the thioester by an active center enzyme base (Scheme 4, step ⓒ). Precedents for generation of such a carbanion have been discussed earlier in this paper. Isomerization of this species would yield a (transient) conjugated diene carbanion which could conceivably be the nucleophile responsible for irreversible modification of the flavin (step ⓓ). While this is clearly speculation at present, it is hoped that elucidation of the structure of the modified flavin will permit detailed conclusions concerning the mechanism of action of the inhibitor to be drawn as proved possible with D-lactate dehydrogenase [21,22].

Scheme 4



A similar powerful inhibitor of GADH is the CoA derivative of 3,4-pentadienoic acid (Table 1). Incubation of the enzyme with a twofold molar excess of this substrate analog leads to a very rapid bleaching of the flavin absorption, which goes along with inactivation, and to formation of a species similar to that observed upon incubation with MCPA-CoA (Fig. 3). In contrast to the latter case, however, a partial recovery of the 450 nm absorbance is observed over several hours indicating a different mode of coenzyme modification. Thus from these criteria also 3,4-pentadienoyl-CoA can be considered to be a suicide inhibitor of acyl-CoA dehydrogenase.

D-Amino Acid Oxidase, and L-Amino Acid Oxidase

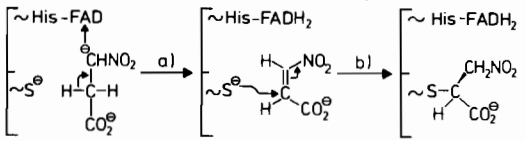
Vinylglycine and Propargylglycine two substrate analogs are oxidized catalytically by these two enzymes, and, most importantly they are complementary suicide substrates [56,57]. In fact L-amino acid oxidase is irreversibly inactivated by vinylglycine, but is not affected by propargylglycine, while with D-amino acid oxidase the reverse is the case. Inactivation occurs in both cases by reaction of the inhibitor with an active center amino acid residue. While the actual mechanisms of inactivation have not been elucidated in detail yet in the case of D-amino acid oxidase 1.5 - 1.7 molecules of inactivator are incorporated into the protein, and the active site His_{171}, Tyr_{259}, and probably Tyr_{263}, are modified [58]. Interestingly, Tyr_{259}, is the target of modification, when the enzyme is inactivated by chlorination with N-chloro-D-leucine [59,60].

Succinate Dehydrogenase

The toxicity of Indigofera endecaphylla to animals has long been suspected to be due to its content of 3-nitroproprionate [61]. This molecule is isoelectronic, in its dianionic form, with succinate, the normal substrate of succinate dehydrogenase. This enzyme was indeed

found to be irreversibly inactivated upon incubation with 3-nitroproprionate [62]. However, the inactivation was found not to result from a reaction of this molecule itself, e.g. by nucleophilic addition of the nitroproprionate ß-position to the flavin acceptor at N(5), as one might reasonably expect from analogy with the inactivation of D-amino acid oxidase with nitroalkane anions [14], and as was first postulated [62]. Instead, the actual inactivator is the oxidation product of 3-nitroproprionate, 3-nitroacrylate, and inactivation arises from alkylation of an active center -SH group via a Michaelis-type addition mechanism [63].

Scheme 5



CONCLUSIONS AND OUTLOOK

In most cases suicide inactivation can be attributed to a covalent modification of the flavin coenzyme (Table 1). In these cases the substrate function subject to oxidation is flanked by a chemical group, which has the capability of stabilizing the developing negative charge of the transient carbanion intermediate (cf. Scheme 1). In the normal catalytic event, the transfer of electrons occurs probably via covalent intermediates between the flavin N(5) position and the substrate Cα [7,9,14,15,16]. In the case of the suicide reaction, on the other hand, the reciprocal orientation of the delocalized suicide substrate carbanion charge (donor), and the oxidized flavin (acceptor) would be sufficiently different so as to lead to attack at other positions, resulting in the formation of stable covalent adducts. This hypothesis is in agreement with the present knowledge of flavin catalysis.

The present status of suicide inhibitors of both flavin and other enzymes could be expanded at will from the presently known systems simply by introduction of the known masked active groups into substrates of different enzyme classes, provided they work by a similar basic mechanism. Alternatively new suicide functions could be created and inserted into known substrates.

Clearly the physiological and pharmacological relevance of studies with suicide substrates does not need emphasis. On the other hand one should keep in mind that their mode of action involves destruction of enzymes rather than decrease of their catalytic rates. This might limit their actual application in therapeutics, where the ultimate goal remains the creation of enzyme inhibitors of very high specificity. A possible approach is to inactivate enzymes by mechanisms analogous to those of suicide substrates, but, however, via a mechanism that is slowly reversible.

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