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On the Definition and Classification of Mechanism-Based Enzyme Inhibitors

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Abstract: A formal mechanistic classification scheme for active site-directed enzyme inhibitors is described in order to provide a framework from which the meaning of the term "mechanism-based inhibitor" can be seen in relief. Attention is called to non-classical mechanism-based inhibitors as under-exploited in inhibitor design.

The drive towards ever more specific and potent enzyme inhibitors was transformed some twenty years ago by the emergence of the concept of the mechanism-based inhibitor.[1] Most of the earliest recognized and designed inhibitors of this type[2] were examples of what might be called the classical variety, where the normal enzyme-catalyzed reaction of the inhibitor as a substrate yielded a normal intermediate which, because of specific latent functionality built into the inhibitor, underwent covalent rearrangement with formation of a reactive functional group. The latter then reacted covalently with an active site component to inhibit the enzyme. More recently, however, other modes of inhibition, mechanistically quite distinct from the classical form, have been, and not unreasonably in many cases, also termed "mechanism-based."[3]

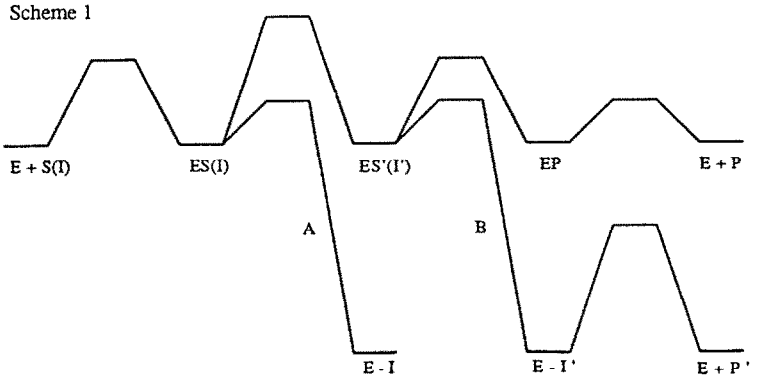
It is the purpose of this brief overview to propose, or, perhaps better, since few of the elements are in themselves novel, to propose to formalize, a mechanistic classification of inhibitors, such that the nature and range of mechanism-based inhibitors can be clearly defined. The basis for the proposed scheme is, first, the relationship between the inhibition pathway and the reaction coordinate describing the progress of a normal substrate, and, second, the nature of the chemistry, covalent or non-covalent, primarily responsible for the enzyme inhibition.

First, enzyme-specific inhibitors should be divided into two major and very different groups, Active Site-Directed Inhibitors and Allosteric Effectors. The former term is meant to imply that the designated inhibitors specifically interact with the enzyme active site because of their resemblance to substrates -- functional group modifying reagents that are not enzyme-specific are excluded. The latter include all inhibitors (or activators) whose action is effected through their interaction with sites other than the active site -- their action is of course mediated by networks of non-covalent interactions. This paper addresses classification of the former group.

Active Site-Directed Inhibitors

These are subdivided mechanistically with reference to the reaction-coordinate diagram of Scheme 1. According to this Scheme, a normal substrate (S) proceeds by way of what will be referred to as a linear reaction coordinate through ES, its first specifically-bound (non-covalent) complex with enzyme (E), the Michaelis complex, to EP the analogous product (P) complex (otherwise seen as the ES complex of the reverse reaction). ES′ corresponds to any one of the potentially many complexes between ES and EP,

Scheme 1



representing intermediate stages in the normal enzyme-catalyzed reaction. EI, EI′ E-I and E-I′ are complexes of the enzyme with the active site-directed inhibitor I; P′, which may differ from P, is a product of I via E-I′.

Two major classes of active site-directed inhibitor are then distinguished:

1. **Linear Reaction Coordinate Inhibitors** In this category, the inhibited enzyme species, EI or EI′, have structures which mimic those of the ES or ES′ complexes respectively, i.e. complexes in the direct linear progression from ES via ES′ to EP. Further subdivision is helpful:

(a) Covalent EI′ species formed from this group of inhibitors have undergone, at the active site, part of the covalent chemistry involved in the conversion of ES to EP, i.e. they mimic the structures ES′ or associated transition states. This group includes the "reaction coordinate inhibitors" of Christianson and Lipscomb.[4] They are, in general, incomplete or deactivated substrates, lacking some element of a real substrate that would enable them to proceed further to EP.

(b) Non-covalent Here only non-covalent interactions are found between E and I in EI or EI′. Three subclasses can be distinguished:

(i) S (or P) Analogs These are the classical fast-binding, reversible,[5] competitive inhibitors, including multisubstrate inhibitors.[7]

(ii) S′ Analogs

(iii) Transition State Analogs

In variants (ii) and (iii), the pathway of I to EI′ will not, in general, be that of S to ES′ since no enzyme-catalyzed covalent chemistry would occur. This difference may give rise to slow-binding phenomena with these inhibitors;[8] in addition, (ii), if ES′ is a high energy intermediate, and (iii) should be tight-binding inhibitors.[5]

2. **Branched Reaction Coordinate Inhibitors** E-I and E-I′, generated from these inhibitors via a branched pathway, differ structurally from ES and ES′ respectively in ways significant to the inhibition. It is useful to subdivide this group on the basis of the position of the branch, at EI or at EI′ (see Scheme 1), and the nature of the reactions giving rise to inhibition.

A. EI Branch E-I derives from EI prior to normal enzyme-catalyzed covalent chemistry, via path A in Scheme 1.

(a) Covalent These are classical active-site directed covalent inhibitors.

(b) Non-covalent These derive from a conformational rearrangement of EI, although not one related to catalysis. Slow-binding might be observed.

B. EI′ Branch Inert complexes E-I′ derive from EI′ subsequent to partial catalysis: path B in Scheme 1.[9] They may (slowly) yield a product P′. This group is subdivided on the basis of whether a covalent or non-covalent reaction, occurring en route between EI′ and E-I′, is directly responsible for the inactivation, and whether this reaction produces an intermediate that attacks and modifies the enzyme ("active") or not ("passive").

(a) Covalent

(i) Active In this group, formation of EI′, or facile covalent rearrangement of EI′, creates a reactive functional group that covalently reacts with the enzyme or an enzyme-bound cofactor. These are the "classical" mechanism-based inhibitors.[2]

(ii) Passive Here, covalent rearrangement of I′ in EI′ occurs to give a structure inert to further enzyme-catalyzed reaction. Conformational adaptation by the enzyme may also occur but the inhibition does not require this.[10]

(b) Non-covalent

(i) Active From EI′, I′ transforms into a structure that causes non-covalent (conformational) rearrangement of EI′ into E-I′. The transformation of I′ alone does not explain the inhibition.[11]

(ii) Passive Direct conformational relaxation of EI′ to E-I′.[9]

Thus, an attempt has been made to formally classify all active site-directed, in the sense defined above, enzyme inhibitors. It will be noticed that the term "mechanism-based" does not appear in the scheme. It could be included, if desired, in more than one way, either broadly, encompassing all of the above inhibitors -- defining mechanism-based as synonymous with active site-directed -- or more narrowly, either as a collective descriptor of class 1(a) plus class 2B, of class 2B alone, or perhaps even further restricted, to class 2B (a)(i), a return to its initial position.[12] The present author would prefer the class 2B option, where the term continues to describe a variety of branched mechanisms stemming from EI′. This would seem to be in the spirit of the original concept, broadened slightly to exclude from the definition the nature of the chemistry between EI′ and E-I′.

The available space does not permit an exhaustive assignment of specific inhibitors to these classes although some examples are mentioned in the footnotes. Familiarity with such assignment is readily achieved, however, by thoughtful perusal of previous reviews on mechanism-based inhibitors.[2,13] The latter, in general, have emphasized, and often classified mechanism-based inhibitors on the basis of, the enzymes inhibited, the specific chemical transformations involved in the inhibition, and the pharmaceutical application of mechanism-based inhibitors as drugs; class 2B (a)(i) has dominated the examples presented. The decision as to which class to assign an inhibitor would normally require detailed mechanistic studies and, in some cases, the crystal structure of the enzyme/inhibitor complex.

It is hoped that the perspective afforded by the classification Scheme suggested in this paper will help

focus impressions from the previous reviews, providing a useful basis for judgement as to whether a given inhibitor is best thought of as mechanism-based or not; at present there appears to be little generally-accepted explicit guidance available on this point. More importantly, it is hoped that this contribution will help provide a conceptual framework to assist future inhibitor design. In particular, it has recently become increasingly clear, from both experimental and theoretical advances, that very effective modulation of protein structure and dynamics, and thus of enzyme activity, can be achieved non-covalently. Since theoretical methods of treatment of non-covalent interactions in proteins are becoming so effective, it seems that the non-covalent and passive modes of mechanism-based inhibition [classes 2B(a)(ii) and 2B(b)] are fertile, although largely barren at present, fields for exploration and exploitation. Of these, class 2B(a)(ii) does not require prior detailed knowledge of enzyme structure and dynamics for inhibitor design, while class 2B(b) does.

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References and Notes

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2. (a) Rando, R. R. *Acc. Chem. Res.* **1975**, *8*, 281-288. (b) Abeles, R. H.; Maycock, A. L. *Acc. Chem. Res.* **1976**, *9*, 313-319; (c) Walsh, C. T. *Tetrahedron* **1982**, *38*, 871-909.
3. The relative merits of various synonyms for "mechanism-based inhibitor" have been discussed elsewhere[13c] and will not be addressed in this paper.
4. Christianson, D. W.; Lipscomb, W. N. *Acc. Chem. Res.* **1989**, *22*, 62-69. 5-Fluoro-2'-deoxyuridine monophosphate as a thymidylate synthetase inhibitor (Santi, D. V.; McHenry, C. S. *Proc. Natl. Acad. Sci. U.S.A.* **1972**, *69*, 1855-1857), 3-fluoro-3-deoxycitrate as a bacterial citrate lyase inhibitor (Rokita, S.; Srere, P.; Walsh, C. T. *Biochemistry* **1982**, *21*, 3765-3774), 2-deoxy-2-fluoro-D-glycosyl fluorides as glycosidase inhibitors (Withers, S. G.; Rupitz, K.; Street, I. P. *J. Biol. Chem.* **1988**, *263*, 7929-2932), and methionine sulfoximine as an inhibitor of glutamine synthetase (Meister, A.; Tate, S. S. *Annu. Rev. Biochem.* **1976**, *45*, 559-604), all often referred to in the literature as mechanism-based inhibitors, represent further examples.
5. The phenomenological and subjective terms fast- and slow-binding, reversible and irreversible, and tight-binding have no unique mechanistic meaning, and without further qualification, seem to this author to be unhelpful in classification except on purely practical grounds. "Slow-binding inhibitors" as reviewed by Morrison and Walsh,[6] for example, appear to include several of the classes of inhibitors proposed here.
6. Morrison, J. F.; Walsh, C. T. *Adv. Enzymol. Relat. Areas Mol Biol.* **1988**, *61*, 201-301.
7. Broom, A. D. *J. Med. Chem.* **1989**, *32*, 2-7.
8. Schloss, J. V. *Acc. Chem. Res.* **1988**, *21*, 348-355.
9. ß-Lactamase inhibitors appear to rather uniquely span the range of variants in this class. Reviews where the classification described in this paper is employed are available. (Pratt, R. F. *Design of Enzyme Inhibitors as Drugs*; Sandler, M.; Smith, H. J. Eds.; Oxford University Press: Oxford, 1989; pp. 178-205. Pratt, R. F. *The Chemistry of ß-Lactams*; Page, I. M., Ed.; Blackie: Glasgow; in press.)
10. Well-known examples are isatoic anhydride and other oxazinediones as chymotrypsin inhibitors (Moorman, A. R.; Abeles, R. H. *J. Am. Chem Soc.* **1982**, *104*, 6785-6787. Weideman, B.; Abeles, R. H. *Biochemistry* **1984**, *23*, 2373-2376).
11. An excellent example of this class is 3-benzyl-6-chloropyrone acting as a chymotrypsin inhibitor (Ringe, D.; Mottonen, J. M.; Gelb, M. H.; Abeles, R. H. *Biochemistry* **1986**, *25*, 5633-5638).
12. A combination of classes 2A(b) and 2B(a)(i) has been referred to as "branched-pathway inhibitors" with respect to ß-lactamases (Cartwright, S. J.; Waley, S. G. *Med Res. Rev.* **1983**, *3*, 341-382) and of classes 1(a), 2B(a)(ii) and 2B(b) as "crippled substrates" (Bey, P. *Actual. Chim. Ther.*, **1989**, *16*, 111-122). The existence of non-covalent mechanism-based inhibitors has been remarked, in passing, by many authors, most notably by Silverman and Hoffman (Silverman, R. B.; Hoffman, S. J. *Med. Res. Rev.* **1984**, *4*, 415-447).
13. (a) Palfreyman, M. G.; Bey, P.; Sjoerdsma, A. *Essays in Biochem* **1987**, *23*, 28-81. (b) Silverman, R. B. *J Enzyme. Inhib* **1988**, 2, 73-90. (c) Silverman, R. B. *Mechanism-Based Enzyme Inactivation: Chemistry and Enzymology*; CRC Press: Boca Raton, FL, 1988; Vols. 1 and 2. (d) Ator, M. A.; Ortiz de Montellano, P. R. *The Enzymes*, 3rd Ed.; Sigman, D. S.; Boyer, P D..; Eds.; Academic Press: San Diego, CA; Vol. XIX, 1990; pp. 214-282.