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COMMENTARY

ESTERASES: PROBLEMS OF IDENTIFICATION AND CLASSIFICATION

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Abstract—The problems of esterase classification are discussed and some ideas presented to clarify the present confused state of the classification systems.

Strictly speaking, the term esterase refers only to enzymes which are concerned with the hydrolysis of esters. Examples of ester hydrolysis are shown in Fig. 1.

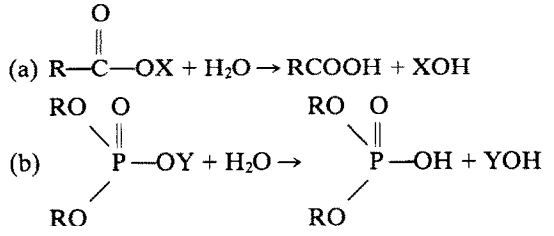

Fig. 1. Examples of ester hydrolysis. R = Alkyl group. X and Y = "leaving" groups.

Esterases are classified as hydrolases [1], a large and diverse group of enzymes which can hydrolyse peptides, amides and halides in addition to esters. The fact that enzymes which show esterase activity can hydrolyse non-ester bonds as well, raises serious problems over terminology and classification [2]. It is now clear that some esterases at least are of wide substrate specificity and are capable of hydrolysing both endogenous and exogenous esters of widely differing structures [3]. This raises the question of what the normal physiological role of these esterases is. What are their normal substrates? To what extent is their function to hydrolyse foreign esters? The broad specificity of some of them suggests a significant role in degrading exogenous esters.

The present discussion will be concerned mainly with the problems of characterising and classifying those esterases which are involved in the metabolism of esters which are foreign compounds.

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ESTERASE ACTIVITY TOWARDS FOREIGN SUBSTRATES

Many pesticides, drugs and other man-made chemicals are esters. Indeed, three of the four main classes of insecticides fall into this category, namely, the organophosphorus, carbamate and pyrethroid groups.

The cleavage of esters has often been observed in complex systems; in homogenates, sub-cellular fractions or *in vivo*. Frequently, such degradation has been attributed to esterases, largely or entirely on the evidence of the overall chemical change. The enzyme(s) responsible have not been isolated and the mechanism of degradation has not been tested. The pitfalls associated with this approach are obvious.

The removal of both alkyl groups and leaving groups (X and Y in Fig. 1) from organophosphates such as paraoxon and malaoxon was often attributed to 'phosphatases', or 'arylesterases' in the earlier literature. Although the products of such organophosphate metabolism are the same as would be produced by esterase attack, it has been shown that both types of reaction can be mediated by entirely different enzyme systems: Microsomal monoxygenase can remove alkyl groups (O-dealkylation) and leaving groups, from many organophosphates [4, 5]. Similarly, glutathione-S-transferases present in the soluble fraction of mammalian liver homogenates or insect homogenates can also remove both alkyl groups and leaving groups [6, 7]. Thus, much of the "apparent" hydrolysis of an organophosphate in a complex system may be due to non-hydrolysing enzymes.

Some confusion has also arisen from the use of the term "DFPase". DFP'ase is used to describe the enzyme(s) responsible for the hydrolysis of DFP (diisopropylfluorophosphate) (Fig. 2).

The enzyme(s) responsible for DFP hydrolysis is classified as an 'A'-esterase because it catalyses the hydrolysis of an organophosphate, although it differs from other 'A'-esterases in its requirement for Mn^{2+}

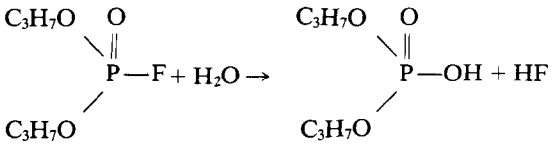

Fig. 2. DFP hydrolysis.

or Co^{2+} and its non-dependence upon Ca^{2+}. It is also debatable as to whether the reaction can be classified alongside other ester hydrolyses. The products of "classical" ester hydrolysis are the constituent acid and alcohol, in the case of DFP, however, two acid products result from hydrolysis.

PURIFICATION

In spite of their toxicological significance very few esterases which hydrolyse foreign compounds have actually been purified. Purification has for a long time been limited to the identification of esterase bands separated by electrophoresis. A few attempts have been made to purify toxicologically active esterases, some of which are shown in Table 1.

Table 1 illustrates the somewhat haphazard approach to the purification and characterization of esterases by workers in the field, little attempt being made to fully characterise the purified enzymes (hence the gaps evident in Table 1) with the possible exception of Arndt *et al.* [3] and Lombardo *et al.* [12, 13]. In the majority of cases quoted in Table 1, little or no investigation into the toxicological significance of the purified esterases was undertaken. When pure samples of esterases are available it is possible to test activity over a wide range of substrates so aiding the classification of them. It is also possible to test the activity *in vivo* by injecting the enzyme into animals to assess its affects on detoxication of foreign esters. Such a study was undertaken by Main [17] on the effects of A-esterase injection on organophosphate toxicity.

ATTEMPTS TO CLASSIFY ESTERASES

The classification of esterases (which hydrolyse xenobiotics) is in a confused state. Sometimes terms are used which describe only particular reactions. Thus for example "phosphatases" hydrolyse P–O–C bonds, "amidases" hydrolyse amide bonds and "carboxyesterases" hydrolyse carboxy–ester bonds. Even disregarding the cases where such reactions are mediated by enzymes other than esterases, this approach is an unsatisfactory one. It is becoming increasingly clear that esterases tend to have broad and overlapping substrate specificities [2]. Thus, one enzyme can be both an amidase and a phosphatase [2] or a phosphatase and a carboxyesterase. Furthermore, a single esteratic reaction is frequently mediated by several enzymes.

Augustinsson [18, 19] separated esterases by simple electrophoresis and developed an esterase classification based on the hydrolysis of simple substrates such as phenylacetate, triacetin and butyrycholine by the fractions so obtained. This classification distinguishes between aliesterases (hydrolysing aliphatic substrates), arylesterases (hydrolysing aromatic substrates) and cholinesterases (hydrolysing choline esters). It soon became apparent that there was no clear distinction between ali- and arylesterases. Aliesterase preparations hydrolyse arylesters [2] and vice versa.

Aldridge [20] produced a classification based upon the interaction of esterases with organophosphates. 'A'-esterases hydrolyse organophosphates, B-esterases are inhibited by them and C-esterases (added some time later [21]) do not interact with them. Although a very simplistic approach, this has some virtues. According to Aldridge the basic mechanism of interaction is essentially the same with both 'A' and 'B' esterases. The only difference is that the rate of reactivation of phosphorylated 'B'-esterases is very much slower than that of 'A'-esterases. In 'B'-esterases a serine residue at the active site is phosphorylated, whereas this does not appear to occur with 'A'-esterases. It has been suggested that during the evolution of esterases, the serine hydroxyl group of 'B'-esterases, has been replaced by an —SH group at the active centre [22]. A further development of the system of Aldridge could be to divide 'A'-esterases into DFP'ases and the rest, to take into account the essentially different characteristics of DFP'ases.

The classification of Aldridge has some practical value in toxicology. Cholinesterases (a type of B-esterase) represent the site of action of organophosphorus and carbamate pesticides. Also 'A'-esterases show an interesting phylogenetic distribution. There is high 'A'-esterase activity in mammalian plasma associated with high density lipoprotein [23], but hardly any in the plasma of birds [24]. This provides an explanation for the hypersensitivity of birds to organophosphates such as diazinon, pirimiphos-methyl, pirimiphos-ethyl and coumaphos.

It appears that certain insects lack 'A'-esterase. In a study of three strains of *Tribolium castaneum*, no 'A'-esterase activity was detected when paraoxon and pirimiphos-methyloxon were used as substrates [25]. Devonshire [16] conducted a detailed study upon the 'carboxylesterase' present at high levels in certain resistant strains of aphids. After treatment with paraoxon, the reactivation rate of this enzyme was very slow, clearly indicating that it was a 'B'-esterase according to the classification of Aldridge. If 'A'-esterase activity is absent from many strains of insect, this could provide at least a partial explanation of their very high sensitivity to organophosphorus insecticides in comparison to mammals.

Both methods of classification of toxicologically important esterases described above are far from real. The terms ali- and arylesterases do not take into account the wide substrate specificity and overlap of many esterases. On the other hand the method of Aldridge takes little account of substrate specificity apart from that towards organophosphates and (perhaps) carbamates. This classification, however, does not appear to have problems of overlap each of the esterases so far investigated fitting into just one of the three categories. Thus, by dividing esterases into three groups this method of classification could provide the basis upon which a more elaborate system of classification could be built.

In discussing their investigation of unspecific car-

Table 1. Some examples of esterase purification, with properties of the purified enzymes

Enzyme	Source	Substrates	pH Optima	Co-factors	Inhibitors	Molecular weight	Comments	Ref.
Paraoxonase	Sheep serum	Paraoxon	—	—	Barium EDTA	—	Only partially purified	[8]
Carboxyesterase	Rat liver microsomes	*trans*-Pyrethroids Malathion Phenthoate *p*-Nitrophenylacetate	—	—	Organophosphates Carbamates	74,000	Has the character of a 'B'-esterase	[9]
Carboxyesterase	Human liver	β-Naphthylacetate Aspirin Clofibrate Procaine	6.5		Paraoxon DFP	180,000	Has the character of a 'B'-esterase	[10]
DFP'ase	Hog kidney	Dialkylfluorophosphates Tabun, Sarin		Mn^{2+}/Co^{2+} Two unknown co-factors	*p*-Chloro-mercuribenzoate	—		[11]
Carboxyesterase	Human pancreatic juice	*p*-Nitrophenylacetate Vitamin E acetate Cholesterololeate	8.0			100,000	A glycoprotein	[12, 13]
Carboxyesterase isoenzymes	Rat liver microsomes	Anilides Aliphatic esters Triglycerides	9–10	—	Paraoxon	180,000	2 enzyme forms each composed of 3 sub-units	[31]
Arylesterase	Bovine plasma	Phenylacetate				440,000	Postulated to be a HDL_2 particle	[14]
A-esterase multiple forms	Sheep serum	Paraoxon Pirimiphosmethyloxon	8.0–8.5	Ca^{2+} plus unknown co-factor(s)	EGTA Triton X-100	200,000+	4 forms, postulated to be HDL_2 particles	[15, 23]
Carboxyesterase	Aphid (*Myzus persicae*) homogenate	α-Naphthylacetate			Paraoxon		See text [16]	

boxylesterase, Junge and Krisch [2] raise some important issues. In the first place, the pure enzyme has very wide substrate specificity, and will hydrolyse amides in addition to true carboxylesters. They go on to argue that this wide specificity makes certain aspects of I.U.B. classification [26] untenable.

This proposes the following groups:

3.1.1.1 Carboxylesterase (synonymous with aliesterase, 'B'-esterase), systematic name carboxylic-ester hydrolase.

3.1.1.2 Arylesterase (synonymous with 'A'-esterase and paraoxonase), systematic name aryl-ester hydrolase.

However, 3.1.1.1 does hydrolyse aryl-esters and 3.1.1.2 does hydrolyse ali-esters, so there is no logical basis for the terms aliesterase and arylesterase. Furthermore, it is clear from recent work that the term arylesterase is not synonymous with 'A'-esterase or paraoxonase [15, 25]. Preparations without paraoxonase activity show arylesterase activity. Thus there are strong reasons for removing the I.U.B. categories 3.1.1.1 and 3.1.1.2 as they stand at present.

When discussing esterase classification it is relevant to ask the question 'how active must a protein be in catalysing a hydrolysis before it can be termed an esterase?' Several proteins will hydrolyse esters, e.g. α-chymotrypsin and aldehyde dehydrogenase will both hydrolyse *p*-nitrophenylacetate although the turnover rates at 0.9 and 54 per enzyme molecule respectively are very much lower than for the same substrate with pig liver esterase at 41,000 [2]. Albumin will hydrolyse paraoxon [27] and α-naphthylacetate (Mackness, unpublished data) although very slowly compared to other enzymic components of the serum.

With this in mind a definition of what is a true esterase is required. This definition should include a turnover number in the order of many thousands for any purified enzyme with ester substrates before the enzyme can be called a true esterase to distinguish the esterases from other proteins which are able to hydrolyse some esters but at a slower rate than this. Such an approach would also clarify the point that organophosphates are not true substrates of B-esterases even though they are turned over very slowly.

CONCLUSION

Much of the work upon the apparent hydrolytic metabolism of foreign esters, including the organophosphorus insecticides, has been performed upon complex systems where it has not even been clear to what extent esterases are involved in the transformations. To resolve this problem and to properly characterise the enzymes involved it is necessary to purify them. Progress in this field has been very slow and relatively few esterases have been purified.

Following this approach it should be possible to develop a more logical and systematic classification of the esterases than is possible at present. The system of Aldridge may still provide a useful starting point in that it appears to be fairly clear cut, and based upon a real difference between three groups of esterases. It is also clear, however, that it throws little light upon the substrate specificity of the esterases which are encountered in biochemical toxicology. One of the problems of characterisation, however, is that certain forms of esterases (e.g. unspecific carboxylesterase) have very wide substrate specificity. Indeed, this is to be expected if esterases have been developed to detoxify a wide range of esters of exogenous origin.

Any new system of classification should start with a characterisation of purified forms of the enzymes and be wide ranging in its approach. It should extend as widely as possible through the different groups of the animal kingdom. A phylogenetic approach such as this may lead to some understanding of the evolution—and the function—of different forms of esterases which metabolise xenobiotics. The characterization of esterases which provide a resistance mechanism against insecticides should throw new light on the question of the evolution of these enzymes in response to the selective pressure of exogenous esters.

Recent work with esterases, as with other enzymes concerned with xenobiotic metabolism has produced evidence of multiple forms. Sheep serum contains at least four peaks of 'A'-esterase activity toward paraoxon which can be isolated by preparative polyacrylamide gel electrophoresis [15]. Two of these paraoxonase peaks also hydrolyse pirimiphos-methyloxon and all four hydrolyse the classical "arylesterase" substrate phenylacetate. Indeed some current ideas on the development of xenobiotic metabolizing enzymes [28] suggest that the duplication of genes leading to modification may be important in their evolution. The use of cloning techniques for genes should promote the study of such groups of enzymes, and, if applicable to esterases, should aid the development of a better system of classification.

REFERENCES

1. M. Dixon and E. C. Webb, *Enzymes*, 3rd edition. Longman, London (1979).
2. W. Junge and K. Krisch, *Mol. cell. Biochem.* **1**, 41 (1973).
3. R. Arndt, H. E. Schlaak, D. Uschtrin, D. Sudi, K. Michelssen and W. Junge, *Hoppe-Seyler's Z. fur Physiol. Chem.* **359**, 641 (1978).
4. R. S. H. Yang, E. Hodgson and W. C. Dauterman, *J. agric. fd Chem.* **19**, 10 (1971).
5. C. Donninger, D. H. Hutson and B. Pickering, *Biochem. J.* **102**, 26p (1967).
6. N. Motoyama, and W. C. Dauterman, *Pestic. biochem. Physiol.* **2**, 170 (1972).
7. N. Motoyama and W. C. Dauterman, *Pestic. biochem. Physiol.* **2**, 113 (1972).
8. A. R. Main, *Biochem. J.* **74**, 10 (1960).
9. T. Suzuki and J. Miyamoto, *Pestic. biochem. Physiol.* **8**, 186 (1978).
10. M. Inoue, M. Morikawa, M. Tsuboi, Y. Ito and M. Sugiura, *Jap. J. Pharmac.* **30**, 529 (1980).
11. J. A. Cohen and M. G. P. J. Warringa, *Biochim. biophys. Acta* **26**, 29 (1957).
12. D. Lombardo, O. Guy and C. Figarella, *Biochim. biophys. Acta* **527**, 142 (1978).
13. D. Lombardo, P Deprez and O. Guy, *Biochimie* **62**, 427 (1980).

14. M. M. Don, C. J. Masters and D. J. Winzor, *Biochem. J.* **151**, 625 (1975).
15. M. I. Mackness and C. H. Walker, *Biochem. Pharmac.* (in press).
16. A. L. Devonshire, *Biochem. J.* **167**, 675 (1977).
17. A. R. Main, *Can. J. biochem. Physiol.* **34**, 197 (1956).
18. K. B. Augustinsson, *Acta. Chem. Scand.* **13**, 571 (1959).
19. K. B. Augustinsson, *Ann. N.Y. Acad. Sci.* **94**, 844 (1961).
20. W. N. Aldridge, *Biochem. J.* **53**, 110 (1953).
21. F. Bergmann, R. Segal and S. Rimon, *Biochem. J.* **67**, 481 (1957).
22. K. B. Augustinsson, *J. histochem. Cytochem.* **12**, 744 (1968).
23. M. I. Mackness, An investigation into the esterases of sheep and *Tribolium castaneum* (Herbst.) with particular reference to sheet serum 'A'-esterases. PhD Thesis, University of Reading (1982).
24. C. J. Brealey, C. H. Walker and B. C. Baldwin, *Pestic. Sci.* **11**, 546 (1980).
25. M. I. Mackness, C. H. Walker, D. G. Rowlands and N. R. Price, *Comp. biochem. Physiol.* **74C**, 65 (1983).
26. *Enzyme Nomenclature (1978)*—Recommendation 1978 of the Nomenclature Committee of the International Union of Biochemistry. Academic Press, New York (1979).
27. E. G. Erdös and L. E. Boggs, *Nature (London)* **190**, 716 (1961).
28. D. W. Nebert and N. Masahiko, *Biochem. Pharmac.* **31**, 2311 (1982).