Distinction between 'A'-esterases and arylesterases

Implications for esterase classification

Michael I. MACKNESS,*‡ Helen M. THOMPSON,* Anthony R. HARDY† and Colin H. WALKER*
*Department of Physiology and Biochemistry, University of Reading, P.O. Box 228, Whiteknights, Reading RG6 2AJ, Berks., U.K., and †Ministry of Agriculture, Fisheries and Food, Tangley Place, Worplesdon, Surrey, U.K.

'A'-esterase activities (substrates paraoxon and pirimiphos-methyloxon) and arylesterase activities (substrate phenyl acetate) were assayed in the sera of 14 species of birds representing seven different orders and 11 species of mammal representing five different orders. Ten species of birds had no detectable 'A'-esterase, and the remaining four species only low activity, yet all birds showed considerable arylesterase activity (16.8–99.3 μmol/min per ml of serum). Ten species of mammal showed both 'A'- and 'aryl'-esterase activities. In humans, gel filtration of serum completely separated peaks representing paraoxonase and arylesterase activities. Thus, in both birds and humans, serum enzymes exist that express arylesterase activity but not 'A'-esterase activity. These findings suggest that a distinction should be made between these two types of esterase in future classifications.

INTRODUCTION

Recently several authors have drawn attention to inconsistencies in the present NC-IUB (Nomenclature Committee of the International Union of Biochemistry) classification of general esterases (Pen & Beintema, 1986; Walker & Mackness, 1983; Junge & Krisch, 1973). Problems have arisen in attempting to make a satisfactory classification because very few of the enzymes have been purified, because of their wide substrate specificities and because of the failure, so far, to identify them with particular 'in vivo' substrates.

An important group of enzymes falling into this category is that of the so-called 'aryl-ester hydrolases' (EC 3.1.1.2; NC-IUB, 1984), henceforth 'arylesterases', which includes the 'A'-esterases that are able to hydrolyse paraoxon (oo-diethyl-op-nitrophenyl phosphate) and other organophosphates (Aldridge, 1953a,b). A consistent classification of this group is a matter of current concern because paraoxonase activity is now known to be related to two pathological conditions: (1) the enzyme(s) is linked genetically to cystic fibrosis (Eiberg et al., 1985; Schmiegelow et al., 1986), and (2) relatively low levels of serum paraoxonase have been found in cases of myocardial infarction (McElveen et al., 1986).

Certain lines of evidence suggest that 'A'-esterase activity and arylesterase activity may be expressed by different enzymes. Thus, after preparative polyacryl-amide-gel electrophoresis of sheep serum, 'A'-esterase activity (paraoxon substrate) and arylesterase activity (phenyl acetate substrate) were found to be distributed differently in the resulting fractions (Mackness & Walker, 1983). By using the same substrates, human serum 'A'-esterase activity was found to have a biphasic distribution in several studies of Caucasian populations (Krisch, 1968; Carro-Ciampi et al., 1981; Eckerson et al., 1983; McElveen et al., 1986; Reiner et al., 1987), whereas arylesterase activity had a unimodal distribution in two of these populations (Simpson, 1971; Lorentz et al., 1979; Reiner et al., 1987).

In spite of these differences, some authors still describe paraoxon and phenyl acetate as two substrates of the same enzyme (La Du & Eckerson, 1984a,b). We report here some investigations which throw further light on the classification of esterases of this type.

MATERIALS AND METHODS

Sources of serum

Serum was prepared by centrifugation from un-heparinized blood collected from mammals and birds as previously described (Brealey et al., 1980). Blood was obtained from Wistar rats, New Zealand White rabbits, white mice (strain CD) and sheep (Finnish Land-race × Dorset Horn), chickens and Japanese quail kept at the University of Reading. Blood from goat, ox and pig was obtained from Newbury Abattoir (Newbury, Berks., U.K.). Avian blood samples were obtained from live specimens of cormorant, shag, puffin, razorbill and guillemot (collected under licence as part of a National Environmental Research Council-sponsored project) and Canada goose, pigeon and starling, supplied by the Ministry of Agriculture, Fisheries and Food, Tolworth, Surrey, U.K. Samples of serum from mute swan, great tit, tree sparrow, bee-eater, capybara, cat and badger were gifts from J. Blakey (Edward Gray Institute, University of Oxford, Oxford, U.K.). Human serum samples were obtained from the Royal Berkshire Hospital, Reading, Berks., U.K. All serum samples were stored at -20 °C before assay for enzyme activity. Esterase activity was stable for 6 months at this temperature.

Assays

Enzyme assays were performed by the methods described in the cited references: 'A'-esterase activity towards paraoxon by monitoring of generated p-nitrophenol with a recording spectrophotometer (see McElveen et al., 1986), and pirimiphos-methyloxon by

---

‡ To whom correspondence and reprint requests should be addressed.

Table 1. 'A'-esterase and arylesterase activities in the sera of birds and mammals

Assays were conducted on individual serum samples. The sources of sera and information on animal strains are given in the Materials and methods section. The Practical limits of detection of the assays were: 1 μmol of p-nitrophenol generated/min in the paraoxon assay, 0.05 nmol of hydroxypyrimidine generated/min in the pirimiphos-methyloxon assay, and 120 nmol of phenol generated/min in the phenyl acetate assay. Abbreviations/symbols: *, sex unknown; N.D., not detectable; −, not determined.

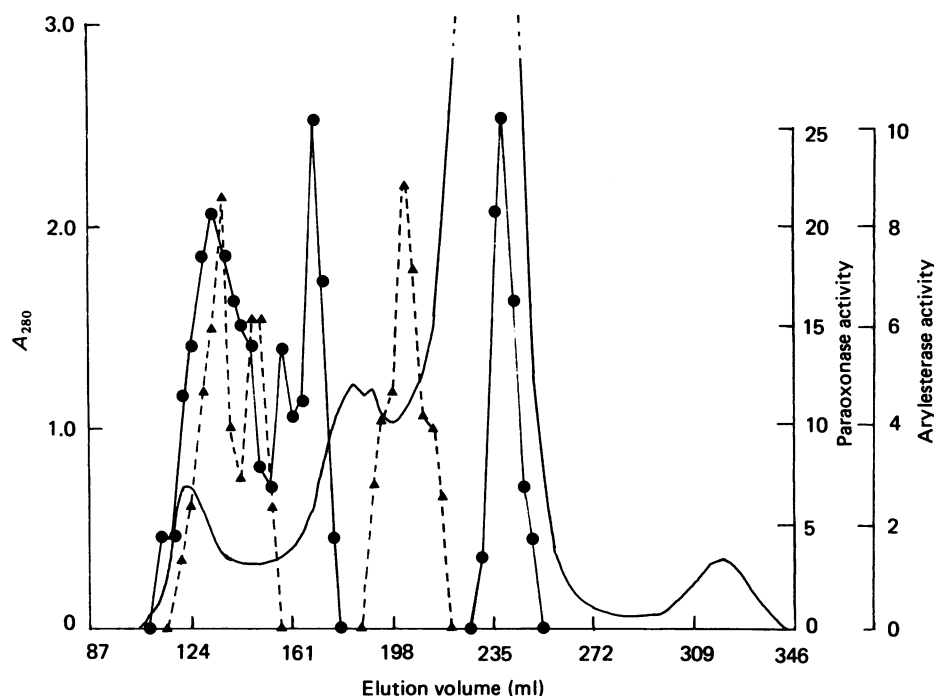|					Substrate concn. (mM)….	Paraoxonase (nmol/min per ml of serum) 2	Pirimiphos-methyloxonase (nmol/min per ml of serum) 4	Phenyl-acetate esterase (μmol/min per ml of serum) 5
Class	Order	Family	Species	Number and sex				
Birds	Pelicaniformes	Phalacrocoracidae	Cormorant (Phalacrocorax carbo)	3♂		N.D.	2.0±0	−
			Shag (Phalacrocorax aristotelis)	2♀, 1♂		N.D.	50	31.9±10.6
	Anseriformes	Anatidae	Canada goose (Branta canadensis)	4♀, 4♂		N.D.	N.D.	99.3±6.4
			Mute swan (Cygnus olor)	2♂		0.8±0.4	−	96.5±23.7
	Galliformes	Phasianidae	Japanese quail (Coturnix coturnix japonica)	4♀, 4♂		N.D.	N.D.	−
			Chicken (domestic form of Gallus gallus)	3♂ adult		N.D.	N.D.	36.2±6.4
				3♂ juvenile		N.D.	−	16.8±0
	Charadriiformes	Alcidae	Puffin (Fratercula arctica)	3♂		N.D.	N.D.	29.4±4.2
			Razorbill (Alca torda)	3♀		N.D.	N.D.	21.0±3.7
			Guillemot (Uria aalge)	3♀		N.D.	55.5±18.1	44.6±13.3
	Columbiformes	Columbidae	Pigeon (domestic form of Columba livia)	3*		N.D.	N.D.	69.8±2.8
	Passeriformes	Sturnidae	Starling (Sturnus vulgaris)	4*		N.D.	−	86.6±9.6
		Paridae	Great tit (Parus major)	3*		N.D.	−	75.7±0.6
		Fringillidae	Tree sparrow (Passer montanus)	3*		N.D.	−	69.8±10.5
	Coraciformes	Meropidae	Bee-eater (Merops apiaster)	7*		4×N.D. 3 = 5.4±2.5	−	27.5±3.2
Fish			Trout (Salmo gairdneri)	1♂		N.D.	−	26.8±2.0
Mammals	Rodentia		Mouse	5♂		44.2±9.8	920±40	48.8±10.5
			Rat	5♀ 5♂		57.3±3.9	2020±130	37.9±4.2
			Capybara (Hydrochoerus capybara)	6*		4×N.D. 2×0.8	−	70.3±10.7
	Lagamorpha		Rabbit	5♂		675.5±15.3	−	8.1±3.9
		Bovidae	Sheep	5♀		68.7±1.5	2910±50	31.1±16.6
			Goat	5♀		79.3±0.5	−	44.6±14.8
			Ox	5♂		65.6±4.3	−	27.7±2.1
	Suidae		Pig	5♀		31.6±0	−	95.0±2.1
	Carnivora		Cat, feral	3*		71.5±0.6	−	16.8±7.6
			Badger (Meles taxus)	2*		23.8±0.4	−	113.5±12.5
			Human	Pool of 2♀ and 4♂		41.1±3	−	71.5±14.2

Fig. 1. Separation of human serum 'A'-esterase and arylesterase activities by gel filtration on sepharose 6B

The method used is described in the Materials and methods section. The total volume of the column was 480 ml and the void volume 124 ml. ——, Protein; ▲, 'A'-esterase activity towards paraoxon (nmol of p-nitrophenol generated/min per fraction); ●, arylesterase activity (μmol of phenol generated/min per fraction).

separation of product from substrate by reverse-phase h.p.l.c. (Brealey *et al.*, 1980). Arylesterase activity was assayed using phenyl acetate as substrate and subsequent chromogenic determination of the phenol released (Lorentz *et al.*, 1979).

Gel filtration of human serum

Human serum [6 ml, containing 5% (w/v) sucrose] was applied to a column (30 cm × 4.5 cm; Amicon Corp.) containing Sepharose 6B (Pharmacia) gel-filtration medium equilibrated with 0.02 M-Tris/HCl buffer, pH 8.0, containing 1 mM-$CaCl_2$. Elution of the column was at 20 ml/h with the above buffer, and 5 ml fractions were collected. Individual fractions were assayed for protein (A_{280}), paraoxonase and arylesterase activities.

RESULTS AND DISCUSSION

Sera of 14 species of bird, representing seven orders and nine families, hydrolysed phenyl acetate and therefore had arylesterase activity (Table 1). A wide range of arylesterase activities was found, ranging from 16.8 μmol of phenol produced/ml of serum in the juvenile chicken to 99.3 μmol in the Canada goose. However, no 'A'-esterase activity could be found in the serum of ten species of bird, with only very low activities in the remaining four species.

Mammalian sera, on the other hand, showed both 'A'-esterase and arylesterase activities (Table 1), with the exception of that of the capybara, where four individuals out of six had no 'A'-esterase activity. The ratios of activities indicate, however, that different enzymes are responsible for these activities, i.e. high 'A'-esterase

activity (as in the rabbit), does not necessarily correspond to high arylesterase activity.

Gel filtration of human serum (Fig. 1) showed the presence of two major peaks of paraoxonase activity and three of arylesterase activity. None of the peaks of arylesterase activity correspond with those of para-oxonase activity; two peaks of arylesterase activity and one of paraoxonase activity did not overlap at all, indicating that different enzymes are responsible for these two activities in human serum. Thus there are esterases in the serum of birds and humans which show marked arylesterase activity, yet no measurable 'A'-esterase activity, towards paraoxon and/or pirimiphos-methyloxon. Also, the arylesterases of birds such as the starling, guillemot and tree sparrow are completely inhibited by a concentration of 10^{-4} M-paraoxon, and thus are 'B'-esterases, not 'A'-esterases, according to the classification of Aldridge (1953a,b).

It is concluded that the serum arylesterase of at least ten of the species of birds possess no measurable 'A'-esterase activity, so a satisfactory classification will need to distinguish between these two types of activity. Further, there are distinct enzymes in human serum showing arylesterase and paraoxonase activities respectively, an observation leading to the same conclusion.

It is noteworthy that no 'A'-esterase activity was detected in organophosphate-resistant strains of the aphid *Myzus persicae* (Devonshire, 1977), or in one organophosphate-susceptible and two organophosphate-resistant strains of the rust-red flour beetle (*Tribolium castaneum*), which showed high arylesterase activity (Mackness *et al.*, 1983).

The division into 'A'- and 'B'-esterases may still be useful with regard to the development of a more

satisfactory classification. Organophosphates evidently inhibit 'B'-esterases, e.g. cholinesterase, by phosphorylation of serine groups at the active site (Eto, 1974), although this has yet to be proved for all enzymes of the group. It is believed that no such phosphorylation occurs at the active site of 'A'-esterases, which can effectively hydrolyse organophosphates. If it can be shown that the difference between 'A' and 'B'-esterases with regard to their interaction with organophosphates is the consequence of a fundamental difference in their active sites, then it may provide a useful distinction in a new classification of esterases. Such an approach could get round a major problem with esterase classification. Some of these enzymes have wide substrate specificities and may be concerned with the detoxication of naturally occurring lipophilic esters. If this interpretation is correct, such esterases have no 'in vivo' substrates upon which a classification as proposed by Pen & Beintema (1986) could be based. Other criteria (e.g. structure of the active site) would need to be used to develop a satisfactory classification.

REFERENCES

Aldridge, W. N. (1953a) Biochem. J. 53, 110–117
Aldridge, W. N. (1953b) Biochem. J. 53, 117–124
Brealey, C. J., Walker, C. H. & Baldwin, B. C. (1980) Pestic. Sci. 11, 546–554

Carro-Ciampi, G., Kadar, D. & Kalow, W. (1981) Can. J. Physiol. Pharmacol. 59, 904–907
Devonshire, A. L. (1977) Biochem. J. 167, 675–683
Eckerson, M. W., Romson, J., Wyte, C. & La Du, B. N. (1983) Am. J. Hum. Genet. 35, 214–227
Eiberg, H., Mohr, J., Schmiegelow, K., Nielson, L. S. & Williamson, R. (1985) Clin. Genet. 28, 265–271
Eto, M. (1974) Organophosphorus Pesticides: Organic and Biological Chemistry, CRC Press, Cleveland, OH
Junge, W. & Krisch, K. (1973) Mol. Cell. Biochem. 1, 41–52
Krisch, K. (1968) Z. Klin. Chem. Klin. Biochem. 1, 41–45
La Du, B. N. & Eckerson, H. W. (1984a) Fed. Proc. Fed. Am. Soc. Exp. Biol. 43, 2338–2341
La Du, B. N. & Eckerson, H. W. (1984b) in Genetic Variability in Responses to Chemical Exposure (Banbury Report No. 16) (Omenn, G. S., ed.), pp. 167–177, Cold Spring Harbor Laboratory Publishers, Cold Spring Harbor
Lorentz, J. K., Flatter, B. & Augustin, E. (1979) Clin. Chem. 25, 1714–1720
Mackness, M. I. & Walker, C. H. (1983) Biochem. Pharmacol. 32, 2291–2296
Mackness, M. I., Walker, C. H., Rowlands, D. G. & Price, N. R. (1983) Comp. Biochem. Physiol. 74C, 65–68
McElveen, J., Mackness, M. I., Colley, C. M., Peard, T., Warner, S. & Walker, C. H. (1986) Clin. Chem. 32, 671–673
NC-IUB (1984) Enzyme Nomenclature 1984, pp. 271–278, Academic Press, Orlando.
Pen, J. & Beintema, J. J. (1986) Biochem. J. 240, 933
Reiner, E., Radic, Z., Buntic, A. & Kralj, M. (1987) Biochem. Soc. Trans. in the press
Schmiegelow, K., Eiberg, H., Tsui, L.-C., Buchwald, M., Phelen, P. D., Williamson, R., Warwick, W., Niebuhr, E., Mohr, J., Schwartz, M. & Koch, C. (1986) Clin. Genet. 29, 374–377
Simpson, N. E. (1971) Am. J. Hum. Genet. 23, 375–382
Walker, C. H. & Mackness, M. I. (1983) Biochem. Pharmacol. 32, 3265–3269