Chem.-Biol. Interactions, 87 (1993) 25 – 34
Elsevier Scientific Publishers Ireland Ltd.

STUDIES ON HUMAN SERUM PARAOXONASE/ARYLESTERASE

BERT N. LA DU[a], STEVE ADKINS[b], CHUNG-LIANG KUO[b] and DAVID LIPSIG[a]

[a]*Departments of Pharmacology and Anesthesiology, Medical School, University of Michigan, Ann Arbor, MI 48109-0572 and* [b]*Department of Environmental and Industrial Health, School of Public Health, University of Michigan, Ann Arbor, MI 48109-2029 (USA)*

SUMMARY

The complete amino acid sequence of human serum paraoxonase/arylesterase and the DNA sequence coding for that protein have recently been determined in two independent laboratories. There is now considerable evidence that the esterase exists in two genetically determined allozymic forms, and these A and B allozymes possess both paraoxonase and arylesterase activities. The B-type esterase has relatively higher paraoxonase activity and is stimulated to a greater degree by 1 M NaCl than the A allozyme. The structural basis for the distinctive isozymic properties is a single nucleotide base at position 572. Codon 191 is CAA (for glutamine) in the A-type esterase, and CGA (for arginine) in the B-type enzyme. There is a second polymorphic site which affects amino acid 54; this can be either methionine or leucine, but these alternatives have not been found to affect either the level or the quality of the allozymes. Purified A or B-type esterases are stimulated by the addition of phosphatidylcholine. The latter addition increases the maximum velocity rate, but does not alter the K_m of the reaction with either paraoxon or phenylacetate. In serum, the esterase is tightly bound to the high density lipoproteins, particularly apo A-1, but the importance of this association as far as the stability and catalytic properties of the esterase is not clear, and still under study. No physiological role of the esterase has been established, but its ability to hydrolyze several potent organophosphates may be of some significance in protecting against organophosphate toxicity.

Key words: Paraoxonase purification — Serum arylesterase characteristics — Organophosphate hydrolysis — Polymorphic isozymes

Correspondence to: Bert N. La Du, Department of Anesthesiology, Research Laboratories, R4038 Kresge Medical Research II, University of Michigan Medical School, Ann Arbor, MI 48109-0572, USA.

0009-2797/93/$06.00

INTRODUCTION

Molecular biology studies on paraoxonase/arylesterase over the last two years in our laboratory [1] and by Furlong's group [2] have supported the model we proposed in 1983 [3]: that the human serum esterase exists in two closely related isozymic forms (A and B); that the two allozymes can hydrolyze both organophosphates and aromatic esters such as phenylacetate; and that isozyme B has a much higher turnover number for paraoxon than isozyme A. The affinity of the esterase in serum for binding tightly with other lipoproteins and to aggregate with such components may explain why another laboratory described several molecular forms of the esterase in serum, some of which appeared to have only paraoxonase or arylesterase activity [4]. However, the cloning and sequencing of the cDNA coding for the enzyme in the two laboratories leaves little doubt that two major isozymic forms (A and B) must be responsible in some way for what appear to be contrary observations from other laboratories (see La Du [5]).

The enzyme has been highly purified from human serum in our laboratory and has a molecular weight of about 43.0 kDa [6]. It requires calcium for stability as well as its catalytic activity with either phenylacetate or paraoxon. Our method of purification of the enzyme and the basic characteristics of the isozymes have recently been published [6,7]. Several laboratories have estimated the allelic frequencies of the paraoxonase polymorphism in ethnic groups in different parts of the world [8–11], but most of these estimates are based on measuring paraoxonase levels, alone, rather than by a method which distinguishes individual phenotypes (see La Du [5]). The recent progress in the molecular analyses of the polymorphism should make it simpler to relate phenotypes and genotypes, and estimate allelic frequencies more accurately. The molecular studies have not yet helped us to understand the possible physiological function of the esterase, its significance in endogenous metabolism, or its close association with the HDL complex.

This report will summarize some of the recent molecular and catalytic studies on human serum paraoxonase/arylesterase in our laboratory.

MATERIALS AND METHODS

Paraoxonase/arylesterase was purified from human serum previously phenotyped so that individual units of the same A, AB, or B phenotype could be pooled. The purification method was developed in our laboratory [6], and we were able to obtain large quantities of enzyme of over 50% purity, with small amounts of albumin and apolipoprotein A-1 as impurities. Estimates of paraoxonase and arylesterase activities and the phenotyping of individual sera were carried out as described previously [3,12]. Ratios of paraoxonase activity in the presence of 1 M NaCl divided by the arylesterase activity were used to identify individual phenotypes, as follows: type A, 1.21 ± 0.19; type AB, 4.68 ± 0.85, and type B, 8.36 ± 0.07 (means ± S.D.).

For sequencing of the DNA in the polymorphic regions, DNA was isolated

from blood samples (buffy coat) by the salt-chloroform extraction method of Mullenbach [13]. Both polymorphic sites were near intron-exon junctions, so PCR primers were used which bridged the junctions to amplify the regions corresponding to amino acids 54 and 191. These gave products of about 180 and 230 base pairs (bp), respectively. Sequencing primers synthesized by the University of Michigan DNA Sequencing Facility and Oligos Etc. (Wilson, OR) were then used for direct sequencing of the polymorphic segments after PCR amplification to determine individual genotypes, as we recently reported [14].

Phospholipids tested for their effects on arylesterase and paraoxonase activities with purified serum esterase were obtained from Sigma Chemical Co., St. Louis, MO. These were mixed in a buffer and dispersed by sonication, then premixed with preparations of the esterase at 37 °C for about 30 min before the enzymatic assays.

RESULTS AND DISCUSSION

Amino acid sequence of human serum paraoxonase/arylesterase

The amino acid sequence of the mature protein and the location of the two polymorphic sites of human serum paraoxonase/arylesterase are shown in Fig. 1. Although the amino acid sequence shown has been deduced from our analyses of genomic DNA and liver cDNA nucleotide sequences, nearly 45% of the total amino acid sequence was confirmed in our laboratory by direct sequencing of tryptic and peptic digest peptides derived from the purified esterase [14]. Evidence for the polymorphism at position 191 was also obtained from high pressure liquid chromatography analysis of unique peptides identified in the tryptic digest profiles from purified A- and B-type esterases [14,15].

One interesting feature of the esterase structure, as first noted by Hassett et al. [2], is the unusual concentration of hydrophobic amino acid residues at the amino terminal end of the protein. It appears to represent the retention of a modified leader sequence. Presumably, a mutation altered the leader sequence and prevented removal of this hydrophobic region. No doubt, its presence helps account for the tendency of this protein to bind to other proteins, and aggregate by itself.

There are 354 amino acids in the mature protein with only 3 cysteine residues. Two of these are near the ends of the protein; the third is residue number 283. In our sequencing of tryptic digest peptides from purified esterase without reduction, a disulfide bridge linking two tryptic peptides was found. One of these was at the amino terminal end, and the other was near the carboxyl terminal position (Fig. 2). These were subsequently sequenced after reduction and separation to verify their locations. These results suggest that the ends of the native protein are linked by a disulfide bond, and this would leave only the cysteine at position 283 as the likely candidate for being a key component in the active center of this esterase.

Analysis of the structural polymorphisms

Variations in the amino acid residues at positions 54 and 191 were noticed by

```
                    5                                  10                                  15                                  20                                  25
Ala Lys Leu Ile Ala Leu Thr Leu Leu Gly Met Gly Leu Ala Leu Phe Arg Asn His Gln Ser Ser Tyr Gln Thr
GCG AAG CTG ATT GCG CTC ACC CTC TTG GGG ATG GGA CTG GCA CTC TTC AGG AAC CAC CAG TCT TCT TAC CAA ACA

                   30                                  35                                  40                                  45                                  50
Arg Leu Asn Ala Leu Arg Glu Val Gln Pro Val Glu Leu Pro Asn Cys Asn Leu Val Lys Gly Ile Glu Thr Gly
CGA CTT AAT GCT CTC CGA GAG GTA CAA CCC GTA GAA CTT CCT AAC TGT AAT TTA GTT AAA GGA ATC GAA ACT GGC

                |Met| 55                                  60                                  65                                  70                                  75
Ser Glu Asp |Leu| Glu Ile Leu Pro Asn Gly Leu Ala Phe Ile Ser Ser Gly Leu Lys Tyr Pro Gly Ile Lys Ser
TCT GAA GAC |ATG| GAG ATA CTG CCT AAT GGA CTG GCT TTC ATT AGC TCT GGA TTA AAG TAT CCT GGA ATA AAG AGC
            |TTG|
                    80                                  85                                  90                                  95                                  100
Phe Asn Pro Asn Ser Pro Gly Lys Ile Leu Leu Met Asp Leu Asn Glu Glu Asp Pro Thr Val Leu Glu Leu Gly
TTC AAC CCC AAC AGT CCT GGA AAA ATA CTT CTG ATG GAC CTG AAT GAA GAA GAT CCA ACA GTG TTG GAA TTG GGG

                   105                                 110                                 115                                 120                                 125
Ile Thr Gly Ser Lys Phe Asp Val Ser Ser Phe Asn Pro His Gly Ile Ser Thr Phe Thr Asp Glu Asp Asn Ala
ATC ACT GGA AGT AAA TTT GAT GTA TCT TCA TTT AAC CCT CAT GGG ATT AGC ACA TTC ACA GAT GAA GAT AAT GCC

                   130                                 135                                 140                                 145                                 150
Met Tyr Leu Leu Val Val Asn His Pro Asp Ala Lys Ser Thr Val Glu Leu Phe Lys Phe Gln Glu Glu Glu Lys
ATG TAC CTC CTG GTG GTG AAC CAT CCA GAT GCC AAG TCC ACA GTG GAG TTG TTT AAA TTT CAA GAA GAA GAA AAA

                   155                                 160                                 165                                 170                                 175
Ser Leu Leu His Leu Lys Thr Ile Arg His Lys Leu Leu Pro Asn Leu Asn Asp Ile Val Ala Val Gly Pro Glu
TCG CTT TTG CAT CTA AAA ACC ATC AGA CAT AAA CTT CTG CCT AAT TTG AAT GAT ATT GTT GCT GTG GGA CCT GAG

                   180                                 185                                 190 |Arg|                            195                                 200
His Phe Tyr Gly Thr Asn Asp His Tyr Phe Leu Asp Pro Tyr Leu |Gln| Ser Trp Glu Met Tyr Leu Gly Leu Ala
CAC TTT TAT GGC ACA AAT GAT CAC TAT TTT CTT GAC CCC TAC TTA |CGA| TCC TGG GAG ATG TAT TTG GGT TTA GCG
                                                           |CAA|
                   205                                 210                                 215                                 220                                 225
Trp Ser Tyr Val Val Tyr Tyr Ser Pro Ser Glu Val Arg Val Val Ala Glu Gly Phe Asp Phe Ala Asn Gly Ile
TGG TCG TAT GTT GTC TAC TAT AGT CCA AGT GAA GTT CGA GTG GTG GCA GAA GGA TTT GAT TTT GCT AAT GGA ATC

                   230                                 235                                 240                                 245                                 250
Asn Ile Ser Pro Asp Gly Lys Tyr Val Tyr Ile Ala Glu Leu Leu Ala His Lys Ile His Val Tyr Glu Lys His
AAC ATT TCA CCC GAT GGC AAG TAT GTC TAT ATA GCT GAG TTG CTG GCT CAT AAG ATT CAT GTG TAT GAA AAG CAT

                   255                                 260                                 265                                 270                                 275
Ala Asn Trp Thr Leu Thr Pro Leu Lys Ser Leu Asp Phe Asn Thr Leu Val Asp Asn Ile Ser Val Asp Pro Glu
GCT AAT TGG ACT TTA ACT CCA TTG AAG TCC CTT GAC TTT AAT ACC CTC GTG GAT AAC ATA TCT GTG GAT CCT GAG

                   280                                 285                                 290                                 295                                 300
Thr Gly Asp Leu Trp Val Gly Cys His Pro Asn Gly Met Lys Ile Phe Phe Tyr Asp Ser Glu Asn Pro Pro Ala
ACA GGA GAC CTT TGG GTT GGA TGC CAT CCC AAT GGC ATG AAA ATC TTC TTC TAT GAC TCA GAG AAT CCT CCT GCA

                   305                                 310                                 315                                 320                                 325
Ser Glu Val Leu Arg Ile Gln Asn Ile Leu Thr Glu Glu Pro Lys Val Thr Gln Val Tyr Ala Glu Asn Gly Thr
TCA GAG GTG CTT CGA ATC CAG AAC ATT CTA ACA GAA GAA CCT AAA GTG ACA CAG GTT TAT GCA GAA AAT GGC ACA

                   330                                 335                                 340                                 345                                 350
Val Leu Gln Gly Ser Thr Val Ala Ser Val Tyr Lys Gly Lys Leu Leu Ile Gly Thr Val Phe His Lys Ala Leu
GTG TTG CAA GGC AGT ACA GTT GCC TCT GTG TAC AAA GGG AAA CTG CTG ATT GGC ACA GTG TTT CAC AAA GCT CTT

Tyr Cys Glu Leu
TAC TGT GAG CTC
```

Fig. 1. Amino acid sequence and codons for human serum paraoxonase/arylesterase. The locations of the two polymorphic sites at residues 54 and 191 are indicated. (modified from Ref. 14).

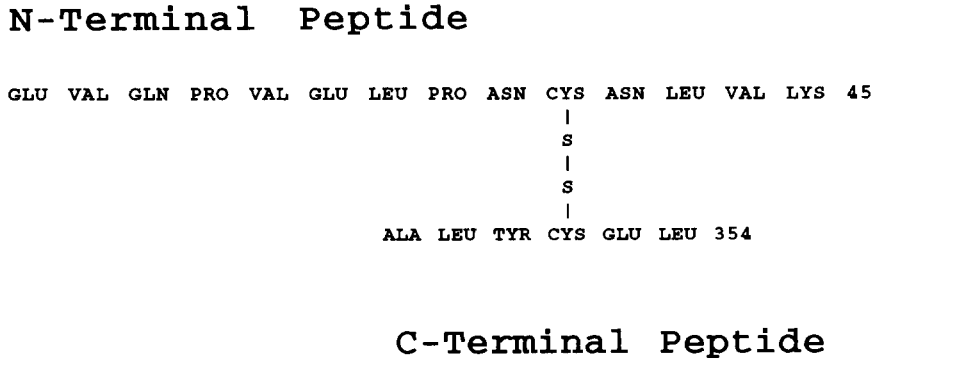

Fig. 2. Amino acid sequences of disulfide-linked peptides obtained by tryptic digestion of purified human serum paraoxonase/arylesterase.

Furlong's laboratory [2] and our laboratory [14,15]. At amino acid residue 54, it was methionine or leucine, and at position 191 it was glutamine or arginine. The two residues at one of these positions, alone, might explain the paraoxonase/arylesterase polymorphism, or certain combinations within the four isomers could be required. It was thus necessary to compare the genotypes obtained by direct sequencing of the PCR amplified genomic DNA at the two polymorphic sites with phenotyping results from serum of the same individuals.

Blood samples from twenty-seven unrelated volunteers were phenotyped by their ratios of paraoxonase activity in the presence of 1 M NaCl divided by their arylesterase activity (Fig. 3), and their DNA samples were used to sequence the two polymorphic regions after PCR amplification. The results of these analyses

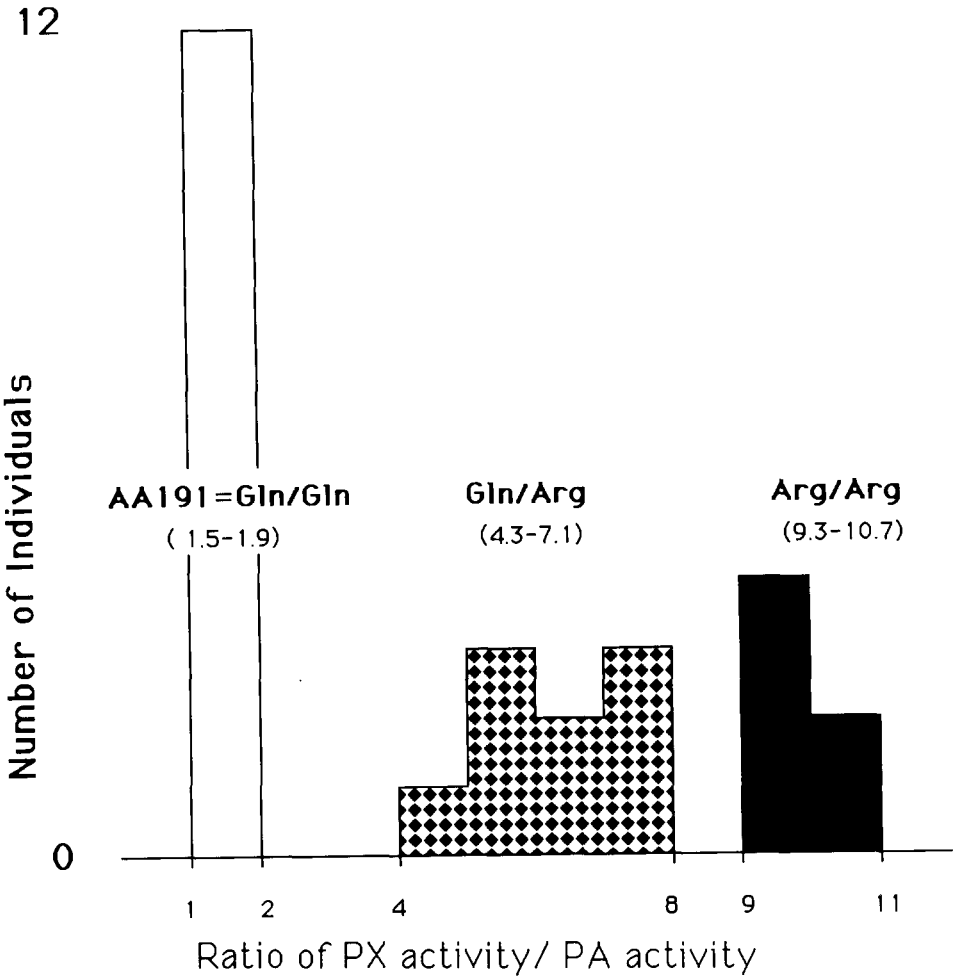

Fig. 3. Distribution of ratios of paraoxonase activity in the presence of 1 M NaCl divided by the arylesterase activity of 27 unrelated individuals. The three modes correspond to the A, AB and B phenotypes.

are shown in Table I. Comparison of the serum esterase phenotypes and the DNA analyses showed that the amino acid at position 191 coincided exactly with the serum enzyme phenotype in every individual. All those of phenotype A had glutamine, and those of phenotype B all had arginine at position 191; heterozygous individuals (AB) had both of these amino acids represented. On the other hand, the methionine/leucine polymorphism determined by the codon for position 54 did not affect the serum phenotype in any obvious way. It is of interest that all B-type individuals were homozygous for leucine at the latter site, but this may simply be the result of linkage disequilibrium since these genetic markers are very close. The perfect agreement between the amino acids at position 191 and the serum esterase phenotypes in the individuals indicates that gene frequencies estimated from either of these traits would be the same. In contrast,

TABLE I

CORRELATION OF SERUM PARAOXONASE PHENOTYPES AND DNA GENOTYPES IN 27 UNRELATED INDIVIDUALS

Serum phenotype	Individual	μMoles PA[a] / Min × ml	nMoles PX[b] / Min × ml	PX/PA ratio	Amino acid 191	Amino acid 54
A	D.Li	95	155	1.6	GLN,GLN	MET,MET
A	L.S.	100	169	1.7	GLN,GLN	MET,MET
A	R.S.	100	191	1.9	GLN,GLN	MET,MET
A	S.V.	82	152	1.9	GLN,GLN	MET,MET
A	S.P.	98	169	1.7	GLN,GLN	MET,LEU
A	J.K.	62	105	1.7	GLN,GLN	MET,LEU
A	A.H	122	212	1.7	GLN,GLN	MET,LEU
A	W.W.	89	157	1.8	GLN,GLN	MET,LEU
A	S.L.	134	223	1.7	GLN,GLN	LEU,LEU
A	D.La	86	162	1.9	GLN,GLN	LEU,LEU
A	P.C.	129	201	1.6	GLN,GLN	LEU,LEU
A	E.L.	118	176	1.5	GLN,GLN	LEU,LEU
AB	S.A.	58	321	5.6	GLN,ARG	MET,MET
AB	K.D.	100	627	6.3	GLN,ARG	MET,MET
AB	B.D.	81	496	6.1	GLN,ARG	MET,LEU
AB	J.De	141	784	5.6	GLN,ARG	MET,LEU
AB	T.I.	57	398	7.0	GLN,ARG	MET,LEU
AB	H.C.	51	355	7.0	GLN,ARG	MET,LEU
AB	K.C.	114	496	4.3	GLN,ARG	LEU,LEU
AB	L.Z.	129	758	5.9	GLN,ARG	LEU,LEU
AB	M.G.	110	780	7.1	GLN,ARG	LEU,LEU
B	M.S.	91	846	9.3	ARG,ARG	LEU,LEU
B	B.L.	86	890	10.3	ARG,ARG	LEU,LEU
B	A.T.	106	1006	9.5	ARG,ARG	LEU,LEU
B	J.Da	76	737	9.7	ARG,ARG	LEU,LEU
B	A.C.	100	1063	10.7	ARG,ARG	LEU,LEU
B	J.V.	106	1020	9.7	ARG,ARG	LEU,LEU

[a] Phenylacetate.

[b] Paraoxon in the presence of 1 M NaCl.

the frequencies of the methionine/leucine polymorphic choices at residue 54 did not show a similar agreement. It is, none the less, a very common polymorphism, and its importance must still be determined.

A schematic representation of the enzyme structure showing the free sulfhydryl group (cysteine), the single internal disulfide bridge, the two polymorphic sites, and the location of four possible carbohydrate chains based on the requisite amino acid sequence is shown in Fig. 4.

Stimulation of esterase activity by phospholipids

It has been observed that with higher purification of the serum paraox-

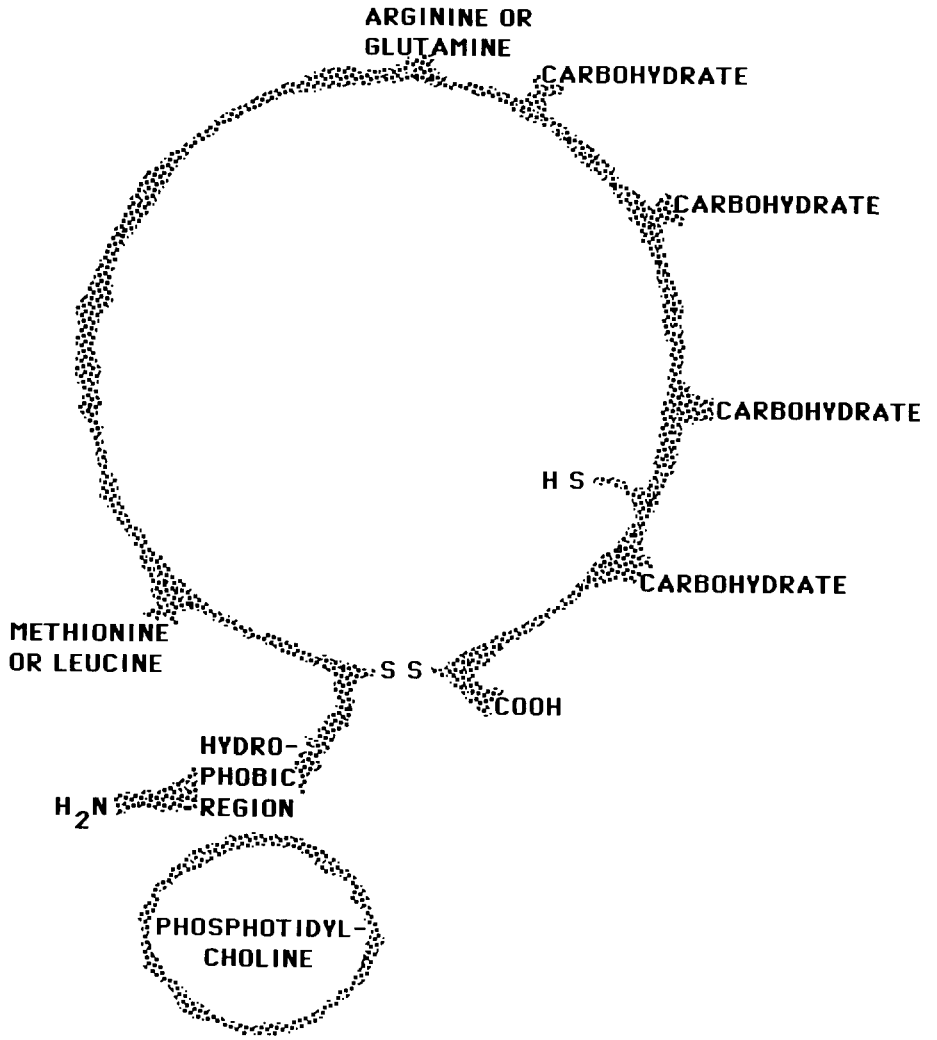

Fig. 4. Schematic representation of the paraoxonase/arylesterase enzyme showing the polymorphic sites, hydrophobic N-terminal segment, the disulfide bridge and single free sulfhydryl residue.

onase/arylesterase some loss of activity regularly takes place, but this loss can be largely regained by adding back certain fractions obtained during the DEAE column chromatography step. We analyzed these fractions and found that they were enriched in phospholipids. When we substituted purified dilauroyl phosphatidylcholine, a maximal degree of stimulation was obtained (Table II). Commercial lecithin preparations and most other phospholipids were somewhat less effective; phosphatidyl-serine was inhibitory. A comparison of the effectiveness of the compounds shows that there is some degree of specificity for this effect, and saturated medium length fatty acids were more effective than the other analogs. We now suspect that some of our earlier observations on protective or stimulatory effects obtained by combining apolipoprotein A-1 with the purified esterase were probably due to the phospholipid content of the mixture. We have yet to see a clear beneficial effect of apolipoprotein A-1 with the purified esterase when there is also an optimal concentration of choline-containing phospholipids. Among the compounds not effective in place of the phosphatidylcholine were tripalmitin, sphingomyelin, and phosphatidylinositol. Simple lipids, then, are not all stimulatory like selective phospholipids. Dilauroyl phosphatidylcholine does not change the K_m appreciably, but it increases the V_{max} of the reaction. Thus, the phosphatidylcholine is not simply facilitating the interaction of substrate with the enzyme, but is making the enzyme a more efficient catalyst, and presumably is increasing the turnover number of the esterase with either paraoxon or phenylacetate (Fig. 5).

The very close association of serum paraoxonase/arylesterase with apolipoprotein A-1 and with phosphatidylcholine may take place through the hydrophobic region at the amino terminus of the enzyme. However, a more exact understanding of the interactions of the esterase with these components will be possible after we obtain the three-dimensional characteristics of the molecule by crystallographic analysis.

TABLE II

STIMULATION OF PHENYLACETATE HYDROLYSIS BY PHOSPHATIDYLCHOLINE AND ANALOGUES

Compound	% Inhibition	% Stimulation
Dilauroyl-PC		65.0
Dioleoyl-PC		56.3
Lecithin		41.1
Lysolecithin		31.6
Phosphatidylethanolamine		18.6
Phosphatidylglycerol		9.6
Phosphatidylserine	33.3	
6-palmityl-ascorbic acid	37.4	

No effect: Tripalmitin, cholesterylacetate, sphingomyelin, cardiolipid, phosphatidylinositol, vitamin K3, glycerophosphorylcholine. (Control activity: 64.4 U/ml.)

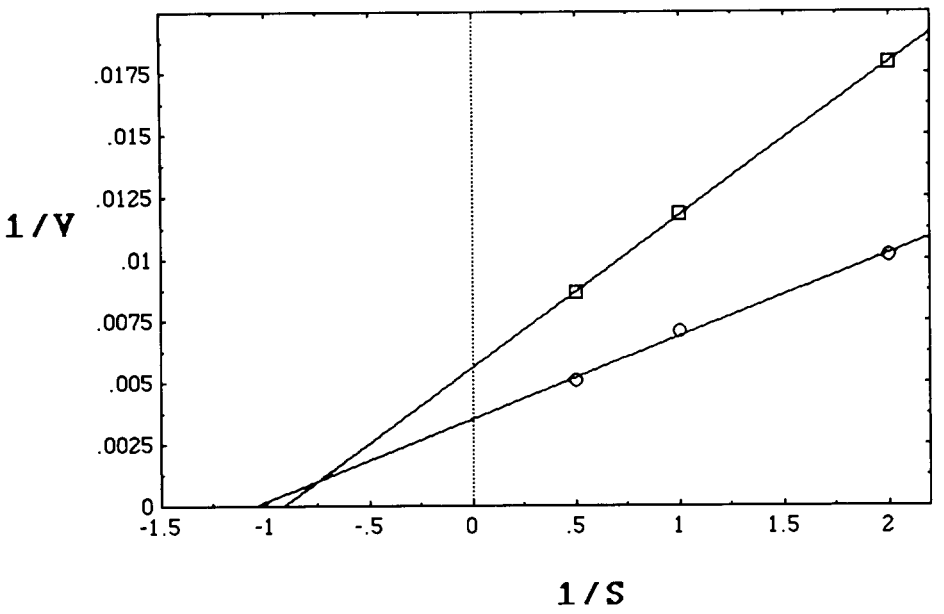

Fig. 5. Effect of dilauroyl phosphatidylcholine on arylesterase activity of purified human serum paraoxonase/arylesterase. Squares indicate enzyme alone and circles indicate enzyme with dilauroyl phosphatidylcholine.

ACKNOWLEDGEMENTS

The support of Grant GM 46979 from the National Institute of General Medical Sciences is gratefully acknowledged.

REFERENCES

1 S. Adkins, K.N. Gan, M. Mody and B.N. La Du, Purification and cloning of human serum paraoxonase/arylesterase, FASEB J., 5 (1991) A648.
2 C.Hassett, R.J. Richter, R. Humbert, C. Chapline, J.W. Crabb, C.J. Omiecinski and C.E. Furlong, Characterization of cDNA clones encoding rabbit and human serum paraoxonase: the mature protein retains its signal sequence, Biochemistry, 30 (1991) 10141–10149.
3 H. W. Eckerson, C.M. Wyte and B.N. La Du, The human serum paraoxonase/arylesterase polymorphism, Am. J. Hum. Genet., 35 (1983) 1126–1138.
4 M.I. Mackness, H.M. Thompson, A.R. Hardy and C.H. Walker, Distinction between 'A'-esterases and arylesterases, Biochem. J., 245 (1987) 293–296.
5 B.N. La Du, Human serum paraoxonase/arylesterase, in: W. Kalow (Ed.), Pharmacogenetics of Drug Metabolism, Pergamon Press, New York, 1992, pp. 51–91.
6 K.N. Gan, A. Smolen, H.W. Eckerson and B.N. La Du, Purification of human serum paraoxonase/arylesterase, evidence for one esterase catalyzing both activities, Drug Metab. Dispos., 19 (1991) 100–106.
7 A. Smolen, H.W. Eckerson, K.N. Gan and B.N. La Du, Characteristics of the genetically determined allozymic forms of human serum paraoxonase/arylesterase, Drug Metab. Dispos., 19 (1991) 107–112.

8 J.R. Playfer, L.C. Eze, M.F. Bullen and D.A.P. Evans, Genetic polymorphism and interethnic variability of plasma paraoxonase activity, J. Med. Genet., 13 (1976) 337–342.
9 H.W. Goedde, H.G. Rothhammer and P. Bogdanski, Ecogenetic studies in Atacameno Indians, Hum. Genet., 67 (1984) 343–346.
10 B.N. La Du, S. Adkins and R.A.-L. Bayoumi, Analysis of the serum paraoxonase/arylesterase polymorphism in some Sudanese families, in: W. Kalow, H.W. Goedde and D.P. Agarwal (Eds.), Ethnic Differences in Reactions to Drugs and Xenobiotics, Alan R. Liss, New York, 1986, pp. 87–98.
11 M. Geldmacher-v, Mallinckrodt and T.L. Deipgen, The human paraoxonase polymorphism and specificity, Toxicol. Environ. Chem., 18 (1988) 79–196.
12 H.W. Eckerson, J. Romson, C. Wyte and B.N. La Du, The human serum paraoxonase polymorphism: identification of phenotypes by their response to salts, Am. J. Hum. Genet., 35 (1983) 214–227.
13 R. Mullenbach, P.J.L. Lagoda and C. Welter, An efficient salt-chloroform extraction of DNA from blood and tissues, Trends Genet., 5 (1989) 391.
14 S. Adkins, K.N. Gan, M. Mody and B.N. La Du, Molecular basis for the polymorphic forms of human serum paraoxonase/arylesterase: glutamine or arginine at position 191 for the respective A or B allozymes, Am. J. Hum. Genet., 52 (1993) 598–608.
15 M. Mody, S. Adkins, K. Gan and B. La Du, Direct evidence from peptide sequencing for one amino acid difference in human serum paraoxonase/ arylesterase A and B isozymes, FASEB J., 6 (1992) A3741.