Biochemical Pharmacology, Vol. 30, No. 8, pp. 903-906, 1981.
Printed in Great Britain.

0006-2952/81/080903-04$02.00/0
Pergamon Press Ltd.

'A'-ESTERASE ACTIVITY IN THE LIPOPROTEIN FRACTION OF SHEEP SERUM

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In 1953 Aldridge defined two classes of esterases according to their interaction with organophosphate anticholinesterases. 'A'-esterases hydrolyse such substances whilst 'B'-esterases are inhibited by them [1]. The active forms of organophosphorus pesticides are inhibitors of 'B'-esterases and are in many cases deactivated by 'A'-esterases [2]. Mammals tend to have high levels of 'A'-esterases in the blood and the liver, and this is apparently an important factor in determining their relatively low susceptibility to such organophosphates as diazinon and pirimiphos-methyl. By comparison birds have very low levels of 'A'-esterases and are relatively susceptible to these two compounds [3]. The present work was initiated to obtain further characterisation of mammalian 'A'-esterases which are but poorly understood in spite of their toxicological importance. This involved a preliminary investigation of the 'A'-esterase activity in the lipoprotein fraction of sheep serum.

Methods

Sheep blood was obtained unheparinised from Reading Abattoir and serum prepared from it by centrifuging at 2,100g for one hour at 4°C. The lipoprotein fraction was prepared by adding potassium bromide to serum (324.6 mg per ml serum) to give a specific gravity of 1.21 and then centrifuging at 135,000g for 24 hours [4]. The lipoproteins (high and low density) separated as a pale yellow layer from the remainder of the serum and this was removed with a Pasteur pipette. This method of collecting the lipoprotein fraction unfortunately leads to errors in enzyme recovery (see Table 1), as the interface between lipoprotein and non-lipoprotein fractions is not easily distinguishable, and accordingly the positioning of the tip of the sampling pipette is not exactly reproducible.

Serum, lipoprotein and non-lipoprotein fractions were assayed for 'A'-esterase using paraoxon and pirimiphos-methyloxon as substrates. The methods used were as described by Brealey *et al* [3,5] except in the paraoxonase assay, where the Sørensen's phosphate assay buffer was changed to 0.02M pH 7.6 Tris/HCl. The effects of EGTA (ethyleneglycol-bis-(β-amino-ethyl ether)N,N'-tetra acetic acid) and pH on the paraoxon hydrolysing activity

(paraoxonase) of the lipoprotein fraction were determined by the following methods:-

i) EGTA - an aliquot of assay buffer was replaced by an aliquot of EGTA solution to give a final EGTA concentration of 134 μM in the assay, the lipoprotein fraction added and preincubated at 37°C for 15 min before assaying for paraoxonase activity. Assays were performed on five lipoprotein preparations.

ii) pH Dependency - Assay buffer was replaced by universal buffer [6] at a variety of pH between 2.0 and 9.0. Assays were performed on 4 lipoprotein and 3 non-lipoprotein preparations and the average activity at each pH value was expressed as a percentage of the activity at pH 7.0.

Results and Discussion

The distribution of 'A'-esterase activity assayed by paraoxon (paraoxonase) and pirimiphos-methyloxon (pirimiphos-methyloxonase) after fractionation of sheep serum into lipoprotein and non-lipoprotein fractions is given in Table 1.

Enzyme	% Activity Recovery in the Lipoprotein Fraction	Purification Factor of Activity in the Lipoprotein Fraction	% Activity Recovery in the Non-lipoprotein Fraction	Total % Activity Recovery
Paraoxonase	52.6 ± 12.3	8.37 ± 2.9	20.6 ± 7.1	73.2 ± 12.3
Pirimiphos-methyloxonase	24.8 ± 9.3	4.52 ± 1.42	27.3 ± 6.7	51.1 ± 9.3

Table 1. Recovery of 'A'-esterase activity after fractionation of serum

The results show that the majority of paraoxonase activity is found in the lipoprotein fraction with a relatively small amount in the non-lipoprotein fraction, indicating that paraoxonase is probably a lipoprotein or a lipoprotein-associated enzyme as is the case with bovine plasma arylesterase (E.C. 3.1.1.2) [7,8]. This method represents a useful first stage in paraoxonase purification on account of its simplicity of operation and the high purification factor involved. Pirimiphos-methyl oxonase activity however is distributed almost equally between lipoprotein and non-lipoprotein fractions. The ratio of paraoxonase to pirimiphos-methyl oxonase activity changes from 6.82 (± 0.23) in serum to 6.64 (± 1.81) in the lipoprotein fraction and 11.18 (± 0.44) in the non-lipoprotein fraction. These observations, coupled with the differing purification factors and total activity recoveries for the two assays, and differences in the pH curve between lipoprotein and non-lipoprotein fractions (see later) tend to suggest that two or more enzymes exist with differing substrate specificities. It should be emphasised, however, that due to the difficulties of sampling, the composition of the lipoprotein and non-lipoprotein fractions vary between experiments, as shown in Table 1. It is possible that the lipophilicity of the substrate is important in determining the distribution of activity. Pirimiphos-methyloxon, which is the more polar of the two substrates, may show

less affinity for the enzyme(s) which predominates in the lipoprotein fraction.

The effects of EGTA (ethyleneglycol-bis-(β-amino-ethyl-ether)N,N'-tetra-acetic acid) and EGTA followed by $CaCl_2$ on paraoxonase activity in the lipoprotein fraction are shown in Table 2.

Effector	Paraoxonase Specific Activity nmoles/min/mg protein Control	with Effector	Paraoxonase Activity as a % of Control
134 μM EGTA	1.78 ± 0.66	0	0
134 μM EGTA followed by 67 μM $CaCl_2$*	1.78 ± 0.66	1.88 ± 0.66	107

Table 2. The effects of EGTA and EGTA followed by $CaCl_2$ on lipoprotein fraction paraoxonase activity

*Preliminary studies showed that this concentration of $CaCl_2$ provided optimal reactivation of paraoxonase after inhibition with EGTA.

EGTA (134 μM) causes complete inhibition of paraoxonase activity. This inhibition can however be completely reversed by making the assay medium 67 μM with respect to $CaCl_2$, and it would appear that paraoxonase in the lipoprotein fraction of sheep serum, like the enzyme in sheep serum itself [9], requires Ca^{2+} as a co-factor.

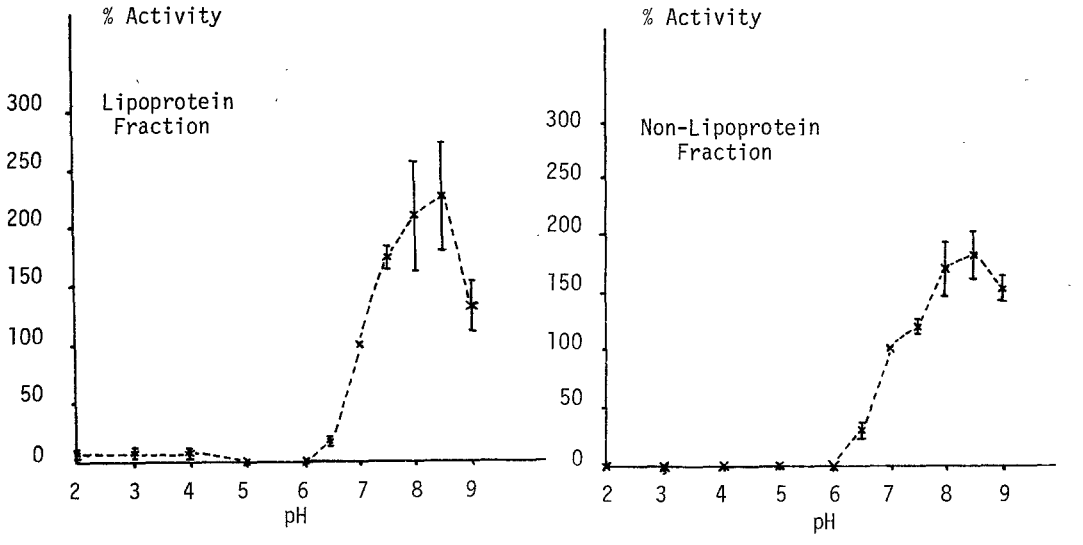

Figure 1. pH Dependency of sheep serum lipoprotein and non-lipoprotein fraction paraoxonase activity

Activities are expressed as a percentage of the activity at pH 7.0

Paraoxonase in the lipoprotein fraction of sheep serum has a pH optimum of about pH 8.5 (Figure 1) while that in the non-lipoprotein fraction of serum shows a pH optimum of similar magnitude. The two pH curves however are not identical. Paraoxonase in the

lipoprotein fraction shows activity between pH 2.0-4.0 but the paraoxonase in the non-lipoprotein fraction shows no activity in this region. (This result is highly significant at the level $P < 0.01$). This slight difference in the pH activity profile between the two fractions may be an indication of the presence of different enzyme forms in the two fractions.

Work is in progress to characterise the 'A'-esterase activity in sheep serum lipoprotein fraction.

Conclusion

The results indicate that much of the paraoxonase activity of sheep serum is due to either a lipoprotein or lipoprotein-associated enzyme, with a pH optimum of about pH 8.5, which requires Ca^{2+} as a cofactor. Relatively little activity is found in the non-lipoprotein fraction. In contrast pirimiphos-methyloxonase activity is distributed almost equally between the lipoprotein and non-lipoprotein fractions of sheep serum. There may be two or more forms of 'A'-esterase with different substrate specificities towards paraoxon and pirimiphos-methyloxon. This active 'A'-esterase of sheep serum appears to be an important factor in determining the low susceptibility of this species to certain organophosphates e.g. diazinon and pirimiphos-methyl [3].

Acknowledgement

The authors are grateful to S.D. Hallam for technical assistance and to D.G. Rowlands and N.R. Price for helpful discussions during the course of the work.

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