Peacock Plasma, A Useful Cholinesterase Source for Inhibition Residue Analysis of Insecticidal Carbamates

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Plasma cholinesterases of warm-blooded animals are known to differ widely in their reaction with substrates and inhibitors (1, 2, 3). Recent investigations on certain interspecies differences performed in this laboratory with many mammalian and avian plasma cholinesterases showed that the enzyme of the peacock was particularly sensitive to some insecticidal carbamates and thus suitable for cholinesterase inhibition residue analysis of these compounds. This finding allows the automated procedure previously described for the analysis of enolphosphates (4) to be extended to carbamates by substituting human plasma cholinesterase by the corresponding peacock enzyme.

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Materials and Methods

All experiments performed were based on an automated procedure (4) in which esters of thiocholine are used as substrates for cholinesterases and 5,5-dithiobis-2-nitro benzoic acid (DTNB, Aldrich Chem. Co., Milwaukee, USA) as the reagent for the enzymatic hydrolysis product thiocholine (5). Figure 1 presents the flow diagram of the automated system. The enzyme working solution was obtained by diluting one part of peacock plasma with 150 to 200 parts of 0.067 M Soerensen phosphate buffer pH 8.0 [1]. Acetylthiocholine (Fluka AG., Buchs, Switzerland), was chosen as the substrate [2] and used at a final concentration of 4×10^{-4} M. For comparative purposes cholinesterases of outdated human plasma

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1) At the present time we have experience with plasma samples of two peacocks. The first one was kindly provided by the Zoological Garden of Basle, Switzerland, whereas the second was taken from an animal which is kept on an experimental farm of CIBA Ltd. Blood is taken from the animal's wing veins and centrifuged at 3000 rpm for 15 minutes. The plasma (4-5 ml) may be kept for several weeks if frozen.

2) In contrast to many other plasma cholinesterases the peacock plasma preparation hydrolyzed propionylthiocholine more rapidly than acetylthiocholine and butyrylthiocholine. However, the difference observed was small (10-15 %), so that all three substrates may be used for inhibition experiments.

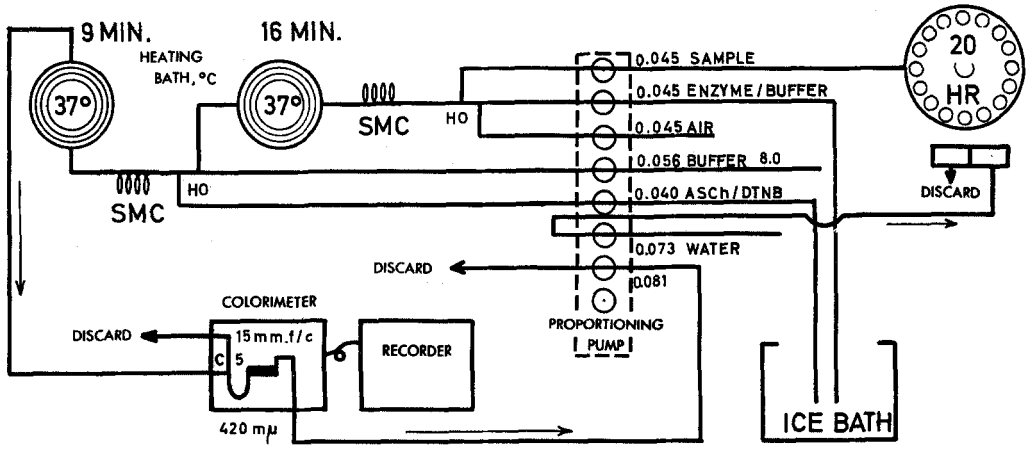

Figure 1. Flow diagram of the automated cholinesterase inhibition procedure [4] described by Voss and Geissbühler (4).

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[4] Reprints of this publication, in which the automated procedure has been described in detail, may be obtained from the Agrochemical Division of CIBA Ltd., Basle, Switzerland.

and bovine erythrocytes (commercial sample of Serva Entwicklungslabor, Heidelberg, Germany) were also taken together with the peacock plasma for I_{50} determinations with the following insecticides: carbaryl, N-methyl-1-naphthyl-carbamate; propoxur, N-methyl-2-isopropoxyphenyl-carbamate; C-8353, 2-(1,3-dioxolan-2-yl)-phenyl-N-methyl-carbamate; C-9643, 2-(4-methyl-1,3-dioxolan-2-yl)-phenyl-N-methyl-carbamate; C-10015, 2-(4,5-methyl-1,3-dioxolan-2-yl)-phenyl-N-methyl-carbamate; phosphamidon, 0,0-dimethyl-0-(2-chloro-2-diethyl-carbamoyl-1-methyl)-vinyl phosphate; monocrotophos [3] 0,0-dimethyl-0-(2-methyl-carbamoyl-1-methyl)-vinyl phosphate; dicrotophos, 0,0-dimethyl-0-(2-dimethylcarbamoyl-1-methyl)-vinyl phosphate.

Results

The results obtained in parallel experiments with the three different cholinesterase preparations and eight inhibitors (carbamates and organophosphates) are presented in Table 1. It is evident that the peacock enzyme is more sensitive to all compounds tested, when compared with human plasma cholinesterase. The differences are particularly pronounced with the carbamates, towards which the peacock cholinesterase is approximately ten times more sensitive.

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3) proposed common name

TABLE 1

I_{50}-VALUES (final molar concentration during pre-inhibition) OF CERTAIN CARBAMATES AND ORGANOPHOSPHATES, DETERMINED WITH THREE DIFFERENT CHOLINESTERASES BY MEANS OF AN AUTOMATED PROCEDURE.

	purified bovine AChE	human plasma ChE	peacock plasma ChE
Carbamates			
carbaryl	3.2×10^{-6}	1.2×10^{-5}	1.5×10^{-6}
propoxur	9.5×10^{-7}	2.0×10^{-5}	2.8×10^{-6}
C-8353	5.5×10^{-6}	9.0×10^{-6}	9.0×10^{-7}
C-9643	1.1×10^{-5}	5.5×10^{-6}	5.5×10^{-7}
C-10015	1.0×10^{-5}	2.4×10^{-6}	2.0×10^{-7}
Organophosphates			
phosphamidon	1.2×10^{-5}	6.7×10^{-7}	1.3×10^{-7}
monocrotophos	2.4×10^{-5}	8.0×10^{-7}	5.3×10^{-7}
dicrotophos	9.0×10^{-6}	3.0×10^{-7}	1.2×10^{-7}

However, purified bovine erythrocyte cholinesterase is the most sensitive enzyme when propoxur is the inhibitor. The inhibitor potencies of the dioxolane carbamates decrease with molecular size in the case of bovine erythrocyte cholinesterase, whereas in the case of the two plasma enzymes an increase is observed.

The usefulness of peacock plasma for residue analyses of certain carbamates was then examined with C-8353, a new insecticidal compound of CIBA Ltd. The I_{50}-values of this substance against bovine erythrocyte and human plasma cholinesterase were too high for the application of the standard automated residue method routinely used for the determination of insecticidal enolphosphates in this laboratory. However, by using peacock plasma the limit of detection can be lowered to 0.05 ppm without complicating the extraction and clean-up procedures [5]. The results of a few recovery experiments on four crops fortified with different amount of C-8353 are summarized in Table 2.

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5) The plant material (fruits, vegetables) is macerated and extracted with acetonitrile. After evaporation of the organic solvent the aqueous residue is extracted with chloroform. The chloroform extract is then transferred into hexane by repeated evaporations. The insecticide is removed from the organic solvent by shaking with water and is then determined on the AutoAnalyzer. Dry plant materials, such as grains, are directly extracted with chloroform, then transferred into hexane and finally partitioned into water.

TABLE 2

RESULTS OF RECOVERY EXPERIMENTS WITH THE CARBAMATE C-8353 ON VARIOUS CROPS.

Crop	Fortified with C-8353	Percent C-8353 recovered
apples, fruits	1.0 ppm	110
cabbage, leaves	0.2 and 1.0 ppm	88 ± 9
rice, grains	0.4, 0.8 and 10 ppm	100 ± 12
wheat, grains	0.4 and 0.8 ppm	95 ± 5

In addition to the recovery experiments the inhibition of peacock plasma cholinesterase by extracts of untreated plant materials (controls) was also checked. The control values were not higher than those found with human plasma cholinesterase (10-15 % inhibition). Therefore the particular sensitivity of the peacock enzyme towards carbamates does not seem to be connected with a general sensitivity towards unspecific substances present in plant materials.

Discussion

Cholinesterase inhibition residue methods are well established for many of the insecticidal organophosphates, whose inhibition potencies are often greater than those of the carbamates. This may be the reason for the rather limited number of publications on enzyme inhibition procedures

for the latter group of insecticides. Zweig and Archer (6) reported horse plasma cholinesterase to be suitable for the determination of carbaryl residues.

According to our experience with an automated method, the I_{50}-value of a cholinesterase inhibiting insecticide towards any particular type of cholinesterase should be at least in the order of 10^{-6} M. Such an I_{50}-value allows a final aqueous extract to be used, of which one milliliter corresponds to one gram of plant material. Most control inhibition values are small or even negligible at this level and the limit of detection will be in the order of magnitude of 0.05 to 0.1 ppm. Plant materials treated with insecticides of lower inhibition potencies would have to be subjected to time consuming and sophisticated clean-up procedures in order to reduce or exclude interferences by plant substances. Complicated clean-up methods may also reduce recovery percentages. As long as completely automated extraction and purification techniques are not available, the automation of the final determination step must be combined with the use of sensitive cholinesterases, which permit simple and straightforward extraction methods to be applied. This is especially true for serial analyses of crops, which have been treated with a single cholinesterase inhibiting insecticide. A first approach in this direction has been described in the present paper.

Although cholinesterase inhibition methods are usually regarded to be unspecific, a certain degree of specifity may be obtained by including a thin-layer chromatography step into the clean-up procedure. Even

closely related compounds may be completely separated from each other prior to a quantitative determination by cholinesterase inhibition (4). A great advantage of cholinesterase inhibition procedures is the co-determination of enzyme inhibiting toxic metabolites, which are not only known for organophosphates, but also for insecticidal carbamates (7).

Summary

The plasma cholinesterase of the peacock was found to be particularly sensitive to inhibition by certain insecticidal carbamates. This property was made use of in an automated procedure, which was originally developed for the analysis of enolphosphates. When human plasma solution was replaced by diluted peacock plasma, the limit of detection of these carbamates was lowered by a factor of ten. The principle of a simple clean-up procedure as well as recovery values and the limit of detection are given for the carbamate C-8353.

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