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An Amperometric Biosensor for the Determination of Organophosphorus Pesticides

Azmi TELEFONCU
Ege University, Faculty of Science, Department of Biochemistry,
35100 Bornova İzmir-TURKEY

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Organophosphorus pesticides are inhibitors of cholinesterase and this enzyme is used as a monitoring system for pesticides containing organophosphorus compounds. The decrease in activity of cholinesterase is monitored by the choline sensor and is correlated to the concentration of pesticide present in solution. According to this observation a biosensor for pesticides was determined. This biosensor consists of a Clark oxygen electrode, modified with cellulose acetate and dialysis membranes. Between the two membranes there are two immobilized enzymes: acetyl-cholinesterase and choline oxidase.

Introduction

Organophosphorus compounds are widely used in agriculture, medicine and industry, and include some highly toxic chemical warfare agents. Organophosphorus pesticides have been applied to a variety of crops including green vegetables. These applications leave residue on the crops and also contaminate surface water draining from the cropland. The presence of these pesticides in water, food and animal feedstuffs presents a potential hazard owing to their high mammalian toxicity[1,2].

Organophosphorus pesticides inhibit both cholinesterases: red blood cel(RBC) cholinesterase and plasma cholinesterase. The inhibition of this enzyme is a confirmatory test for organophosphate poisoning, but is not diagnostic when used alone. Plasma cholinesterase levels both decline and regenerate more quickly than RBC cholinesterase levels. The principal mechanism of action of the organophosphorus pesticides is the inhibition of activity of acetylcholinesterase (AchE), the enzyme performing the hydrolysis of acetylcholine to choline and acetic acid. Specific ChE(EC 3.1. 1.7) is located in the nervous ganglionic synapses of neuromouscular structures and in erythrocytes, and nonspescific ChE(EC 3.1.1.8) occurs mainly in the plasma and liver. Organophosphorus pesticides generally inhibit both enzymes. The cholinestrerase inhibition reaction is often utilized for the sensitive detection of organophosphorus pesticides and carbamates[3-6]. The use of this enzyme is easy and the low cost of equipment make this method highly attractive for laboratories with limited resources.

The analysis of pesticides is generally carried out using gas chromatographic or HPLC methods[7-10] which identify single compounds in complex matrices and reach detection limits compatible with those imposed by law for pesticide residues in the environment. These methods generally require sample pretreatment and preconcentration steps and are not suitable for field or on-line analysis.

Potentiometric or amperometric methods based on the inhibition of acetylcholinesterase have been reported[11-15]. In this paper an amperometric method is described based upon the property of organophosphorus pesticides to inhibit the enzyme acetylcholinesterase.

Experimental

Materials

The daialysis membrane(Cat.No. D-9652), acetylcholinesterase(EC 3.1.1.7), choline oxidase (1.1.3.17), acetylcholine, choline, and all other chemicals were obtained from the Sigma Chemical Co.,St. Louise, MO, USA. Cellulose acetate membrane was prepared in our laboratory according to the procedure described in ref.16. Paraoxon and parathion were purchased from Reidel, Hannover, Germany.

Apparatus

Amperometric measurements were carried out in a 50 ml glass cell with stirring the oxygen electrode used was a Schott electrode, which was connected to a CG 867 model oxygenmeter (Schott, Hofheim, Germany).

Biosensor Assembly

The enzymes, acetylcholinesterase and choline oxidase were co-immobilized on the Clark oxygen electrode surface by entrapment between the cellulose acetate and the dialysis membrane. The biosensor assembly in shown in Figure 1.

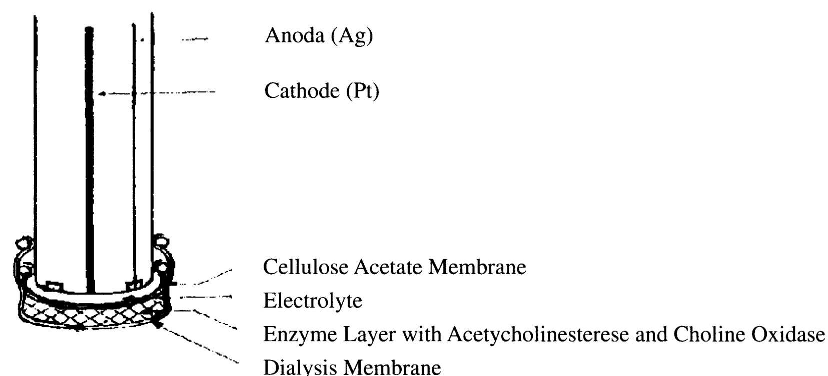
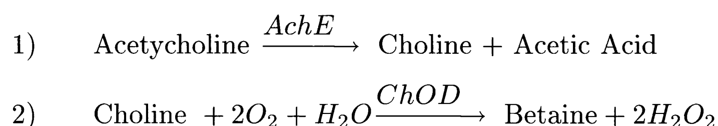


Figure 1. Scheme of the organophosphorus pesticide biosensor

Procedure

The measurement of acetylcholine is based up the catalysed hydrolysis acetylcholinesterase to choline and acetate, follolwed by the choline oxidase catalysed oxidation of choline to betaine and hydrogen preoxide.



$$1) \quad \text{Acetycholine} \xrightarrow{AchE} \text{Choline} + \text{Acetic Acid}$$

$$2) \quad \text{Choline} + 2O_2 + H_2O \xrightarrow{ChOD} \text{Betaine} + 2H_2O_2$$

reaction(1) is catalysed by acetylcholinesterase(AchE); reaction(2) is catalysed by the enzyme choline oxidase(ChOD). The consumption of the dissolved oxygen(DO) is correlated to the concentration of choline esters present in solution.

The enzyme electrode was stabilized in 0.1 M glycine buffer (pH 8.0) contanining acetylcholine(0.2 mM). A decrease of the dissolved oxygen concentration was immediately observed. The probe was equilibrated(about three minutes) and then a known amount of organophosphorus pesticide was added. An increase in the oxygen concentration was observed. This increase in the oxygen concentration, measured after the new oxygen gas equilibrium conditions was found to be proportional to the pesticide concentration in the solution.

The determination of the organophosphorus compounds was performed by measuring the decrease of the rate of oxygen consumption, because organosphosphorus pesticide inhibitis acetylcholinesterase. For each measurement, is was necessary to repeat this sequence of dipping and maintaining the biosensor in the glycine buffer solution while stirring for 20 minutes, between subsequent measurements. using the calibaration graph, obtained from these measurements, the organophosphorus pesticide concentration of the samples can be determined.

Results and Discussion

The use of a choline probe has been previously described[11–15,17,18–21]. For the analysis of organophosphorus pesticides calibration curves for the biosensor with O_2, choline and acetylcholine were executed. Calibration curves of paraoxon and parathion with immobilized AchE and ChOD are given in Figure 2.

As a hydrolase group enzyme, the acetylcholinesterase is greattly infulenced by the pH of the testing solution. Because of the enzym immobilization by entrapment with a pH neutral dialysis membrane, immobilized and native acetylcholinesterase show the same pH dependence. Therefore we measured only native enzyme activity in the glycine buffer(0.1 M) at different pH values. The optimum activity was found to be at approxiamately pH 8.0.

The incubation time is dependent on the degree of inhibition of the acetylcholinesterase. It plays an important role in defining the detection limit for the analysis and has to be selected based on the expected concentration levels of the pesticide in solution. The analysis of paraoxon at different incubation times is repoted in Figure 3. At a fixed concentration of pesticide, the degree of inhibition increases with the incubation time but this correlation is not linear. On the other hand, at a fixed incubation time it is linear with the concentration of pesticide. However, when the incubation time is too long the increase of inhibition becomes negligible. It is important to know the range to be analyzed, so that the right incubation time can be selected and the analysis carried out at its maximum sensitivity.

The most significant advantage of this analytical method is due to the use of an enzyme choline sensor as the indicator electrode and consequently from its high specificity to choline that represents on of the products of acetylcholine enzymatic hydrolysis. Different indicator electrodes, such as pH[11], FET[22] or piezoelectric sensors[23] are not as specific as the choline sensor: only immunochemical sensors[24] are more specific than the choline sensor. The development of a method; based on the inhibition of the immobilized acetylcholinesterase, starts results from the contact between the enzyme and the organophosphorus pesticide during a short time, but, if the contact time is prolonged and a high concentartion of the pesticide is used, the inhibition becomes permanent[25]. The use of this choline sensor displays great advantages for its practicality and its possible use *in situ* of the sonsor. Furthermore due to the elimination of the incubation time and thus, in a shorter analysis time the sensor can be used for rapid and emergency on-site analysis of

pollution levels[26]. The reproducibility of the biosensor was also searched for a paraoxon concentration of 3.0 mg/1(n=10); average value (x), standard deviation(SD and variation coefficient (CV) as 2.985 mg/1, ± 0.116 mg/1 and 3.9 % , respectively. The regression lines were estimated as y=0.57x-0.046 and y= 0.50x-0.032 for paraoxon and parathion, respectively.

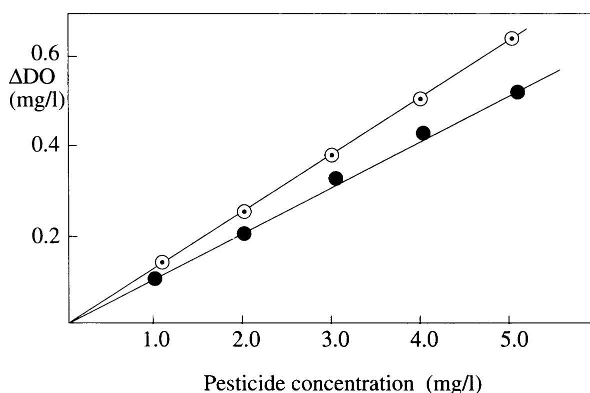


Figure 2. Calibration curves for pesticides with immobilized acetylcholiesterase and choline oxidase(Glycine buffer, pH 8.0, Temp.: 25°C, incubation time: 20 minutes, Acetylcholine concentration: 0.2 mM). (○): Paraoxon, (●): Parathion

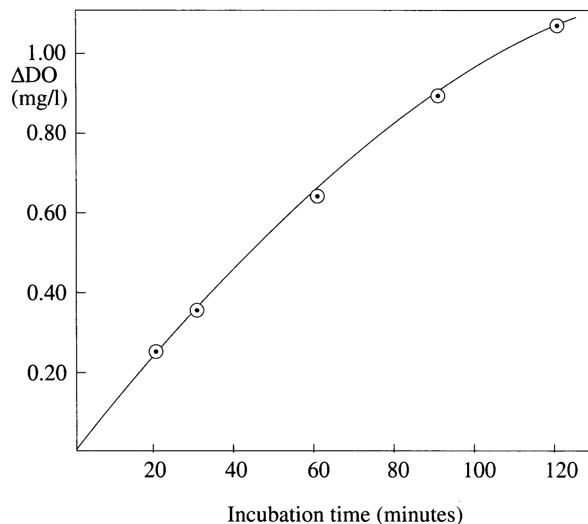


Figure 3. The effect of incubation time on the inhibition of the immobilized acetylcholinesterase(Acetylcholine concentration: 0.3 mM)

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