# KINETICS OF THE ACETYLCHOLINESTERASE (AchE) INHIBITION

**HENDRY YANUAR**
*Chemistry Dept - FMIPA, Cenderawasih State University, Jayapura- Papua*

**ABSTRACT**

*Acetylcholinesterase (AchE) is an enzyme, which work on acetylcholine hydrolysis. Some insecticides can inhibit the activities of this enzyme. The purpose of this research was determined the kinetics of the AchE inhibition over the organophosphate and carbamat inhibitors with the methylindoxylacetate (MIA) as a substrate. The AchE extract was obtained from the local honeybee head (100 heads on the 5 mL of phosphate buffer 0.05 M). Based on the preliminary analysis, the volume of the enzyme extract for the reaction rate was 100 $\mu$L on 1-5 mL of the substrate. Monocrothopos, Carbophenathion, Baycarb and MIPC were used as inhibitors which the concentration were 0.0018, 0.0030, and 0.0042 mg/mL respectively. The reaction rate were measured by Fluorescence HPLC Monitor (Shimadzu RF 535) at 540 nm, and some computational program were used on data analysis. The result of this research showed that the maximum rate of MIA hydrolysis by AchE without the presence of inhibitor was 5.16 mL/s and the hydrolysis constant ($K_m$) was 3.49, and the inhibitors did not influence the maximum rate of substrate hydrolysis. It was finally concluded that the kinetics of AchE inhibition on MIA hydrolysis over the organophosphate and carbamat inhibitors was the competitive inhibition.*

Keyword*: acetylcholinesterase, inhibition, organophosphate, carbamat*

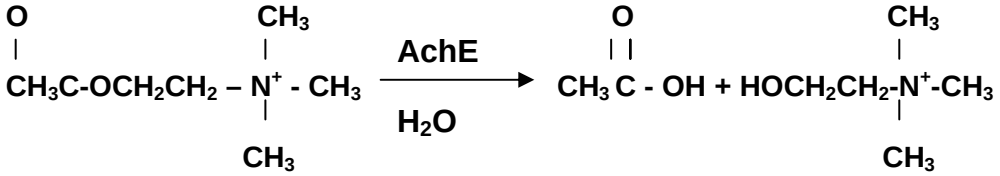
## INTRODUCTION

Acetylcholinesterase (AchE) is an enzyme, which work on the acetylcholine hydrolysis to produce the acetic acid and choline. The reaction was written on scheme 1.

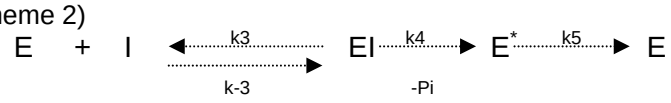
AchE has two kinds of active sites, that were anionic and estheratic sites. The anionic site (carboxylic acid from the glutamic or aspartate) has two negative charges and bonded to the nitrogen from the acetylcholine. The estheratic site was involved on the substrate hydrolysis and have the base (imidazole from the histidine) and acid groups (aromatic hydroxyl from the tyrosine). The imidazolin group was formed to activate the serine hydroxyl, and then the partial negative charge of the serine oxygen will be increasing the nucleofilic attack to the carboxyl group of acetylcholine.

Fallah [1] reported that the acetylation of AchE would make this enzyme unstable and easy to hydrolysis. In the presence of the carbamat, decarbamilation step due around of 40 minutes (carbamate is the reversible inhibitor for the AchE). The inhibition of AchE over the organophosphate is irreversible, because the hydrolysis rate of posphorilated AchE is lower than carbamilated AchE.

(Scheme 1)




(Scheme 2)



The schematic diagram of enzyme carbamilation can be describe on scheme 2 [2]. Where: E = active enzyme, EI= reversible enzyme complex, $E^*$ = carbamilated enzyme (inactive)

The formation of EI complex was depending on the affinity of the inhibitor to the enzyme and dissociation constant.

$$K_a = \frac{[E]\ [I]}{[EI]} \quad (1)$$

The rate of irreversible inhibition was determined by phosporilation constant, $k_4$. If the enzyme was reversible activated to $E^*$ by the inhibitor ($k_5$=0), the formation rate of EI can be describe as follow:

$$\delta[EI]/\delta t = k_3 [E]\ [I] - k_{-3} [EI] - k_4 [EI] \quad (2)$$

and

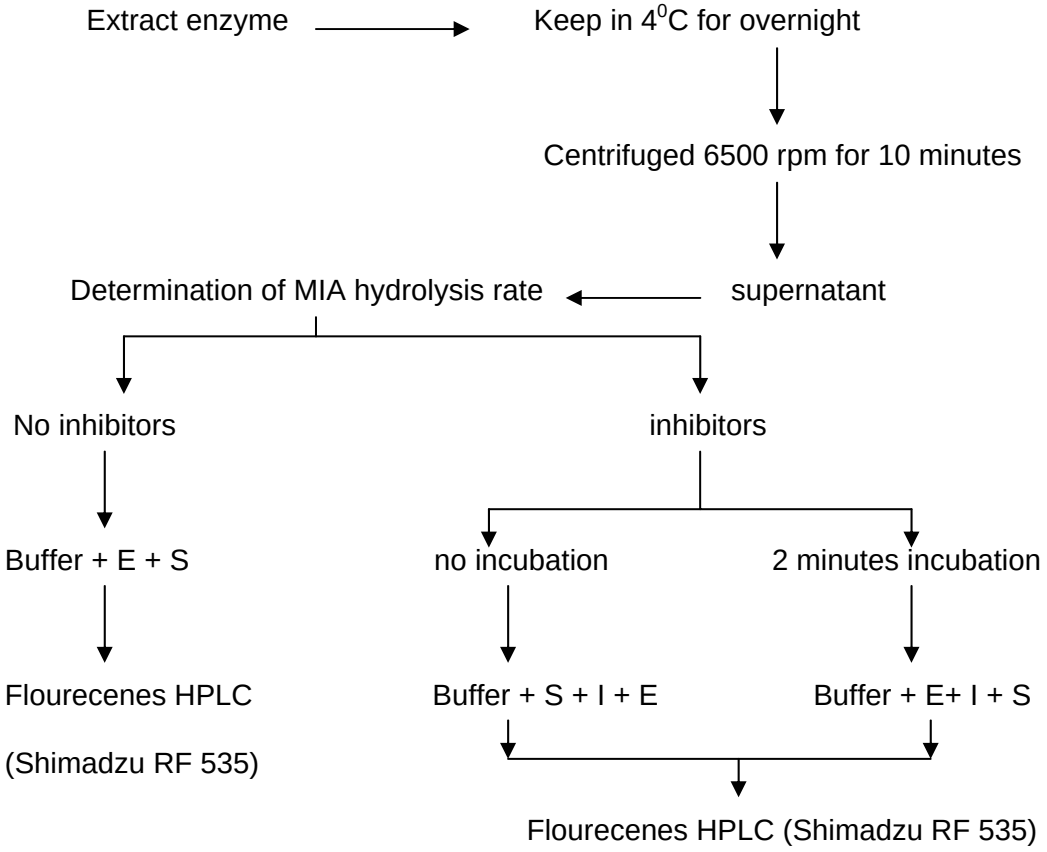
$$E^* = \delta[E^*] = k_4 [EI] \quad (3)$$

In this research the AchE enzyme was extracted from the local honeybee head (Local strain from Wamena Region-Papua Prov.), according to [3], that the AchE from the bee head has the lowest impurities than the others AchE sources. The objectives of this research are to observe the kinetics of AchE inhibition on MIA hydrolysis and to develop the techniques of enzyme kinetic experiment.

## EXPERIMENTAL SECTION

### AchE extraction

The AchE extract was obtained from honeybee head, 100 heads on the 5 mL phosphate buffer 0.01M. The schematic diagram of enzyme extraction and substrate hydrolysis rate measurement were as follow.



## RESULT AND DISCUSSION

1) MIA hydrolysis without the inhibitors

The result of MIA hydrolysis without the organophosphate and carbamat inhibitors was given in Table 1. The $V_{max}$ and $K_m$ were obtained from plot of reaction rate vs. substrate concentration as given in figure 1.

Based on the powerfit program (order one) calculation, it was obtained that the value of $V_{max}$ was 5.16 and Km was 3.49, so the value of $(1/V_{max})$ and $(-1/K_m)$ were 1.94 and –0.29 respectively. The value of $K_m$ that obtained from this graphic was not represent the true value of $K_m$. Palmer (1985), reported that the value of $K_m$ that obtained from the graphic was quite different with the value of $K_m$ that obtained from the reaction rate determination.

Figure 1, showed that the activities of AchE still in increased by substrate concentration increasing. The temperature increasing during enzyme extraction and crude enzyme centrifugation was caused the enzyme activity decreased. So that, in this research the activity of AchE was not optimal on MIA hydrolysis.

Table 1 the result of MIA hydrolysis without the inhibitors

| [ MIA ] mL/s | $\delta P_s / \delta t$ mL/s | | | 1/v |
|---|---|---|---|---|
| | I | II | Mean | |
| 1 | 1.16 | 1.13 | 1.14 | 0.88 |
| 2 | 1.93 | 1.93 | 1.93 | 0.52 |
| 3 | 2.44 | 2.50 | 2.42 | 0.40 |
| 4 | 2.64 | 2.90 | 2.77 | 0.36 |
| 5 | 2.86 | 2.86 | 2.86 | 0.35 |

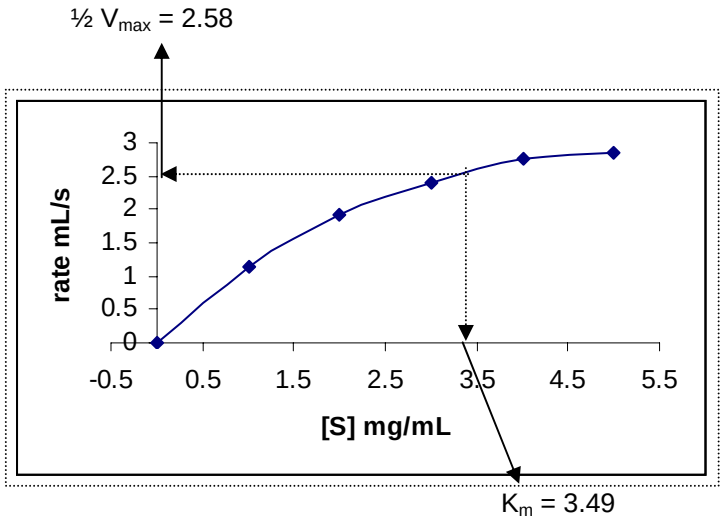
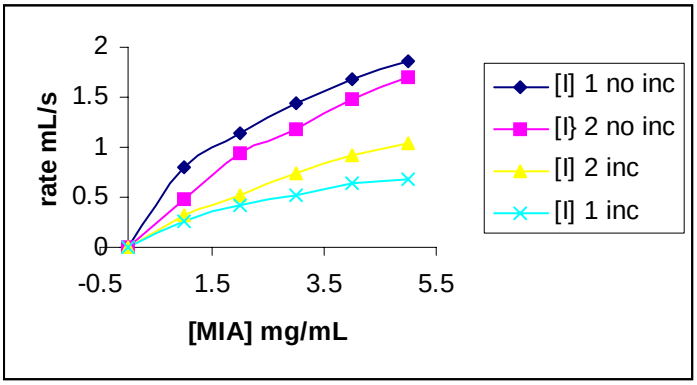



Figure 1 Plot of reaction rate vs substrate concentration

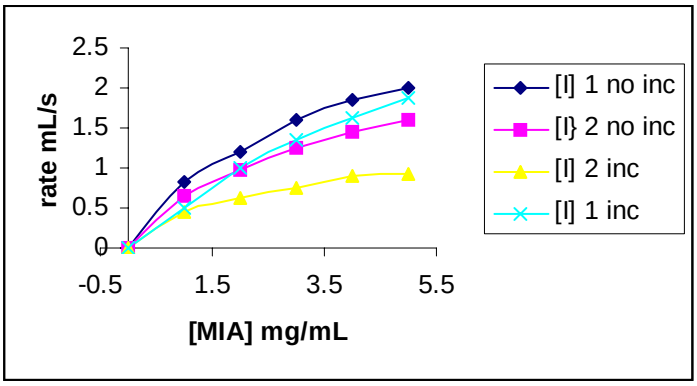
Table 2 The value of $V_{max}$ and $K_m$ of MIA hydrolysis by AchE over organophosphate inhibitors

| Organophosphate | $V_{max}$ | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| Monocrothopos | 4.73 | 4.04 | 4.73 | 5.51 |
| Carbophenanthion | 4.79 | 5.05 | 4.96 | 4.88 |
| | $K_m$ | | | |
| Monocrothopos | 11.14 | 16.67 | 28.01 | 51.55 |
| Carbophenathion | 8.76 | 19.88 | 17.86 | 45.45 |

Note:1 [Inhibitor] = 0.0018 mg/mL, no incubation, 2 [Inhibitor] = 0.0018 mg/mL, 2 minutes incubation, 3 [Inhibitor] = 0.003 mg/mL, no incubation, 4 [Inhibitor] = 0.003 mg/mL, 2 minutes incubation,




(a) inhibition by monocrotophos




(b) inhibition by carbophenanthion

Figure 2 Plot of reaction rate vs. substrate concentration in the presence of inhibitor

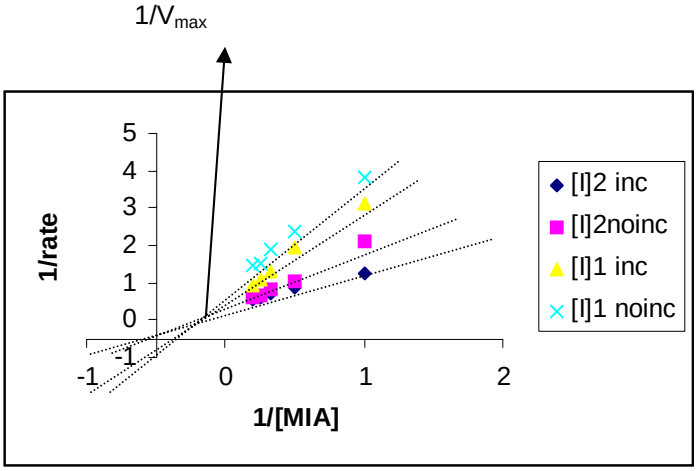



Figure 3 Lineweaver-Burk Plot of organophosphate inhibitor

2) MIA hydrolysis over organophosphate inhibitor

The organophosphate was used as inhibitors in this research were monocrothopos and carbophenanthion, which the concentration were 0.0018 and 0.003 mg/mL respectively. The results of MIA hydrolysis by AchE over organophosphate inhibitors was given in table 2. This experiment was divided into two conditions: no incubation of enzyme-substrate-inhibitor and 2 minutes incubation of enzyme-substrate inhibitor.

The statistical test of $V_{max}$ showed that the value of $V_{max}$ was obtained from this experiment was not different with the value of $V_{max}$ was obtained from the experiment of MIA hydrolysis without the inhibitors, but the value of $K_m$ was changed. It was showed that the increasing of inhibitors concentration was increased the value of $K_m$. The value of $K_m$ that obtained from the experiment with 2 minutes incubation was higher than the value of $K_m$ that obtained from the experiment with no incubation. This phenomenon can be explain that during the incubation the bonding between the enzyme and the inhibitor was established, so its need of more time for breaking the bonding. This condition was caused the decreased of substrate rate hydrolysis, and the rate determining steps were the concentration and affinity of substrate to the AchE.

Plot of reaction rate vs. substrate concentration in the presence of inhibitor is shown in Figure 2. In order to know the type of enzyme inhibition, it is importance to observe the plot of (1/rate) vs. (1/substrate concentration); the plot was given in figure 3. Figured 3 showed that the type of enzyme inhibition was competitive inhibition, it was proved by unchanged value of Vmax (the line was crossed at one point).

**3) MIA hydrolysis over carbamat inhibitor**

MIPC and baycarb were used as carbamat inhibitors in this research, which the concentration were 0.0042 and 0.0030 mg/mL respectively. The results of MIA hydrolysis over organophosphate inhibitors was given in table 3. This experiment was divided into two conditions: no incubation of enzyme-substrate and 2 minutes incubation of enzyme-substrate.

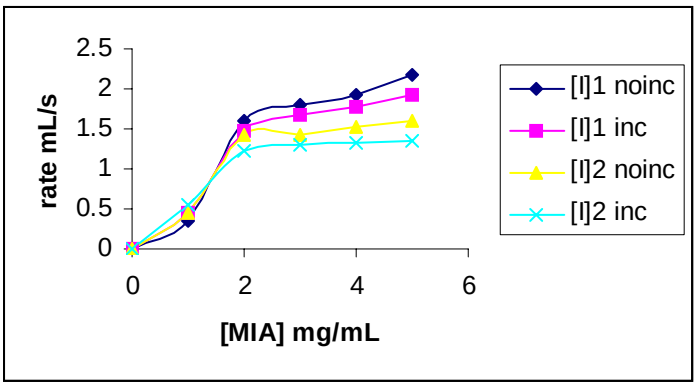
Figure 4 showed that the carbamat inhibitor has lower influence on the rate hydrolysis of MIA than the organophospate. It was proved by the rate of MIA hydrolysis of each condition was almost in the same value. This condition showed that the organophosphate has bigger affinity to the AchE than the carbamat. The value of $V_{max}$ was obtained in this experiment was unchanged, the inhibitor just influence the value of $K_m$. The changed value of $K_m$ will observe by plot of (1/rate) vs. (1/substrate concentration).

Figure 5 showed that the type of enzyme inhibition by carbamat was competitive inhibition, it was proved by the unchanged value of $V_{max}$ and variable value of $K_m$.

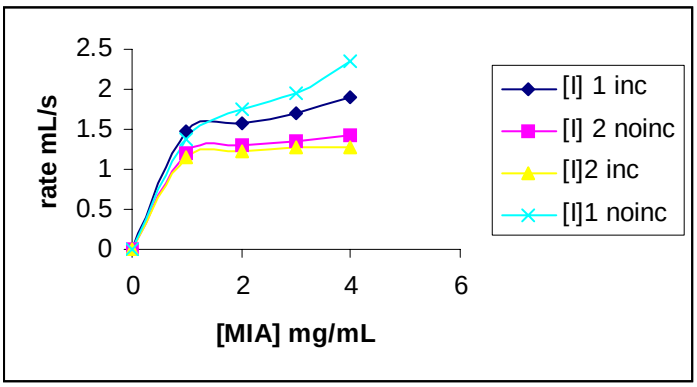
Table 3 The value of $V_{max}$ and $K_m$ of MIA hydrolysis by AchE over carbamat inhibitors

| Carbamat | $V_{max}$ | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| MIPC | 4.69 | 4.97 | 4.59 | 4.68 |
| Baycarb | 4.87 | 4.64 | 4.76 | 4.78 |
| | $K_m$ | | | |
| MIPC | 16.06 | 21.93 | 43.33 | 47.62 |
| Baycarp | 20.49 | 27.39 | 33.67 | 30.86 |

Note:1 [Inhibitor] = 0.0030 mg/mL, no incubation, 2 [Inhibitor] = 0.0030 mg/mL, 2 minutes incubation, 3 [Inhibitor] = 0.0042 mg/mL, no incubation, 4 [Inhibitor] = 0.0042 mg/mL, 2 minutes incubation,




(a) inhibition by MIPC




(b) inhibition by baycarb

Figure 4 Plot of reaction rate vs. substrate concentration in the presence of inhibitor

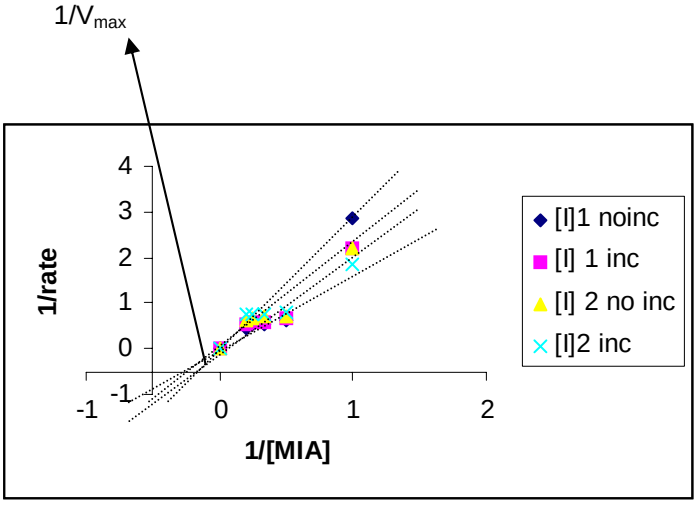



Figure 5 Lineweaver-Burk Plot of carbamat inhibitor

## CONCLUSION

It was concluded that the $V_{max}$ of MIA hydrolysis by AchE without the presence of inhibitor was 5.16 mL/s and the value of $K_m$ was 3.49. The inhibitor did not change the value of $V_{max,}$ but it was changed the value of $K_m$. The kinetic inhibition of MIA hydrolysis by AchE over organophosphate and carbamat inhibitor was competitive inhibition.

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