Detoxification of Organophosphate Pesticides Using an Immobilized Phosphotriesterase from *Pseudomonas diminuta*

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A purified phosphotriesterase was successfully immobilized onto trityl agarose in a fixed bed reactor. A total of up to 9200 units of enzyme activity was immobilized onto 2.0 mL of trityl agarose (65 μmol trityl groups/mL agarose), where one unit is the amount of enzyme required to catalyze the hydrolysis of one micromole of paraoxon in one min. The immobilized enzyme was shown to behave chemically and kinetically similar to the free enzyme when paraoxon was utilized as a substrate. Several organophosphate pesticides, methyl parathion, ethyl parathion, diazinon, and coumaphos were also hydrolyzed by the immobilized phosphotriesterase. However, all substrates exhibited an affinity for the trityl agarose matrix. For increased solubility and reduction in the affinity of these pesticides for the trityl agarose matrix, methanol/water mixtures were utilized. The effect of methanol was not deleterious when concentrations of less than 20% were present. However, higher concentrations resulted in elution of enzyme from the reactor. With a 10-unit reactor, a 1.0 m*M* paraoxon solution was hydrolyzed completely at a flow rate of 45 mL/h. Kinetic parameters were measured with a 0.1-unit reactor with paraoxon as a substrate at a flow rate of 22 mL/h. The apparent K_m for the immobilized enzyme was 3–4 times greater than the K_m (0.1 m*M*) for the soluble enzyme. Immobilization limited the maximum rate of substrate hydrolysis to 40% of the value observed for the soluble enzyme. The pH–rate profiles of the soluble and immobilized enzymes were very similar. The immobilization of phosphotriesterase onto trityl agarose provides an effective method for hydrolyzing and thus detoxifying organophosphate pesticides and mammalian acetylcholinesterase inhibitors.

INTRODUCTION

The current environmental problems associated with pesticide usage and contamination require serious attention. It is estimated that up to 800,000 individuals each year are affected by pesticide poisoning.[1] Of particular interest are the organophosphate pesticides since they comprise the major proportion of the agricultural pesticides utilized in today's agricultural industry (E. Brandt, EPA, Washington, DC, personal communication). Contamination results from either occupational or incidental exposure where adsorption occurs by direct contact with air, food, and water. This class of agricultural insecticides dominate as the leading cause of pesticide poisoning cases in the United States.[2]

Of equal importance is the environment, where the need for safe, convenient, and economically feasible methods for pesticide disposal must be developed. There are several methods of disposal currently available,[3,4] but most of these have some undesirable limitations. Incineration offers a reliable method but is economically restrictive, and the proper treatment of the noxious gaseous effluents are of concern. Chemical methods, although viable, leave the problem of disposal of large volumes of caustics and acids. Landfills function adequately, but leaching into the soil and water is a problematic consideration. To avoid some of these limitations, the use of enzymatic detoxification is one method receiving serious attention. Biological methods such as the use of activated sludge and anaerobic digestion have proved successful. However, these methods require the maintenance of a biological system and rely on the production of enzymes for detoxifying contaminants.

Recently, an enzyme has been purified and characterized as a phosphotriesterase with a very broad substrate specificity.[5] This enzyme has been shown to hydrolyze a substantial number of the most widely used organophosphate pesticides including methyl and ethyl parathion, diazinon, fensulfothion, dursban, and coumaphos.[5] In addition, this phosphotriesterase is able to degrade deadly nerve toxins such as sarin and

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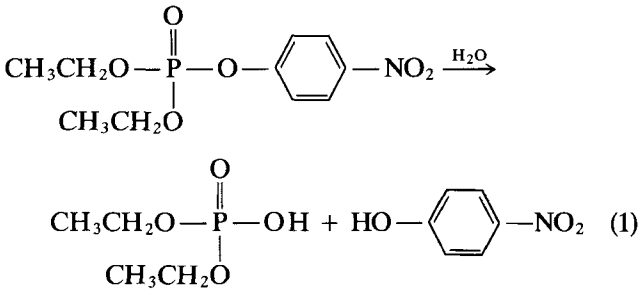
soman.[6] The most characteristic reaction of this enzyme involves the hydrolysis of paraoxon[7]:



The immobilization of such an enzyme may provide the means whereby toxic substances can be efficiently degraded in a continuous process. Immobilization avoids the limitation of maintaining a biological system and usually increases the stability of the enzymatic activity. The utilization of an immobilized enzyme for degradation of pesticides has been described in one instance and involves the immobilization of a parathion hydrolase from a crude cell extract onto porous silica for the hydrolysis of parathion.[8] Because the phosphotriesterase from *Pseudomonas diminuta* is capable of hydrolyzing many different organophosphate pesticides, its immobilization may allow a stable system where its substrates can be hydrolyzed in a continuous fashion under mild environmental conditions.

Of the various methods of protein immobilization, it has been suggested that the physical adsorption of an enzyme is the simplest to perform and least affects enzyme activity.[9] In this study, the specific method of immobilization was based on the demonstrated hydrophobic nature of phosphotriesterase.[5] Trityl agarose was chosen as the water insoluble matrix because of the reported high coupling properties and the high retention of activity upon the immobilization of many different enzymes.[9] In this report, the immobilization of the phosphotriesterase from *P. diminuta* is described. The characteristics of this immobilized enzyme are defined, and the potential ability of this immobilized phosphotriesterase to hydrolyze and thus detoxify several organophosphate pesticides is investigated.

MATERIALS AND METHODS

Materials

Phosphotriesterase was purified to a specific activity of 2500 units/mg of protein from *Spodoptera frugiperda* (sf9) cells transfected with a recombinant baculovirus system containing the cloned gene for the enzyme.[5] Trityl agarose (65 μmol trityl groups/mL of agarose) was obtained from Sigma Chemical Co. [3-Cyclohexylamino]-1-propane sulfonic acid (CAPS), (2-[*N*-cyclohexylamino] ethanesulfonic acid (CHES), (*N*-2-hydroxylethyl piperazine)-*N*-2 ethanesulfonic acid (HEPES), ((2-*N*-morpholino) ethanesulfonic acid (MES), piperazine-*N*,*N*-bis [2-ethanesulfonic acid] (PIPES), (tris[hydroxymethyl] methylaminopropane sulfonic acid (TAPS), dithiothreitol (DTT), diisopropyl fluorophosphate (DFP), and paraoxon were also purchased from Sigma Chemical Companyl. Methyl parathion, ethyl parathion, diazinon, and coumaphos were purchased from Chem Service.

Preparation of the Immobilized Phosphotriesterase

The immobilization of phosphotriesterase was accomplished by a procedure similar to that of Cashion et al.[9] To a slurry of trityl agarose in 125 m*M* CHES, pH 9.0, was added a predetermined amount of enzyme activity. After gently swirling for at least 10 min, the mixture was poured into a glass column reactor. After the reactors were prepared, they were washed with at least 30 mL of 125 m*M* CHES, pH 9.0, and fractions of the effluent were collected and assayed for the presence of enzyme activity. The extent of coupling of the free enzyme to the trityl agarose was determined by measuring the amount of enzyme activity before it was applied to the column and then subtracting the catalytic activity that was recovered in the wash fractions during and after the reactor preparation. A general schematic of the experimental system is shown in Figure 1.

Enzymatic hydrolysis was measured in two different packed bed reactors. A 280-unit enzyme reactor was prepared in a glass column (0.7 × 8 cm) with a final volume of 3.0 mL. The other reactor system was prepared in a glass column (0.5 × 10 cm) with a final volume of 2.0 mL. Several independent columns were prepared using the latter of the two reactor systems with various amounts of enzyme activity applied (0.1, 1.0, 10.0, 100, 1000, and 9200 units of enzyme activity). These reactors were stored at 4°C in 125 m*M* CHES, pH 9.0, between experiments.

Activity Measurements

Enzyme activity is defined in units where one unit is the amount of enzyme required to catalyze the hydrolysis of one micromole of paraoxon in one minute. The amount of enzyme activity was determined by monitor-

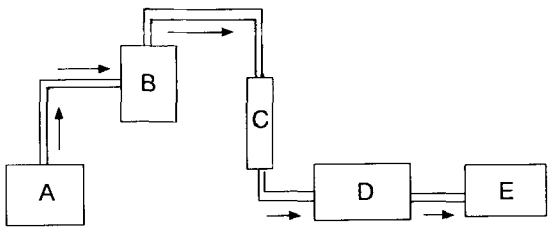


Figure 1. A general schematic for the experimental system, (A) substrate reservoir, (B) Rainin peristaltic pump, (C) fixed bed reactor, (D) Gilson Model HM Holochrome UV–visible detector, and (E) effluent collector.

ing the absorbance of *p*-nitrophenol at 400 mm (ε = 17,000 M^{-1}) produced by the hydrolysis of paraoxon at pH 9.0 with a Gilford 260 spectrophotometer. The hydrolysis of the organophosphates by the immobilized enzyme was performed at continuous flow rates. In all cases, fractions were collected in tubes containing 1.0 m*M* dithiothreitol, a competitive inhibitor for the enzyme (K_i = 8.8 μM),[5] to ensure protection from further hydrolysis of any unreacted substrate. The hydrolysis of methyl parathion and ethyl parathion was monitored by measuring spectrophotometrically the concentration of *p*-nitrophenol produced in the collected fractions. The hydrolysis of diisopropylfluorophosphate was determined using an Orion Model 94-09-00 fluoride sensitive electrode by measuring the concentration of fluoride anion. The fractions collected from the hydrolysis of coumaphos were monitored spectrophotometrically at 348 nm (ε = 9100 M^{-1}) for the release of 3-chloro-7-hydroxy-4-methyl coumarin. The product from the hydrolysis of diazinon was measured spectrophotometrically at 228 nm for the presence of 2-isopropyl-4-methyl-6-hydroxy pyrimidine.

Solvent Effects

The effect of organic solvent on the affinity of the immobilized enzyme for the trityl agarose matrix was determined by washing a 280-unit reactor (0.7 × 8 cm) with 125 m*M* CHES, pH 9.0, with various concentrations of organic solvent. At least 10 mL of effluent were collected at a flow rate of 22 mL/h at each concentration and the amount of enzyme activity eluted was assayed as previously described.

Thermostability of the Immobilized Phosphotriesterase

The effect of temperature on the stability of the immobilized phosphotriesterase was determined using a 1-unit reactor which was eluted with 0.3 m*M* paraoxon in 125 m*M* CHES, pH 9.0, at a constant flow rate of 20 mL/h at 25°C. After steady state hydrolysis was achieved, the temperature was elevated in 5°C increments. At each controlled temperature the steady state hydrolysis was measured and monitored for at least 1 h. The amount of *p*-nitrophenol produced at steady state was determined as above. The thermostability of the free enzyme was determined by incubating identical enzyme solutions buffered at pH 9.0 with 50 m*M* triethanolamine for a period of at least 1 h at controlled temperatures. Aliquots were removed at timed intervals and assayed for enzyme activity.

Kinetic Analysis

Kinetic measurements were made with a 0.1-unit reactor at 25°C using paraoxon with concentrations ranging from 0.03 to 0.92 m*M* in 10% methanol at pH 9.0. Steady state hydrolysis was measured by monitoring the concentration of *p*-nitrophenol in the effluent at each flow rate for at least 30 min with a Gilson Model HM Holochrome detector equipped with a 40-μL cuvette and a Linear chart recorder precalibrated at 400 and 460 nm. Flow rates were kept constant with a Rainin peristaltic pump. Fractions were collected and the amount of *p*-nitrophenol at pH 9.0 was measured at 400 nm with a Gilford 260 spectrophotometer to corroborate the chart recorder data. In all cases, the fractions collected contained DTT (final concentration 1.0 m*M*).

pH Profile

The extent of steady state hydrolysis of 1.0 m*M* paraoxon was determined in the pH range of 6–10 at intervals of approximately 0.5 pH units utilizing several independently prepared 1.0-unit immobilized phosphotriesterase reactors. Each reactor was equilibrated with the appropriate buffer (50 m*M* CHES, pH 9.5, pH 9.0; 50 m*M* TAPS, pH 8.5, pH 8.0; 50 m*M* PIPES, pH 7.5, pH 7.0; and 50 m*M* MES, pH 6.5, pH 6.0) before the elution of paraoxon. At a constant flow rate of 21 mL/h, fractions were collected and the pH measured. The pH of the fractions was adjusted to pH 9 and the amount of *p*-nitrophenol was determined spectrophotometrically at 400 nm.

Data Analysis

Kinetic data were analyzed using the least squares method of Cleland.[10] Data were applied to the appropriate equation to give the kinetic parameters and estimates of the standard errors. The apparent values for K_m and V_s were calculated from a fit to the data to Equation (2):

$$v = V_s A/(K_m + A) \quad (2)$$

where v is the initial velocity in micromoles per minute, V_s is highest possible velocity in micromoles per minute, A is the initial paraoxon concentration, and K_m is the apparent Michaelis constant. The pK_a value from the pH–rate profile was determined from a fit to Equation (3):

$$\log y = \log[c/(1 + H/K_a)] \quad (3)$$

where y is the amount of paraoxon hydrolyzed, c is the pH independent value of y, H is the hydrogen ion concentration, and K_a is the dissociation constant of the group that ionizes.

RESULTS AND DISCUSSION

Immobilization of Phosphotriesterase

Phosphotriesterase was successfully immobilized onto trityl agarose with a very simple and easily performed procedure. A series of columns containing from 0.1 to 9200 units of enzyme activity was prepared. These

columns were defined by the activity of the soluble enzyme before immobilization and in no manner infer the activity of the bound enzyme. During the preparation and washing with 125 m*M* CHES buffer, each column lost an insignificant amount of enzyme activity (<0.006 units) in the effluent, except for the 9200-unit reactor, which lost 1.2 units of enzyme activity. The large amount of enzyme applied to the reactor may be approaching the functional limits of this particular reactor (65 μmol trityl groups/mL agarose). However, the high enzymatic activity of this enzyme[7] does not necessarily require the preparation of such a high activity column.

Organophosphate pesticides are not very soluble in aqueous systems and thus require an organic solvent to achieve higher concentrations. Previous kinetic analysis with a variety of organophosphate pesticides hydrolyzed by phosphotriesterase necessitated the use of at least 10% methanol to improve solubility.[5] Since the coupling of phosphotriesterase is based on hydrophobic interactions, it was necessary to determine the effect of organic solvent on the coupling of the enzyme to the trityl agarose. Figure 2 shows that with the 280-unit fixed bed reactor, increasing the methanol concentration by 10% increments in the eluting buffer results in approximately a 10-fold increase in enzyme leakage from the reactor. The use of dimethylformamide (DMF) had an even more severe effect, as shown in Figure 2. Any concentration of DMF over 5% led to significant losses greater than 0.3 units/mL. The use of organic solvents imposes some restrictions in the use of the immobilized phosphotriesterase for the hydrolysis of substrates with extreme low solubility. With this particular reactor system, limits are set at 20% methanol in the eluting buffer before deleterious effects are observed while DMF is not a suitable solvent.

Hydrolysis of Organophosphate Pesticides

The application of the immobilized phosphotriesterase as a method for degrading various pesticides was evaluated. Shown in Table I are the initial experiments performed with a 280-unit reactor which demonstrates the ability of the immobilized phosphotriesterase to degrade the same substrates as the soluble enzyme, namely paraoxon, DFP, methyl parathion, ethyl parathion, and coumaphos at a flow rate of 22 mL/h. This table reflects the extent of hydrolysis for a limited amount of substrate applied to the immobilized enzyme column. The "recovered volume" is the amount of effluent required to collect the indicated percentage of the "recovered product." Near quantitative hydrolysis of paraoxon is obtained. However, the kinetically slower substrates[5] were not completely hydrolyzed under the conditions imposed. In the case of coumaphos and ethyl parathion only a low concentration of the unhydrolyzed substrate was collected and measured in the fractions. Moreover, progressively larger volumes of effluent were required to collect the hydrolysis products. Therefore, trityl agarose either showed an affinity for the substrates or to the hydrolysis products. The latter possibility appears very unlikely since, at pH 9.0, *p*-nitrophenol (pK_a 7.2) exists primarily in its ionized form and the fluoride anion produced from DFP hydrolysis should not be retarded hydrophobically. The affinity of the substrates and the products for the column matrix were analyzed by eluting paraoxon or *p*-nitrophenol from a trityl agarose column containing no bound enzyme. The results are shown in Table II and clearly indicate that *p*-nitrophenol is not retarded. Paraoxon did show a significant affinity for the trityl agarose as indicated by the large elution volumes required for complete recovery of the substrate. Furthermore, the addition of methanol to the elution buffer facilitates a smaller volume for recovery of this substrate where methanol serves to diminish the hydrophobic interaction. These experiments illustrate the affinity of the hydrophobic substrates for the trityl agarose matrix which results in greater elution volumes required for recovery of the hydrolysis products. The lack of a significant recoverable unhydrolyzed substrate also supports the affinity of the substrates to the matrix. The affinity of a hydrophobic substrate on a hydrophobic matrix is not unusual and has been observed in other systems.[11] This affinity does not pose a serious threat to the utilization of this system for pesticide degradation since in a continuous process of hydrolysis, a steady state would eventually be realized. However, this substrate partitioning may be expected to affect the kinetics of the immobilized enzyme.

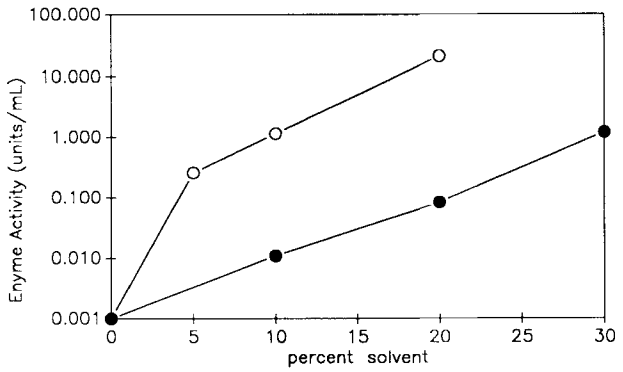


Figure 2. The effect of solvent on the elution of the immobilized phosphotriesterase from trityl agarose (280-unit reactor). Methanol (●), DMF (○). Additional details are given in the text.

Because some of the substrates shown in Table I were not completely hydrolyzed due to the affinity of the compounds for the matrix, experiments were conducted using the higher enzyme activity columns. Using the 1000-unit reactor at a flow rate of 11.5 mL/h, 54% of a 0.25 m*M* methyl parathion solution could be hydrolyzed in a continuous process for at least 2 h. This is a marked improvement in hydrolysis over the 34% hydrolyzed and recovered with the 280-unit reactor. Diazinon (0.07 m*M*) at pH 9.0 in 10% methanol was hydrolyzed to greater than 99% completion at a flow

Table I. Enzymatic hydrolysis of substrates with immobilized phosphotriesterase (280-unit reactor).[a]

Substrate	Concentration (m*M*)	Applied volume (mL)	Hydrolyzed product (%)	Recovery volume (mL)	Recovered product (%)
Paraoxon	0.1	3	99	27	99
Paraoxon[b]	0.1	2.8	99	13	99
Methyl parathion[b]	0.1	3	98	28	35
Ethyl parathion[b]	0.1	5	92	27	44
Diisopropyl fluorophosphate[c]	5	2.8	99	20	63
Coumaphos[d]	0.06	3.4	97	30	30

[a] Substrates were added at a constant flow rate of 22 mL/h. Elution of the product and/or unreacted substrate was performed with 125 m*M* CHES, pH 9, except where otherwise noted.
[b] 10% Methanol.
[c] 10% Methanol in 125 m*M* HEPES, pH 7.
[d] 20% Methanol.

Table II. The affinity of substrates for the trityl agarose matrix.

Substrate	Concentration (m*M*)	Volume (mL)	Recovery (%)	50% Recovery volume (mL)	Total recovery volume (mL)
p-Nitrophenol	2	3	100	2.7	6
Paraoxon	0.1	3	92	28	45
Paraoxon[a]	0.1	2.8	100	18	25

[a] 10% Methanol.

rate of 55.5 mL/h. These results indicate that by increasing the amount of enzyme activity or changing the flow rates, improved hydrolysis of these organophosphate pesticides can be realized with little difficulty. To further define the properties of the immobilized phosphotriesterase, different concentrations of paraoxon were loaded onto a 0.1-unit reactor. Figure 3 illustrates that as the flow rate and the initial paraoxon concentration are increased, the percentage of hydrolysis decreases. Only 50% hydrolysis could be achieved at 11.5 mL/h with 0.03 m*M* paraoxon. Similar experiments were performed with the 1.0 and 10-unit reactors. Escalating to the higher immobilized enzyme reactor results in a noticeable improvement in effective hydrolysis. For example, near quantitative hydrolysis is observed at concentrations of 0.97 m*M* paraoxon at a much faster flow rate of 45 mL/h with the 10-unit reactor. Under these conditions, the complete hydrolysis of paraoxon was effectively achieved for at least 2 h at steady state. As would be expected, the 1.0-unit reactor is intermediate in its effectiveness toward the hydrolysis of paraoxon. These experiments demonstrate the minimal requirements for near quantitative hydrolysis of paraoxon with this immobilized phosphotriesterase.

Thermostability of the Immobilized Enzyme

Figure 4 shows the effect of increased temperature on the activity of the enzyme. At 55°C, the half-life of 95 min for the immobilized enzyme is significantly greater than 16 min for the soluble enzyme. Temperatures at or below 45°C did not result in the inactivation

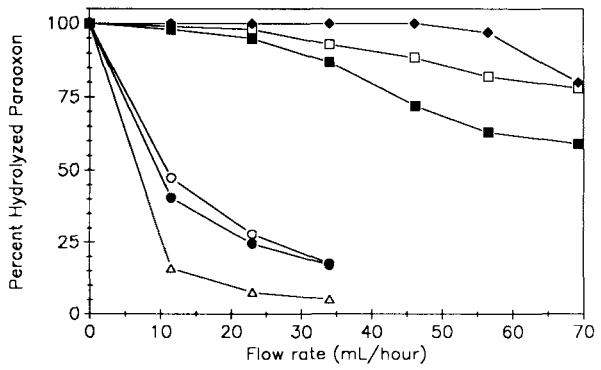


Figure 3. The hydrolysis of paraoxon in 125 m*M* CHES, 10% methanol at varied flow rates with an immobilized phosphotriesterase fixed bed reactor (0.1 unit) (△, 0.92 m*M*; ●, 0.1 m*M*; ○, 0.046 m*M*); (1.0 unit) (■, 0.17 m*M*; □, 0.087 m*M*); (10.0 unit) (◆, 0.97 m*M*).

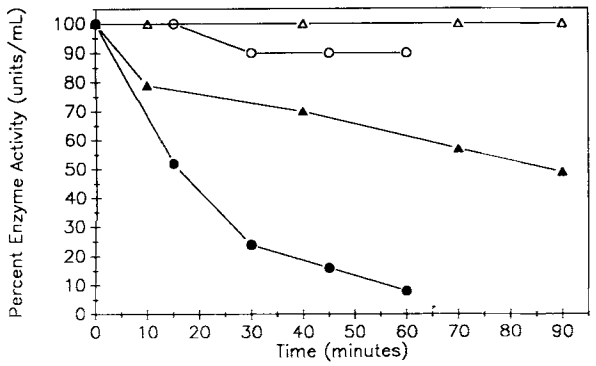


Figure 4. Thermostability of (○) soluble and (△) immobilized enzyme at 45°C (open symbols) and 55°C (shaded symbols).

of either the soluble or immobilized enzyme over periods of at least 1 h. Immobilization of phosphotriesterase has resulted in increased stability, which is consistent with previous observations that immobilization of enzymes can increase stability.[12] However, even with this relative increased stability at 55°C, there is a continued decline in activity making the utilization of this immobilized enzyme unfeasible at this temperature.

Kinetic Analysis

The kinetic analysis of the immobilized phosphotriesterase is shown in the Lineweaver-Burk plots in Figure 5. Linear plots are observed over a wide range of paraoxon concentration (0.03–0.97 mM) at varied flow rates. The linearity of these data suggest that the immobilized enzyme follows Michaelis-Menten kinetics in this concentration range. Utilizing the FORTRAN programs of Cleland, a best fit analysis gave the apparent Michaelis constant and V_s values shown in Table III. The apparent K_m values of 0.29–0.45 mM are only 3–4 times greater than that of the free soluble enzyme (K_m = 0.1 mM). These higher K_m values are in accord with many other immobilized enzyme systems where higher apparent K_m values can be attributed to diffusional limits on the substrate by the matrix.[11,13] This could also be the result of steric or conformational effects. As shown in Table III, the differences in the K_m values are not substantial, but there is a slight increase in the apparent K_m with decreasing flow rates, suggesting external diffusional effects.[14] Internal diffusional effects have been attributed to enzymes possessing high catalytic turnover rates.[12,14] Since the soluble phosphotriesterase exhibits a high catalytic rate at diffusion-limited rates for paraoxon hydrolysis (unpublished results of S. R. Caldwell and F. M. Raushel), a larger apparent K_m relative to the soluble enzyme would be expected. However, this effect on K_m may have been minimized by substrate partitioning and the affinity of the substrates for the trityl agarose.[14]

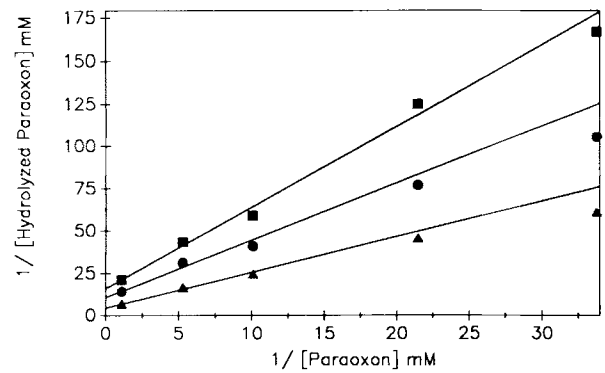


Figure 5. Lineweaver–Burk plots for the immobilized phosphotriesterase (0.1 unit) with varying concentrations of paraoxon at different flow rates: (▲), 11.5 mL/h; (●), 22 mL/h; (■), 34 mL/h.

Table III. The apparent kinetic parameters and the effect of flow rates on the hydrolysis of paraoxon with the immobilized phosphotriesterase (0.1 unit).

Flow (mL/h)	K_m (mM)	μmol/min
11.5	0.45 ±0.05	0.042
23	0.31 ±0.04	0.038
34	0.29 ±0.03	0.034

Shown in Table III is the effectiveness of the immobilized enzyme (0.1-unit reactor) in degrading paraoxon. Regardless of the flow rate, the maximum performance of this reactor is about 40% that of the free soluble enzyme. The value of V_{max} for the enzymatic hydrolysis of paraoxon in the free soluble system is 2300 s^{-1}.[5] This lower effectiveness may be attributed to steric or conformational effects imposed by the immobilization of the enzyme. The lower effectiveness may also be a result of internal diffusional limits and/or the inherent high catalytic efficiency of the soluble phosphotriesterase.[14]

pH Profile

The pH activity profile for the immobilized phosphotriesterase when compared to the kinetic pH profile for the soluble enzyme[7] is shown in Figure 6. In this figure the values for log V_{max} are plotted in arbitrary velocity units.[7] Since the pH activity curve at saturating concentrations of paraoxon for the soluble enzyme is similar to the corresponding log V_{max} and log V/K profiles for the soluble enzyme, such a comparison is justified. The kinetic pK_a for the group that must be unprotonated for activity has been shifted from 6.1 ±0.1 for the soluble enzyme[7] to 6.8 ±0.1 for the immobilized enzyme. Shifts of the pK_a value in the pH profiles of immobilized enzymes are generally expected with a charged matrix support.[15] However, in the experiments reported here there is no charged support, and the substrate solution is buffered. The shift might be explained on the basis of the highly hydrophobic environment to which the enzyme has been immobilized where the microenvironment of the essential amino acids have been affected. The slight variation might also be attributed to possible conformational effects on the active site.

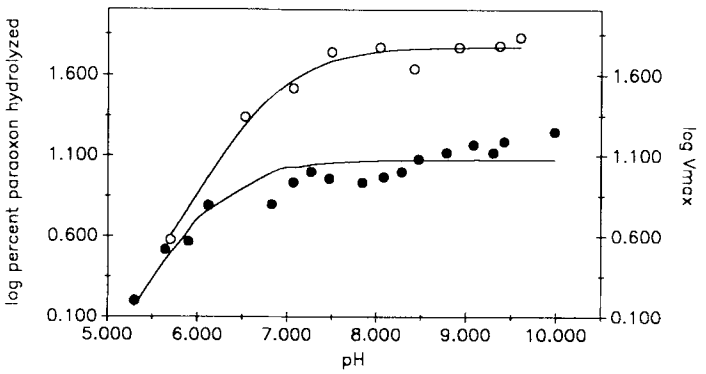


Figure 6. The effect of pH on the hydrolysis of saturating paraoxon by the soluble enzyme (●) and the immobilized phosphotriesterase (○). The values for V_{max} of the soluble enzyme are in arbitary units.[7]

Regardless, a comparison of the two profiles serve to underline the similarity of the catalytic performance of the immobilized enzyme in relation to the free enzyme.

The immobilized phosphotriesterase on trityl agarose provides an effective system for the degradation of some organophosphate pesticides and a mammalian acetylcholinesterase inhibitor. The chemical and kinetic parameters of the immobilized phosphotriesterase are comparable to those of the free enzyme. However, one of the primary limitations of the immobilized phosphotriesterase system is the negative effect of high concentrations of organic solvent, which results in the elution of enzyme activity from the reactor. The effect of organic solvents (>1%) was also shown to be deleterious to the immobilized parathion hydrolase described by Munnecke.[8] In this case the negative impact was the inhibition of the enzyme, not the direct loss of enzyme activity from the reactor, and restoration of the enzymatic activity was possible by removing the solvent from the reactor. To realize the effective hydrolysis of high concentrations of organophosphate pesticides by the immobilized phosphotriesterase, a solubilizing agent with minimal effects on the retention of the immobilized enzyme or the inhibition of enzyme activity will be required. At present, these types of reagents are being evaluated.

Another limitation of the immobilized phosphotriesterase system involves economic considerations. Though the immobilization of this enzyme onto trityl agarose is quite simple, the cost of using trityl agarose as a support matrix on a very large scale may become cost preventative. For this reason, other methods of immobilizing phosphotriesterase are being developed. Preliminary experiments have indicated that the immobilization of this phosphotriesterase onto a nylon membrane is functional and has the extra advantage of being relatively cost effective. Current studies are now being directed at this system.

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