Enzyme-Catalyzed P-F Bond Hydrolysis of Monofluorophosphate as a Simple Model of Sarin Detoxification

Norimasa Yoza,* Sachiko Nakashima, and Tetsuya Nakazato
Department of Chemistry, Faculty of Science, Kyushu University, Hakozaki, Fukuoka 812

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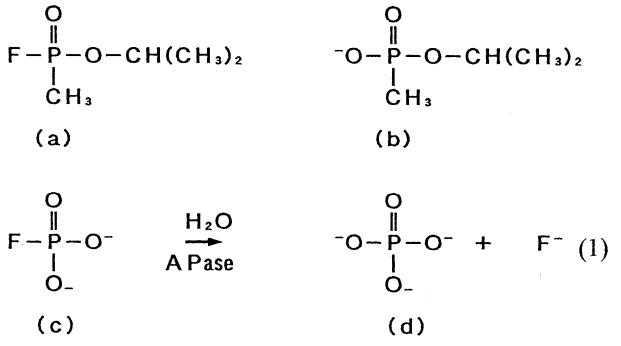
The catalytic ability of alkaline phosphatase (EC3.1.3.1) to promote P-F bond hydrolysis was examined with a simple nontoxic P-F compound, monofluorophosphate (Na_2PO_3F), to obtain an insight into the enzymatic degradation of Sarin, a toxic P-F compound. The enzyme recognized inorganic monofluorophosphate as a substrate and accelerated its hydrolytic conversion to orthophosphate and fluoride ions at pH 7.2 - 9.0 and 30 °C by a factor of ten billion (10^{10}).

Sarin (**a**) is a toxic organophosphorus compound with a P-F bond.[1] Since the lethal tragedy on the Tokyo metro in 1995 much attention has been focused on the detoxification method of Sarin.[2] The presence of P-F bond is likely to be an essential factor of its toxic function. It has been suggested[1,2] that the detoxification of Sarin might be achieved by its hydrolysis; the displacement of fluorine by OH from a water molecule with resultant formation of a nontoxic compound(**b**). However, the practical problem to achieve the hydrolytic reaction in a short time remains unsolved. Little information is available on enzymes that may promote the hydrolysis of Sarin. Therefore, the catalytic ability of alkaline phosphatase (APase, EC3.1.3.1)[3] to promote P-F bond degradation was examined in this work with a simple nontoxic analogue of Sarin.



(1)

Monofluorophosphate (MFP, **c**) used as the model of Sarin is a fluorine derivative of orthophosphate (**d**). Its disodium salt, Na_2PO_3F, has been chemically prepared, commercially available and widely used as a caries-preventive additive in dentifrice.[4] Alkaline phosphatase used as the hydrolytic enzyme is widely distributed in nature and catalyzes the P-O-C bond hydrolysis of phosphate monoesters.[3] Our attempt using APase to accelerate the P-F hydrolysis of inorganic MFP might appear to be curious, but provided unexpectedly interesting results. Kinetic processes were visualized by employing flow injection analysis (FIA).[5-7] Kinetic data from high-performance liquid chromatography (HPLC)[5,6] and P-31 NMR[8] experiments were also provided for cross reference to the FIA data.

The enzymatic conversion of MFP to orthophosphate and fluoride ions in equation 1 was initiated by mixing a pH-buffered solution of magnesium chloride (activator),[6] an MFP solution (substrate)[5] and an alkaline phosphatase solution (enzyme, Sigma P-5521).[6] Aliquots of the incubated reaction mixture were then analyzed at appropriate intervals. Prior to the kinetic experiments with the FIA technique, P-31 NMR method[8] was employed to ascertain that orthophosphate was

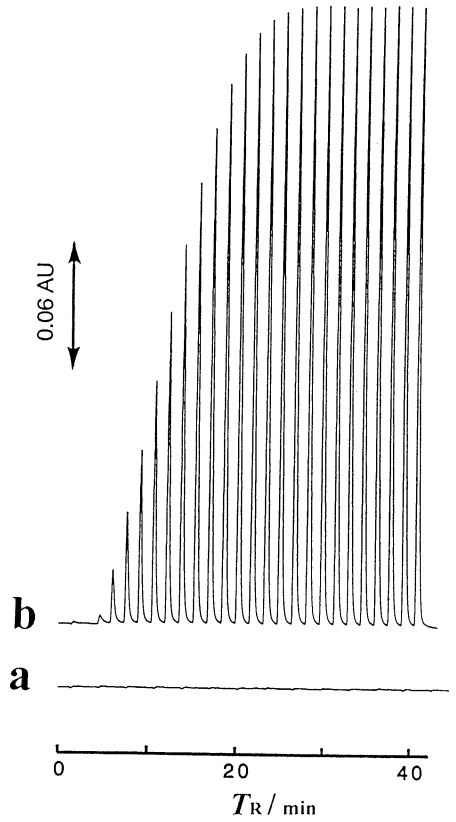


Figure 1. Kinetic FIA profile indicating the hydrolytic conversion of MFP to orthophosphate at pH 7.2 and 30 °C. The sample for non-enzymatic hydrolysis (a) consists of 0.5 mM MFP and 1 mM Mg^{2+}. The sample for enzymatic hydrolysis (b) was prepared by adding alkaline phosphatase to the sample (a) to be 6.3 x10^{-9} M APase.

the sole phosphorus product of MFP. When $1x10^{-2}$ M MFP (1 M = 1 mol dm^{-3}) and $1x10^{-7}$M enzyme were used an NMR signal of MFP (doublet)[8] decreased gradually with time, with consequent increase of orthophosphate signal (singlet). After 60 min no signal of MFP was observed, indicating the completion of hydrolysis. Similar conclusion was also provided from an HPLC experiment by which both MFP and orthophosphate could be analyzed at 6 min intervals.[5]

FIA[6] was employed to achieve the rapid analysis of the hydrolytic process at a more short interval. By using a chromogenic Mo(VI) reagent only orthophosphate could be detected selectively.[6] In the absence of an enzyme very small peak of orthophosphate, due to the contaminant in the original MFP sample, was observed throughout multiple injections at 1.5 min intervals(Figure1a). On addition of the enzyme to the MFP solution, however, the orthophosphate peak increased linearly with reaction time, T_R, in accordance with the zero-order reaction rate (Figure 1b).[6] Towards the completion of enzymatic hydrolysis the peak height became constant. The slope in Figure 2b is a measure of APase activity and is related to the maximum velocity, k_3[APase], in Michaelis-Menten equation.[6] The catalytic rate constant, k_3, can be calculated when the enzyme concentration, [APase], is given on the assumption that the molecular weight of the enzyme is 80000.[3]

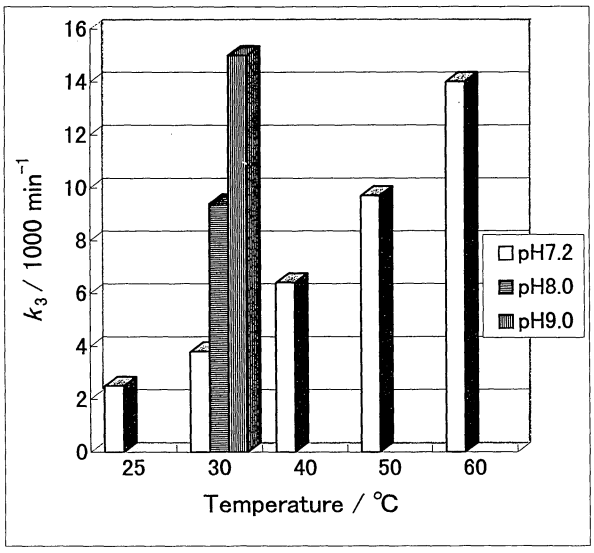


Figure 2. Effects of pH and temperature on catalytic rate constant of enzymatic hydrolysis of MFP.

The pH dependence of the rate constant at 30 °C is shown in Figure 2. In the buffered region, pH7.2 - 9.0, where a Tris-HCl buffer was effective the k_3 values increased from $3.8x10^3$ to $1.5x10^4$ min^{-1} with increasing pH. This type of pH dependency of APase activity can also be seen in the P-O-C bond hydrolysis of orthophosphoric monoesters.[3]

From the temperature dependence of the k_3 values at pH 7.2 (Figure 2) the activation energy of enzymatic P-F hydrolysis was calculated to be ca. 36 $kJmol^{-1}$. This value is smaller than that of non-enzymatic P-F bond hydrolysis of MFP in an acidic medium,[8] 65 $kJmol^{-1}$, but is comparable to that of the enzymatic P-O-P bond hydrolysis of pyrophosphate (diphosphate),[6] 38 $kJmol^{-1}$. Michaelis constant, K_m, of the enzymatic MFP hydrolysis at pH 7.2 was also estimated by separate experimental runs with lower substrate concentrations. Its value at 30 - 50 °C, 28±3 μM, was considerably larger than that of pyrophosphate hydrolysis,[6] 5 μM.

MFP, as well as Sarin, are artificially prepared substances and their existence in nature is unlikely. A central interest is that alkaline phosphatase, a natural metalloenzyme for P-O-C bond cleavage,[3] is able to recognize inorganic MFP as a substrate and accelerate its P-F bond hydrolysis. An acceleration factor by the enzyme at neutral pH is roughly calculated to be as high as ten billion (10^{10}) ; the rate constants of non-enzymatic hydrolysis[8] and enzymatic hydrolysis (Figure 2) are nearly 10^{-6} and 10^4 min^{-1}, respectively. Little information is available about enzymatic degradation or biodegradation of Sarin that is too toxic to be used at academic laboratory. We hope the basic kinetic data with nontoxic MFP will be valuable for designing the degradation methodologies of Sarin.

References and Notes

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