ISOLATION OF SERINE PHOSPHATE FROM THE ACTIVE SITE OF HUMAN PROSTATIC ACID PHOSPHATASE; INHIBITION OF THE ENZYME BY DFP

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A characteristic feature of certain esterases appears to be the presence of serine in their active sites (1). Many of these enzymes are inhibited irreversibly by organophosphorus compounds which have the general formula $(RO)_2(X)P=O$, such as diisopropyl phosphorofluoridate (DFP), isopropyl methylphosphonofluoridate (Sarin), O, O-diethyl O-p-nitrophenyl-thiophosphate (Parathione), and many others. The reaction of the organophosphate with the enzyme is an S_N2 reaction in which the electronegative group, (X), is split off and a covalent bond is formed between the phosphorus atom and a group in the active site of the enzyme. On hydrolysis of the inhibited enzymes the phosphoryl group has always been found as O-phosphorylserine (SerOP). Whether the hydroxyl group of serine is actually the nucleophilic group with which the primary reaction takes place is still an open question. SerOP was also isolated from other types of enzymes, as for instance phosphoglucomutase (2) and intestinal and bacterial alkaline phosphatases (3), after interaction of the enzymes with glucose-1-P and phosphoric acid, respectively. It thus appeared of interest to determine whether human prostatic acid phosphatase exhibits properties characteristic of other hydrolytic enzymes.

The procedure used in the present experiments was based on the assumption that organophosphates may react with the enzyme by the same mechanism as found with other hydrolytic enzymes. Therefore, DFP inhibition of human prostatic acid phosphatase was studied. The enzyme was purified according to Boman (4) to a degree that its specific activity was 130 μmoles hydrolyzed/mg protein/min. It has been found that the enzyme activity is indeed inhibited by DFP and that this inhibition is irreversible. The inhibition rises linearly with the time of incubation of the enzyme with DFP (Fig. I). The rate of inhibition increases with the concentration of the inhibitor. In the presence of substrate the rate of inhibition is decreased since the substrate protects the active site of the enzyme from the inhibitor. Even with the rather high concentrations of DFP used, the maximum percent inhibition observed was only 60% when

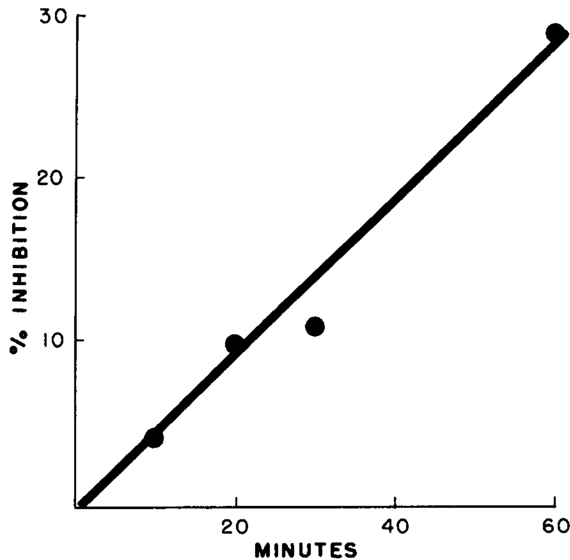

Fig. 1. Inhibition of human prostatic acid phosphatase by DFP as a function of incubation time.

The enzyme was assayed in 4 ml of 0.1 M acetate buffer at pH 5.0. p-nitrophenylphosphate was used as a substrate at a concentration of 10^{-3} M. Aliquotes of 0.5 ml were removed at different time intervals and put into 1.0 ml of 0.1 N NaOH. The liberated p-nitrophenol was measured colorimetrically at 420 mμ.

the protein concentration was 0.76 mg/ml. However, as may be seen in Table I, at lower protein concentrations or at higher temperatures more inhibition was obtained in shorter periods of time. The irreversible nature of the inhibition was shown in two ways: dilution of the reaction mixture 10,000 fold did not affect the percent inhibition; reassay of incubation mixtures left standing for eight days showed no gain in activity. These data indicate that DFP may be a suitable reagent for the analysis of the active site of human acid phosphatase.

TABLE I

THE EFFECT OF DFP ON ACID PHOSPHATASE ACTIVITY

$t^{o}C$	Protein mg/ml	DFP $x10^{-3}M$	Time of incubation	% Inhib.
4	5	1	24 hrs	35
	0.76	1	"	29
	"	2	"	50
	"	4	"	50
25	0.76	2	30 min	11
	"	4	"	27
37	0.76	1	120	38
	"	1	240	47
	"	2	120	56
37	0.0074	1	30	40
	"	1	50	59
	"	2	35	60

Experiments with phosphoric acid-P^{32} yielded radioactive SerOP. The procedure used was as follows: after incubation of the enzyme at pH 5 with 10^{-6} M phosphate-P^{32} (1 mC) for 30 min at 37°C, the enzyme was denatured with trichloracetic acid and boiled for 2 min at 100° C. The insoluble protein was washed 3 times with water and hydrolyzed by refluxing with 6N HCl for 24 hrs. Carrier SerOP was added and the hydrolysate was chromatographed on a Dowex 50-X8 column. The radioactive SerOP fraction was rechromatographed on Dowex 1-X8 and subjected to paper

electrophoresis. Radioactivity remained associated with the SerOP. The results with DFP^{32}, although suggestive, were not yet conclusive and further experiments are presently in progress.

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