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PHOSPHORYLPHOSPHATASE AND OXIMES

BY

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The effects of pralidoxime iodide, 1,1-trimethylenebis(4-formylpyridinium bromide) dioxime and diacetyl monoxime on the activity of phosphorylphosphatase prepared from pig kidney *in vitro* have been studied. It was found that these oximes, even at high concentrations, did not affect the activity of the enzyme.

Oximes have been shown to be effective antidotes against poisoning by organophosphates, including diisopropyl phosphorofluoridate (dyflos) (Kewitz, Wilson & Nachmansohn, 1956; Edery & Schatzberg-Porath, 1958; Hobbiger & Sadler, 1959). The most remarkable effect is their ability to reactivate phosphorylated acetyl cholinesterase; proteolytic enzymes with esterase activities, such as chymotrypsin (Green & Nicholls, 1959) and trypsin (Edery, to be published), can also be reactivated by high concentrations of the oximes.

Phosphorylphosphatase is an enzyme which appears to play an important role in the detoxification of dyflos in animals (Mounter, 1960) and possibly in human beings as well (Cohen & Warringa, 1954). Since there is no information about the effect of oximes on phosphorylphosphatase, it seemed of interest to examine this problem. It should be remembered that hydroxylamine, the parent compound of the oximes, has inhibitory effects on phosphorylphosphatase (Mounter, Floyd & Chanutin, 1953).

METHODS

A purified preparation of phosphorylphosphatase was obtained from pig kidney according to the method of Bergmann, Segal & Rimon (1957). The activity was determined by a manometric method described by the same authors.

The oximes used were: pralidoxime iodide (pyridine-2-aldoxime methiodide; 2-hydroxyiminomethyl-*N*-methylpyridinium iodide); 1,1-trimethylenebis(4-formylpyridinium bromide) dioxime {*N,N'*-trimethylenebis(4-hydroxyiminomethylpyridinium bromide)}; and diacetyl monoxime. A purified specimen of dyflos served as substrate for phosphorylphosphatase. Mn^{++} and Co^{++} were used as chlorides. Fresh solutions of all these substances were made in a buffer of the following composition: 0.1 M NaCl, 0.04 M $MgCl_2$, 0.026 M $NaHCO_3$ and 0.1% gelatin.

RESULTS

The oximes neither increased nor inhibited the activity of phosphorylphosphatase. The previous observations of Mounter *et al.* (1953), that Mn^{++} and Co^{++} ions

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potentiate phosphorylphosphatase activity, are confirmed; pralidoxime does not affect this potentiation. Table 1 summarizes most of the results obtained.

TABLE 1

EFFECTS OF OXIMES ON PHOSPHORYLPHOSPHATASE ACTIVITY

Phosphorylphosphatase incubated with pralidoxime (PAM), 1,1-trimethylenebis(4-formylpyridinium bromide) dioxime (TMB-4), diacetyl monoxime (DAM), Mn^{++} or Co^{++}, for 20 min in the side arm of a Warburg vessel before tipping into 5×10^{-3} M dyflos after equilibration of the system. Activity expressed as ml. CO_2/ml. undiluted enzyme/hr. Figures were corrected for non-enzymic hydrolysis and represent the mean of two separate experiments

Control activity	PAM 1×10^{-2}M	PAM 1×10^{-5}M	TMB-4 1×10^{-2}M	DAM 1×10^{-2}M	Mn^{++} 1×10^{-3}M	Mn^{++} 1×10^{-3}M and PAM 1×10^{-3}M	Co^{++} 1×10^{-3}M	Co^{++} 1×10^{-3}M and PAM 1×10^{-3}M
22·5	22·3	22·0	22·5	20·0	42·7	41·6	34·9	35·3

In other experiments phosphorylphosphatase was incubated with pralidoxime, 1,1-trimethylenebis(4-formylpyridinium bromide) dioxime, or diacetyl monoxime at a concentration of 1×10^{-1} M for 2 hr prior to the introduction of dyflos into the system. Under these conditions, too, phosphorylphosphatase activity was not affected.

DISCUSSION

The experiments show that oximes do not affect phosphorylphosphatase activity. It therefore seems reasonable to assume that they can be used in the treatment of dyflos poisoning without the risk of their interference with the natural enzymic process of detoxification.

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