Liberation of Hydrofluoric Acid by Enzymatic Hydrolysis of Dialkyl Fluorophosphates. (21278)

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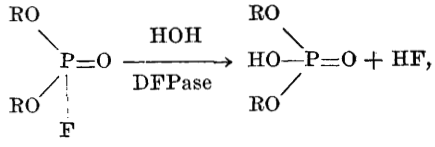
Mazur(1) first demonstrated that an enzyme in rabbit serum hydrolyzed diisopropyl fluorophosphate (DFP) to dialkyl phosphoric acid, hydrogen and fluoride ions. He also showed that enzymes (DFPases) which hydrolyzed dialkyl fluorophosphates were present in a number of rabbit and human tissues and assumed that the mode of action of these enzymes was similar to that of rabbit serum. It has been shown that the characteristics of the DFPases from rabbit serum and hog kidney differ markedly(2,3,4,5). This investigation was undertaken to determine if hog kidney DFPase was specific in hydrolyzing the P-F linkage of diisopropyl-, dibutyl- and diethyl fluorophosphates.

Materials and Methods. A purified preparation (Fraction A-2) obtained from hog kidney was used as the source of DFPase(2). Enzyme activity was determined by measurement of CO_2 evolution with the Warburg apparatus(1,2). When Mn++ or Mn++ and a cofactor were employed to activate DFPase, they were first incubated with the enzyme preparation for 15 minutes before mixing with the substrate. At different intervals, pairs of flasks, one with and one without enzyme, were removed from the water bath and their contents were immediately washed out with 30 ml of 60% ethanol and with 4 ml of chloroacetic acid/sodium hydroxide buffer (pH 3.8) into an Erlenmeyer flask. These additions stopped the enzyme action and the fluoride was titrated with thorium nitrate using sodium alizarin sulfonate as indicator(6). The solutions in the Warburg flasks usually contained less than 0.1 mg protein nitrogen which did not interfere with the fluoride estimation. When larger amounts of protein were present silver nitrate was used as the precipitating agent(1). Qualitative tests were also made for isopropyl alcohol(7) and inorganic phosphate(8). Diisopropyl fluorophosphate (DFP) was a Merck product. Dibutyl fluorophosphate (DBFP) and diethyl fluorophosphate (DEFP) were prepared from the respective chlorophosphates* by an exchange reaction with sodium fluoride(9).

Experimental. Varying amounts of the enzyme preparation, in the presence and absence of activators, were used to study the hydrolysis of DFP, DBFP and DEFP. According to the equation,



two moles of CO_2 should be liberated from the bicarbonate buffer by the hydrolysis of each

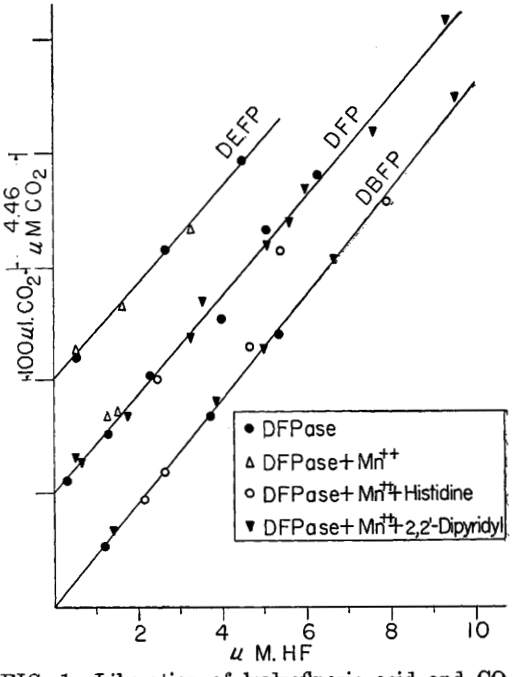


FIG. 1. Liberation of hydrofluoric acid and CO_2 during hydrolysis of diisopropyl fluorophosphate (DFP) dibutyl fluorophosphate (DBFP) and diethyl fluorophosphate (DEFP) by hog kidney DFPase.

* The chlorophosphates were kindly donated by the Victor Chemical Co., Chicago.

mole of dialkyl fluorophosphate. The plots in Fig. 1 show the relationship between CO_2 evolved and inorganic fluoride liberated after the hydrolysis of three dialkyl fluorophosphates. According to the slopes of the lines, the CO_2/HF ratios for DFP, DEFP and DBFP are 43.5, 42.5 and 46.0, respectively, as compared to a theoretical ratio of 44.8. Within the experimental error of the methods, these data indicate that 1 mole of HF is liberated for each 2 moles of CO_2 evolved. This ratio differs from that obtained by Mazur(1) with rabbit serum (1:1.49) which may be explained by the retention of CO_2 in the presence of comparatively large amounts of plasma. These results indicate that the products of hydrolysis of dialkyl fluorophosphates by enzymes of differing characteristics are the same.

Qualitative tests for inorganic phosphate and isopropyl alcohol were negative after complete hydrolysis of DFP, both by the enzyme alone and by the enzyme in the presence of cofactors.

Cohen and Warringa(10) studied the metabolism of DFP^{32} (diisopropyl fluorophosphate labelled with P^{32}) after intramuscular injection of nontoxic doses into human subjects. They observed that 50 to 60% of the injected DFP is excreted in the urine as diisopropyl phosphoric (DIP) acid during the first 10 days. The residual DFP probably remains firmly bound as a DIP-protein complex(11). It is probable that the *in vivo* and *in vitro* hydrolyses of DFP are similar.

Summary. Evidence has been presented to show that hog kidney dialkyl-fluorophosphatase (DFPase) liberates 1 mole of hydrofluoric acid and 1 mole of dialkyl phosphoric acid in the enzymatic hydrolysis of a mole of diisopropyl fluorophosphate, diethyl fluorophosphate or dibutyl fluorophosphate.

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Received July 26, 1954. P.S.E.B.M., 1954, v87.

Heat-Stability of Antibodies in Tuberculous Sera. (21279)

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The presence of hemagglutinins and hemolysins in sera of tuberculin-negative cattle has been reported from these laboratories(1). The persistence of these antibodies in low titers over a 2- to 3-year period suggested that they might be naturally occurring or "normal" antibodies with specificities for antigens similar to those present in *Mycobacterium tuberculosis*.

Preliminary results showed that antibodies in these tuberculin-negative bovine sera were eliminated by exposure to 70°C for 10 minutes. When tuberculous bovine or human sera were similarly treated, "heat-stable" antibodies could still be detected in some samples. The possibility of greater correlation of "heat-stable" antibody with tuberculous infection prompted a further study of the heat-stability of antibodies produced in response to injections of *M. tuberculosis*, B.C.G., P.P.D., and