DIALKYLFLUOROPHOSPHATASES OF MICROORGANISMS

BY L. A. MOUNTER, ROBERT F. BAXTER,* AND ALFRED CHANUTIN

(From the Department of Biochemistry, School of Medicine, University of Virginia, Charlottesville, Virginia)

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It has been found that enzymes (DFPases) which hydrolyze dialkyl fluorophosphates are present in every tissue of six species studied (1). Since these enzymes may play a rôle in the normal metabolism of tissues, it was decided to study their distribution in microorganisms. These studies demonstrate the presence of DFPases in microorganisms and describe some of their characteristics.

Methods

Organisms were grown for periods varying from 20 to 48 hours in a peptone-yeast extract-glucose medium buffered at pH 6.8 to 7.0 with 0.05 Mr phosphate buffer. Representative types which grew satisfactorily on this medium were selected for study. Cells were harvested by centrifugation and washed three times with water. The washed cells were suspended in a small volume of water, lyophilized, and stored in a desiccator over P2O5 in a cold room (−10°C). Lyophilized material was used in all experiments because the results with a given culture were more reproducible and corrections for endogenous metabolism were relatively low.

Known amounts of cells were suspended in 0.025 Mr NaHCO3, and 20 to 40 mg. of the lyophilized organisms in 0.2 to 0.3 ml. were pipetted into the side arms of the Warburg flasks. The procedures previously described (2, 3) for measuring the hydrolysis of diisopropyl fluorophosphate (DFP) were used. Control experiments were carried out for non-enzymatic hydrolysis (NaHCO3 + DFP) and for the acid production as measured by metabolic CO2 evolution (NaHCO3 + microorganisms). Results for DFP hydrolysis were corrected for these two blanks. When activators were added to the cell suspensions, they were incubated together for 15 minutes before mixing with the substrate.

Results

The growth of Proteus vulgaris, Pseudomonas aeruginosa, or Neisseria sicca does not appear to be affected when cultured in media adjusted to a final concentration of 10 mM DFP. These organisms are capable of hy-

* Lederle Medical Student Research Fellow, 1954.
drolizing DFP, with the liberation of fluoride ion (2, 4). In the inoculated media, almost all of the DFP originally present could be accounted for as free inorganic fluoride; only partial hydrolysis was noted in the uninoculated tubes. Furthermore, the addition of $5 \times 10^{-3}$ M NaF to the reaction mixture in the Warburg flasks had a negligible effect on the observed DFPase activities.

The DFPase activities of lyophilized microorganisms were determined with and without added Mn$^{2+}$, Co$^{2+}$, or Mg$^{2+}$. DFP was hydrolyzed by all organisms with the exception of two types of micrococci (Table I). These data indicate that the highest activities are possessed by gram-negative organisms. The DFPases in ten of fourteen species were definitely activated by Mn$^{2+}$; this effect was particularly marked in the case of Salmonella pullorum and Shigella alkalescens. Co$^{2+}$ appreciably activated the DFPase of three species, but in most of the organisms this metal ion

### Table I

**Hydrolysis of DFP by Microorganisms**

<table>
<thead>
<tr>
<th>Species</th>
<th>Activity, µL CO$_2$ per 100 mg. per hr.</th>
<th>Effect of metal ions*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mn$^{2+}$</td>
</tr>
<tr>
<td>Proteus vulgaris (-)</td>
<td>1400</td>
<td>+</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa (-)</td>
<td>1100</td>
<td>+</td>
</tr>
<tr>
<td>Serratia marcescens (-)</td>
<td>900</td>
<td>0</td>
</tr>
<tr>
<td>Salmonella pullorum (-)</td>
<td>650</td>
<td>+</td>
</tr>
<tr>
<td>Pasteurella avicida (-)</td>
<td>470</td>
<td>+</td>
</tr>
<tr>
<td>Escherichia coli (-)</td>
<td>400</td>
<td>+</td>
</tr>
<tr>
<td>Shigella alkalescens (-)</td>
<td>270</td>
<td>+</td>
</tr>
<tr>
<td>Aerobacter aerogenes (-)</td>
<td>280</td>
<td>+</td>
</tr>
<tr>
<td>Staphylococcus albus (+)</td>
<td>180</td>
<td>+</td>
</tr>
<tr>
<td>Clostridium sporogenes (+)</td>
<td>170</td>
<td>-</td>
</tr>
<tr>
<td>Corynebacterium zereus (+)</td>
<td>100</td>
<td>+</td>
</tr>
<tr>
<td>Hemophilus pertussis (-)</td>
<td>60</td>
<td>+</td>
</tr>
<tr>
<td>Bacillus subtilis (+)</td>
<td>50</td>
<td>+</td>
</tr>
<tr>
<td>Neisseria sicca (-)</td>
<td>30</td>
<td>+</td>
</tr>
<tr>
<td>Mycobacterium phlei (+)</td>
<td>&lt;25</td>
<td>+</td>
</tr>
<tr>
<td>Streptococcus faecalis (folic acid var.) (+)</td>
<td>&lt;25</td>
<td>+</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot; (+)</td>
<td>&quot;</td>
</tr>
<tr>
<td>Micrococcus pyogenes (+)</td>
<td>&quot;</td>
<td>Negligible</td>
</tr>
<tr>
<td>&quot;</td>
<td>citreus (+)</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

The symbols in parentheses indicate gram-negative or gram-positive organisms.
* † = activation; - = inhibition; 0 = negligible effect.
† Concentration in the side arm; final concentration after mixing with substrate, $10^{-4}$ M.
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exerted a marked inhibitory effect. Mg\textsuperscript{2+} had negligible effects in most cases, but inhibited *Aerobacter aerogenes* DFPase.

![Graph showing the effect of varying concentrations of metal ions on the activity of DFPase](image)

**Fig. 1.** Effect of varying concentrations of metal ions on the activity of DFPase.

![Graphs showing the effect of varying concentrations of metal ions on selected organisms](image)

**Fig. 2.** Effect of varying concentrations of metal ions on the activity of DFPase. Symbols as in Fig. 1.

After this preliminary survey of the DFPase activities, the effects of varying amounts of Mn\textsuperscript{2+}, Co\textsuperscript{2+}, and Mg\textsuperscript{2+} on six selected organisms were studied (Figs. 1 and 2). It can be seen that the degree of activation of the
enzymes by Mn$^{++}$ varies considerably in each case. The DFPases of four microorganisms reached optimal Mn$^{++}$ concentrations between $10^{-3}$ and $10^{-4}$ M. The results with Clostridium sporogenes are particularly striking, since maximal activation and marked inhibition are noted within a small range of Mn$^{++}$ concentration. Shigella alcalsecens and Salmonella pullorum have maximal activities at relatively high concentrations of Mn$^{++}$ ($10^{-3}$ M). Four of the six organisms are inhibited by Co$^{++}$. Mg$^{++}$ acts as a
powerful inhibitor for the DFPase of *Aerobacter aerogenes* and has slight activation or negligible effects on the remaining organisms.

It has been reported that DFP is hydrolyzed in the presence of microorganisms and that there are species variations in the activation characteristics of the hydrolytic reaction. In order to prove that the observed results are due to enzymes and not to non-specific catalytic effects, a series of kinetic studies was carried out with *Pseudomonas aeruginosa* and *Aerobacter aerogenes*. Linear relationships are observed between CO₂ liberation and both cell concentration and time of reaction (Fig. 3). The effect of substrate concentration on the rate of hydrolysis was determined and, within the limits of experimental accuracy, the points lie on rectangular hyperbolae, which are characteristic of enzyme reactions obeying classical Michaelis-Menten kinetics (Fig. 4). A number of substances which inhibit the DFPases of tissues (3) have similar effects on the enzymes of the microorganisms (Table II).

DFPase activity in the presence of Mn⁺⁺ and histidine, 2,2'-dipyridyl, or picolinic acid revealed that these cofactors had negligible potentiating effects.

**DISCUSSION**

It has now been shown that DFPases appear to be universally distributed in vertebrate tissues (1) and in microorganisms. A number of different DFPases are present in microorganisms as judged by their response to metal ion activators and inhibitors. As a result of previous experience with these enzymes, it appears that the characterization of DFPases cannot be made until purified fractions of cell-free extracts are obtained.

According to Goldstein and Goldstein (5), cholinesterases are absent in
most microorganisms. It is noteworthy that bacterial DFPases have the ability to hydrolyze alkyl fluorophosphates, which are powerful inhibitors of cholinesterases. These observations suggest that the two enzymes are not related functionally to each other in tissues.

SUMMARY

Microorganisms are capable of hydrolyzing diisopropyl fluorophosphate, and this reaction may be potentiated or inhibited by Mn++, Co++, or Mg++. Large differences in activity are observed with various types of microorganisms.

Kinetic studies show that DFP is hydrolyzed by enzymes which are similar in many respects to those present in tissues.

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