Nevertheless, we note the possibility that some promoters may have the reverse requirements for transcription start or stop operations, compared with the classical lac operon of *E. coli.*


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**Inhibition of Acid Proteinase from Dictyostelium discoideum**

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The cellular slime mould *Dictyostelium discoideum* possesses an acid proteinase activity that is present throughout the life cycle (Susman & Susman, 1969; Wiener & Ashworth, 1970). Although this activity is probably of great importance for the processing of food and for mobilization of endogenous protein during development, its properties have not been studied in any detail. Many acid proteinases from fungal, plant and mammalian sources display common features (Takahashi et al., 1974; Takahashi & Chang, 1976; Rickert & McBride-Warren, 1977), in particular inactivation by three specific agents, the pentapeptide pepstatin (Umezawa et al., 1970), diazoacetyl-DL-norleucine methyl ester (Rajagopalan et al., 1966) and 1,2-epoxy-3-(p-nitrophenoxy)-propane (Tang, 1971). As part of an investigation of the *Dictyostelium* acid proteinase the effect of these inhibitors on the activity in crude cell extracts and partially purified preparations has been examined.

Crude cell extracts of washed myxamoebae of strain AX 2 were prepared by addition of 0.1% (v/v) Triton X-100; cell debris was removed by centrifugation at 7500g for 10 min. Acid proteinase was purified by a procedure involving (i) (NH$_4$)$_2$SO$_4$ fractionation, (ii) treatment with DEAE-cellulose and (iii) gel filtration on Sephadex G-200.

Proteolytic activity was assayed by using Hide Powder Azure (Calbiochem, Los Angeles, CA, U.S.A.) as substrate (Rinderknecht et al., 1968). Up to 100 μl of sample was added to a suspension of 5 mg of substrate in 1 ml of 0.1 M-glycine/HCl buffer, pH 3.0, and shaken at 25°C. The reaction was stopped by adding 0.2 ml of 50% (w/v) trichloroacetic acid.
Preincubation was carried out as described in the text with methanol only, (○), diazoacetyl-DL-norleucine methyl ester (■) or diazoacetylglycine ethyl ester (●). (a) Pepsin (0.2%)(Sigma, Kingston upon Thames, U.K.; 0.11 unit/ml), (b) crude cell extract from *D. discoideum* (0.13 unit/ml), (c) purified preparation from *D. discoideum* (0.03 units/ml).

Fig. 1. *Inactivation by diazoacetyl-DL-norleucine methyl ester and diazoacetylglycine ethyl ester*

Preincubation was carried out as described in the text with methanol only, (○), diazoacetyl-DL-norleucine methyl ester (■) or diazoacetylglycine ethyl ester (0.2 mg in 10 μl of methanol) (●). (a) Pepsin (0.2%)(Sigma, Kingston upon Thames, U.K.; 0.11 unit/ml), (b) crude cell extract from *D. discoideum* (0.13 unit/ml), (c) purified preparation from *D. discoideum* (0.03 units/ml).

Activity (%)  
Preincubation time (min)
Table 1. Effect of salts on acid proteinase activity in crude extract of *D. discoideum*

Assays were carried out as described in the text in the presence of salts at the concentration shown. The extract contained 0.51 unit/ml.

<table>
<thead>
<tr>
<th>Salt</th>
<th>Concentration (mM)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDTA (sodium salt)</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>NaCl</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Na$_2$SO$_4$</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>MgCl$_2$</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>FeCl$_2$</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>ZnSO$_4$</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>CoCl$_2$</td>
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<td>4</td>
</tr>
<tr>
<td>MnCl$_2$</td>
<td>10</td>
<td>12.5</td>
</tr>
<tr>
<td>CuCl$_2$</td>
<td>10</td>
<td>24.5</td>
</tr>
<tr>
<td>CuCl$_2$</td>
<td>1</td>
<td>24.0</td>
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<tr>
<td>SnCl$_2$</td>
<td>10</td>
<td>98.4</td>
</tr>
<tr>
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<td>75.5</td>
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<tr>
<td>FeCl$_3$</td>
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<td>1</td>
<td>96.1</td>
</tr>
<tr>
<td>HgCl$_2$</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>HgCl$_2$</td>
<td>1</td>
<td>100</td>
</tr>
</tbody>
</table>

Inactivation of the purified proteinase by diazoacetyl-DL-norleucine methyl ester was prevented by the addition of crude extract heat-treated to inactivate endogenous proteinase. The inhibitory factor responsible was not removed by dialysis and was present in material precipitated by trichloroacetic acid. Since inactivation of pepsin was not inhibited by crude *Dictyostelium* extracts, the inhibitor was specific for the *Dictyostelium* enzyme. It is possible that the presence of an excess of endogenous protein, which has been found to be an excellent substrate for the proteinase, inhibits competitively the reaction with diazoacetyl-DL-norleucine methyl ester, and this would explain the insensitivity to diazoacetyl-DL-norleucine methyl ester of the proteinase in crude extracts. Acid proteinases in crude extracts from other sources are inactivated by diazoacetyl-DL-norleucine methyl ester, however (Takahashi *et al.*, 1974).

Crude extracts have also been used for studies with pepstatin (Takahashi *et al.*, 1974; Marks *et al.*, 1973), but even the purified preparation from *D. discoideum* was insensitive to this inhibitor. Since the purified proteinase is free to react with diazoacetyl-DL-norleucine, the lack of effect of both pepstatin and 1,2-epoxy-3-(p-nitrophenoxy)propane may indicate a fundamental difference between *Dictyostelium* acid proteinase and similar enzymes from other sources.

The only effective inhibitors of the activity in crude extracts were metal ions (Table 1), in particular Fe$^{3+}$, Sn$^{2+}$ and Hg$^{2+}$ ions. Pepsin was also inhibited by Fe$^{3+}$ and Sn$^{2+}$ ions, but with respect to its sensitivity to Hg$^{2+}$ ions the *Dictyostelium* proteinase would appear to differ from most other acid proteinases.

The enzyme purification was undertaken by Kevin Kelly. I thank the Science Research Council for financial support.


Lactose Metabolism in *Klebsiella pneumoniae* Strains Growing in Chemostat Culture

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Smith & Dean (1972) showed that when *Klebsiella aerogenes* N.C.I.B. 418 (now called *Klebsiella pneumoniae* N.C.I.B. 418) was subjected to protracted growth in a lactose-limited chemostat its β-galactosidase specific activity eventually reached a very high value, which represented a considerable excess over the calculated minimum activity required to maintain growth. This has been confirmed in the same organism by Calcott & Postgate (1974), and similar β-galactosidase hyperactivity has also been observed in various strains of *Escherichia coli* (Horiuchi *et al.*, 1962; Silver & Mateles, 1969; Macleod *et al.*, 1975). We report here a study of the development of β-galactosidase hyperactivity in five other N.C.I.B. strains of *K. pneumoniae*. The activities of some enzymes involved in the metabolism of the glucose liberated by the hydrolysis of the lactose have also been determined.

The organisms were grown aerobically at 37°C in lactose-limited chemostats and before introduction into the chemostats were given 20 daily subcultures in lactose-limited medium. The chemostat, the composition of the minimal growth medium and the β-galactosidase assay were described by Smith & Dean (1972). As in their experiments, the activity determined with intact organisms is taken as a measure of β-galactoside permease activity and the activity of organisms disrupted by ultrasonication as the total β-galactosidase activity. β-Galactosidase specific activity is expressed as nmol of o-nitrophenol liberated from o-nitrophenyl β-D-galactopyranoside/min per mg of protein. Protein was determined by the method of Warburg & Christian (1941). Glucose phosphate isomerase, 6-phosphofructokinase, glucose 6-phosphate dehydrogenase, phosphogluconate dehydrogenase (decarboxylating) and pyruvate kinase were also determined in ultrasonically disrupted organisms by standard methods involving the rates of oxidation or reduction of adenine dinucleotides (Bergmeyer, 1965) and enzyme specific activities are expressed as nmol of NADP⁺ (NADH) reduced (oxidized)/min per mg of protein.

After the changeover from batch culture to chemostat culture in lactose-limited medium Smith & Dean (1972) observed that the total β-galactosidase specific activity of *K. pneumoniae* N.C.I.B. 418 rose rapidly and soon settled at a quasi-steady-state value, which persisted for about 15 generations of growth. Thereafter the enzyme specific activity increased gradually as chemostat operation continued, and 85 generations later reached a final value, which corresponded to a 3-fold increase over the activity in the quasi-steady state and an 11-fold increase over that obtained in batch culture. The rate of substrate transport (as measured by the activity of intact organisms) increased approximately in parallel with the total β-galactosidase specific activity during the progression from batch culture to the final steady state. We found a very similar pattern of behaviour when strains N.C.I.B. 8258, 8805 and 9261 of *K. pneumoniae* were grown at a dilution rate (D) of 0.5 h⁻¹ in lactose-limited chemostats, between 70 and 100 generations elapsing, depending on the strain, before the final steady state was attained. In sharp contrast, the final steady state was reached in strains N.C.I.B. 8017 and 8153.