Deoxyribonucleic Acid Polymerase from Ehrlich Ascites-Tumour Cells and Other Sources

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The properties of a DNA polymerase (EC 2.7.7.7) preparation from mouse Ehrlich ascites-tumour cells have been investigated. As found in other systems there was an absolute requirement for magnesium ions, the optimum concentration being 6mM. In the presence of tris buffer the activity was maximal at pH 7·6–7·8. As expected there was a requirement for a heat-denatured DNA primer, native DNA being inactive, and also for the triphosphates of deoxyadenosine, deoxycytidine, deoxyguanosine and deoxythymidine. In contrast with some other systems it did not prove possible to saturate the enzyme with these substances, even at concentrations of up to 0·4mg./ml. DNA and 0·2mM-deoxynucleoside triphosphates. The apparent K_m with respect to total deoxynucleoside triphosphates was 0·078mM.

The DNA polymerase activity was maximal in the presence of 60mM-potassium or sodium chloride, but the activity was much less if these were replaced by ammonium ions. EDTA, RNA, and ribonuclease all had no effect on the DNA polymerase activity. Sodium fluoride (100mM) produced a complete inhibition, but this effect was apparently not due to the binding of magnesium ions.

From its behaviour on Sephadex columns it could be inferred that the mol. wt. of the Ehrlich ascites enzyme was near 200000: a similar value to those quoted for polymerases from other sources (Keir, 1965; Bollum, 1966).

The activity of the terminal DNA polymerase in Ehrlich ascites cells was approximately 50% of the replicative activity, which contrasts with the very low terminal activity found in Landschütz ascites cells (Keir, Omura & Shepherd, 1963).

Sulphydryl reagents such as *p*-chloromercuribenzoate, *N*-ethylmaleimide and iodoacetate inhibited the polymerase. The simple thiols 2-mercaptoethanol, dithiothreitol or reduced glutathione had no inhibitory effect, but such disulphides as cystine and oxidized glutathione completely inhibited the enzyme (Slater, 1966).

These observations will be compared with those obtained by other workers on DNA polymerases from a variety of sources.

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Dissimilation of Aromatic Substrates by *Rhodopseudomonas palustris*

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The utilization of benzoate by a *Rhodopseudomonas* sp. has been reported using cells grown anaerobically in the light, i.e. photosynthetically, on a benzoate–mineral salts medium (Proctor & Scher, 1960). Such cells, using standard manometric techniques, were capable of oxidizing, in the dark, benzoate through a sequence claimed to be:
benzoate → protocatechuate → catechol → an α-oxo acid.

The implication was made that molecular oxygen could replace the proposed photochemically generated oxidant, assumed to be involved in the photometabolism of benzoate (Scher & Proctor, 1960).

We wish to report that the patterns of oxygen consumption exhibited by photosynthetically grown cells bear little relationship to those of utilization of aromatic substrates observed anaerobically in the light.

Rhodopseudomonas palustris grown photosynthetically, with benzoate as sole carbon source, showed no significant oxygen uptake with benzoate, protocatechuate, catechol, or any of the monohydroxybenzoates. Photosynthetically, these cells assimilated without a detectable lag-period the following, in order of decreasing rates: benzoate, *m*-hydroxybenzoate, *o*- and *p*-hydroxybenzoate and protocatechuate. Catechol was not utilized over 4hr., neither were the other dihydroxybenzoates except the 2,3-dihydroxybenzoate (assimilated slowly, and ceasing after about 25% of the substrate had disappeared).

The same organism grown photosynthetically on *p*-hydroxybenzoate consumed oxygen with *p*-hydroxybenzoate, or protocatechuate, but not with benzoate, *o*- or *m*- hydroxybenzoate, or catechol, when these were used as substrates. Photosynthetically, the monohydroxybenzoates and benzoate were metabolized at similar rates, whilst protocatechuate disappeared more slowly.

With cells grown photosynthetically on *m*-hydroxybenzoate, oxygen consumption was observed with protocatechuate, but not with *m*-hydroxybenzoate or benzoate. Incubated photosynthetically, the cells utilized the following

substrates in order of decreasing rates: *m*-hydroxybenzoate, benzoate, and protocatechuate.

Cells grown photosynthetically on acetate did not photometabolize benzoate, *m*- or *p*-hydroxybenzoate when incubated for an experimental period of 2hr.

Attempts to grow *Rhodopseudomonas palustris* under aerobic conditions with benzoate, *o*- or *m*-hydroxybenzoate as substrates, failed; *p*-hydroxybenzoate or protocatechuate were, however, capable of supporting good growth. Similar observations have been made by Leadbetter & Hawk (1965). We have established that the aerobic dissimilation of *p*-hydroxybenzoate proceeds via protocatechuate, and scission of the ring by the 4,5-oxygenase pathway (Dagley, Evans & Ribbons, 1960).

Oxygen uptake by cells grown photosynthetically was exhibited only when the substrate in question could support aerobic growth. The introduction of air into a cell system photometabolizing benzoate caused a complete cessation of benzoate utilization; re-establishment of anaerobic conditions allowed benzoate metabolism to continue.

These observations do not support the suggestion that molecular oxygen can substitute for the photogenerated entity involved in the cleavage of the aromatic nucleus. The oxygen uptake observed with photosynthetically grown cells acting on various aromatic substrates, probably reflects the presence of low levels of the enzymes which normally operate in the aerobic metabolism of these compounds. The anaerobic photosynthetic aromatic pathway employed by the Athiorhodaceae is under investigation.

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Detection of Multiple β-Galactosidases in Ox Liver with Natural and Synthetic Substrates

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Enzymes hydrolysing synthetic substrates such as *o*- or *p*-nitrophenyl β-D-galactosides, have been found in many mammalian tissues. In a study of human intestinal lactases (Hsia, Mahler, Semenza & Prader, 1966) it was shown that two β-galactosidases (β-D-galactoside galactohydrolase EC 3.2.1.23) were present, one which hydrolysed lactose but not 6-bromo-2-naphthyl β-D-galactoside, while the second hydrolysed the synthetic substrate but lactose only to a lesser extent.

We have now found enzymes of a similar nature in ox liver, one of which is concerned in the degradation of glycoprotein carbohydrate.

Fractionation of ox liver homogenate by ammonium sulphate (Langley & Jevons, 1966) and Sephadex G-200 completely separated *N*-acetyl-β-glucosaminidase (β-2-acetamido-2-deoxy-D-glucoside acetamidodeoxyglucohydrolase, EC 3.2.1.30) and β-galactosidase as identified by the action on the synthetic substrates *p*-nitrophenyl *N*-acetyl-β-D-glucosamine and *o*-nitrophenyl β-D-galactoside respectively. A further fractionation of these enzyme preparations on DEAE-cellulose separated two protein fractions for each enzyme, the K_m values for the *N*-acetyl-β-glucosaminidases I and II being the same (1·6mM), while those for the β-galactosidases differed (3·4mM for I and 8·2mM for II).

When these four enzyme preparations were incubated separately with lactose and sialic acid-free α_1-acid glycoprotein, no galactose was released from either substrate by β-galactosidase I and II, even after prolonged incubation at 37°, even though galactose was released from a similar α_1-acid glycoprotein preparation by *Diplococcus pneumoniae* β-galactosidase (Hughes & Jeanloz, 1964). However, both lactose and sialic acid-free α_1-acid glycoprotein were hydrolysed by the fractions rich in *N*-acetyl-β-glucosaminidase I and II, releasing galactose from both substrates, as well as *N*-acetylglucosamine from the glycoprotein. Sugars were detected by gas–liquid chromatography after silylation (Brobst & Lott, 1966) on a 1·5% S.E. 30 column.

Many glycosidases are thought to be specific for their respective glycone and the position of the linking glycosidic bond. However, the results obtained point to the possibility that the specificity of the lactase identified in *N*-acetyl-β-glucosaminidase fractions I and II, resides in the next carbohydrate residue and not the galactose residue alone.

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