with a 10% decrease in ATP (γ -P) radioactivity. By contrast adenosine and AMP, added at 0.125mM concentration for 5 min, had no effect on the turnover of protein-bound phosphoserine, despite their more potent effect in increasing the cyclic AMP content of the tissue (Sattin & Rall, 1970). However, rather higher concentrations of adenosine inhibit protein kinase activity in preparations of membrane fragments from brain (Weller & Rodnight, 1971), and it is possible that the compound may exert a similar effect on protein phosphorylation in the intact tissue.

These observations support the hypothesis that some post-synaptic responses to neurotransmitter release may be mediated through cyclic-AMP-dependent protein kinase activity in the receptor cell. Whether these are events occurring at the post-synaptic membrane or intracellularly remains to be investigated.

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Heald, P. J. (1957) *Biochem. J.* **66**, 659

Jones, D. A. & Rodnight, R. (1971) *Biochem. J.* **121**, 597

Kakiuchi, S., Rall, T. W. & McIlwain, H. (1969) *J. Neurochem.* **16**, 485

Sattin, A. & Rall, T. W. (1970) *Mol. Pharmacol.* **6**, 13

Shimizu, H., Creveling, C. R. & Daly, J. (1970) *Proc. Nat. Acad. Sci. U.S.A.* **65**, 1033

Trevor, A. J. & Rodnight, R. (1965) *Biochem. J.* **95**, 889

Weller, M. & Rodnight, R. (1970) *Nature (London)* **225**, 187

Weller, M. & Rodnight, R. (1971) *Biochem. J.* **124**, 393

Partial Reactions of D-Glucose 6-phosphate-1L-myoInositol 1-Phosphate Cyclase from Rat Testis

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From a study of hydrogen movements during the cyclization, we have previously proposed (Barnett & Corina, 1968) that the sequence of events in the enzyme reaction is oxidation by NAD^+ of C-5 of D-glucose 6-phosphate to give 5-oxo-D-glucose (D-xylulo-5-hexosulose) 6-phosphate, an aldol condensation between C-6 and C-1 of this molecule to give 1L-myoinosose-2 1-phosphate, and, finally, reduction by the NADH first formed to give 1L-myoinositol 1-phosphate. The NADH and oxidized intermediates were tightly bound to the enzyme and could not be isolated.

We have found that the NAD^+ can be removed from the enzyme by using charcoal, and that on readdition of NAD^+ (K_m 5×10^{-5} M) the enzyme retains 75% of the original activity, but has modified

properties. By using the charcoal-treated enzyme lacking NAD^+ we have been able to incorporate hydrogen from $[^3\text{H}]\text{NADH}$ into 5-oxo-D-glucose 6-phosphate. The label was located in the D-[5- ^3H]-glucose 6-phosphate formed by the methods previously described (Barnett & Corina, 1966).

For kinetic measurements carrier glucose 6-phosphate was added, the reaction mixture was reduced with sodium borohydride, the D-glucitol 6-phosphate was hydrolysed, separated by paper chromatography, benzoylated and purified on t.l.c., and the D-glucitol hexabenzoylate was assayed by its E_{232} value and its radioactivity counted.

The incorporation of radioactivity was linear with time up to 2h, and after 4h about 7% of the label from the NADH was incorporated. The incorporation was abolished by pretreatment with trichloroacetic acid, and 90% inhibited by 0.2mM-2-deoxy-D-glucose 6-phosphate, which has K_i 2×10^{-5} M for the cyclization of glucose 6-phosphate. No label was incorporated if untreated enzyme containing bound NAD^+ was used.

Incubation of the substrate analogue 5-oxo-D-glucitol 6-phosphate (L-sorbose 1-phosphate) with the charcoal-treated enzyme and $[^3\text{H}]\text{NADH}$ gave D-[5- ^3H]-glucitol 6-phosphate.

The enzyme-catalysed reaction of the hypothetical intermediates of the enzyme to give substrate strongly suggests that the mechanism previously proposed for the enzyme is correct.

Barnett, J. E. G. & Corina, D. L. (1966) *Carbohydr. Res.* **3**, 134

Barnett, J. E. G. & Corina, D. L. (1968) *Biochem. J.* **108**, 125

Pullulanase Synthesis in *Klebsiella (Aerobacter) aerogenes*

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Bacterial pullulanase, an inducible enzyme, has proved valuable in the analysis of starch-type polysaccharides (Manners, 1966) and has potential industrial uses. Initially reported by Bender & Wallenfels (1961) in a strain of *Klebsiella aerogenes* that they had isolated, pullulanase has also been detected in *K. aerogenes* A.T.C.C. 9621 (N.C.I.B. 8021) grown in appropriate conditions (Brown *et al.*, 1965). We have investigated the precise conditions necessary for its optimum synthesis by *K. aerogenes* N.C.I.B. 418 grown in chemostat continuous culture. In these conditions the enzyme remains entirely cell-bound. When maltose, maltotriose or pullulan was used as sole carbon source in carbon-limited conditions the steady-state values of pullulanase specific

activity (μmol of maltotriose liberated from pullulan/min per mg dry wt.) of the cells reached maximum values of 0.25–0.30 unit at a dilution (growth) rate (D) of 0.2h^{-1} at 30°C at pH 7.1. In other nutrient-limited conditions the activity was negligible (cf. Bender & Wallenfels, 1961). Intact and disrupted cells had similar activities, in general, and in pullulan-limited conditions at $D = 0.2\text{h}^{-1}$ the specific activity observed is in considerable excess of that required to hydrolyse the carbon substrate at the rate necessary to maintain growth. Culturing of maltose-adapted cells in amylopectin-limited medium gave higher activities, which reached a maximum value of 0.45 unit at $D = 0.35\text{h}^{-1}$, but growth could not be maintained in glycogen-limited conditions even at $D = 0.1\text{h}^{-1}$.

Fifteen other N.C.I.B. strains of *K. aerogenes* have also been examined in maltose-limited medium at $D = 0.2\text{h}^{-1}$. Strains N.C.I.B. 5938 and N.C.I.B. 8017 had pullulanase activities of 0.86 and 0.83 unit respectively, strains N.C.I.B. 8153 and N.C.I.B. 9146 showed negligible activity even after disruption (but were inducible with pullulan), and the others were similar to strain N.C.I.B. 418.

Although understandable in cells utilizing pullulan and amylopectin, the function of pullulanase in maltose-utilizing cells is not clear. Abdullah & French (1966) report that, besides hydrolysing oligosaccharides, pullulanase can synthesize an α -1,6-dimer and even a trimer from maltose. The formation of polysaccharides in this way provides a possible function. However, no clear correlation is apparent between the total polysaccharide content of cells and their pullulanase activity.

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Abdullah, M. & French, D. (1966) *Nature (London)* **210**, 200

Bender, H. & Wallenfels, K. (1961) *Biochem. Z.* **334**, 79

Brown, D. H., Illingworth, B. & Kornfield, R. (1965) *Biochemistry* **4**, 486

Manners, D. J. (1966) *Annu. Rep. Chem. Soc.* **63**, 590

Separation of α -L-Fucosidases in Extracts of *Trichomonas foetus*

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The destruction of human blood-group H and Le^a serological activity by extracts of the protozoan *Trichomonas foetus* has been attributed to the action of two different α -L-fucosidases (Tyler & Watkins, 1960; Watkins, 1962). Electrophoresis of the crude *T. foetus* extracts on starch columns gave partial separation of the enzymes destroying blood-group

H and Le^a activities; from their action on low-molecular-weight oligosaccharides, together with the known structures of the blood-group H and Le^a determinants (cf. Watkins, 1966), the enzymes were tentatively characterized as α -1,2- and α -1,4-L-fucosidase respectively. This communication reports attempts to further purify and characterize the enzyme that destroys blood-group Le^a activity.

Extracts of the organisms, prepared as described previously (Watkins, 1966), were fractionated with ammonium sulphate and the material that was precipitated at 55–80% saturation was, after dialysis, subjected to starch column electrophoresis on an LKB Uniphor apparatus at 8V/cm at pH 6.2 for 24h. A single peak with H-destroying activity was eluted from the column, followed by a peak with Le^a -destroying activity. The fractions with Le^a -destroying activity were pooled, dialysed, concentrated and applied to a DEAE-cellulose (Whatman DE52) column in 0.04M-phosphate buffer, pH 6.0. Elution with a linear salt gradient (0–1.0M-NaCl in 0.04M-phosphate buffer, pH 6.0) separated the Le^a -destroying enzyme from a small amount of residual H-destroying enzyme.

Tested on low-molecular-weight substrates, the Le^a -destroying enzyme preparation released fucose from lacto-*N*-fucopentaose II, in which the fucose is attached by an α -(1 $\rightarrow$ 4)-linkage to *N*-acetylglucosamine, but did not hydrolyse lacto-*N*-fucopentaose I or 2'-fucosyl-lactose, in which the fucose is linked α -(1 $\rightarrow$ 2) to galactose (cf. Kuhn, 1957). Although free from α -1,2-L-fucosidase activity, the Le^a -destroying enzyme preparation released fucose from 3-fucosyl-lactose (cf. Montreuil, 1960), lactodifucotetraose and lacto-*N*-difucohexaose I (cf. Kuhn 1957), results that indicate the presence of an α -1,3-L-fucosidase in the preparation in addition to the α -1,4-L-fucosidase. Whether these two activities are both associated with the same enzyme, or result from two different enzymes in the preparation, remains to be established. The H-destroying enzyme, separated from the Le^a -destroying enzyme by DEAE-cellulose chromatography, hydrolysed α -(1 $\rightarrow$ 2)-fucosyl linkages in low-molecular-weight substrates, but had no action on α -(1 $\rightarrow$ 3)- or α -(1 $\rightarrow$ 4)-linkages.

The Le^a -destroying enzyme destroys both blood-group Le^a and blood-group Le^b activities in glycoproteins. When tested on blood-group-H/ Le^b -active glycoproteins the decrease in blood-group Le^b activity is accompanied by an enhancement of blood-group H activity. The blood-group Le^b determinant is a difucosyl structure formed as an interaction product of the H and *Le* blood-group genes (Marr *et al.* 1967); removal of the α -(1 $\rightarrow$ 4)-linked fucose by the Le^a -destroying enzyme would therefore be expected to result in a loss of blood-group Le^b activity and the exposure of blood-group-H-active groupings.