cells harvested from cultures containing butan-1-ol and ethanol was examined by following the reduction of NAD^+ by the method of Vallee & Hoch (1955). The activity of the enzyme preparation was examined with respect to the following primary and secondary alcohols: ethanol, propan-1-ol, propan-2-ol, butan-1-ol, 2-methylpropan-1-ol, butan-2-ol, 2-methylbutan-1-ol and 3-methylbutan-1-ol. Results obtained indicate that the alcohol dehydrogenase of *C. acetobutylicum* is non-specific with regard to substrate. The synthesis of 2-methylpropan-1-ol, propan-1-ol and any of the above-mentioned alcohols is therefore possible on provision of a suitable precursor. Further studies on the properties of this enzyme preparation are in progress.

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The Alkylation of Yeast Hexokinase A

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Hexokinase A is one of the two isoenzymes isolated from baker's yeast by a method that avoids proteolysis (Lazarus, Ramel, Rustum & Barnard, 1966). Evidence suggests that 4 out of the 8 thiol groups/molecule of mol.wt. 100 000 are not essential for catalytic activity (Lazarus, Derechin & Barnard, 1968). However, inactivation of hexokinase A by iodoacetate at low ionic strength is accompanied by carboxymethylation of all 8 thiol groups (Lazarus *et al.* 1968).

Hexokinase A was treated with iodoacetate (10–700 mM) at pH 8.4 in tris-HCl buffer, *I* 0.1, when the enzyme is presumed to exist as a dimer of mol.wt. 50 000 (see Lazarus *et al.* 1968). At 25°C inactivation of the enzyme is first-order with respect to the concentration of each reactant with a rate constant of $0.9 \times 10^{-3} \text{ l} \cdot \text{mol}^{-1} \cdot \text{s}^{-1}$. At 30°C inactivation of hexokinase A is first-order with respect to the concentration of the enzyme, but the relationship between reaction rate and the concentration of iodoacetate is hyperbolic. The data are consistent with a reversible equilibrium between protein and inhibitor (K_i 0.03 M) before alkylation of the enzyme. At low concentrations of iodoacetate and at pH 8.4 the apparent second-order rate constant for inactivation at 30°C is $28 \times 10^{-3} \text{ l} \cdot \text{mol}^{-1} \cdot \text{s}^{-1}$ and it is very sensitive to changes in pH or temperature. These changes may reflect the conformational state of the protein (see also Jones, 1970).

Inactivation (86%) of hexokinase A at 30°C by 16 mM-iodo[^{14}C]acetate leads to the incorporation of 3.2 g-atoms of ^{14}C /mol of enzyme. Glucose (20 mM) prevents this inactivation but 0.8 g-atom of ^{14}C is still incorporated/mol of enzyme. When this latter experiment was repeated with 0.2 M-iodoacetate the enzyme still retained over 80% of its activity. After being desalted this preparation could be inactivated by fresh iodoacetate at 25°C, the kinetics of inactivation being identical with that described earlier for native hexokinase A. In one experiment the partially alkylated active enzyme described above was treated with iodo[^{14}C]acetate, when complete inactivation was accompanied by the incorporation of 1.9 g-atoms of ^{14}C /mol of enzyme.

The collective results confirm that hexokinase A possesses both essential and non-essential thiol groups. Hence glucose prevents inactivation but only decreases the degree of alkylation of enzyme by iodoacetate.

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The Hydrolysis of Glycine Oligopeptides by Guinea-Pig Intestinal Brush Borders

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Previous studies have shown that, whereas diglycine peptide hydrolase (EC 3.4.3.1) occurs predominantly in the cytosol from intestinal mucosa, significant amounts of triglycine peptide hydrolase (EC 3.4.1.3) are localized in the brush-border fraction (Peters, 1968). It was therefore decided to study the specificity of oligopeptide hydrolysis in further detail.

Brush borders were prepared from guinea-pig intestinal mucosa (Eichholz & Crane, 1965) and were demonstrated to be substantially free of other subcellular organelles. The brush borders were shown to hydrolyse glycine oligopeptides up to and including hexaglycine, but did not hydrolyse polyglycine (mol.wt. approx. 10 000). The oligopeptides studied were not hydrolysed by pepsin, trypsin, α -chymotrypsin, elastase, carboxypeptidase

A or crude pancreatin, indicating that the brush-border peptidase activity was not due to adsorbed gastric or pancreatic enzymes.

Analysis of the products of hydrolysis indicated that the oligopeptides were hydrolysed sequentially. For example, when pentaglycine was used as a substrate, stoichiometric amounts of tetraglycine and free glycine were released. It was also found that the pH optimum for the hydrolysis of tetraglycine was the same as that for the release of smaller peptides and of free glycine. Studies of the relative rates of hydrolysis of the glycine peptides indicated that the longer peptides were hydrolysed more rapidly than the shorter.

Studies were performed to determine whether the glycine residues were cleaved from the *N*- or the *C*-terminal of the peptide. A series of *N*- and *C*-terminal-protected glycine derivatives were incubated with brush borders to select suitable blocking groups. Free glycine was liberated from the methyl, ethyl, *tert*-butyl and benzyl esters of glycine, but not from glycine amide, benzyl-oxy-carbonylglycine and benzoylglycine. Incubation of peptide derivatives showed that free glycine was liberated from triglycine amide, but not from benzyloxycarbonyltriglycine, benzoyltriglycine or benzyloxycarbonyltetraglycine, suggesting that the glycine residues were cleaved sequentially from the *N*-terminal of the peptide.

This conclusion was confirmed by synthesizing two tetraglycine peptides, labelled at either the *N*- or the *C*-terminal glycine residue (Merrifield, 1964; Gutte & Merrifield, 1969). When the [$1-\alpha\text{-}^3\text{H}_2\text{-glycine}$]tetraglycine was incubated with brush borders labelled glycine was released but the liberated diglycine and triglycine were unlabelled. When the [$4-\alpha\text{-}^3\text{H}_2\text{-glycine}$]tetraglycine was hydrolysed no labelled glycine was released but the diglycine and triglycine were radioactive. These experiments indicate that the brush-border hydrolase is an amino-oligopeptidase.

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Partial Purification and Characterization of an Iodide Peroxidase from *Enteromorpha linza*

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The occurrence of iodinated compounds in marine algae is well established (Roche & Yagi, 1952; Scott, 1954), but the mechanism by which they are formed is not fully understood (Tong & Chaikoff, 1955; Roche, Thoai & Lafon, 1949). A peroxidase is responsible for the iodination of tyrosyl groups of thyroglobulin (Coval & Taurog, 1967; Hosoya & Morrison, 1967), but similar activity could not be detected with red-algal peroxidases (Murphy & Ó hEocha, 1969). However, iodination is associated with a peroxidase that we have partially purified from the green alga *Enteromorpha linza*.

The alga, which was collected on the shores of Galway Bay, was ground in the presence of liquid N_2 and extracted overnight in 10 mM- NaHCO_3 , pH 8.3. The extract catalysed the iodination of tyrosine in the presence of iodide and hydrogen peroxide. In the absence of tyrosine, iodide was peroxidized to iodine, a process that can be conveniently followed spectrophotometrically in the presence of excess of iodide (Hosoya, Kondo & Ui, 1962; Alexander, 1962), thus providing a sensitive and rapid assay procedure for monitoring the activity. The extract was centrifuged at 35000g for 90 min. The supernatant, which contained most of the activity, was decanted and fractionated with ammonium sulphate. The fraction precipitated by between 0.5 and 0.7 saturation with ammonium sulphate was redissolved in 0.1 M-glycine-NaOH buffer, pH 9.0, dialysed against the same buffer and further fractionated on a column of Sephadex G-200.

Starch-gel electrophoresis of the peroxidase-containing fraction of the purified preparation in tris-citrate buffer, pH 7.0 (*I* 0.018 in gel and 0.27 in electrode), resolved two components, detected with a staining system that contained iodide as electron donor (Murphy, 1970). Isoelectric focusing of this fraction in a sucrose gradient also revealed two components; the major component had isoelectric point 4.45, and the minor one isoelectric point 4.7.

The peroxidase activity, as determined with *o*-dianisidine as electron donor (Worthington Biochemical Corp. Descriptive Manual), ran parallel with the iodide peroxidation activity during the above purification steps including starch-gel electrophoresis and isoelectric focusing, suggesting that one enzyme is responsible for both reactions.