DEAE-cellulose and eluted with a gradient of pH and phosphate buffer the main laminarinase component was divided into three parts (A, B and C); the first (A) contained all the luteanase. The third peak (C) contained most of the laminarinase but did not lyse cell walls, either alone or in the presence of a purified luteanase. Fractions A and B both lysed cell walls and had little or no chitinase activity, which was found mainly in fraction C.

Fractions A, B and C are all endoglucanases, but, whereas fraction C produces mainly glucose, laminaribiose and laminaritriose from laminarin, fractions B and C do not seem to degrade this slightly branched polysaccharide (degree of polymerization about 25; Annan, Hirst & Manners, 1965) beyond the pentasaccharide stage.

It remains to be proved that the glucanase in fraction B is responsible for lysis of the cell walls, but evidently neither luteanase nor chitinase needs to be present. This fits the idea that the cell-wall β -(1 $\rightarrow$ 3)-glucan has practically no β -(1 $\rightarrow$ 6)-interchain linkages (Manners & Masson, 1969).

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Multiple Forms of Human Brain Monoamine Oxidase: Substrate Specificities

By G. G. S. COLLINS and M. B. H. YODIM. (*Bernard Baron Memorial Research Laboratories and Institute of Obstetrics and Gynaecology, Queen Charlotte's Maternity Hospital, Goldhawk Road, London W.6, U.K.*)

Although pig brain monoamine oxidase (EC 1.4.3.4) is a single enzyme (Tipton & Spire, 1968), rat brain monoamine oxidase appears to exist in multiple forms (Johnson, 1968; Youdim, Collins & Sandler, 1969). Human brain mitochondrial monoamine oxidase has now been purified by the method described by Youdim *et al.* (1969); in all brain areas investigated (whole brain, cerebral cortex, cerebellum, basal ganglia, hypothalamus, pons and centrum ovale) multiple forms of the enzyme were detected. Four bands of activity separated by electrophoresis on 5% polyacrylamide gel (Youdim, Collins & Sandler, 1968); two bands migrated towards the anode (MAO₂ and MAO₃), one remained at the origin (MAO₁) and one migrated toward the cathode (MAO₄).

The specific activities (expressed as nmol of substrate deaminated/10 min per mg of protein) of the intact mitochondrial enzyme and of the bands of enzyme activity, both prepared from the various

brain areas, were measured with [¹⁴C]tryptamine, [¹⁴C]tyramine, [¹⁴C]dopamine (3,4-dihydroxy[¹⁴C]-phenethylamine) and [¹⁴C]benzylamine as substrates (Robinson, Lovenberg, Keiser & Sjoerdsma, 1968). The activities of the mitochondrial preparations for the four substrates were 0.73, 1.74, 2.36 and 5.61 respectively. Each band of activity also differed in its substrate specificities; for example, the activity of MAO₁ prepared from whole brain homogenates towards dopamine and tyramine was 2.09 and 1.66 respectively whereas that of MAO₄ was 58.54 and 0.45. In addition, comparison of the specific activities of the enzyme forms isolated from the various brain areas investigated showed marked differences. The activity towards dopamine of MAO₄ isolated from basal ganglia, a brain area rich in this amine (Bertler & Rosengren, 1959), was 338.03 whereas that of the same band found in the cerebellum was 4.46. The corresponding activities of MAO₁ were 1.45 and 1.33 respectively. However, not all the substrates showed such variation. The specific activity of MAO₄ towards tryptamine was 0.71 in the basal ganglia and 0.78 in the cerebellum. These variations in specific activity were not accompanied by differences in the corresponding Michaelis constants.

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Insoluble Pronase

By P. CRESSWELL and A. R. SANDERSON. (*McIndoe Research Unit, Queen Victoria Hospital, East Grinstead, Sussex, U.K.*)

Insoluble pronase (B grade; Calbiochem Ltd., London W.1, U.K.) was prepared by the method successfully used for papain (Cebra, Givol, Silman & Katchalski, 1961) and other proteases.

A synthetic polypeptide of *p*-aminophenylalanine and leucine (25 mg) was diazotized and precipitated from solution at alkaline pH after partial self-coupling. The precipitate was washed with 50 mM-tris-HCl buffer, pH 8.0, containing CaCl₂ (25 mM), and a solution (1 ml) of pronase (8 mg) was added. The

reaction proceeded at 0°C for 20min and was terminated by addition of 0.4% phenol (5ml), which rapidly exhausts any remaining diazonium groups. CaCl₂ appears to be necessary to preserve enzymic activity.

The insoluble product is almost identical in pH and reaction temperature optima with the soluble enzyme, but is much more stable. It has retained complete activity during storage for several months at 4°C, compared with a decline of about 25%/day for the soluble enzyme stored under identical conditions.

Apparent recovery of enzymic activity depends on the size of substrate used to measure proteolysis. High-molecular-weight substrates appear to have sterically restricted access to the catalytic sites. Such restriction is progressively decreased as the molecular weight (or shape and size) of a substrate falls. With the exception of one substrate, all activity was measured by determination of trichloroacetic acid-soluble peptide released after 30min at 37°C. The exception was that of L-leucine *p*-nitroanilide [Sigma (London) Chemical Co. Ltd., London S.W.6, U.K.], where release of *p*-nitroaniline was followed at 400nm.

A simple expression (1) seems to relate the molecular weight of a substrate to the soluble enzyme/insoluble enzyme reaction-rate ratio, and applies over a wide size range from proteins to simple peptides:

$$\frac{\text{Reaction rate with insoluble enzyme}}{\text{Reaction rate with soluble enzyme}} = \frac{K + k \cdot \log(\text{mol.wt. of substrate})}{1} \quad (1)$$

With low-molecular-weight substrates approx. 75% of catalytic activity is retained.

The use of insoluble catalyst has two principal advantages over the soluble protein. First, the stability referred to above obviates the necessity for repeated addition of enzyme to combat self-destruction. Secondly, its simple removal from reaction mixtures by centrifugation overcomes the problem of contamination of digests by enzyme-derived peptides.

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Glycoproteins from Yeast Cell Wall

By T. P. LYONS and J. S. HOUGH. (*Sub-Department of Malting and Brewing, Department of Biochemistry, University of Birmingham, Edgbaston, Birmingham 15, U.K.*)

Yeast cells, of various strains and at various stages of growth, were suspended in water and treated for

2min in a Braun disintegrator, and cell walls were isolated by differential centrifugation. Fractionation with ethylenediamine gave the fractions A, B and C described by Korn & Northcote (1960). Chemical analyses of the fractions from the different strains showed little variation, although walls with a high affinity for calcium had a fraction A with high phosphorus content. Polyacrylamide-gel electrophoresis at pH 8.3 in miniature columns (Davis, 1964) separated fraction A into two sharp bands. Both bands took up stains for protein (Amido Black) and carbohydrate (Schiff-periodate), and the relative proportions of the bands depended on the yeast strain and the stage of growth. From yeast cell walls labelled with [³²P]phosphate it was shown that no phosphorus was present in one of the bands. Preparative polyacrylamide-gel electrophoresis was used to isolate quantities of both band components. For fraction B, a complex insoluble in water, gel electrophoresis at pH 8.6 in 10% Cyanogum and 8M-urea gave two components staining for protein and carbohydrate.

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ε-N-Monomethyl-lysine and Trimethyl-lysine in Myosin

By M. HARDY, I. HARRIS, S. V. PERRY and D. STONE. (*Department of Biochemistry, University of Birmingham, Edgbaston, Birmingham 15, U.K.*)

It has been reported by Hardy & Perry (1969) that subfragment I of myosin and the total protein fraction of myofibrils contain ε-N-monomethyl-lysine. In addition to the latter and 3-methylhistidine, Hardy & Perry (1969) reported the separation of a fraction containing an unidentified methylated amino acid. This has now been identified as ε-N-trimethyl-lysine (DeLange, Glazer & Smith, 1969) by comparison of its chromatographic behaviour in the Unicrome Beckman amino acid analyser (0.35M-sodium citrate buffer, pH 5.79, 28°C) with a known sample of the methylated amino acid (kindly supplied by Dr R. J. DeLange). No dimethyl-lysine could be detected in subfragment I. The presence of ε-N-monomethyl-lysine in subfragment I received confirmation from the independent observations by Huszar & Elzinga (1969), who obtained a single peak of elution characteristics identical with those of ε-N-monomethyl-lysine, although their analytical procedure did not resolve the N-methyl-lysines.

Myosin and subfragment I contain 0.24mol and 0.59mol of ε-N-monomethyl-lysine respectively and 0.72mol and 1.75mol of ε-N-trimethyl-lysine respectively/10⁵g. Whereas 3-methylhistidine, the other methylated amino acid present in adult skeletal-muscle myosin, is absent from myosin isolated from