Sanwal & Lata, 1961), we consider that this activity has little physiological significance compared with the activity in the presence of the substrates L-glutamate and 2-oxoglutarate. Specificity for ammonia appears to be absolute, as a number of aliphatic amines gave no detectable activity.

Similar experiments with NADP derivatives showed that glutamate dehydrogenase has the ability to utilize oxidized 3-acetylpyridine-adenine dinucleotide phosphate and deamino-NADP⁺, both with lower $V_{\max}$ values, but deamino-NADP⁺ had a lower K_m value than NADP. Nicotinamide, adenine, AMP, ADP and NAD⁺ did not inhibit the deamination of L-glutamate with NADP⁺, but adenosine 2'-phosphate did, indicating the necessity of the phosphate group for binding. *p*-Chloromercuribenzoate (10 μ M) was found to inhibit both forward and back reactions, although NADP⁺ and NADPH offered protection. The inhibition appears to be due to combination at or near the active site and not to gross structural change.

The native enzyme is highly fluorescent, having an excitation maximum at 287 nm with a peak emission wavelength of 335 nm (all spectra uncorrected). NADPH quenches the protein fluorescence with a concomitant enhancement of its own fluorescence, the emission maximum of which is also shifted from 465 to 450 nm. Quantitative titrations of the protein with NADPH by monitoring nucleotide fluorescence enhancement have been performed and values for the dissociation constant, K_d , of the binary complex have been obtained. These values are subject to considerable variation depending on the pH and ionic strength of the medium, as indeed might be expected. K_d is lowered with decreasing pH, but increases proportionately with ionic strength over a wide range. Preincubation of the enzyme with *p*-chloromercuribenzoate prevents the characteristic enhancement obtained on addition of NADPH to native enzyme, presumably by preventing the formation of the binary complex. Addition of *p*-chloromercuribenzoate (10 μ M) to the enzyme-NADPH complex caused a gradual decrease in the observed fluorescence of the binary complex.

In addition, these experiments have yielded a value of 6 equivalent NADPH-binding sites/molecule of protein of molecular weight 267 000 (Barratt & Strickland, 1963). On this basis the monomeric molecular weight would thus appear to be about 44 000, which is at variance with the value of eight to nine subunits of 30 000 molecular weight obtained from amino acid analysis.

By comparing protein fluorescence quench and the nucleotide enhancement it was found that in the presence of NADPH at low protein concentrations and pH 6.0 the presumed hexamer dissociated into subunits. Gel-filtration studies with columns of Sephadex G-100, equilibrated at pH 6.0 with

NADPH, yielded values of about 40 000 for the smallest unit molecular weight.

Barratt, R. W. & Strickland, W. N. (1963) *Arch. Biochem. Biophys.* **102**, 66.

Sanwal, B. D. & Lata, M. (1961) *Can. J. Microbiol.* **7**, 319

The Subunit Structure of the Nicotinamide-Adenine Dinucleotide Phosphate-Dependent Glutamate Dehydrogenase of *Neurospora crassa*

By J. C. WOOTTON, J. G. TAYLOR and J. R. S. FINCHAM
(Department of Genetics, University of Leeds, Leeds LS2 9JT, U.K.)

The number of subunits in the molecule of the NADP-dependent glutamate dehydrogenase [L-glutamate-NADP oxidoreductase (deaminating), EC 1.4.1.4] of *Neurospora crassa* has been reported as eight (Jacobson *et al.*, 1969) and as about eight to ten (Fincham & Coddington, 1963). Our evidence now supports a structure of six identical subunits each of molecular weight 45 000–50 000.

Enzyme preparations were pure by criteria of polyacrylamide-gel electrophoresis and chromatography on Sephadex G-200 and DEAE-Sephadex, and have been crystallized. Ultracentrifugal examination of the native enzyme by sedimentation to equilibrium (Yphantis, 1964) gave a mean molecular weight of 275 000, based on a partial specific volume of 0.731 ml/g as calculated from the amino acid composition. Taking the plausible limits of the partial specific volume as 0.71–0.75 ml/g gives a molecular-weight range of 250 000–305 000. Equilibrium sedimentation of the subunit in 6M-guanidinium chloride gave a molecular weight close to one-sixth that of the native enzyme for a given value of partial specific volume, and a plausible molecular-weight range of 39 000–50 000.

Electrophoresis in polyacrylamide gels containing 0.1% sodium dodecyl sulphate, in comparison with proteins of known molecular weight, suggested a subunit molecular weight of approx. 46 000, and a plausible molecular-weight range of 42 000–50 000, allowing for uncertainties in the method.

From digests with trypsin of the *S*-carboxymethylated or the performic acid-oxidized subunit, over 40 peptides of distinct amino acid composition have been purified in yields of up to 65%. The sequences of most of these, amounting to over 200 residues, have been determined, and they include nine unique sequences containing histidine and six containing tryptophan. The total number of residues in peptides of probably unique sequence is over 420. These results and the amino acid composition of the enzyme are incompatible with a subunit molecular weight of less than approx. 46 000. Peptide 'maps' of digests and

analyses of insoluble material produced during digestion show that very few if any peptides have been overlooked. Analyses of the enzyme for carbohydrate suggest that little or none is present.

Digestion of the intact subunit with carboxypeptidases revealed a single C-terminal sequence, -Lys-Asn. The amount of asparagine released is compatible with a polypeptide molecular weight described above.

Fincham, J. R. S. & Coddington, A. (1963) *Cold Spring Harbor Symp. Quant. Biol.* **28**, 517

Jacobson, J. W., Strickland, W. N. & Barratt, R. W. (1969) *Biochim. Biophys. Acta* **188**, 283

Yphantis, D. A. (1964) *Biochemistry* **3**, 297

The Production of Protein for Non-ruminant Feeding by Growing Filamentous Fungi on Barley

By A. E. READE, R. H. SMITH and R. M. PALMER
(Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB, U.K.)

Barley, the chief staple in pig feeding, contains usually 10–12% crude protein ($N \times 6.25$). Because the young pig's requirement for protein is about 16–20% of the diet (Agricultural Research Council, 1967), the deficit is generally made good by adding soya bean or fishmeal. Microbial fermentation in which added non-protein N is converted into protein N at the expense of barley carbohydrate is another way of improving the protein content. We thus sought a non-pathogenic organism giving rapid dominant growth that would ferment barley grain under non-aseptic conditions.

Twenty-three fungi were screened in shake-flask cultures grown for 3 days in a medium containing 5% barley, 0.1% urea and 0.2% KH_2PO_4 . Contents of crude protein (dry basis) in the biomasses ranged from 8% (*Geotrichum candidum*) to 23% (*Aspergillus oryzae*). Comparison of two high-yielding organisms, *A. oryzae* and *Rhizopus arrhizus*, was made in 7-litre stirred cultures. With 1% barley and 0.1% urea, pH 4.0, *Rhizopus* grew at a much lower rate than *Aspergillus*, where mycelial growth stopped at 24h (3.5g/l, dry basis), whereas *Rhizopus* was still growing at 60h (4.0g/l); mycelial N contents were maximal at about 7% during early growth. With $(NH_4)_2SO_4$ as N source both organisms gave higher mycelial and protein yields within 24h (*Rhizopus*, 3g/l; *Aspergillus*, 5g/l). Growth, which ceased at 24h, was limited by carbohydrate; 50% only of the added N was utilized.

With 2% barley, *A. oryzae* gave a mycelial yield of 7.4g/l at 24h with a maximum at 34h of 8.5g/l; the mycelial N content was 7–7.5% and utilization of

carbohydrate and $(NH_4)_2SO_4$ was almost complete. With 3% barley and 0.42% $(NH_4)_2SO_4$ the mycelial yield was 8.5g/l at 24h and 10.5g/l at 34h; the N content was 7.5%. At 34h 90% of carbohydrate and 80% of N source were used up and uptake ceased. With both 2% and 3% barley the concentration of reducing sugar reached a high value during the rapid phase of growth.

A. oryzae has been successfully grown under non-aseptic conditions in 1000-litre culture in a modified pig-feed mixing vessel. With 20kg of barley, 1kg of urea, 2kg of KH_2PO_4 , pH 3.5, in 24h, 9.3kg of biomass was recovered at 31% crude protein (dry basis); this contained (g/16g of N): lysine, 4.4; methionine, 1.2; cysteine, 1.5; leucine, 5.8; isoleucine, 3.2; valine, 4.8; threonine, 3.4; phenylalanine+tyrosine, 6.4; arginine, 5.9; hexosamine, about 7%. The biomass, fed to rats, gave a net protein utilization value of 56; mixed with barley it was readily eaten by pigs, which showed satisfactory weight gains.

Agricultural Research Council (1967) *The Nutrient Requirements of Farm Livestock no. 3, Pigs*, p. 95, Agricultural Research Council, London

The Purification and Physical Properties of 3-Carboxy-*cis-cis*-muconate Cycloisomerase

By D. R. THATCHER* and R. B. CAIN† (Department of Botany, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU, U.K.)

Cell-free extracts of *Aspergillus niger*, grown on a *p*-hydroxybenzoate carbon source, contain an enzyme that catalyses the lactonization of the product of aromatic ring fission (3-carboxy-*cis-cis*-muconate) to 3-carboxymuconolactone (Cain *et al.*, 1968). The reaction is similar to that catalysed by the enzyme muconate cycloisomerase (EC 5.5.1.1), which has been characterized in the pathway responsible for the degradation of catechol in *Pseudomonas putida* A3.12 (Ornston, 1966). The *A. niger* enzyme has therefore by analogy been called 3-carboxy-*cis-cis*-muconate cycloisomerase.

The enzyme was purified by a combination of ammonium sulphate fractionation, DEAE-cellulose chromatography and gel filtration. The 30–50%-saturation- $(NH_4)_2SO_4$ fraction of 10.5 litres of cell-free extract was purified first by batch elution on Whatman DE11 anion-exchange DEAE-cellulose at pH 6.5 followed by gradient elution of the enzyme from a column of Whatman DE52 DEAE-cellulose at pH 7.5. A final separation on a column of Sephadex

* Present address: Department of Molecular Biology, University of Edinburgh, Edinburgh EH9 3JR, U.K.

† Present address: Biological Laboratories, University of Kent, Canterbury, Kent, U.K.