purity than those obtained by other means. Preliminary experiments with other plant ribonucleases suggest that the media described will be of general application and that, when used in combination with other nucleotide-containing media, they should simplify the search for base-specific nucleases.

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Separation of Nucleoside Deoxyribosyltransferase into Four Active Components by Affinity Chromatography

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Nucleoside deoxyribosyltransferase (EC 2.4.2.6) was first described by McNutt (1952). This enzyme has been found in only a few micro-organisms and transfers the deoxyribosyl moiety from a purine (or pyrimidine) deoxyribonucleoside to a purine (or pyrimidine) base according to the equation:

abbreviated as (Pu → Pu'). Attempts to purify the enzyme by conventional methods were only partially successful; moreover, an observation made by Roush & Betz (1958) indicated that more than one enzymic protein could transfer the deoxyribosyl moiety. This hypothesis was confirmed in our laboratory by several independent methods. The technique of affinity chromatography was used to purify the enzyme and to separate the different enzymic proteins classified as EC 2.4.2.6.

The following deoxyribonucleoside analogues were synthesized: thymine-1-acetylhydrazine (I), 5-(*p*-aminophenyl-*n*-propyl)uracil (II), hypoxanthine-9-acetylhydrazine mixed with hypoxanthine-7-acetylhydrazine (III), 8-(2,4-diaminophenylazo)xanthine (IV), 6-(aminophenyl-*n*-hexylamino)purine (V) and 6-(*p*-aminobenzylamino)purine (VI). A rapid study of the affinity of these substances for the enzyme showed that compounds (II), (V) and (VI) were good candidates. A first attempt was made with a Sepharose column, activated with CNBr as described by Cuatrecasas *et al.* (1968), to which compound (VI) was attached.

This column had a limited capacity and gave only a partial purification. The link between the Sepharose matrix and the base was lengthened by the use of

compound (V). In this case the retention was nil, probably owing to the fact the flexible chain folds back on itself, bringing the base portion of the molecule too close to the matrix. To obtain a more rigid link, *m*-phenylenediamine was attached to the activated Sepharose, and to this modified matrix compound (VI) was coupled after diazotization. The capacity of this column was 100 times that of the first one. All four transfer activities were retained (Pu → Pu, Pu → Py, Py → Pu and Py → Py), but near saturation the (Pu → Pu) activity seems to predominate.

Elution could be obtained with alkaline buffers (tris above pH 7.3), 1 M-guanidine hydrochloride, 1 M-urea, 1–5 mM-deoxyinosine or 1 mM-adenine. Alkaline buffers and urea elute all four activities without any fractionation, whereas deoxyinosine elutes only (Pu → Pu) activity. Adenine elutes (Pu → Pu) and (Pu → Py) activities rapidly; (Py → Pu) activity is significantly retarded. A column prepared with 5-(*p*-aminophenyl-*n*-propyl)uracil (generously given by Dr. B. R. Baker, Department of Chemistry, University of California, Santa Barbara, Calif., U.S.A.) retained only (Py → Py) activity. With these columns it was possible to establish without ambiguity the occurrence of four specific enzymic proteins, and the protein responsible for the (Pu → Pu) activity was obtained with 1400-fold purification in a single step.

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Studies on the Glutamate Dehydrogenase from *Neurospora crassa*

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The enzyme glutamate dehydrogenase [L-glutamate-NADP⁺ oxidoreductase (deaminating), EC 1.4.1.4] from wild-type *Neurospora crassa* (WT 74A) has been obtained from mycelia grown on minimal media with forced aeration (J. R. S. Fincham, personal communication). The purification procedure was essentially as described by Barratt & Strickland (1963), except that Sephadex G-200 column fractionation replaced the use of the above authors' final DEAE-cellulose column.

Although we have observed that a number of amino acids and keto acids are oxidatively deaminated and reductively aminated in the presence of glutamate dehydrogenase (cf. Barratt & Strickland, 1963;

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.

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

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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