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.

- Axén, R., Porath, J. & Ernback, S. (1967) *Nature (London)* **214**, 1302
 Barnard, E. A. (1969) *Annu. Rev. Biochem.* **38**, 677
 Cuatrecasas, P. (1970) *J. Biol. Chem.* **245**, 3059
 Wilchek, M. & Gorecki, M. (1969) *Eur. J. Biochem.* **11**, 491
 Wyen, N. V., Udvardy, J., Solymosy, F., Marré, E. & Farkas, G. L. (1969) *Biochim. biophys. Acta* **191**, 588

Separation of Nucleoside Deoxyribosyltransferase into Four Active Components by Affinity Chromatography

By R. CARDINAUD and J. HOLGUIN (*Département de Biologie, Service de Biophysique, B.P. no. 2, 91-Gif-sur-Yvette, France*)

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.

- Cuatrecasas, P., Wilchek, M. & Anfinsen, C. B. (1968) *Proc. Nat. Acad. Sci. U.S.* **61**, 636
 McNutt, W. S. (1952) *Biochem. J.* **50**, 384
 Roush, A. H. & Betz, R. F. (1958) *J. Biol. Chem.* **233**, 261

Studies on the Glutamate Dehydrogenase from *Neurospora crassa*

By M. G. GORE and C. GREENWOOD (*School of Biological Sciences, University of East Anglia, Norwich NOR 88C, U.K.*) and J. J. HOLBROOK (*Molecular Enzymology Laboratory, Department of Biochemistry, University of Bristol, Bristol BS8 1TD, U.K.*)

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;