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Promotion of Deoxyribonucleic Acid-Dependent Ribonucleic Acid Synthesis by Protein Isolated on a Plant Hormone Affinity Column

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Although it has long been considered that there may be specific proteins mediating the primary action of plant hormones, activity in this area has been relatively unproductive. Recently preliminary reports have indicated that, in the presence of certain protein fractions, auxins (Matthyse & Phillips, 1969) and cytokinins (Matthyse & Abrams, 1970) can stimulate RNA synthesis directed by isolated pea chromatin or DNA. Similarly it appears that both gibberellic acid (Johri & Varner, 1968) and abscisic acid (Pearson & Wareing, 1969) effects on RNA synthesis *in vitro* may require the presence of factors that are rapidly lost from nuclei or chromatin during the isolation procedures.

An attempt has been made to obtain possible protein mediators of this kind by the affinity-chromatography principle, by using an agarose column to which a derivative of the synthetic auxin 2,4-dichlorophenoxyacetic acid is attached by the method of Cuatrecasas *et al.* (1968). Such a column retains small amounts of protein from crude extracts of pea or maize shoots. Elution with 2mM-KOH gives a protein factor that enhances DNA-dependent RNA synthesis (supported by *Escherichia coli* polymerase) by 50–300% in different preparations. Activity is not due to inhibition of adenosine triphosphatase or ribonuclease action, nor is it an endonuclease effect. Time-course experiments involving rifampicin suggest the factor acts, partially at least, on RNA chain initiation.

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Purification of Tobacco Ribonuclease by Affinity Chromatography

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The number of plant ribonucleases that have been studied in detail is small in comparison with ribonucleases from other sources (Barnard, 1969). Those plant ribonucleases that have been studied exhibit no absolute base-specificity, but do show a marked preference for purine bases. They will hydrolyse purine cyclic nucleotides and are inhibited by purine mononucleotides (Wyen *et al.*, 1969; L. Jervis, unpublished work). Wilchek & Gorecki (1969) have purified bovine pancreatic ribonuclease on an affinity medium based on the inhibitory properties of uridine 2′(3′)-monophosphate. Several affinity media have been developed that will purify tobacco ribonuclease. These media make use of the inhibition of tobacco ribonuclease by guanosine 2′(3′)-monophosphate.

Adsorbent 1 was prepared by coupling 5′-(4-aminophenylphosphoryl)guanosine 2′(3′)-monophosphate to Sepharose 2B by the CNBr procedure (Axén *et al.*, 1967). Absorbent 2 was obtained by binding guanosine 2′(3′)-monophosphate to Sepharose 2B–aminohexanoic acid by the water-soluble carbodi-imide method (Cuatrecasas, 1970). Adsorbent 3 was made by coupling guanosine 2′(3′)-monophosphate to CNBr-activated Sepharose 2B.

Adsorbents 1 and 2 bind tobacco ribonuclease strongly. This can be released either by substrate elution or by changing the buffer conditions. Adsorbent 3 does not bind ribonuclease, presumably owing to steric hindrance. It does, however, bind other proteins that are bound by adsorbents 1 and 2, and is consequently useful when crude enzyme preparations are being used. Adsorbent 2 exhibits ion-exchange properties in addition to specific adsorption. This has been shown to be largely due to free carboxyl groups remaining on the Sepharose–aminohexanoic acid after reaction with GMP. This ion-exchange behaviour can be avoided by careful control over the coupling conditions or by blocking the free carboxyl groups after reaction with GMP. In addition to binding ribonuclease, the affinity media described also adsorb some phosphomonoesterase activity and phosphodiesterase from crude preparations. These enzymes can be selectively removed by substrate elution, leaving the ribonuclease still bound to the column.

Use of these affinity media has given tobacco ribonuclease preparations of substantially greater

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:

$$PudR + Pu' \rightarrow Pu + Pu'dR$$

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;