precisely the protein under scrutiny. This very specificity, however, clearly limits the scope of applicability of many immobilized purification systems, the synthesis of which often involves elaborate chemistry. We in Lund have therefore focused our attention on the development of generalized affinity chromatography by using matrix-bound ligands with a broad 'enzyme spectrum' (Mosbach *et al.*, 1971).

The ligands we selected to bind to separate gel matrices were NAD^+ and an AMP analogue. For preparation of immobilized NAD^+ , 6-aminohexanoic acid has been coupled to Sepharose 4B by the CNBr method (Axén *et al.*, 1967), and subsequently dicyclohexylcarbodi-imide was used to attach NAD^+ , probably via ester linkages (Larsson & Mosbach, 1971). These preparations have been shown to retain some of the cozymic function of the free nicotinamide nucleotide (Mosbach, 1971; Larsson & Mosbach, 1971). The use of similarly immobilized cofactors in affinity chromatography has also been reported by other workers in this field (Lowe & Dean, 1971).

For preparation of the immobilized AMP analogue, N^6 -(6-aminoethyl)-AMP (H. Guilford, P.-O. Larsson & K. Mosbach, unpublished work) has been linked directly to activated Sepharose 4B. In both cases the 'spacing' of the cofactor and inhibitor moieties from the matrix by a hexacarbon 'chain' of length about 0.7 nm ($\text{\AA}$) was used to increase the steric availability of the ligands to the enzymes studied, e.g. glyceraldehyde 3-phosphate dehydrogenase (EC 1.2.1.12) and lactate dehydrogenase (EC 1.1.1.27).

On application of a mixture of bovine serum albumin, glyceraldehyde 3-phosphate dehydrogenase and lactate dehydrogenase to columns packed with either gel matrix, only the two enzymes were retained. A 0.15 mM solution of NAD^+ eluted only glyceraldehyde 3-phosphate dehydrogenase, and subsequent application of 0.15 mM-NADH eluted only lactate dehydrogenase. The observed elution pattern is consistent with the affinities of NAD^+ and NADH reported for the two dehydrogenases (McPherson, 1970, and references cited therein). Subsequent more detailed studies with the AMP-Sepharose preparation gave the following results.

(1) The enzymes could not be eluted by a 0.0–0.5 M-KCl gradient, indicating strong binding of the enzyme to the nucleotide.

(2) Elution of glyceraldehyde 3-phosphate dehydrogenase followed by lactate dehydrogenase was achieved by application of a gradient of 0.0–0.15 M-salicylate, which is known to be an inhibitor competing with NAD^+ for both enzymes (Hines & Smith, 1964).

(3) Batchwise elution studies of bound lactate dehydrogenase under specified conditions revealed the following approximate relative elution efficiencies

with different eluents: lactate+semicarbazide, 0; 1 mM- NAD^+ , 0; lactate+ NAD^+ , 17; lactate+semicarbazide+ NAD^+ , 21; NADH, 21. We ascribe the closely comparable efficiency of NADH and of lactate+semicarbazide+ NAD^+ to the enzymic formation of reduced cofactor taking place in the latter system under the experimental conditions. The rather high efficiency of lactate+ NAD^+ is surprising in view of the fact that the equilibrium is far to the side of the oxidized form of the cofactor. The cumulative effect in enzyme elution due to the presence of the substrate, lactate, will be discussed.

(4) Commercially available crude pyruvate kinase having no affinity for AMP (Reynard *et al.*, 1961), but highly contaminated with lactate dehydrogenase activity, was completely separated from the dehydrogenase in a single-step procedure: all the pyruvate kinase appeared in the early fractions, leaving the lactate dehydrogenase bound.

The general applicability to affinity chromatography of the bound nucleotide preparations described will be discussed together with aspects of selective (co)substrate elution.

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Molecular Properties of Immobilized Proteins

By D. GABEL and J. PORATH (*Institute of Biochemistry, University of Uppsala, Uppsala, Sweden*)

A method is presented that allows us to follow conformational changes in immobilized proteins (Gabel *et al.*, 1972). The method is based on the fact that a gel bed of an immobilized protein can be considered as a fluorescent surface. A special cell has been devised that fits into most commercial and home-made spectrofluorometers. As it can be operated as a small chromatographic column, it allows rapid dialysis and change of liquid.

It was established that gels of Sephadex and agarose represent an environment for tryptophan similar to that of water. Changes in the observed fluorescent properties of proteins can thus be attributed to changes in their conformation. The inherent limitations of the method are discussed.

With this method it was shown that the covalent binding of trypsin to CNBr-activated Sephadex stabilized the protein against the denaturing effect of concentrated urea solutions, in contradistinction to the similar agarose-bound trypsin, which behaved as the free trypsin. This agrees with activity measurements (Gabel *et al.*, 1970), the latter derivative being inactive in 8M-urea, as is the free enzyme, whereas Sephadex-bound trypsin retains most of its activity even after long storage in urea solutions.

The stabilization of the active surface of trypsin by Ca^{2+} , found for the free enzyme (Sipos & Merkel, 1970), was not observed with the Sephadex-bound enzyme. The protein in solution unfolds at 60°C in the presence of Ca^{2+} , as reflected by a red shift of the tryptophan fluorescence, whereas the fluorescence properties of Sephadex-bound trypsin did not change up to 70°C. At that temperature the fluorescence intensity increased, but no red shift was observable. This increase coincided with the loss of activity. It is suggested that the temperature-induced conforma-

tional transitions that inactivate trypsin in the absence and in the presence of Ca^{2+} , as well as the Ca^{2+} -dependent transition leading from native trypsin to a more compact structure (Sipos & Merkel, 1970), are not possible in Sephadex-bound trypsin owing to a more-or-less extensive cross-linking between the protein and the rigid carrier.

Attempts were made to identify those ϵ -amino groups that contribute to the stabilization of Sephadex-bound trypsin against denaturing influences.

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COMMUNICATIONS

Biosynthesis of Fatty Acids and Phenols by Stationary-Phase Cultures of *Aspergillus fumigatus*

By N. M. PACKTER and A. C. WARD (*Department of Biochemistry, University of Leeds, Leeds LS2 9LS, U.K.*)

Many fungi respond to deprivation of essential nutrients by enhancement of lipid synthesis (Borrow *et al.*, 1961) or secretion of acetate-derived phenols. *Aspergillus fumigatus* secretes 2,3,5-trihydroxytoluene under submerged cultural conditions. Synthesis of this metabolite does not involve a reductive step (but an oxygenase is required) and the complex concerned may possibly be a 'defective' fatty acid synthetase. However, Dimroth *et al.* (1970) have established that the synthetases responsible for forming 6-methylsalicylate and fatty acids are distinct in *Penicillium patulum*. Certain facets of fatty acid and phenol synthesis in *A. fumigatus* were therefore examined.

Homogeneous colonies of mycelium were transferred (before phenol secretion) to 5% (w/v) glucose solution. [$1\text{-}^{14}\text{C}$]Acetate (10 μCi) was added to each flask; three flasks (150 ml) were removed after a further 3 h, 7 h, 21 h and 28 h incubation. Lipids were isolated from the mycelium and saponified to generate the constituent fatty acids. They were purified and determined spectrophotometrically (Duncombe, 1963). Phenols were extracted as described by Packter

(1965); trihydroxytoluene was rechromatographed and crystallized. Fatty acid and ergosterol contents doubled during the test period. [^{14}C]Acetate was efficiently incorporated into these lipids (20 and 0.7%) within 3 h, but gave rise to insignificant labelling (0.05%) in the phenols from either medium or mycelium; carrier material was added to the early groups.

Next, [$1\text{-}^{14}\text{C}$]acetate was added to mycelium at 0.5, 5, 17, 28 and 48 h after transfer; each group was harvested 1 h later. Incorporation into fatty acids was 16.8, 32.5, 16.9, 20.1 and 18.2% for the individual groups; values for ergosterol were 0.7, 1.6, 0.4, 1.3 and 1.2%. Again, lipid and ergosterol content increased. Incorporation into phenols was low for groups 1 and 2 (0.3 and 0.7%), but considerable conversion occurred in the later groups (14.3, 13.6 and 13.1%).

The ability to form fatty acids at a late stage in the growth period on normal medium was verified by assay of the synthetase (Lynen, 1969). Its activity fell after exhaustion of nitrogen, but only to a value comparable with that of many other enzymes.

These results indicate that initiation of phenol synthesis is not a simple response to lack of nutrients. Presumably metabolic events that ensue later are responsible for the expression of 'aromatic synthetase' activity. However, fatty acid synthetase remains active.

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