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Insolubilized Enzymes for Food Synthesis

The synthesis of edible foods from metabolic wastes can be made possible by use of certain enzymecatalyzed reactions, but the enzymes must be made insoluble and recoverable so that they can be used repeatedly.

Methods which have been developed thus far for the insolubilization of enzymes can be classified into four major groups: (1) Covalent attachment to organic and inorganic carriers; (2) Entrapment within polymeric matrices; (3) Adsorption onto insoluble carriers such as colloidal silica or ion-exchange resins; (4) Crosslinking with bifunctional reagents. Unfortunately, because each method has one or more disadvantages, it fails to meet the desired features of wide applicability, large retention of activity per gram of carrier, low cost, simplicity, and physical properties compatible with a wide variety of operational procedures. For example, the most attractive technique, adsorption onto colloidal silica, produces particles of such fine size that they cannot be used in columns.

A study to investigate synthesis of starch from glucose combined the best features of each of the noted methods; a novel cellulose derivative was produced that had high activity and possessed the requisite physical characteristics for filtration or column operations. The product also allows the coupling step in the synthesis to proceed under mild conditions.

Enzyme-coated silica particles were prepared according to the procedure of Haynes and Walsh (see Reference). Effective adsorption requires that the net

charge of the protein be opposite to that of the silica particle; for some proteins (e.g., those with a net negative charge at the pH at which adsorption is to occur), it is necessary to change the character of the silica particles by first causing them to adsorb a layer of polyethylenimine.

The insolubilized enzymes on silica substrates were then allowed to react covalently with a unique supporting material precursor, aminoethyl cellulose which had been converted into a novel "active ester" derivative:

Cellulose-O-C₂H₄-NH-CO-C₂H₄-CO₂-C₆H₄-SO₂-CH₃(-p) \downarrow +Protein-Silica Cellulose-O-C₂H₄-NH-CO-C₂H₄-CO-NH-Protein-Silica

Thus, the final product consisted essentially of a cellulosic matrix with numerous enzyme-coated silica particles of colloidal size permanently bound at various sites within the matrix.

Reference:

Haynes, R., and Walsh, K. A.; Enzyme Envelopes on Colloidal Particles. Biochem. Biophys. Res. Commun., vol. 36, page 235, 1969.

Note:

Requests for further information may be directed to:

> **Technology Utilization Officer** Ames Research Center Moffett Field, California 94035 Reference: TSP 72-10247

> > (continued overleaf)

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