Journal of Macromolecular Science, Part A
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597274

Immobilized Proteins and Peptides

R. D. Falb[a]; G. A. Grode[a]
[a] Department of Biology and Medical Sciences, Battelle-Columbus Laboratories Columbus, Ohio

To cite this Article Falb, R. D. and Grode, G. A.(1976) 'Immobilized Proteins and Peptides', Journal of Macromolecular Science, Part A, 10: 1, 197 — 221
To link to this Article: DOI: 10.1080/00222337608068096
URL: http://dx.doi.org/10.1080/00222337608068096

J. MACROMOL. SCI.--CHEM., A10(1&2), pp. 197-221 (1976)

IMMOBILIZED PROTEINS AND PEPTIDES

R. D. Falb and G. A. Grode

Department of Biology and Medical Sciences
Battelle-Columbus Laboratories
Columbus, Ohio 43201

In recent years a great deal has been learned about the structure and function of proteins and about the roles they play in the complex process of a living organism. The classical approach of the biochemist in studying a given protein is to free it from the cellular species. The properties and structure of the protein in solution are then examined and analyzed. While this approach is logical and has yielded much information about proteins, one should always keep in mind that in nature a great many proteins function not in solution, but at an interface or within solid state assemblages in cells. The protein removed from a surface is thus often not in its natural environment and can display an altered reactivity and stability.

The attachment of proteins and peptides to solids has progressed rapidly in recent years from a laboratory curiosity to a widely useful technique in both laboratory research as well as for medical and industrial applications. Proteins and enzymes immobilized in various ways now find increasing use in giving valuable insight into basic interactions of proteins and, in addition, have many applications in medicine, clinical analysis, and synthetic chemistry. Many different types of proteins including enzymes, antibodies, enzyme inhibitors, proteinaceous antigens, and peptide hormones have

been shown to possess biological activity. More than 50 enzymes have been attached to polymers to make preparations which have many applications because their insolubility makes them easily separable from reaction mixtures and generally renders them more stable than the corresponding soluble polymers. Solid-phase antibodies now find use in diagnostic methods and also are valuable laboratory tools for purification of antigens. Conversely, immobilized antigens can also be used for analytical and preparative purposes. In general, the basic rational for the attachment of a protein or peptide to a solid phase is twofold: (1) to provide a method for conveniently manipulating the protein while making use of its biological properties, or (2) altering or localizing the behavior of the biopolymer.

The field of immobilized proteins requires the skills of both polymer chemists and biochemists. In attaching proteins to the surfaces of polymers, the most common approach is to form a covalent bond between a reactive group on the surface of the polymer and a group on the protein. A very large number of synthetic polymers including polystyrene, ethylene-maleic anhydride, phenolic resins, polyacrylamide, nylon, silicone rubber, polyethylene, polyaminoacids, and polyhydroxyethyl acrylamide have been used as matrices for protein attachment. In general, the polymer is derivatized to contain an active species such as an aliphatic or aromatic amino, hydroxyl, carboxylic, or sulfhydryl group. The proteins contain amino, carboxyl, sulfhydryl, hydroxyphenyl, and hydroxyl groups. By the appropriate choice of activating of coupling agents, these moieties can be joined to appropriate groups on the polymers. For example, one of the most common methods for protein attachment involves utilization of the protein's free amino groups which act as nucleophiles to attack surface-bound activated carboxyl groups (i.e., acid halides, acid azides), activated aliphatic or aromatic halides, and cyclic iminocarbonate esters. In coupling reactions, care must be taken to avoid inactivation of the protein and thus one must carefully select appropriate solvent systems and utilize reactive moieties which do not block the active site of the protein molecule.

Research activity in methods of attachment and applications for immobilized proteins and peptides has been very active in the last 5 years. In the field of immobilized enzymes alone, a literature survey conducted by the authors disclosed over 400 references from June 1973 until the end of 1974. Much of the work on immobilized enzymes is conducted in industrial laboratories and is not available in the open literature. It is beyond the scope of this review to discuss all of the methods available for protein immobilization. The reader is referred to a monography by Zaborsky [1] for a comprehensive review of the methods of preparation and properties of immobilized enzymes. Numerous reviews important to surveying this field have been written and are referenced [2-18].

When the protein has been attached, the composite has many interesting and even exciting properties. Applications for immobilized enzymes are being rapidly developed for food and beverage processing, synthesis of drugs and fine chemicals, and analysis of constituents of body fluids. For synthetic purposes, the polymer-enzyme adduct may be regarded as a heterogeneous catalyst and placed in a column, in a suspension, or coated on the walls of reaction vessels and conduits.

The attachment of a protein to a solid surface may be done in such a way to ensure that the resulting adduct preserves the biological activity of the protein. This requires that the active site of the protein be accessible to the surrounding milieu and not be buried in a pore or blocked by some other component of the surface. It also requires that the reactive groups that comprise the active site of the protein not be modified chemically during the attachment reaction. Another feature which is desirable in most cases is that the protein be irreversibly bonded to the solid phase under the conditions in which the adduct will function. For example, the accuracy of a solid-phase antibody used in an immunoassay system is severely compromised if the antibody desorbs from the solid. An immobilized enzyme system will lose activity over a time period, and the product could be contaminated by the enzyme if the enzyme is not irreversibly attached to the solid phase.

GENERAL METHODS OF IMMOBILIZATION

Proteins have been attached to solid phases in a variety of configurations:

(1) Covalent bonding

(2) Physical adsorption

(3) Cross-linking at solid surfaces

(4) Cross-linking with difunctional reagents

(5) Inclusion in a gel phase

(6) Encapsulation

Because of the desirability of irreversible attachment, most of the work done with protein immobilization has been with covalent bonding. For this purpose, a very large number of synthetic and natural polymers and inorganic materials have been used. Generally, covalent attachment proceeds through a functional group on the surface reacting with a reactive site on the protein, usually a carboxyl, amino, hydroxyl, sulfhydryl, or hydroxyphenyl group. The surfaces to which the proteins are to be attached can be modified in numerous ways to obtain the desired functional groups. Thus a very large number of approaches can usually be used in the attachment of a given protein to a surface. The choice of the method of attachment depends upon the reaction conditions which can be tolerated by the protein, the ease of the attachment sequence, and the availability of reactive groups on the protein for attachment. With respect to the latter point, one usually tries to avoid reaction with a group at the active site which is essential for biological activity. Thus, if an amino group is an essential element of the protein's active site, attachment reactions which involve other groups on the enzyme are usually utilized for the attachment or the amine groups are reversibly blocked during the attachment sequence.

As mentioned previously, one of the primary requirements for utilizing proteins bonded to solids is that the active site be accessible. To achieve this, sometimes it is necessary to place the attached protein a certain distance away from the solid surface.

This is usually done by means of a tether linkage, one end of which is attached to the surface and the other end of which is attached to the protein. Tethers of varying lengths have been used in the attachment of proteins, and very often the length of the tether has a profound effect on the biological activity of the proteins.

The desirable amount of protein per given volume of solid varies according to the intended use of the solid-phase adduct. Generally for immobilized enzymes, one wishes to have as large an amount of active enzyme per given weight as possible. The same is true for solid-phase antibodies and antigens which are used for adsorption and purification processes. If an adduct has a relatively low amount of protein per given weight, a column of this material used for synthetic or preparative purposes will be unnecessarily large and the process will thus be inefficient. As a rule of thumb, it is desirable to have at least 100 units of enzyme activity per gram of solid to avoid excessive bulkiness. (A unit of enzyme activity is defined as 1 micromole converted per minute.)

In cases where the solid-phase proteins are used for analytical purposes, the most important consideration is usually the reproducibility of protein binding per given unit surface area. For example, in solid-phase radioimmunoassay the antigen being assayed is usually present in very small amounts. The antibody used to bind the antigen at the surface needs to be present in corresponding amounts and thus the surface coverage of the antibody is quite small. One needs to know very accurately the amount of antibody per given area or the test will not be accurate.

A number of solid-phase materials specifically prepared for protein attachment are now available commercially. These include derivatized porous glass beads, various types of derivatized cellulose, Sephadex and agarose particles, and acrylamide gels. One of the most commonly used methods at present involves attachment to materials containing adjacent hydroxyl groups such as cellulose, Sephadex, or agarose by use of cyanogen bromide activation. This

method has been used for a very large number of different proteins and appears to have good general applicability. In the attachment of protein to any solid, an important considration is the amount of active protein which can be recovered in the solid-phase adduct. Large amounts of biological activity are frequently lost in the attachment sequence, and the process can be thus unfeasible economically. The cyanogen bromide process usually gives good retention of biological activity in the adduct.

Immobilized enzymes are now being used in the synthesis and resolution of amino acids, and contemplated for use in starch saccarification, beer deproteinization, fruit juice clarification, preparation of invert sugars, and carbohydrate synthesis. For the last several years our laboratory has been engaged in research on the use of immobilized enzymes for the synthesis of carbohydrates in space cabin environments [19,20]. The utilization of enzymes for many of the above applications has generated a need for polymer materials which are relatively inexpensive, can contain a high amount of enzyme, and are amenable for use as column packings, vessel surfaces, fibers, etc.

Immobilization of enzymes may also provide a method of efficiently utilizing the inherent high specificity of many enzymes for the sensitive detection of constituents of body tissue and fluids. An enzyme may be attached to the surface of a pH or ion-specific electrode and catalyze a reaction with a specific substrate which can be detected by that electrode.

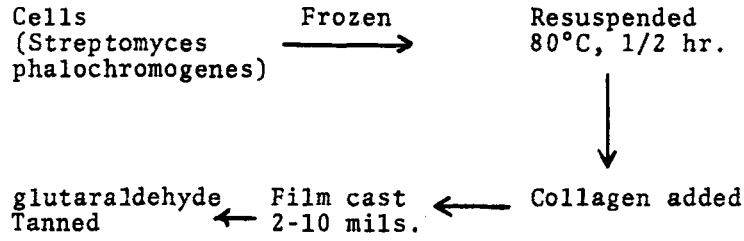
The immobilization of antigens and antibodies has many possible practical consequences. Antigens attached to solid particles can be used for the preparation and purification of purified antibodies. Conversely, immobilized antibodies can be used to purify antigens. Such techniques will have future potential in removing undesirable antigens and antibodies in patients with certain diseases or possibly patients with transplanted organs. Immobilized antibodies also

find use in clinical chemistry where they can be used to detect very small amounts of a given substance in blood, urine, or other body tissues. For example, our laboratory has been working on solid-phase assay methods for insulin and a gonadotrophic hormone which are based on an immobilized antibody. In the assay for insulin, antibody to insulin is covalently attached to the surface of a 20 μl glass capillary. The sample containing insulin is spiked with a measured amount of radiolabeled insulin and drawn into the capillary. The insulin originally present in the sample competes with the added radiolabeled insulin for antibody sites present on the surface of the capillary. By measuring the radioactivity of the capillaries after rinsing, one can determine the amount of insulin in the sample. This assay is sensitive to a few picograms of insulin. At present, however, the poor reproducibility of the assay resulting from varying amounts of attached antibody limits the usefulness of the assay.

In addition to enzymes, antibodies, and antigens, peptide hormones have been attached to polymers with retention of biological activity. For example, insulin has been immobilized and this adduct has been shown to be effective in stimulating glucose transport in cell membranes [21]. While the results of this work are now being disputed, they do point out the possibility of therapeutic effects of various immobilized hormones and enzymes.

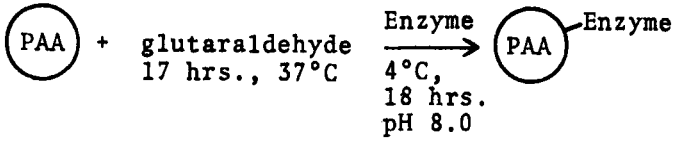
It is not intended that this brief discussion be construed in any sense as a thorough and complete survey of this rapidly burgeoning field with its many exciting scientific and technological thrusts. It is our objective, however, to present at least a cross section of recent significant developments. Hopefully this will be useful to those new to the field as well as those active in immobilized protein research. The following highlights are presented in chart form for clarity and to minimize verbiage. In addition to these highlights, the Bibliography includes many additional references which are separated into a few major categories for clarity.

CHART I. Immobilization of cells in a collagen membrane. [W. R. Vieth, S. S. Wang, and R. Saini, Biotechnol. Bioeng., 15, 565 (1973).]



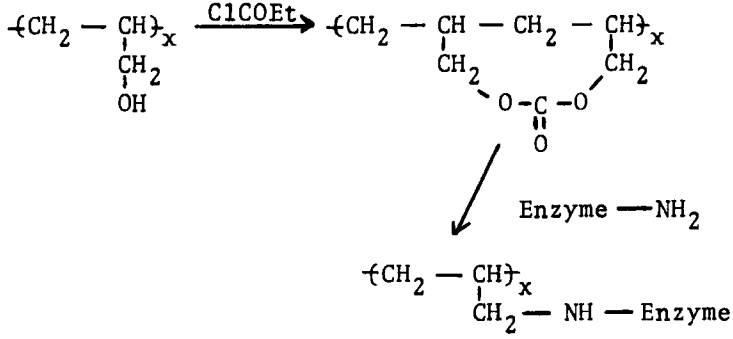
(131 μ/g glucose isomerase activity)

CHART II. Polyacrylamide/glutaraldehyde beads. [P. D. Weston and S. Avrameas, Biochem. Biophys. Res. Commun., 45, 1974 (1971).]



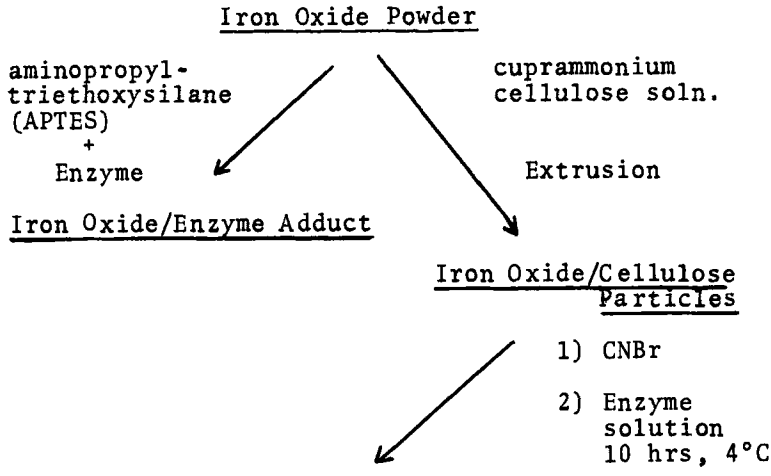
	mg. enzyme/g. polymer	% activity
Acid Phosphatase	18	81
Ribonuclease	20	30
Glucose Oxidase	7	56
Trypsin	18	58
Chymotrypsin	19	65

CHART III. Attachment to poly(allylcarbonate). [J. F. Kennedy, S. A. Barker, and A. Rosevear, J. Chem. Soc., 1971, 2468.]



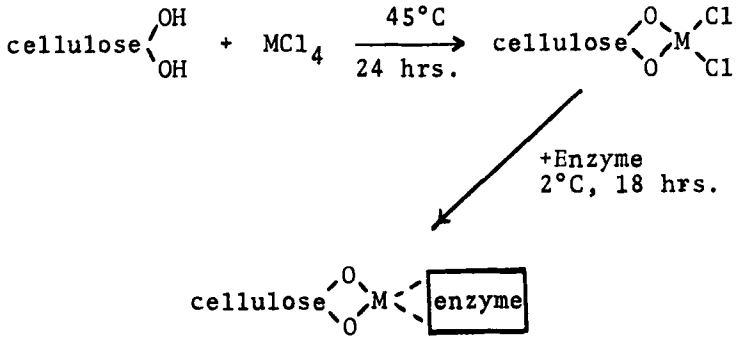
Enzyme	Concentration	Activity
α-Amylase:	2-6 μ/g polymer	2%
β-D-Glucosidase:	6-12 μ/g polymer	30%
Trypsin:	0.25-0.95 μ/g polymer	8%

CHART IV. Magnetic supports for enzymes. [P. J. Robinson, P. Dunhill, and M. D. Lilly, Biotechnol. Bioeng., 15, 603 (1973).]



Enzyme	Cellulose	Silane
β-Galactosidase	900 μ/g	1825 μ/g
α-Chymotrypsin	309 μ/g	800 μ/g

CHART V. Enzyme coupling via metal salts. [A. N. Emery et al., Chem. Eng. (London), 1972, 71.]

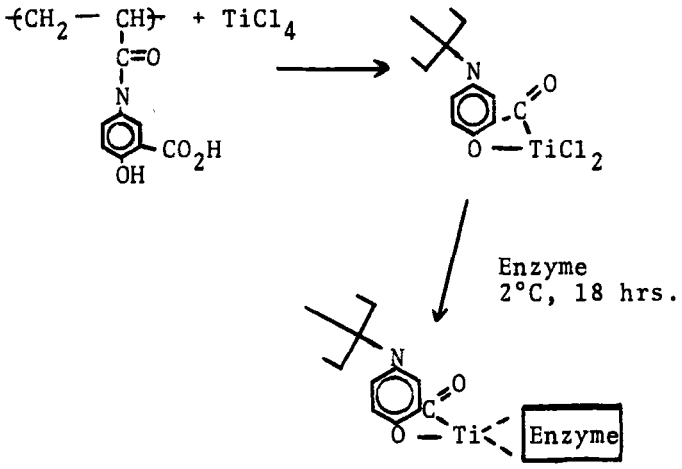


(Metal Salts: $TiCl_4$, $SnCl_4$, $SnCl_2$, $ZrCl_4$, VCl_3, $FeCl_2$)

CHART VI. Enzyme coupling via metal salts.

Enzymes Bound:	Amyloglucosidase	730-2780 μ/g
	Invertase	13-147 mg/g
	Trypsin	44-73% activity
	Amylase	
	Urease	
	Uricase	
	Glucose Oxidase	
Supports:	Borosilicate Glass	
	Soda Glass Beads	
	Filter Paper	
	Cellulose	
	Nylon-66 Fibers	
	Brewers Yeast	
	B. Subtilis	
	E. Coli	

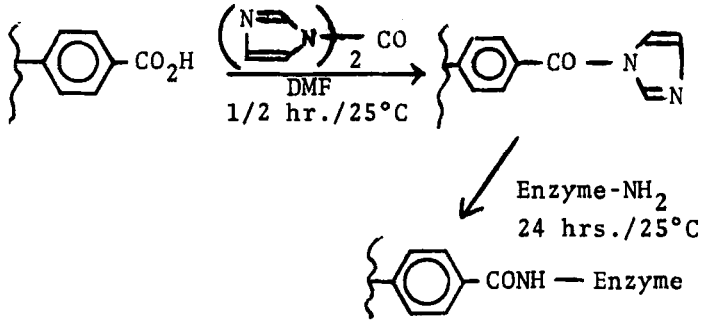
CHART VII. Polyacryloyl-4-and-5-aminosalicylic acid. [J. F. Kennedy and J. Epton, Carbohydr. Res., 27, 11 (1973).]



α-Amylase: 2270-4470 μ/g displaced rapidly

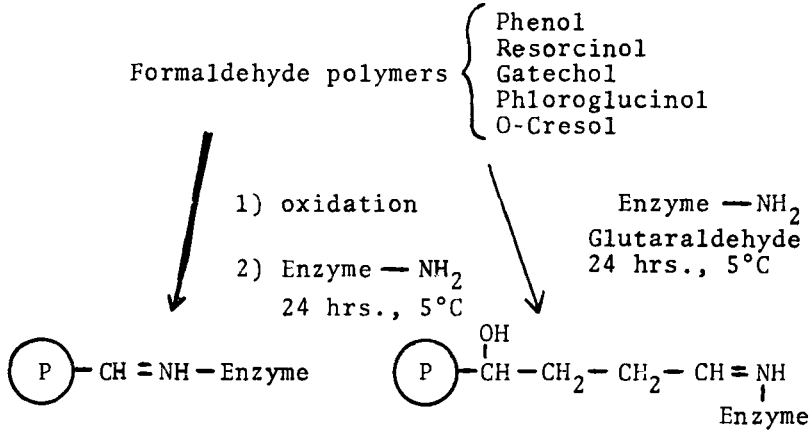
Glucoamylase: 104-469 μ/g stable 24 hrs./37°C

CHART VIII. Immobilization in nonaqueous solvents. [G. J. Bartling, H. D. Brown, and S. K. Chattopadhyay, Nature, 243, 342 (1973).]



(Lysozyme: 25-35 mg./g. polymer, 40% activity)

CHART IX. Immobilization on phenolic resins. [A. C. Olson and W. L. Stanley, J. Agric. Food Chem., 21, 441 (1973); W. L. Stanley and R. Palter, Biotechnol. Bioeng., 15, 597 (1973).]



	μ/g. resin
Lactase	200
Invertase	1200
Glucoamylase	17
α-Chymotrypsin	75
Pronase	33

CHART X. Fiber-entrapped enzymes. [D. Dinelli, Process Biochem., 1972, 9.]

Enzyme solution
Polymer solution
→ Emulsion
→ Spinneret
→ Coagulation Bath
→ Fiber/Enzyme

Polymers:

Cellulose Triacetate
Cellulose Diacetate
Ethyl Cellulose
γ-Methylpolyglutamate

Properties

Invertase: 160-1500 mg./g. polymer, 60-20% activity
1.75×10^5 μ/g polymer, 90% activity after 3 yrs. at 25°C

Penicillin Acylase
β-Tyrosinase
Tryptophan Synthetase
Glucose Oxidase
β-Galactosidase
Catalase

CHART XI. Other methods of enzyme insolubilization. [G. Broun, D. Thomas, G. Gellf, D. Doniurado, A. M. Berjonneau, and C. Guillon, Biotechnol. Bioeng., 15, 359 (1973).]

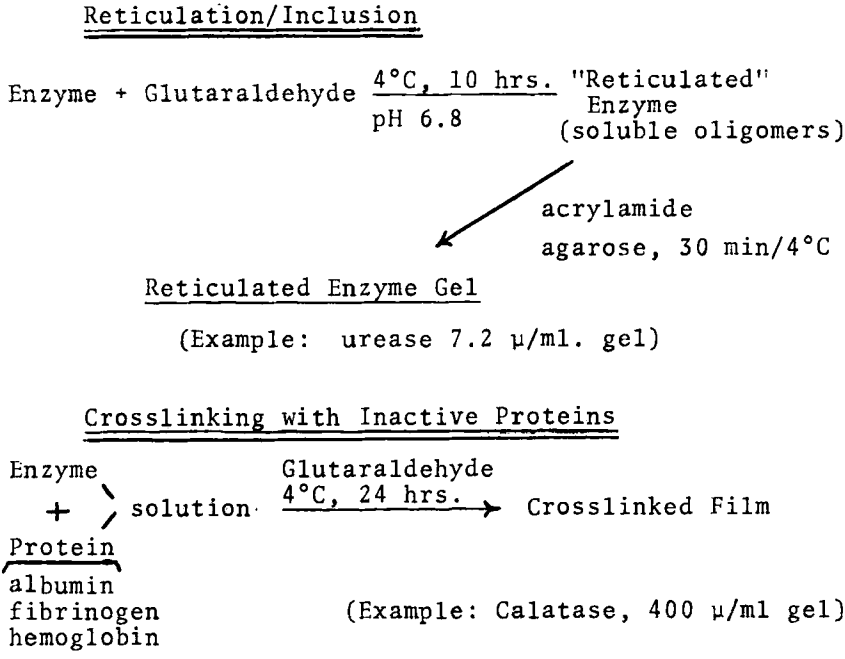
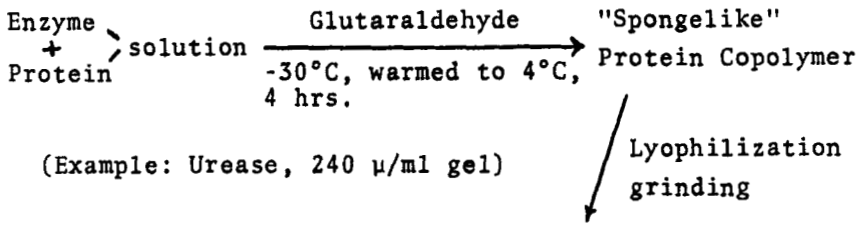


CHART XII. Porous protein particles, Ibid.

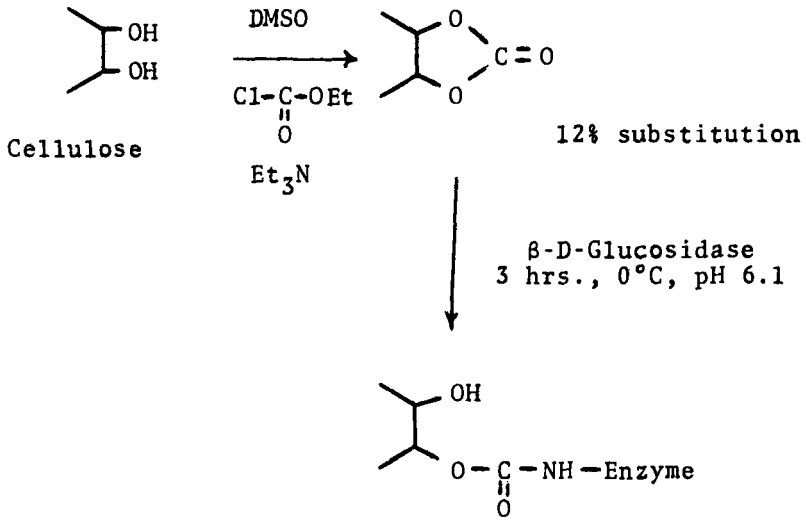


Enzyme/Protein powder

Enzymes

Glucose Oxidase	Asparaginase
Urate Oxidase	Triose-P-Isomerase
L-Aminoacid Oxidase	Carbonic Anhydrase
Xanthine Oxidase	Tyrosine Decarboxylase
Catalase	Phenyl Alanine Decarboxylase
Peroxidase	α-Amylase
Lactate Dehydrogenase	β-Galactosidase
Alcohol Dehydrogenase	Trypsin
Hexokinase	Urease
Ribonuclease	

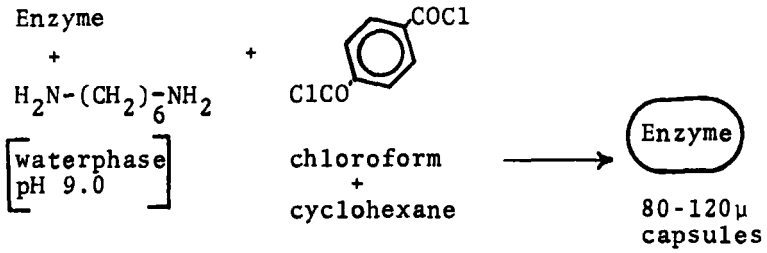
CHART XIII. Cellulose carbonate carriers. [S. A. Barker, S. H. Doss, C. J. Grow, and J. F. Kennedy, Carbohydr. Res., 20, 1 (1971).]



2% protein, 10 μ/gm. carrier

75% retention of activity at 37°C, 50 hrs.

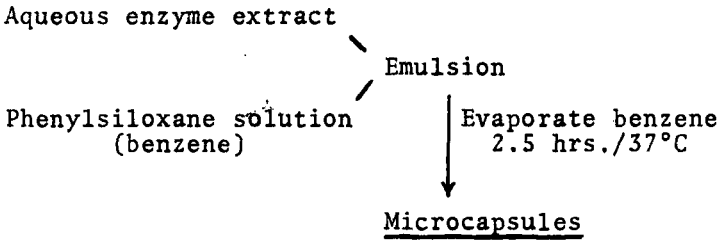
CHART XIV. Microencapsulation via interfacial polymerization. [J.C.W. Ostergaard and S. C. Marting, Biotechnol. Bioeng., 15, 561 (1973).]



β-Galactosidase: 800 μ/g capsules

28% activity after 18 days/37°C

CHART XV. Encapsulation of enzyme extract. M. Kitajima and A. Kondo, Bull. Chem. Soc. Japan, 44, 3201 (1971).



Yeast Extract: Alcohol production, 0.16-0.84 μmole/min g.

Muscle Extract: Lactic Acid production, 0.016 μmole/min g.

CHART XVI. Glass attachment by two different methods. [J. E. Dixon, F. E. Stolzenbach, J. A. Berenson, and N. O. Kaplan, Biochem. Biophys. Res. Commun., 52, 905 (1973).]

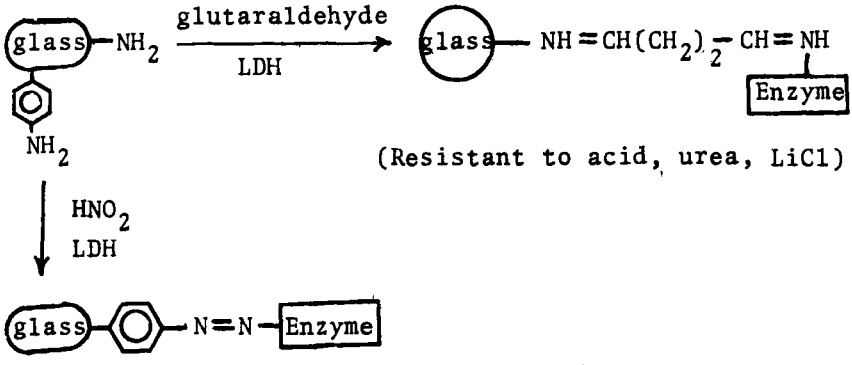
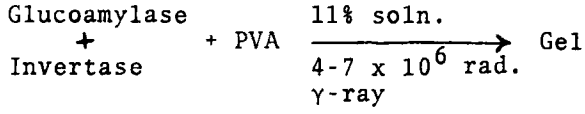
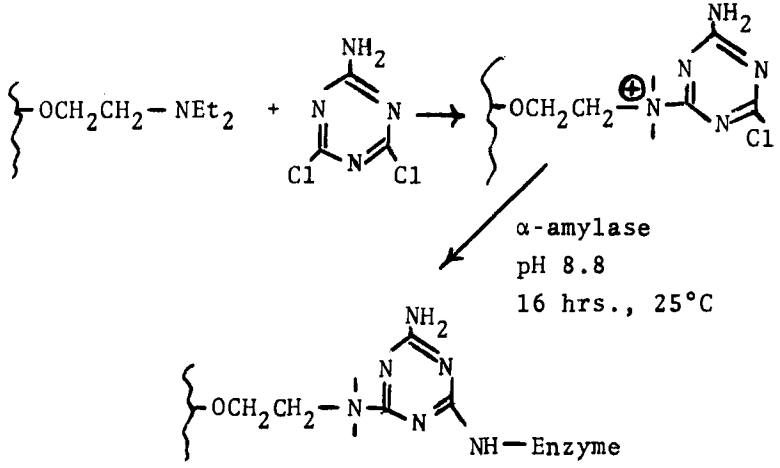


CHART XVII. Entrapment in radiation cross-linked PVA. [H. Maeda, H. Suzuki, and A. Yamauchi, Biotechnol. Bioeng., 15, 607 (1973).]



- 9-24% retention of enzyme activity
- no enzyme leakage at higher crosslinking

CHART XVIII. Soluble support materials. [J. R. Wykes, P. Dunhill, and M. D. Lilly, Biochim. Biophys. Acta, 250, 522 (1971).]



- Protein Content: 0.6-6.1%
- Activity Retention: 25-67%
- Increased Stability at 70°C: 68% ret. after 70 hrs.
- Separation from products by ultracentrifugation.

REFERENCES

[1] O. Zaborsky, Immobilized Enzymes, Chemical Rubber Co., Cleveland, 1973.

[2] I. H. Silman and E. Katchalski, "Water Insoluble Derivatives of Enzymes, Antigens, and Antibodies," Ann. Rev. Biochem., 35, 873 (1966).

[3] G. J. H. Melrose, "Insolubilized Enzymes: Biochemical Applications of Synthetic Polymers," Rev. Pure Appl. Chem., 21, 83 (1971).

[4] G. R. Stark, Biochemical Aspects of Reactions on Solid Supporters, Academic, New York, 1971.

[5] R. D. Falb, "Immobilized Enzymes: An Overview," in Enzyme Engineering, (L. Wingard, ed.), Wiley-Interscience, New York, 1972, p. 177.

[6] R. Goldman, L. Goldstein, and E. Katchalski, "Water-Insoluble Enzyme Derivatives and Artificial Enzyme Membranes," in Biochemical Aspects of Reaction Solid Supports (G. R. Stark, ed.), Academic, New York, 1971, pp. 1-78.

[7] K. Mosbach, "Enzymes and General Biology. Special Reference to Enzyme Synthesis, Purification, and Reactions on Matrixes," J. Dent. Res., 51(2)(Pt.1), 217-223 (1972).

[8] S. J. Updike, "Genetic Engineering, Enzyme Immobilization, and Transplantation," Am. J. Pharm. Educ., 36(5), 718-722 (1972).

[9] A. Wiseman, "Industrial Enzyme Stabilization," Process Biochem., 8(8), 14-15 (1973).

[10] G. P. Royer, P. Andrews, and R. Uy, "Support Materials for Immobilized Enzymes," Enzyme Technol. Dig., 1(3), 99-138 (1973).

[11] M. S. Chang, "Immobilization of Enzymes, Adsorbents, or Both within Semipermeable Microcapsules (Artificial Cells) for Clinical and Experimental Treatment of Metabolite-Related Disorders," Birth Defects, Orig. Artic, Ser., 9(2), 66-76 (1973).

[12] F. Wold, "Chemical Modification of Enzymes," Ibid., 9(2), 46-54 (1973).

[13] H. H. Weetall, "Enzymes Linked by Covalent Bonds to Carriers. Preparation, Properties, and Possibilities of Use," CZ-Chem.-Tech., 1(11), 501-507 (1972).

[14] H. H. Weetall, "Immobilized Enzymes. Applications to Foods and Beverages," Food Prod. Dev., 7(3), 46, 49-50, 52 (1973); 7(4), 94, 96, 98, 100 (1973).

[15] W. Cooreman, A. Lauwers, and S. Scharpe, "Solid-Phase Enzymology," Farm. Tijdschr Belg., 50(3), 170-193 (1973).

[16] W. R. Vieth and K. Venkatasubramanian, "Enzyme Engineering. II. Materials for Immobilized Enzyme Reactions," Chem. Technol., 4(1), 47-55 (1974).

[17] G. Broun, D. Thomas, and M. C. Tran, "Perspectives Offered by the Incorporation of Enzymes into Polymer-Based Membranes," Actual. Nephrol. Hop. Necker, 1972, 113-118.

[18] H. H. Weetall, "Immobilized Enzyme and Its Use in Food and Beverage Industry," Chem. Ztg., 97(11), 611-619 (1973).

[19] D. L. Marshall, "ATP Regeneration Using Immobilized Carbamyl Phosphatase," Biotechnol. Bioeng., 15, 447 (1973).

[20] D. L. Marshall, "Polysaccharide Synthesis on Immobilized Phosphorylase," Carbohydr. Res., 25, 489 (1972).

[21] P. Custrecasas, "Interaction of Insulin with the Cell Membrane: The Primary Action of Insulin," Proc. Natl. Acad. Sci., U.S.A., 63, 450 (1969).

BIBLIOGRAPHY

COVALENTLY BOUND PROTEINS

Chan, W. W. C., "Protein Subunits. V. Specific Interaction Between Matrix-Bound Subunits of Aldolase and Soluble Aldolase Subunits," Can. J. Biochem., 51(9), 1240-1247 (1973).

Cherkasov, I. A., and N. A. Kravchenko, "Structurally Directed Addition of Lysozyme to a Polyacrylamide Support," Izv. Akad. Nauk SSR, Ser. Khim., 1972(10), 2374 (in Russian).

Coughlan, M., and D. B. Johnson, "Preparation and Properties of Immobilized Xanthine Oxidase," Biochim. Biophys. Acta, 320(2), 200-204 (1973).

Flemming, Ch., A. Gabert, and P. Roth, "Synthesis and Properties of Carrier Fixed Enzymes. II. Covalent Coupling of Trypsin to Dialdehyde Epidex, Dialdehyde Sepharose, and 3-Chloro-2-Hydroxypropyl Epidex," Acta Biol. Med. Ger., 31(3), 365-373 (1973).

Forgione, P. S., and R. A. Polistina, "Water-Insoluble Carrier Bound Enzymes," U.S. Patent 3,766,013 (October 16, 1973).

Goldstein, L., "Immobilized Enzymes. Coupling of Biologically Active Proteins to Ethylene-Maleic Anhydride Copolymers of Different Hydride Content," Anal. Biochem., 50(1), 40-46 (1972).

Goldstein, L., "Immobilized Enzymes. Synthesis of a New Type of Polyanionic and Polycationic Resins and Their Utilization for the Preparation of Water-Insoluble Enzyme Derivatives," Biochim. Biophys. Acta, 315(1), 1-17 (1973).

Goldstein, L., "New Polyamine Carrier for the Immobilization of Proteins. Water-Insoluble Derivatives of Pepsin and Trypsin," Ibid., 327(1), 132-137 (1973).

Gray, C. J., C. M. Livingstone, C. M. Jones, and S. A. Barker, "New and Convenient Method of Enzyme Insolubilization Using Diazotized m-Diaminobenzene," Ibid., 341(2), 457-464 (1974).

Katchalski, E., L. Goldstein, Y. Levin, and S. Blumberg, "Water-Insoluble Enzyme Derivatives," U.S. Patent 3,706,633 (December 19, 1972).

Levin, Y., S. Blumberg, E. Katchalski, and L. Goldstein, "Insoluble Polymer-Enzyme Products," U.S. Patent 3,650,900 (March 21, 1972).

Manecke, G., "Immobilization of Enzymes by Various Synthetic Polymers," Biotechnol. Bioeng. Symp., 3, 185-187 (1972).

Mehltretter, C. L., and F. B. Weakley, "Active Water-Insoluble Enzymes," U.S. Patent 3,745,088 (July 10, 1973).

Valaris, M., and W. J. Harper, "Effect of Carboxymethylcellulose on the Proteolysis of Alpha$_{S}$-Casein by Immobilized Trypsin," J. Food Sci., 38(3), 481-483 (1973).

Vretbald, P., and R. Axen, "Use of Isocyanides for the Immobilization of Biological Molecules," Acta Chem. Scand., 27(8) 2769-2780 (1973).

Weakley, F. B., and C. L. Mehltretter, "Binding of Papain to Dialdehyde Starch," Biotechnol. Bioeng., 15(6), 1189-1192 (1973).

TRAPPED OR SORBED PROTEINS

Broun, G., D. Thomas, G. Gellf, D. Domurado, A. M. Berjonneau, and G. Guillon, "New Methods for Binding Enzyme Molecules Into a Water-Insoluble Matrix. Properties After Insolubilization," Biotechnol. Bioeng., 15(2), 359-375 (1973).

Chibata, I., T. Tosa, and T. Mori, "Insoluble Enzyme Preparations in Polyacrylamide Gel and Microcapsules thus Formed," Fr. Demande 2,157,982 (July 13, 1973).

Hofstee, B. H. J., and N. F. Otillio, "Immobilization of Enzymes through Noncovalent Binding to Substituted Agaroses," Biochem. Biophys. Res. Commun., 53(4), 1137-1144 (1973).

Johansson, A., "Matrix-Bound Enzymes Studied by Flow Microcalorimetry," Protides Biol. Fluids, Proc. Colloq., 20, 567-570 (1972).

Kennedy, J. F., and C. E. Doyle, "Active, Water-Insoluble Derivatives of D-Glucose Oxidase and Alginic Acid, Chitin, and Celite," Carbohydr. Res., 28(1), 89-92 (1973).

Kosugi, Y., and H. Suzuki, "Fixation of Cell-Bound Lipase and Properties of the Fixed Lipase as an Immobilized Enzyme," Hakko Kagaku Zasshi, 51(12), 895-903 (1973).

Park, Y. K., "Enzymic Properties of a Fungal Amyloglucosidase-Resin Complex," Ibid., 52(2), 140-142 (1974).

WATER-SOLUBLE ENZYME GRAFTS

Hixson, H. F., "Water-Soluble Enzyme-Polymer Grafts. Thermal Stabilization of Glucose Oxidase," Biotechnol. Bioeng., 15(5), 1011-1016 (1973).

ENZYME MEMBRANES

Research Corp., "Protein-Enzyme Membranes," Fr. Demande 2,165,832 (September 14, 1973).

Vieth, W. R., S. S. Wang, S. G. Gilbert, and R. Saini, "Immobilized Enzyme-Membrane Complexes by Electrocodeposition," U.S. Patent 3,758,396 (September 11, 1973).

PREPARATIONS USING IRRADIATION

Maeda, H., H. Suzuki, and A. Yamauchi, "Preparation of Immobilized Enzymes by Electron-Beam Irradiation," Biotechnol. Bioeng., 15(4), 827-829 (1973).

Maeda, H., H. Suzuki, and A. Yamauchi, "Preparation of Immobilized Enzymes using Poly(vinyl Alcohol) (with γ-Ray Irradiation)," Ibid., 15(3), 607-610 (1973)

Maeda, H., A. Yamauchi, and H. Suzuki, "Preparation of Immobilized Enzymes γ-Ray Irradiation," Biochim. Biophys. Acta, 315(1), 18-21 (1973).

Walton, H. M., and J. E. Eastman, "Insolubilized Amylases (Using Photopolymerization)," Biotechnol. Bioeng., 15(5) 951-962 (1973).

IMMOBILIZATION ON GLASS

DeJong, P. J., and P. L. Kumler, "Preparation of Immobilized Enzymes, and Determination of Their pH Activity Profile," Integrated Organic-Biology Experiment," J. Chem. Educ., 51(3), 200-201 (1974).

Herring, W. M., R. L. Laurence, and J. R. Kittrell, "Immobilization of Glucose Oxidase on Nickel-Silica Alumina," Biotechnol. Bioeng., 14(6), 975-984 (1972).

Messing, R. A., "Immobilized Papain," Ger. Offen. 2,339,805 (February 14, 1974).

Messing, R. A., and H. H. Weetall, "Silane Coupler for Stabilized Enzyme Preparations," Ger. Offen. 2,036,499 (February 3, 1972).

Monsan, P., and G. Durand, "Enzymes Immobilized by Binding on Supports," French Patent 2,133,370 (December 29, 1972).

Royer, G. P., and J. P. Andrews, "Immobilized Leucine Aminopeptidase Applications in Protein Chemistry," J. Macromol. Sci.-Chem., 7(5), 1167-1179 (1973).

Weetall, H. H., N. D. Havewala, W. H. Pitcher, Jr., C. C. Detar, W. P. Vann, P. Williams, and S. Yaverbaum, "Preparation of Immobilized Lactase and Its Use in the Enzymic Hydrolysis of Acid Whey," Biotechnol. Bioeng., 16(3), 295-313 (1974).

PROPERTIES OF IMMOBILIZED PROTEINS

Brockman, H. L., J. H. Lwa, and F. J. Kezdy, "Catalysis by Adsorbed Enzymes. Hydrolysis of Tripropionin by Pancreatic Lipase Adsorbed to Siliconized Glass Beads," J. Biol. Chem., 248(14), 4965-4970 (1973).

Ford, J. R., R. P. Chambers, and W. Cohen, "Active-Site Titration Method for Immobilized Trypsin," Biochim. Biophys. Acta, 309(1), 175-180 (1973).

Gabel, D., and J. Porath, "Molecular Properties of Immobilized Proteins," Biochem. J., 127(2), 13P-14P (1972).

Gestrelius, S., B. Mattiasson, and K. Mosbach, "Regulation of the Activity of Immobilized Enzymes. Microenvironmental Effects of Enzyme-Generated pH Changes," Eur. J. Biochem., 36(1), 89-96 (1973).

Henry, S., J. Koczan, and T. Richardson, "Bactericidal Effectiveness of Immobilized Peroxidases," Biotechnol. Bioeng., 16(2), 289-291 (1974).

Horigone, T., H. Kasai, and T. Okuyama, "Stability of Taka-amylase A Immobilized on Various Sizes of Matrix," J. Biochem. (Tokyo), 75(2), 299-307 (1974).

Mort, J. S., D. K. K. Chong, and W. W. C. Chan, "Continuous Spectrophotometric Assay of a Sepharose-Bound Enzyme and Its Use to Study Kinetics of Coupling of the Enzyme to Sepharose," Anal. Biochem., 52(1), 162-168 (1973).

Ogiso, T., M. Sugiura, and Y. Kato, "Enzymes, LXX. Bile-sensitive Lipase. X. Preparation and Properties of Carrier-Bound Mucor Lipase," Chem. Pharm. Bull., 20(12) 2542-2550 (1972).

Regan, D. L., P. Dunnill, and M. Lilly, "Immobilized Enzyme Reaction Stability. Attrition of the Support Material," Biotechnol. Bioeng., 16(3), 333-343 (1974).

Royer, G. P., R. Uu, "Evidence for the Induction of a Conformational Change of Bovine Trypsin by a Specific Substrate at pH 8.0," J. Biol. Chem., 248(7), 2627-2629 (1973).

Shuler, M. L., H. M. Tsuchiya, and R. Aris, "Diffusive and Electrostatic Effects with Insolubilized Enzymes Subject to Substrate Inhibition," J. Theor. Biol., 41(2), 347-356 (1973).

Srere, P. A., B. Mattiasson, and K. Mosbach, "Immobilized Three-Enzyme System. Model for Microenvironmental Compartmentation in Mitochondria," Proc. Natl. Acad. Sic. U. S. A., 70(9), 2534-2538 (1973).

ANALYTICAL, DIAGNOSTIC, AND ENGINEERING APPLICATIONS

Gellf, G., and J. Boudrant, "Enzymes Immobilized on a Magnetic Support. Preliminary Study of a Fluidized Bed Enzyme Reaction," Biochim. Biophys. Acta, 334(2), 467-470 (1974).

Gholson, R. K., and P. E. Guire, "Enzymic Removal of Oil Slicks," U.S. Natl. Tech. Inform. Serv., AD Rep. 757071 (1973), 14 pp.

Hinberg, I., A. Kapoulas, R. Korus, and K. O'Driscoll, "Gel Entrapment of Enzymes. Kinetic Studies of Immobilized Glucose Oxidase," Biotechnol. Bioeng., 16(2), 159-168 (1974).

Inman, D. J., and W. E. Hornby, "Immobilization of Enzymes on Nylon Structures and Their Use in Automated Analysis," Biochem. J., 129(2), 255-262 (1972).

Inman, D. J., and W. E. Hornby, Preparation of Immobilized Linked Enzyme Systems and Their Use in the Automated Determination of Disaccharides," Ibid., 137(1), 25-32 (1973).

Lin, S. H., "Nonisothermal Immobilized Enzyme Reaction in a Packed-Bed Reactor," Biophysik, 8(4), 302-309 (1972).

Nachtigal, J., "Stabilized, Enzyme-Containing Dentifrice Paste," Fr. Demande 2,156,149 (June 29, 1973).

Rosalki, S. B., and D. Tarlow, "Amylase Determination Using Insoluble Substrates," Ann. Clin. Biochem., 10(2), 47-52 (1973).

Veith, W. R., S. S. Wang, and R. Saini, "Immobilization of Whole Cells in a Membraneous Form," Biotechnol. Bioeng., 15(3), 565-569 (1973).

Wang, S. S., and W. R. Vieth, "Collagen-Enzyme Complex Membranes and Their Performance in Biocatalytic Modules," Ibid., 15(1), 93-115 (1973).

Weetall, H. H., N. B. Havewala, H. M. Garfinkel, W. M. Buehl, and G. Baum, "Covalent Bond between the Enzyme Amyloglucosidase and a Porous Glass Carrier. Effect of Shearing," Ibid., 16(2), 169-179 (1974).

Weibel, M. K., W. Dritschilo, H. J. Bright, and A. E. Humphrey, "Immobilized Enzymes. Prototype Apparatus for Oxidase Enzymes in Chemical Analysis Utilizing Covalently Boun d Glucose Oxidase," Anal. Biochem., 52(2), 402-414 (1973).