Methods in Enzymology

Volume 137

Immobilized Enzymes and Cells

Part D

EDITED BY

Klaus Mosbach

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Preface

Volumes 135 through 137 of *Methods in Enzymology*, Immobilized Enzymes and Cells, Parts B through D, include the following sections: (1) Immobilization Techniques for Enzymes; (2) Immobilization Techniques for Cells/Organelles; (3) Application of Immobilized Enzymes/Cells to Fundamental Studies; (4) Multistep Enzyme Systems and Coenzymes; (5) Immobilized Enzymes/Cells in Organic Synthesis; (6) Enzyme Engineering (Enzyme Technology); (7) Analytical Applications with Emphasis on Biosensors; (8) Medical Applications; and (9) Novel Techniques for and Aspects of Immobilized Enzymes and Cells. The first three sections appear in Volume 135, the next three in Volume 136, and the last three in Volume 137.

Immobilization techniques for enzymes, Section (1), has already been treated in Volume XLIV of this series. Immobilization techniques for cells/organelles, Section (2), an area which seems to have great potential, especially for the application of immobilized yeast and plant and animal cells, is covered for the first time in these volumes. Sections (3) and (4) have been dealt with previously. Section (5), the use of immobilized enzymes/cells in organic synthesis, has probably not been covered before. It is my firm opinion that in the not too distant future we will see a number of processes employed which are based, in part, on the examples given in this section. Section (6) on industrial uses updates the material presented in Volume XLIV. The examples given are, to the best of my knowledge, in operational use today or, at least, on a pilot plant level. Section (7), analytical applications with emphasis on biosensors, is the subject of a great deal of research at present, and it may very well be that in the not too distant future we will witness a breakthrough, i.e., many applications of a number of such devices. The medical area, covered in Section (8), seems promising, but certainly more research is required to fully exploit any underlying potential. Finally, in Section (9), I have collected a number of contributions that did not seem to fit in any of the other sections, but do address important and novel developments.

I would like to note that although major emphasis in these volumes has been placed on immobilization in its strictest sense, preferentially, covalent attachment of enzymes or entrapment of cells, one should not view immobilized systems in too limited a manner. In fact, bioreactors confined by ultrafilter membranes or hollow fiber systems belong in this category, and the various systems appear to overlap. Immobilization techniques as applied to affinity chromatography or immunoassays such as ELISA are not included to any extent in these volumes since they have

been adequately covered in other volumes of this series (e.g., Volumes XXXIV and 104 on affinity techniques).

An area that was originally scheduled for inclusion is synzymes or artificial enzymes. These include attempts to create catalysts mimicking enzymes by coupling of functional groups to, for instance, cyclodextrin [e.g., D'Souza *et al.* (*Biochem. Biophys. Res. Commun.* **129**, 727–732, 1985) and Breslow *et al.* (*J. Am. Chem. Soc.* **108**, 1969–1986)], to crown ethers [Cram *et al.* (*J. Am. Chem. Soc.* **107**, 3645, 1985)], or to solid matrices [Nilsson and Mosbach (*J. Solid Phase Biochem.* **4**, 271, 1979) and Leonhardt and Mosbach (*Reactive Polymers*, in press)].

Related to these studies are attempts to create cavities in polymers with substrate-binding properties [notably by Wulff *et al.* (e.g., *Reactive Polymers* **3**, 261, 1985; and previous publications by these authors) and Arshady and Mosbach (*Makromol. Chem.* **182**, 687, 1981)]. This exciting area is presently in a rapid state of development, and the methodology involved should soon be made available in a more comprehensive context.

Mention should be made of the developments in the utilization of recombinant DNA technology for the immobilization (and affinity purification) of biomolecules. I refer to the reported fusion of "affinity tails" as polyarginine (Smith *et al.*, *Gene* **32**, 321, 1984), of polycysteine [Bülow and Mosbach, Proceedings of the VIII International Conference on Enzyme Engineering, *Annals of the New York Academy of Sciences*, in press (presented 1985)], or of protein A (Nilsson *et al.*, *EMBO J.* **4**, 1075, 1985) to enzymes facilitating their purification and immobilization. These preparations can be obtained by fusion of the respective groups as "tail" to the NH_2 or COOH termini of the enzyme or by site-directed mutagenesis leading to substitution on the enzyme structure. DNA technology can also be usefully employed to create new multienzyme complexes, fusing enzymes acting in sequence to one another (Bülow *et al.*, *Bio/Technology* **3**, 821, 1985) as an alternative to their co-immobilization on supports; similarly, attachment of "tails" allowing reversible coenzyme binding may be accomplished. The same technology has also been used recently in attempts to prepare esterase mimics from the ground up (Bülow and Mosbach, *FEBS Lett.* **210**, 147, 1987).

Since this is such a rapidly moving area, I advise the reader, apart from the usual standard books in this area, to read the proceedings of the Enzyme Engineering Conferences 1–8 (Wiley, first conference; Plenum Press, second–sixth conferences; and Annals of the New York Academy of Sciences, seventh and eighth conferences); Biochemical Engineering, Volumes I–III and subsequent volumes; Annals of the New York Academy of Sciences, 1983; the patent book "Enzyme Technology, Recent Advances" (S. Torrey, ed.), Noyes Data Corporation, Park Ridge, New

Jersey, 1983; and *Biotechnology Review* no. 2. In addition, in the following journals many articles relating to immobilized enzyme and cell research can be found: *Biotechnology and Bioengineering* (John Wiley & Sons); *Trends in Biotechnology* (Elsevier, The Netherlands); *Bio/Technology* (Nature Publishing Co., U.S.); *Applied Biochemistry and Biotechnology* (The Humana Press, Inc., U.S.); *Applied Biochemistry with Special Emphasis on Biotechnology*; *Biotechnology Letters* (Science and Technology Letters, England); *Applied Microbiology and Biotechnology* (Springer-Verlag, Germany); *Enzyme and Microbial Technology* (Butterworth Scientific Limited, England); *Biosensors* (Elsevier Applied Science Publishing Ltd., England).

In studies with immobilized systems, sometimes useful, not immediately obvious "by-products" may be obtained. I refer to the finding that immobilized *Escherichia coli* cells, when kept in media without selection pressure, show improved plasmid stability (de Taxis du Poët, P., Dhulster, P., Barbotin, J.-N., and Thomas, D., *J. Bact.* **165,** 871, 1986). An additional example would be the improved regeneration of plants using immobilized protoplasts discussed in Section (2).

I would like to express the hope that these volumes present an overview of the various areas in which immobilized enzymes and cells are used, act as a stimulus for further research, and provide methodological "know-how." The proper choice of support and/or immobilization technique for a particular application may not always be easily accomplished, but I hope that guidance to do so is found in these volumes.

Putting these volumes together has been a time-consuming and, at times, frustrating undertaking. Without the coeditors, Drs. Lars Andersson, Peter Brodelius, Bengt Danielsson, Stina Gestrelius, and Mats-Olle Månsson, the volumes would not have materialized. Because of the number of coeditors, some heterogeneity in the editing has resulted. Contributors to the various sections are from substantially different disciplines, and again this has contributed to the heterogeneity that can be found. Part of the editing of the three volumes was carried out in Zürich, where I held a chair in biotechnology at the Swiss Federal Institute of Technology. Without the enormous efforts and skills of the staff of Academic Press, these volumes would never have reached production. I also owe much gratitude to my secretaries, notably Ingrid Nilsson, for their highly qualified help. Finally, I would like to thank the contributors for their efforts.

These volumes are dedicated to the memory of the late Professors N. O. Kaplan and S. P. Colowick, with whom I had highly fruitful discussions, especially at the beginning of this undertaking.

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METHODS IN ENZYMOLOGY

EDITED BY

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