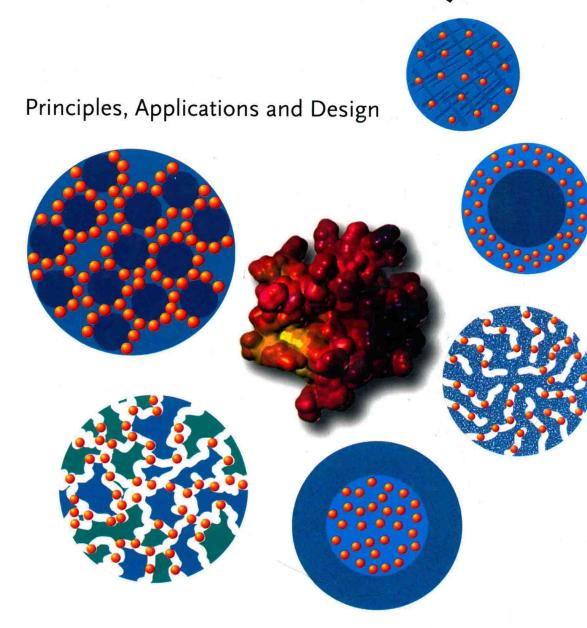
Carrier-bound Immobilized Enzymes



Linqiu Cao

Carrier-bound Immobilized Enzymes

Principles, Applications and Design



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Foreword

Enzymes are the biocatalysts of the living cell. Their excellent performance in cellular metabolism is due to their intrinsic catalytic properties. In addition, their activity may be further enhanced by functioning in distinct cellular compartments, e.g., within or attached to cellular membranes, or as multifunctional enzyme complexes such as a cellulose-degrading cellulosome.

Enzyme technologists have reinvented such natural forms of "enzyme immobilization". They have looked for their own bionic solutions to arrive at immobilized biocatalysts which can be used in analytical devices such as a glucose biosensor or in an industrial plant producing, e. g., chiral amines from racemic precursor material. These initial steps towards a benign "green technology" are presently gaining momentum. In fact, the emerging concept of "white biotechnology" (sustainable chemical processes built on renewable resources and biocatalysts, carried out in "biorefineries") builds not only on fermentation using metabolically engineered microorganisms, but as much on enzymes improved by protein engineering techniques which are used, attached to carrier material, as heterogeneous catalysts in an enzyme reactor. Quite often, the skill to stabilize and re-use an enzyme catalyst through immobilization has proven one of the key steps to render an enzymatic process economically viable.

With this book on "Carrier-bound immobilized enzymes", Linqiu Cao provides a comprehensive survey of this important field, covering both the history and the present state of immobilization procedures used in enzyme technology. After a short introduction to 100 years of enzyme immobilization, he discusses in great detail not only the methods by which enzymes can be adsorbed, covalently bound or entrapped, but also the laws governing their behaviour in these artificial environments. In the concluding chapter of his book, he also adds an authoritative survey of most recent developments such as enzyme immobilization using genetically engineered attachment points, artificial tags or enzymes whose properties have been changed through reversible binding to synthetic polymers. He thus provides both the industrial enzymologist and the researcher in an academic environment with a well-structured, easily accessible choice of options and protocols to solve their individual needs.

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My compliments go to the author for the thorough collection and structuring of a plethora of data, and my wishes to the readers for continuing success in their research on and application of immobilized biocatalysts.

Stuttgart, May 2005

Rolf D. Schmid

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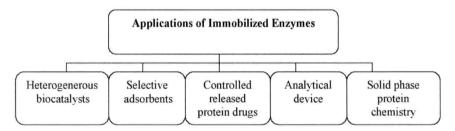
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1 Introduction: Immobilized Enzymes: Past, Present and Prospects

1.1 Introduction

Since the second half of the last century, numerous efforts have been devoted to the development of insoluble immobilized enzymes for a variety of applications [2]; these applications can clearly benefit from use of the immobilized enzymes rather than the soluble counterparts, for instance as reusable heterogeneous biocatalysts, with the aim of reducing production costs by efficient recycling and control of the process [3, 4], as stable and reusable devices for analytical and medical applications [5–11], as selective adsorbents for purification of proteins and enzymes [12], as fundamental tools for solid-phase protein chemistry [13, 14] and as effective microdevices for controlled release of protein drugs [15] (Scheme 1.1).



Scheme 1.1 Range of application of immobilized enzymes.

However, whatever the nature of an immobilized enzyme and no matter how it is prepared, any immobilized enzyme, by definition, must comprise two essential functions, namely the non-catalytic functions (NCF) that are designed to aid separation (e.g. isolation of catalysts from the application environment, reuse of the catalysts and control of the process) and the catalytic functions (CF) that are designed to convert the target compounds (or substrates) within the time and space desired (Scheme 1.2).

NCF are strongly connected with the physical and chemical nature of the noncatalytic part of the immobilized enzymes, especially the geometric properties, e.g. the shape, size, thickness, and length of the selected carrier, whereas the CF are linked to the catalytic properties, for example activity, selectivity, and stability, pH

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