Enzyme Chemistry

Impact and applications

SECOND EDITION

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Edited by
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Preface

As the first edition of this book was going through the publication process, a revolution was taking place in the technologies available for the study of enzymes. The techniques of molecular biology, especially in genetic engineering of organisms and in site specific mutagenesis of genes, were established and were being brought into use to solve many problems in fundamental and applied science, not least in enzymology. Added to these advances the possibility of generating catalysts from antibodies has become a topic of major interest. These major innovations have changed the emphasis of much bioorganic research; whereas in the past, the protein was often the 'sleeping partner' in a study, its detailed function is now the major focus of scientific interest. Similarly in industry, the potential of genetically manipulated organisms to satisfy the needs for the production of chemicals and foodstuffs has been widely recognised. The second edition of 'Enzyme Chemistry, Impact and Applications' takes on board these new developments whilst maintaining the overall aims and views of the first edition. Many of the chapters have been completely rewritten to take account of advances in the last five years especially with regard to the impact of biologically based technologies. Although the book continues to approach its subject matter from the point of view of the chemist, the increased interdisciplinary content of much modern science will be obvious from the discussion. The scope of the book has also been extended to include further discussion of areas of industrial significance in relation to chemical synthesis

and, through a new chapter, the food industry. This chapter has a significantly greater applied scientific bias than many of the others and, because of the macromolecular nature of the substrates involved, is less specific with regard to detailed structural organic chemistry. Such an extension is nevertheless necessary to give an adequate view of the breadth of impact of enzyme chemistry today. For those chemists unfamiliar with the new biological technologies and their associated jargon, an overview is provided through the discussion in the final chapter. I hope that readers will continue to find the articles in this book helpful to their appreciation of an important field of chemical science whether they are seeking an introduction to a topic or are reflecting from a position of experience.

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1 Infant enzyme chemistry

Colin J. Suckling

When this book was first planned, the idea in mind was to review, through a series of personal but related essays, the major impact that the study of enzymes has had upon some important fields of chemistry in the last thirty years. It was therefore something of a surprise to discover in the nineteenth century literature that enzymes had already prompted a great deal of chemical research, some of it with a remarkably modern ring, as I shall try to show in the next few pages. As early as 1833 observations had been made of the phenomenon of the natural hydrolysis of potato starch but with vitalistic concepts still much in people's minds, it was difficult to accept the existence of biological catalysts. The idea that enzymes are chemicals provoked prolonged scepticism and controversy. During the first half of the nineteenth century further naturally occurring reactions were recognized, in particular fermentations involving yeasts. On the one hand, it was held that the enzymic activity responsible for these fermentations was a property inseparable from living cells. Pasteur, amongst others, took this view. On the other hand, Liebig and, not surprisingly, Wöhler, regarded enzymes as chemical catalysts, albeit of unknown constitution, that could be separated from cells. Indeed these two may well have conspired to lampoon vitalism in an anonymous paper in Liebig's *Annalen der Pharmacie* (Anon., 1839). In this amusing article we read of chemical reactions brought about by

> 'small animals which hatch from eggs (yeast) in sugary solution and which on microscopic examination are seen to take the form of a Beindorf

distillation apparatus, without the condenser . . . these animals, which have neither teeth nor eyes, but possess a stomach, a bladder which, when full, looks like a champagne bottle . . . devour the sugar with the production of excrement as alcohol and carbon dioxide.'

Eventually the argument was settled by experiment. In 1897, Buchner demonstrated that a yeast extract was capable of sustaining the fermentation of sugar but a few years earlier, a remarkable series of contributions began to appear from the laboratory of Emil Fischer (1894). The papers make enthralling reading, not only for their scientific content, but also because they convey great enthusiasm, sometimes naïve, but always evident. The main subject to which Fischer addressed his powerful experimental skills and penetrating intellect was stereoselectivity in enzymic catalysis, a field still of current significance; the ability of enzymes to select between stereoisomers has proved one of their most alluring properties.

Fischer's paper (1894) is remarkable for its discoveries themselves and also for the insight of a man of genius into future developments. He was, of course, uniquely well placed to tackle the problem of stereoselectivity because he had available an extensive series of stereoisomeric sugars which he had synthesized to determine their configurations. Derivatives of these compounds served as substrates for glycosidases which even in those days were available in crude cell-free form. His paper begins

'The different properties of the stereoisomeric hexoses with respect to yeast led Thierfelder and I to the hypothesis that the active chemical agent of yeast cells can only attack those sugars to which it possesses a related configuration.'

The hypothesis was supported by demonstrating, amongst other things, that the enzyme that hydrolyses sucrose, called 'invertin' by Fischer, acts only upon α-D-glucosides: β-D-glucosides and L-glucosides were completely untouched. There was no doubt that this was not just a chance phenomenon because a second enzyme, emulsin, was found to hydrolyse β-D-glucosides of both synthetic and natural origin. The complementary nature of these results is conclusive and, of course, still important in modern stereochemical studies. Fischer's assessment of his results is fascinating reading. It also makes an admirable preface to this book because it foreshadows much of what follows. When you have read further, you may be interested to reflect upon these lines:

'But the results suffice in principle to show that enzymes are choosy with respect to the configuration of their substrate, like yeast and other micro-organisms. The analogy between both phenomena appears so complete in this respect that one may assume the same origin for them,

and accordingly, I return to the abovementioned hypothesis of Thierfelder and myself. Invertin and emulsin have many perceptible similarities and consist doubtless of an asymmetrically built molecule . . . To use an image, I would say that the enzyme and glucoside must fit each other like a lock and key to be able to exert a chemical influence upon each other . . . The facts proven for the complex enzymes will soon also be found with simpler asymmetric agents. I scarcely doubt that enzymes will be of use for the determination of configuration of asymmetric substances . . . The earlier much accepted distinction between the chemical ability of living cells and the action of chemical agents with regard to molecular asymmetry does not in fact exist.'

Although the last sentence quoted was directed to his contemporaries, much of the preceding extract reads remarkably freshly to modern chemists nearly a century later. We have the advantage over Fischer in techniques, but some of the concepts that he advanced have still to be realized in perfection as we shall see. However, Fischer was by no means the only scientific prophet in the field of stereochemistry and his work depended much upon the understanding developed by Pasteur. There is little in modern stereochemical research that does not derive something from the experimental and conceptual contribution of these two great scientists (see Robinson, 1974).

It is remarkable how much was achieved in Fischer's time with impure enzyme preparations. A parallel in today's research might be the study of preparations containing unpurified neurotransmitter or hormone receptors, although these too are now amenable to purification by modern chromatographic techniques. As Fischer predicted, enzymes have become widely used for the determination of configuration but it is only in recent years that 'simpler asymmetric agents' have been able to reproduce enzymic stereoselectivity (see Chapters 3 and 4). Not surprisingly, the ever enthusiastic Fischer even had a go at asymmetric synthesis himself (Fischer and Slimmer, 1903, and see Fig. 1.1). Knowing that glucose is chiral, Fischer hoped that the naturally occurring glycoside, helicin, would undergo asymmetric addition at the carbonyl group guided in some way by the asymmetric environment created by the glucose ring. This strategy has since proved successful (Chapter 4) and had Fischer used a more bulky nucleophile, he too might have been successful. His first attempt was to add hydrogen cyanide to helicin and to hydrolyse the product carefully. An optically inactive product resulted. So Fischer tried again using diethyl zinc and this time the product obtained from vacuum distillation was optically active. In the exhilaration of discovery he wrote '. . . with this we thus believed that we had solved the problem of asymmetric synthesis.'! Then came the snag, Gilbertian 'modified rapture'. Rigorous control experiments clearly showed

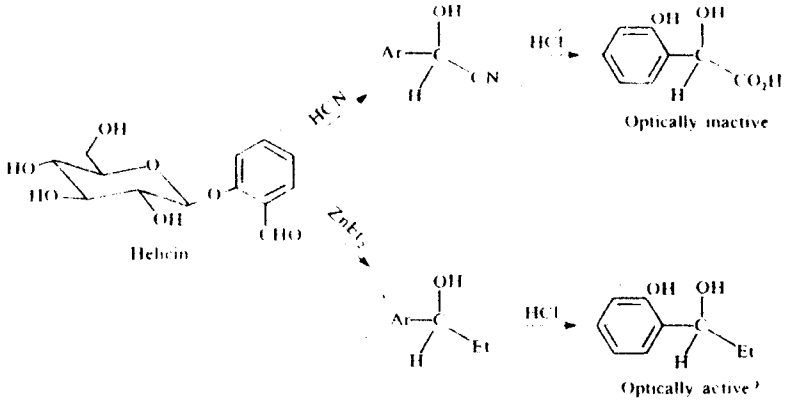

Fig. 1.1

that the apparent asymmetric induction was due to an impurity derived from glucose during distillation and no further attempts were reported. Many people have had similar, but unpublished, experiences.

The turn of the century also marked the first steps in the synthetic use of enzymes. Croft-Hill (1898) demonstrated that yeast enzymes could be used synthetically and Emmerling (1900) reported a synthesis of the glycoside amygdalin using enzymes. These, and other pioneering contributions, are cited by Hoesch (1921) in a special edition of *Berichte* devoted entirely to a biography of Fischer. Many of Hoesch's comments are equally apt today more than sixty years later. For instance, in summarizing Fischer's contribution to enzyme chemistry, Hoesch remarks 'Pure chemists may certainly not feel at home with the enzymatic studies of Emil Fischer'. Another notable comment was that Fischer's lock and key metaphor describing enzymic specificity was much appreciated in his day. From Hoesch's review and Fischer's own writings, it seems possible that Fischer never intended this image to be a scientific hypothesis but used it to illuminate the concept of stereochemical biospecificity to an audience totally unfamiliar with the new idea. Modern work, of course, makes it clear that the physical rigidity of a lock and key do not make an appropriate description of a conformationally mobile enzyme–substrate interaction. Once he had demonstrated bio-specificity with enzymes, similar complementary interactions were enthusiastically discussed for the behaviour of other biosystems such as toxins. However naïve the metaphor, it was certainly seminal.

Yet another part of our story began in the 1890s. Scientists were not only

studying microbial enzymes but mammalian systems were also beginning to be investigated. In 1898, the kidney was shown to contain proteolytic activity (Tigerstedt and Bergmann, 1898). It was further demonstrated that an enzyme named renin hydrolyses a large plasma peptide, which today we know as angiotensinogen, to angiotensin I. We now know that angiotensin I has very little activity in the central or peripheral nervous system; it is further hydrolysed to a smaller peptide, angiotensin II, by an enzyme known as angiotensin converting enzyme. Angiotensin II has powerful effects on the circulatory system and studies of inhibitors of this enzyme have recently developed into one of the classics of modern drug invention (see Chapter 5).

Although much current work was foreshadowed or even initiated at the turn of the century, yet from that time, chemists' contact with enzymes became more remote as for the next five decades, chemists, with some notable exceptions, pursued the systematic study of the reactivity of organic compounds. Sir Robert Robinson was one such exception. Whilst contributing greatly to natural product chemistry and of course to ideas concerning reactivity, he realized that enzymes catalyse reactions under very mild conditions and sought laboratoy analogues in syntheses of alkaloids (Robinson, 1917). Meanwhile, biochemists wanted to find out in detail what enzymes are and set about their purification. The first systematic attempts were begun by Willstaetter in the 1920s but the first substantive success came from Sumner who in 1926 reported the crystallization of urease. Perhaps because he couldn't believe that someone else had done it first, Willstaetter disputed that Sumner actually had an enzyme. Nevertheless, proteolytic enzymes were soon purified to crystallinity and it became clear that enzymes are, as Fischer had surmised, proteins.

Although purified enzymes were available from that time on, chemists were by no means ready to accept the idea of macromolecules, let alone macromolecular catalysts. Staudinger, one of the fathers of polymer chemistry, had great difficulty in persuading the Swiss Chemical Society, at a meeting which ended in uncharacteristic Swiss uproar, that macromolecules can exist. A similar scepticism greeted the ideas of a young physical chemist, McBain, concerning the nature of micelles at a meeting of The Royal Society in London. He was told that his notions of molecular aggregation were 'nonsense'. In Germany too Hans Fischer, who established the structures of porphyrins by degradation and synthesis, as late as 1937 appeared to be unaware of the wide physiological importance of porphyrins although the isolation of the porphyrin-containing proteins, cytochromes, had been described in the mid 1920s by Keilin. Despite their temporary but acute myopia with regard to enzymes, chemists at this time were making great strides in understanding the basis of mechanistic organic chemistry. In time, the synthesis of artificial polymers was demonstrated and natural macromolecules too became respectable. The conceptual basis for a symbiotic

growth of organic chemistry and enzyme chemistry was founded. This book recounts some of the branches of this growth.

What in particular amongst the properties of enzymes has been most significant for chemistry? In the first place, enzymes are such excellent catalysts. Indeed it has been argued that enzymes have evolved to perfect their catalytic function (see Chapter 2). If this is so, then it is a formidable challenge for chemists to understand the chemical basis for enzymic catalysis and a still greater one to mimic it effectively. However, in addition to these purely scientific aims, there are also extremely important practical consequences of the properties of enzymes. Selectivity in catalysis, as Fischer surmised, is one of the most important and it can be applied in a direct sense to perform both regioselective and stereoselective transformations in organic synthesis (Chapter 4). In addition, selective enzyme inhibitors are immensely important as drugs for the treatment of bacterial and viral diseases (Chapter 5).

In the last twenty years, great strides have been made in our understanding of the chemical basis of enzymic catalysis and it is the application of this and related enzyme chemistry that is developing apace. In the chapters that follow, a team of authors from many different countries and backgrounds discuss enzyme chemistry in relation to two broad themes, firstly synthetic organic chemistry. You will read how the study of the mechanism of action of coenzymes has led to a number of novel synthetic reactions (Chapter 3, Seiji Shinkai). Coenzymes are a good starting point for organic chemists because they are relatively small molecules with some innate catalytic activity even in the absence of enzymes. The wide range of reactivity observable is in itself fascinating and Professor Shinkai reviews much recent work for the first time. How conventional synthetic reactions compare in selectivity with enzyme-catalysed reactions and biomimetic systems is the topic of my own contribution (Chapter 4). The second theme, by way of contrast, concerns more biologically significant topics. The relevance of enzyme chemistry to chemotherapy in organic and inorganic aspects is discussed respectively in Chapters 5 (Philip Edwards, Barrie Hesp, Amy Trainor, and Alvin Willard) and 6 (Donald Brown and Ewen Smith). Biosynthetic studies have always built a bridge between organic chemistry and biochemistry and recent developments in this field are reviewed by David Cane in Chapter 7. A major development since the first edition of this book is the impact of genetic engineering and molecular biological techniques in both chemistry and biology. All fields of study described in this book have felt the benefit of the new technology. The basic concepts and methods are outlined in Chapter 9. Enzyme chemistry has borrowed much from and given much to biochemistry and the book ends with a consideration of future interactions between the disciplines (Chapter 9; Keith Suckling). To provide a basis for these discussions and to demonstrate the

depth of thought into catalysis itself that enzyme properties have provoked Ron Kluger begins the essays with some thoughts on the mechanistic basis of enzyme catalysis (Chapter 2). As the scientific understanding of enzymes has improved, so has the extent of the appreciation of their industrial potential. This is recognized in Chapter 4 (synthesis) and in Chapter 8 which concerns the food industry (David Berry and Alistair Paterson). Interestingly and, to some extent, coincidentally several subject areas are discussed from different points of view by several contributors. These topics include enzyme stereochemistry (Chapters 2–5), prostaglandin chemisty (Chapters 4 and 5), β-lactam antibiotics (Chapters 4, 5 and 7), cyclodextrins (Chapters 2–4), and genetic engineering (Chapters 4, 7 and 8). Further areas of chemistry could also have been selected but these seven essays will give the reader insight into the impact of enzyme chemistry upon laboratory and industrial chemistry and the contacts of chemistry with the life sciences.

Now is the time to let each author speak for himself. In editing this book, I have learned much from the thoughts of my fellow contributors. I am sure that they will convince you too that enzyme chemistry has contributed much to chemistry and is still vibrant and vital. Whilst the subject continues to develop, new challenges for biological chemistry are emerging, challenges that can be met all the better because of what the chemist has learned from enzymes in methods, concepts and techniques. As was alluded to earlier, other proteins can now be purified, in particular antibodies and neurotransmitter and hormone receptors. In ten years' time, perhaps someone will be writing the closing lines of an introduction to the impact of receptor and antibody chemistry.

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