Third edition

ENZYME CHEMISTRY

Impact and applications

Edited by
Colin J. Suckling,
Colin L. Gibson
and Andrew R. Pitt

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Enzyme Chemistry

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Preface

Science advances, and in the case of the subject of this book, very greatly – even since the second edition. However, the original concept still appears strongly valid, if only judged by the pressure from the publishers to produce a third edition. I have been pleased and greatly helped to have the expert assistance of two of my colleagues at Strathclyde, Colin Gibson and Andy Pitt, in the planning, writing, and editing of this third edition. We decided to make some changes to the coverage to reflect the development of both the science and its applications. Perhaps the most notable change between today and the time of second edition is the greatly increased penetration of biological methods into the chemist's approach to the study and application of enzymes. For this reason, the contribution of biological methods has been worked into the chapters themselves, omitting the separate chapter. The significance of the detailed structural analysis of proteins and what can be done with the information encouraged us to include a chapter on protein structure to parallel the discussion of catalysis by enzymes and to underpin the later chapters. The increased importance of environmental topics is recognised with a chapter on bioremediation. Elsewhere, there has been some shuffling and recruitment of authors. Overall, as the publisher's blurb recites, we hope that this edition continues to offer the advanced undergraduate and postgraduate student a 'one-stop' entry at reasonable length into the science and application of enzymes from the chemist's point of view.

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1 Infant enzyme chemistry

C.J. SUCKLING

When this book was initially planned in its first edition, 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 past 30 years. It was therefore something of a surprise to discover in the 19th 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 19th 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, among others, took this view. On the other hand, Liebig and, not surprisingly, Wohler, 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 glycosidase 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, among 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 and, like enzymes, have been extensively cloned and expressed using

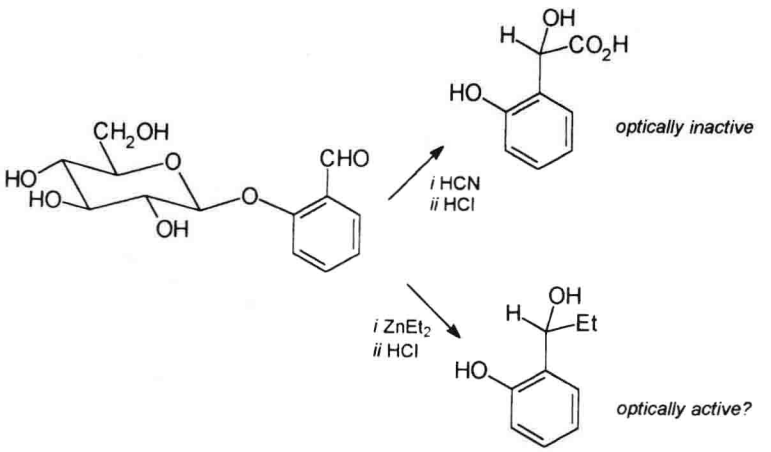

Figure 1.1 Emil Fischer's pioneering attempts at asymmetric synthesis.

the techniques of molecular biology. 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 Chapter 6).

Not surprisingly, the ever-enthusiastic Fischer even had a go at asymmetric synthesis himself (Fischer and Slimmer, 1903, and see Figure 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 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 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 70 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 biospecificity 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. Notable among the extensions was the work of Landsteiner in demonstrating the specificity of recognition of antigens by antibodies; by using a simple series of aromatic amino acids in which the acidic component was sulphonic, arsonic or carboxylic acid (Figure 1.2) he was able to show that antibodies raised to *meta-*

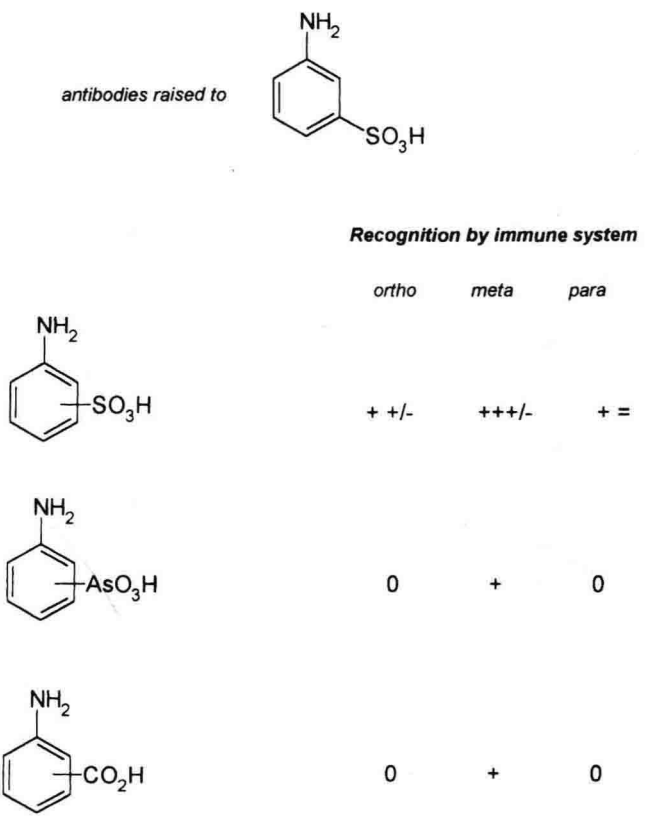

Figure 1.2 Landsteiner's demonstration of the molecular selectivity of antibodies.

aminobenzenesulphonic acid would recognize the *ortho* and *para* isomers of benzenesulphonic acid less strongly but only the *meta* isomer of the analogous arsonic and carboxylic acids (Landsteiner and van der Scheer, 1936).

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. While 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 laboratory 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 among 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). The significance of enzymic selectivity and catalytic efficiency has acquired a new dimension in the past 10 years with the increasing emphasis being placed upon care for the environment. New sub-fields of activity have grown up under such titles as 'Clean Synthesis', 'Biotransformations', and 'Bioremediation', all of which are covered in this book (Chapters 6 to 8). In addition, selective enzyme inhibitors are immensely important as drugs for the treatment of bacterial and viral diseases (Chapter 5).

In the past 20 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. Through the field of catalytic antibodies, scientists have even been able to create novel protein catalysts although their efficiency and availability in quantity are obstacles to applications. Recently contemporaneously with the advances in computing and software, well-known even from the home-based PC, progress in determining the structure of proteins has also been made. Computational methods have served both X-ray crystallographic and nuclear magnetic resonance methods for determining protein structure and the former has also been extended by the availability of synchrotron X-radiation which, at the limit, allows the study of the transformation of a molecule by an enzyme in the crystalline state (see Chapter 3).

Enzyme chemistry has also contributed to more remote fields of scientific endeavour. Structural reasoning combined with molecular biology has made it possible to study some enzymes of animals that have been extinct for millions of years. This contribution to palaentology comes about because it is possible to discern the regularities that underlie mutations between the same enzyme of one modern species and that of another. For example, the sequences of amino acids in the enzyme alcohol dehydrogenase are known for more than 50 species. Using the same logic that a linguist uses to deduce the probable structure of the precursor ancient languages to modern ones, an enzymologist can deduce the probable amino acid sequence for a protein of an ancestor species such as a primitive ruminant or a forerunner of the modern horse. Moreover, once the