Enzyme Chemistry

Impact and applications

Edited by

Colin J. Suckling

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Preface

In the molecular sciences, enzyme chemistry occupies a special niche as one of the major contact points between chemical and biological disciplines. The special properties of enzymes as selective and efficient catalysts are so central to current challenges to chemists that the development of enzyme chemistry in the past thirty years has been a major stimulus to chemical research in general. On the one hand studies of the intrinsic properties of enzymes and, on the other hand, their applications to synthesis, drug design, and biosynthesis have had an immense impact. This book brings together in one volume essays describing several such fields with emphasis on the applications. It would be unnecessarily repetitious to outline the approach and contents of the book in a Preface; the first short chapter is more eloquent than a formal Preface can be. I shall therefore encourage you to begin with the Introduction in Chapter 1 and here I wish to extend my warm thanks to those who have contributed to the production of this book: the authors for their acceptance of the overall concept of the book and for the thoughtfulness of their writing; Dr Charles Suckling, FRS and Professor Hamish Wood for their constructive criticism of the whole book; and Dr John Buckingham and his colleagues at Chapman and Hall for their efficiency and enthusiasm in transforming the typescripts into the book that you now hold.

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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 whi
> on microscopic examination are seen to take the form of a Beind

> 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 naive, 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 microorganisms. 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 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

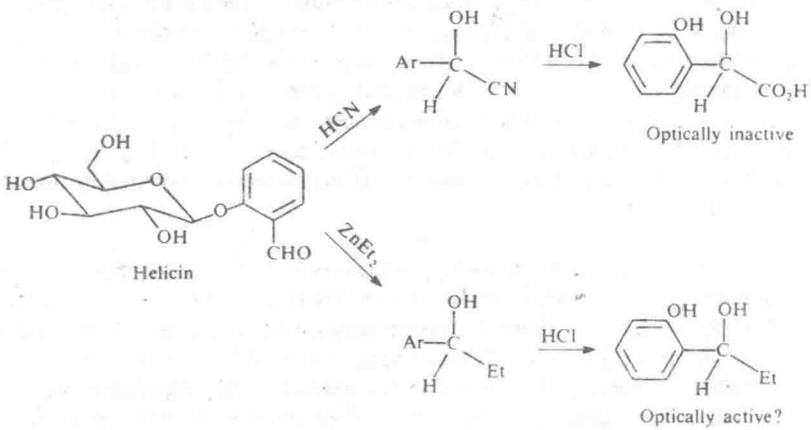

Fig. 1.1

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 biospecificity with enzymes, similar complementary interactions were enthusiastically discussed for the behaviour of other biosystems such as toxins. However naive 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 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 that 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 stides 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 (Barrie Hesp 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. 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 8, 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). 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 chemistry (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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2 The mechanistic basis of enzyme catalysis

Ronald Kluger

2.1 INTRODUCTION

The purpose of this chapter is to provide a connection between the concepts of physical organic chemistry and enzyme chemistry through a survey of some of the most useful general approaches that relate the two areas. However, the topic is very large and we have had to be selective. The references we cite in this chapter are therefore intended to lead the reader to more thorough discussions of the points that are raised here. We have selected reviews or extended discussions, rather than citations of experimental observations and original derivations.

Enzymes are noteworthy catalysts. Through the pressures of evolution, they have developed the ability to process the substrates of metabolism with a superb degree of efficiency and specificity which, although remarkable, to a large extent must be understandable within the context of chemical reaction mechanisms since transformations of organic molecules are occurring. It has become a useful pursuit to elucidate the mechanistic basis of enzyme catalysis because, as we understand how enzymes accomplish their tasks, we often discover new catalytic mechanisms. With this knowledge we can begin to design or modify molecules to be useful catalysts and to use enzymes themselves as catalysts outside of their metabolic functions. Seeking a mechanistic basis for enzymic catalysis really means looking for patterns and explanations that we can comprehend and utilize. Thus, it has become apparent that the study of enzyme catalysis is important not only as a traditional biochemical pursuit but also as a means of developing processes of interest for new catalytic systems. The full significance of these remarks will emerge in the next three chapters.

The mechanistic impact of enzyme chemistry has caused an increased interest in the principles of physical organic chemistry. The physical organic chemist is concerned with the properties of organic molecules in systematic relationship to the structure and reactivity of these molecules. Enzyme chemistry is a logical extension of this interest since catalysis and specificity, the central features of enzymic reactions, are concepts that fall within the realm of physical organic chemistry. We shall review some of the areas of physical organic chemistry that relate in a useful way to problems in enzyme chemistry. With this background, we can examine the influence that research into enzyme chemistry has had upon modern approaches to the study of mechanistic and structural organic chemistry.

2.2 CONCEPTS OF CATALYSIS

A survey of the types of reactions catalysed by enzymes reveals that the common categories of organic and inorganic reactions apply. These categories include, for example, substitution, elimination, addition, oxidation and reduction. Such reaction patterns classify the relationship between the starting materials and products; mechanisms provide the means by which a connection occurs. Although the enzyme-catalysed reaction types are obvious, the mechanisms are often conjectural. Where sufficient information is available, it is useful if the reactions can be systematically divided into mechanistic types (such as the Ingold formulation (Ingold, 1953)). The most common examples of this type of classification are the two general nucleophilic substitution mechanisms, S_N1 and S_N2. Walsh (1979) has written an excellent and extensive survey of enzyme-catalysed reactions in terms of reaction type and mechanisms. In addition, several excellent reviews of the mechanisms of enzymic catalysis in general have been published (Bruice and Benkovic, 1966; Fersht, 1977; Scrimgeour, 1977; Metzler, 1977; Jencks, 1969; Bender, 1971; Cunningham, 1978). With these extensive reviews available, I have decided to attempt a somewhat different approach to the subject. I ask the question what are the key concepts of physical organic chemistry that are relevant to considerations of enzyme mechanisms? Illustrative examples, with an admitted bias toward my own areas of interest, will be given.

2.3 DESCRIBING A MECHANISM

A full description of the events that occur during a chemical reaction, especially one that occurs in solution, whether catalysed or uncatalysed, is unattainable because of the large number of independent and dependent variables which describe it. In addition to changes that the substrates

undergo during the transformation, one may need to be aware of the function of the solvent and the reorganization that it undergoes (Ritchie, 1969). Obviously, a detailed understanding of any reaction at the level of every molecular co-ordinate is not a practical goal. Yet we would like to know the pathway of a reaction in a mechanistic sense. How does the substrate change during the reaction and what is the molecular function of the enzyme in assuring efficiency and specificity? Can the transformation be quantitatively compared to a reaction which does not involve the enzyme? By having such information we can understand in sufficient detail the function of the enzyme in promoting and controlling the reaction. We will also be able to begin to design catalysts which can be based on what we have learned from analysing the enzymic system (see Chapters 3 and 4).

Kinetic methods are probably the most useful tools for providing a basis on which to analyse the effectiveness of an enzyme as a catalyst. We can usually determine an experimental rate law for an enzyme-catalysed reaction and rate constants associated with the rate law can be evaluated by the use of steady-state methods (Segel, 1975). Thus, a logical sequence of events in terms of conversions between stable species and intermediates can be established.

This general description of an enzymic reaction can be further refined by the use of such techniques as isotope exchange (Segel, 1975), pre-steady-state analysis (Fersht, 1977) and other rapid techniques. With this quantitative set of information, we would like to calibrate the effectiveness of the enzyme as a catalyst against a standard. One useful comparison is with a similar reaction in the absence of enzyme. We know that the enzyme probably catalyses the reaction through a series of steps. It is most helpful if the reaction we are comparing can be set up to coincide step by step with the enzyme reaction. Then, by comparing similar enzymic and non-enzymic kinetics, we can describe the function of the enzyme in terms of specific energetics (see Hall and Knowles (1975) for a good example). In order to do this, we need to be able to analyse the enzymic and non-enzymic systems on a common kinetic basis. The comparison must involve considerable information on both processes, including knowledge of the identity of the rate-determining step.

For these reasons, kinetic methods are especially important and we shall begin by reviewing some of the concepts used by physical organic chemists to simplify the analysis of multistep systems. First, the rate at which reactants are converted to products in any multistep reaction is described by a complex rate equation which can often be simplified using the steady-state assumption (see Hammett, 1970, pp. 77–79). If a reactive intermediate or a reactive complex with a catalyst is on the reaction path, then long before the system reaches its final equilibrium destination, it will reach what is called a steady state. Intermediates present in low concentrations in the steady state

undergo *changes* in concentration that are very small compared to those of the observed reactants or products. This method of simplification is particularly appropriate for catalytic systems, since complexes of the substrate and catalyst will usually be present in very low concentrations (Klotz, 1976). In the case of an enzymic reaction, the concentration of enzyme is necessarily low (since its molecular weight is so high). In other reactions, reactive intermediates will also be high in energy and as a result will also be present at low concentrations. A steady state develops as changes in concentrations become undetectable. By this definition, an equilibrium is a steady state that is permanent. However, the steady state we deal with here will be that which is a non-equilibrium condition and will dissipate as components providing the 'fuel' for the steady state is used up. (Life is a steady state; death is an equilibrium.)

2.3.1 *Rate-determining step*

In order to utilize any rate equation it is necessary to identify a rate-determining step. Murdoch (1981) has presented a particularly clear discussion on this subject. Briefly, in any multistep reaction, steps occurring after the rate-determining step have no effect on the rate of conversion of reactants to products, provided that no intermediate is more stable than the reactant or product. If an intermediate is more stable, then the system should be treated as two separate processes and not as a steady-state system. The rate-determining step is the slowest in rate, not the step with the smallest rate constant. Since rate depends on concentration of reactants and the rate constant, the rate-determining step is a function of concentration of species undergoing that step as well as the barrier to reaction from that step. Steps preceding the rate-determining step provide a flux of material to the state prior to the rate-determining transformation. This means that one can identify a rate-determining step by finding the step with the highest energy-transition state. After a mechanism has been established, we can compare the relative rates of any two processes that an intermediate can undergo. The faster process cannot be rate determining. Comparisons are made for each intermediate until a self-consistent answer is found. For an example in which this method is used to analyse a complex set of data, see Kluger and Chin (1982).

2.3.2 *The importance of microscopic reversibility*

The principle of microscopic reversibility requires that the lowest energy path in the forward direction is the same as that of the reverse reaction under the same conditions. If there are two competing paths in the forward direction, they will also compete in the reverse direction and to the same extent. Neglecting to take this into account may lead to erroneous conclusions