ENZYMIC AND NON-ENZYMIC CATALYSIS

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Editors' Preface

This volume collects the presentations and edited discussions of an international symposium entitled 'Enzymic and Non-enzymic Catalysis: Current Status and Industrial Potential for Stereoselective Catalysis'. The meeting, held in April 1978 at the City University, London was organized for the Microbiology, Fermentation and Enzyme Technology Group of the Society of Chemical Industry. As the title suggests, the intention was to focus on enzymic catalysis and those types of chemical catalysis to which it seems to bear a rather close relationship.

The first chapter on 'The Present Knowledge of Enzyme Catalysis' raises questions also on the nature of the forces involved in chemical catalysis which are reflected in the discussion of later chapters. 'The Present Status of Enzyme Technology' reviews some of the successes of recent technology and indicates possible limits. The discussion of 'Enzymes in Synthetic Organic Chemistry' in the third chapter was evidently timely because the discussion with industrialists present indicated the beginnings of commercial activity in this field.

The crown-ether structures which are described in the next chapter convey an impression of the size of a non-enzymic framework which may be required for catalysis of a similar kind to that brought about by enzymes. The chapter on 'Micellar Catalysis', in addition to its chemical significance may, for example, bear upon the behaviour of membrane-associated enzymes.

Two chapters on reactor design show the strong parallels which exist here for chemical and enzyme reactors and the influence of efficient reactor designs on catalyst performance.

The final chapters concern organo-metal catalysts. 'Asymmetric Homogeneous Catalysis' indicates one approach to cheaper chiral ligands, while 'Polymer-attached Homogeneous Catalysis' discusses catalyst retention by attachment to supports. The latter, with quite different origins from enzyme immobilization, nevertheless exhibits a number of strong parallels.

It is hard to escape the conclusion that future catalyst types will draw equally upon the knowledge of both enzymic and chemical catalysis, and the

recent linkage of organo-metal catalysts to proteins suggests that even hybrid molecules may be used. The field illustrates to a striking degree the need for a dialogue between chemist, enzymologist and engineer and it is hoped that this volume has contributed to this dialogue.

We should like to acknowledge the help of Dr. G. Lowe in planning the meeting, of Professor E. M. Crook, Professor H. C. S. Wood, Dr. M. D. Lilly, and Professor C. H. Bamford, who chaired the scientific sessions, and of Miss J. Bovier and Mrs. J. Mealing, whose administrative and organizational support made the meeting possible.

Peter Dunnill
Alan Wiseman
Norman Blakebrough
March, 1979

CHAPTER 1

The Present Knowledge of Enzyme Catalysis

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INTRODUCTION

The first point to consider is whether or not the magnitude of enzymic catalysis may be accounted for in simple terms. The answer is 'yes', but the tortuous path to this conclusion is full of difficulties and false trails that I wish to dispose of first.

The classical approach to understanding the magnitude and nature of enzymic catalysis has been from the extrapolation of the study of simple reactions in solution. This approach leaves us with general feeling that there are no basic problems in accounting for the magnitude of enzymic catalysis. However, the precise, quantitative explanation for the magnitude of enzyme catalysis will eventually come from direct theoretical calculations on proteins. The first real steps are now being made in this direction and I shall intersperse the classical studies with some of the interesting and provocative ideas that are being formulated. If anyone wishes a more general and detailed account, I recommend that they read my recently published book on *Enzyme Structure and Mechanism,* from which most of the topics are taken (Fersht, 1977).

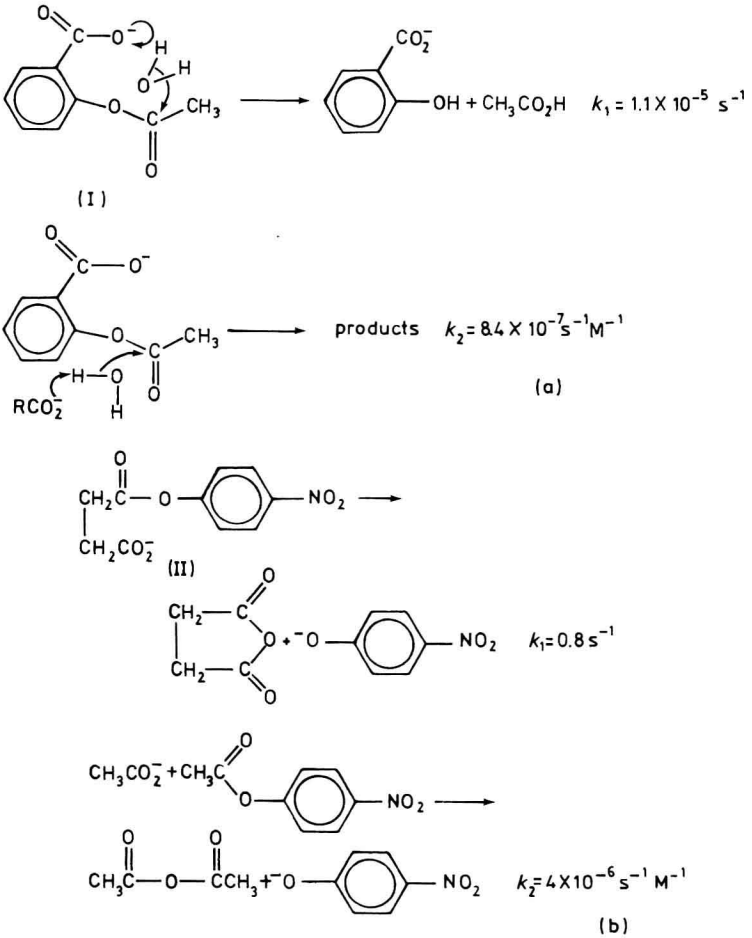
THE CLASSICAL APPROACH

(a) Intramolecular Catalysis

The rate enhancements in simple bi- and trimolecular reactions in solution caused by general acid-base catalysis, nucleophilic catalysis and so on are well documented. There is an inherent difficulty in extrapolating these results to enzymic reactions because the outstanding characteristic of enzymic catalysis is that the reactions here take place in the confines of the enzyme-substrate complex. The catalytic groups of the enzyme are thus effectively part of the same 'molecule' as the substrate and so the reaction rates are first order with respect to their concentration. Estimation of the contribution of this 'proximity' effect has been one of the stumbling blocks in accounting for enzyme catalysis.

Chemists have often attempted to divide proximity into contributions from 'approximation' and 'orientation'. The experimental entry into estimating these effects comes from studying intramolecularly catalyzed reactions and comparing the reaction rates with those of their bimolecular equivalents (Fig. 1.1).

Among the earliest examples are the hydrolysis of aspirin and succinate half esters. The magnitude of the intramolecular effect is given in terms of the effective concentration of the neighbouring group, that is, the concentration of an independent catalyst required to give the same rate. Aspirin is a typical example of a general acid or base catalysed reaction, the effective concentration of the neighbouring carboxylate ion being about 13 M (Fig. 1.1a). Much larger rate enhancements are found for nucleophilic reactions. The effective concentration of the neighbouring carboxylate ion in the succinate half esters is about 10^5M., Fig. 1.1b. This is true for both rate and equilibrium measurements. An even higher value is found for the nucleophilic reaction in the aspirin derivative (III) in Fig. 1.1c. These compounds have unstrained ground states.



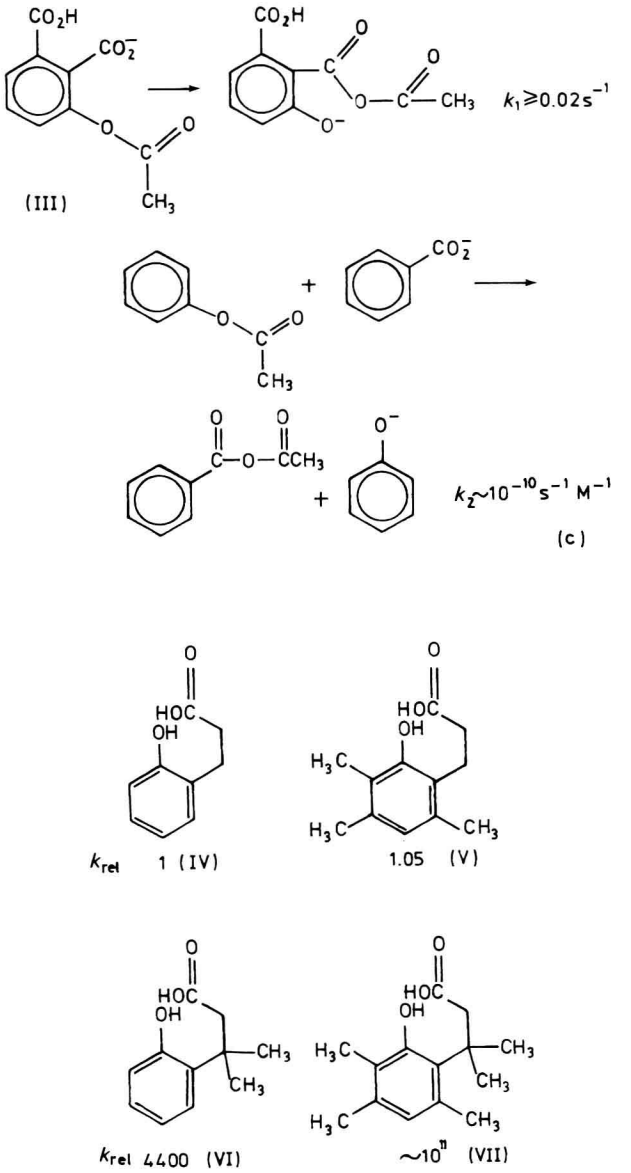


Fig. 1.1 – Comparisons of intramolecular and bimolecular general base or nucleophilic catalysed reactions.

The theory behind these high rate enhancements was provided by Page and Jencks (1971). Until their classic paper, the value of effective concentration due to proximity was thought to be about 10 M, the higher values observed in nucleophilic catalysis caused by orientation or strain effects. However, Page and Jencks showed that the loss of overall rotational and translational entropy on two molecules condensing to form one may give an effective concentration of up to 10^7 to 10^9M. Thus, it is not so much that enzyme reactions are so fast but that bimolecular reactions in solution are slow because of the entropy losses.

SOME FALSE TRAILS

The above analysis of unstrained compounds is the basis of our rationalizing enzyme catalysis based on solution catalysis. The pitfalls have come from over-ambitious attempts of chemists to mimic and estimate orientational factors. Unwittingly, these studies have often been complicated by the preparation of highly strained models whose interest lies in their peculiarities rather than their relevance to enzymic catalysis.

Possibly the widest quoted amongst these is the overalkylated dihydrocoumarinic acid (VII) of Milstien and Cohen (1970). This 'monster' was synthesized to lock the nucleophile and ester bound in close proximity *via* a 'trialkyl lock'. The rationale behind these experiments was to separate the contributions of approximation and orientation.

On going through compounds (IV) to (VII) there is an increase in the rate of lactonization by about 10^{11} fold, to give an effective concentration of the neighbouring group of about 10^{15} M (Page, 1973). The factor of 10^{11} was attributed to an orientation effect dubbed 'stereopopulation control'. This effect has now been shown to be due mainly to the relief of steric strain in the acid. The overalkylated compound is a preposterous artefact of the synthetic chemist. The interaction between the methyl groups is so strong that the benzene ring is distorted. Although retaining planarity, its bond angles deviate by as much as 6° from normal (Karle and Karle, 1972). Of the factor of 10^{11}, 10^7 to 10^8 is attributable to the relief of steric strain and possibly only 10^3 or so to the favourable orientation of reagent groups (Danforth *et al.*, 1976; Winans and Wilcox, 1976).

A series of examples illustrating strain effects occurs in the cyclization of N-methylmaleamic acid derivatives (Table 1.1). As pointed out by Page (1973) the impressive increase in rate on alkylation is due to the relief of steric strain in the acid. The C-C-H bond angle of 118° in maleic acid opens up to 128.5° in maleic anhydride. The H· · · ·H bond distance between the two methyl groups in the dimethyl maleamide increases from about 1.5 Å to 2 Å on forming the anhydride. The consequent relief of steric strain is worth at least a factor of 10^4.

In summary, the proximity of two groups in an enzymic reaction is worth up to an effective concentration of 10^7 to 10^9M for a nucleophilic reaction compared with its bimolecular counterpart. General base catalyzed reactions are