ENZYME-CATALYSED REACTIONS

C. J. GRAY

Department of Chemistry, University of Birmingham

ENZYME-CATALYSED REACTIONS

C. J. GRAY

Department of Chemistry, University of Birmingham

VAN NOSTRAND REINHOLD COMPANY
LONDON
NEW YORK CINCINNATI TORONTO MELBOURNE

VAN NOSTRAND REINHOLD COMPANY
Windsor House, 46 Victoria Street, London S.W.1

INTERNATIONAL OFFICES
New York Cincinnati Toronto Melbourne

Library of Congress Catalog Card No. 75–125951
SBN 442 02808 3

First published 1971

Printed in Great Britain by
Butler & Tanner Ltd, Frome and London

ENZYME-CATALYSED REACTIONS

PREFACE

Recent advances in biology have meant that more attention is being paid to the study of events which take place, at the molecular level, in living systems. For this reason the distinctions between biology, biochemistry and chemistry are becoming increasingly difficult to define. Progressively more biological scientists are finding it necessary to become acquainted with various subjects which were once regarded as primarily the domain of the physical or organic chemist. Similarly, chemists are applying their ideas and methods to the study of biological processes. One field in particular which holds the attention of both these groups of scientists is that of the study of enzyme-catalysed reactions. One aim of this book is to help to bridge the gap between the chemical and biological disciplines even further in this field, by illustrating for the biologist that the enzymes with which he is familiar can be considered in terms of the fundamental principles of organic chemistry, and for the organic chemist that these principles, established for simple molecules, may be extended to the extremely complex molecules upon which life itself depends.

On the whole this book is directed mainly to the senior undergraduate and postgraduate students of both biochemistry and chemistry. In the first chapter some of the basic principles of the theory of reaction mechanisms, including some thermodynamic aspects, are discussed, the treatment being neither very detailed nor too rigorous. This, it is hoped, will provide a suitable introduction for those who are not familiar with the subject. Those who wish to study the subject in more depth should refer to the appropriate textbooks [1, 2, 3, 4]†. The chapter should be of value, too, to those who are familiar with the subject since it is treated from a slightly different point of view which emphasizes the rates of reactions and the ways in which organic compounds can influence those rates.

The purpose of Chapter 2 is to describe some of the methods (mainly chemical) which have been used to study the enzymes and obtain information about their modes of action, so providing a general background to the greater detail contained in the later chapters. These later chapters (Chapters 3–8) describe many of the enzymes individually. In Chapter 9 a brief summary is given of some of the factors which are thought at present to be important to the mechanisms of catalysis by enzymes.

In presenting this kind of material the writer is inevitably confronted with the choice of making his discussion of individual enzymes either

† See references after Chapter 1.

more comprehensive or more selective. Apart from the fact that he is limited by the physical size of the final book, the writer who chooses to be comprehensive in his approach and to present as much information as possible runs the real risk of having the important principles of the subject missed by his reader, because they are clouded by the overwhelming detail. On the other hand, if the writer chooses to be more selective in that he discusses only a very few examples emphasizing the main principles involved, then he is in danger of presenting to the reader only a very small fraction of the vast amount of information which may be available on the subject. In addition, selectivity is of necessity subjective on the part of the writer, and this subjectivity may be undesirable in certain circumstances. Although the field is too large for this book to include a discussion of every enzyme, I have tended towards the more comprehensive, and I hope more objective, approach in that a relatively large number of enzymes have been discussed individually. This allows the reader to see for himself as broad a picture as possible of the whole field. I believe that this more comprehensive treatment fills a gap in the published literature where there are already some good examples of the selective approach.

I am grateful to all those who have given me permission to use their photographs and line drawings in this book, especially those showing the three-dimensional structures of molecules based on X-ray crystallography. I wish to thank Professor S. A. Barker for his invaluable advice and encouragement, Mrs C. M. J. Law for her assistance with drawings and references and Mr. T. H. Yeo for helping with the reading of the proofs.

Thanks are also due to the Efficient Typing Service, Birmingham, for their typing of the manuscript, and especially to the staff of Van Nostrand Reinhold for their advice and help in the preparation of this book.

The jacket design is based on the model of ribonuclease as determined by G. Kartha and co-workers.

1970 C. J. GRAY

CLASSIFICATION OF ENZYMES

On the whole, enzymes are considered in this book in the groups which correspond to the classification recommended by the Commission on Enzymes (1956–61) of the International Union of Biochemistry. The major advantage of this classification system is that the enzymes are considered according to the overall nature of the reactions catalysed. This means that an enzyme can be classified and assigned a systematic name and number as soon as it has been detected since an enzyme is of course detected by means of the reaction which it brings about. An alternative method of classifying the enzyme could be envisaged based on the mechanism of action of each enzyme. In many cases this would coincide with the Commission's classification but there would be certain differences. However, this alternative method would possess the great disadvantage that an enzyme could not be classified, and therefore given a systematic name, until its mechanism of action has been established. Since in only a few cases have we any clear idea at all of the mechanism by which an enzyme functions, an impossible situation would develop. Because we are considering the enzymes from the point of view of their possible reaction mechanisms, in order that as far as possible a unified approach is taken, certain enzymes must be considered out of the order recommended by the Commission. The most important change is that the hydrolases are considered in Chapters 4 and 5 before the transferases. In this way certain principles and concepts are introduced at an earlier stage. Some other exceptions to the classification order are that the thiamine pyrophosphate-dependent *transketolase* is considered in the same section of Chapter 7 as are the thiamine pyrophosphate-dependent *lyases*, and the enzyme *transaldolase* which functions by a mechanism involving an active lysine residue is discussed with the active lysine lyases in Chapter 7. Those *ligases* which contain biotin are also considered out of turn, being taken with the biotin *transferases*. However, it is not a general rule that all enzymes which require one particular coenzyme are considered together. For example the vitamin B_{12} coenzyme transferases are discussed in Chapter 6 while other enzymes requiring this coenzyme are referred to in Chapter 8. Similarly, the pyridoxal-dependent enzymes are not all considered together in the same chapter.

One further difficulty arises from the Enzyme Commission Classification which should be noted; this is that in some cases enzymes which catalyse the same reactions are given the same nomenclature and number,

even where these enzymes may be obtained from different sources. For example, *alcohol dehydrogenase* (alcohol: NAD oxidoreductase, 1.1.1.1) may be obtained from mammalian liver or from yeast. The enzymes from these two sources are not identical and there are certain differences in their mechanisms of action. On the whole, conclusions about the mechanism of action of an enzyme from one source may not be drawn from experiments on a similar enzyme from a different source. This statement does not merely apply to enzymes obtained from different types of organism (such as yeast, bacteria, mammals, etc.) but even to enzymes from different sources within the same organism. *ATPase* (ATP phosphohydrolase, 3.6.1.3) from muscle may differ from the ATPase from cell membranes in the same animal.

The following terms are used in this book without further definition:

Apo-enzyme—The protein-only component of a complex enzyme, from which coenzyme and metal-ion has been removed.

Holo-enzyme—A complete enzyme, comprising apo-enzyme and any required coenzyme or metal-ion.

CONTENTS

PREFACE xi

CLASSIFICATION OF ENZYMES xiii

CHAPTER 1 RATES AND REACTION MECHANISMS 1

Reaction Rates 1
Rates and Rate Constants 1
The Arrhenius Equation and the Collision Theory 3
The Transition-state Theory 5
Acids and Bases 12
Acid–base Catalysis 15
Chemical Processes in Organic Chemistry 16
Energy of Activation for Nucleophilic Substitution 18
The Nucleophile or Lewis Base 19
The Leaving Group 21
The R-group 22
Extreme Cases in Nucleophilic Substitution 23
Elimination Reactions 25
Reactions of Carbonyl Groups 26
Homolytic Bond Cleavage 27
Catalysis 28
Catalysis by Acids and Bases 30
Mutarotation of Glucose and Glucose Derivatives 36
Decarboxylation of β-ketoacids 37
Metal-ions 38
Alternative Pathway Catalysis 43
Proximity Effects 52
Strain 56
Conclusion 56
References 57

CHAPTER 2 ENZYMES AS CATALYSTS 59

Introduction 59
The Active Site 64
Chemical Modification 65
Kinetics of Enzyme-catalysed Reactions 72
Effect of pH on Enzyme Activity 82
Allosteric Effects 86
X-ray Crystallography 88
References 89

CHAPTER 3 OXIDATION AND REDUCTION 93

Oxidation and Reduction 93
Reduction Potentials 95
Hydride Transfer 98

Oxidative Elimination 101
One- and Two-electron Transfer 102
Oxidoreductases 107
Nicotinamide Adenine Dinucleotide Enzymes 107
Alcohol Dehydrogenases 108
Oxidation of aldehydes 121
Flavoenzymes 124
Reduced Nicotinamide Adenine Dinucleotide Dehydrogenases 126
Glucose Oxidase 129
Amino-acid Oxidases 131
Metalloflavoenzymes 134
Hydroxylation and Ring Cleavage 139
Molecular Oxygen as an Electron Acceptor 141
References 142

CHAPTER 4 HYDROLYSIS I 147
Hydrolases which possess Active Serine Residues 147
Chymotrypsin A 148
Chemical Studies with Chymotrypsin 150
The Active Site Environment 153
Kinetic Studies 156
Substrate Binding and Specificity 161
Three-dimensional Structure 162
Mechanism 164
Trypsin and other Active Serine Enzymes 167
Carboxylic Ester Hydrolases 170
Structural Relationships 172
Hydrolases which possess Active Cysteine Residues 173
Structural Relationships 179
Metal-ion-dependent Hydrolases 180
Carboxypeptidase A 181
The Binding of the Zinc-ion of the Apo-enzyme 182
Mechanism of Action 186
Leucine Aminopeptidase 189
Hydrolases which Function at Low pH Values 191
Pepsin 191
References 196

CHAPTER 5 HYDROLYSIS II 204
Hydrolysis of Phosphate Esters 204
Enzyme-catalysed Hydrolysis of Phosphate Esters 209
Hydrolysis of Polyphosphates 211
ATP Phosphohydrolase (ATPase) 213
Hydrolysis of Glycosides 217
Non-enzymic hydrolysis 217
N-Acetylmuramide Glycanohydrolase (muramidase, lysozyme) 218
Other Glycosidases 223
Purine Aminohydrolases 226
References 228

CHAPTER 6 GROUP TRANSFER REACTIONS 232
Methyl Group Transfer 232
Carboxyl Transfer—Biotin Enzymes 237

Acyltransferases 241
Glycosyl Transfer 245
Sucrose Glycosyltransferase 246
Maltose phosphorylase 249
Glycogen phosphorylase 250
Pyridoxal Enzymes 251
Amino Group Transfer 251
Serine Hydroxymethyltransferase 256
Transfer of Phosphorus-containing Groups 259
Kinases 259
Phosphoglucomutase 262
Ribonuclease 264
References 271

CHAPTER 7 LYASES 276
Pyridoxal Phosphate Enzymes 277
Non-enzymic Reactions of Pyridoxal 277
Enzyme-catalysed Reactions 280
Thiamine Pyrophosphate-dependent Enzymes 285
Non-enzymic Thiamine-catalysed Reactions 286
Enzyme-catalysed Reactions 289
'Active-lysine' Lyases 295
Acetoacetate Decarboxylase 295
Aldolases and Transaldolase 298
2-Keto-3-deoxy-L-arabinonate Dehydratase 302
Metal-dependent Lyases 303
Carbonic Anhydrase 304
Malate Synthase 307
References 309

CHAPTER 8 ISOMERASES AND LIGASES 314
Isomerases 314
Amino-acid Racemases 315
Enzymes Requiring the Vitamin B_{12} Coenzyme 316
3-Ketosteroid-Δ^4-Δ^5-isomerases 320
D-Glucose-6-phosphate Isomerase 322
Ligases 324
Glutathione Synthetase 324
γ-Glutamylcysteine Synthetase 327
Amino-acyl-t-RNA Synthetases 329
Arginosuccinate Synthetase 333
Succinyl-CoA Synthetase 334
References 335

CHAPTER 9 THE NATURE OF CATALYSIS BY ENZYMES 338
References 344
INDEX 345

CHAPTER 1

RATES AND REACTION MECHANISMS

REACTION RATES

One of the most important properties of a chemical reaction is the rate at which it proceeds. Among the factors which determine that rate may be listed the type of reaction which is involved, the nature and intrinsic molecular properties of the chemical species which undergo the reaction, and various external factors such as the environment of the molecules (whether or not a solvent is used) and the temperature. In addition, the rates of many chemical reactions may be influenced by the presence of foreign substances, that is, by catalysts.

Rates and Rate Constants

If a chemical reaction actually proceeds according to the equation

$$X + Y \rightarrow P + Q \tag{1.1}$$

that is, at the molecular level a single stage is involved in which one molecule of species X reacts with one of Y to produce one molecule each of P and Q, then experimentally we would find that the rate of the reaction could be expressed by the equation

$$\text{Rate} = k[X][Y], \tag{1.2}$$

where k is a constant known as the *rate constant* for the reaction and the term [X] represents the *activity* of the species X, etc. On the whole we shall be concerned with reactions occurring in solution where the concentrations of the reacting species are low, and in these cases activities become equivalent to concentrations. Hence, [X] refers to the concentration of X, etc. This reaction is said to be first order in X because the concentration term [X] appears in the rate equation (1.2) raised to the power one. Similarly the reaction is first order in Y. This is in fact a reflection of the original statement that one molecule of X reacts with one of Y. If the process had involved two molecules of X reacting with one of Y in a single step,

$$2X + Y \rightarrow \text{Products}, \tag{1.3}$$

then the rate equation would have been as follows:

$$\text{Rate} = k[X]^2[Y] \tag{1.4}$$

in which the reaction would have been second order in X. Thus by studying experimentally the way in which the rate of a reaction depends on the concentrations of the reactants, one may determine the order of the reaction and hence obtain some information about its mechanism. If the reaction proceeds other than by a single step, the situation is more complicated.

One of the factors, therefore, which determine the rate of a chemical reaction is the concentration of each reactant according to the order. All the other rate-determining factors are included in the rate constant k.

Most reactions in organic chemistry, and certainly most of those with which we shall be dealing, are reversible. Thus, for reaction (1.1), the products P and Q can themselves react to re-form the starting materials X and Y. The reaction may be written

$$X + Y \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} P + Q, \tag{1.5}$$

where k_{+1} and k_{-1} are the rate constants for the forward and reverse reactions respectively. The reaction can proceed until the concentrations of the species present are such that the rate of the forward reaction is exactly equalled by that of the reverse process. At this stage,

$$k_{+1}[X][Y] = k_{-1}[P][Q].$$

Hence

$$\frac{k_{+1}}{k_{-1}} = \frac{[P][Q]}{[X][Y]} = K \text{ eq.} \tag{1.6}$$

The system is in a state of dynamic equilibrium, and the ratio of the two rate constants, K eq, is known as the *equilibrium constant*. This constant is of great importance from the thermodynamic point of view because it can be related to the *free energy* change which accompanies the reaction. If ΔG^0 is the standard free energy change, that is, the change in free energy when one mole of X and one mole of Y react together to give one mole each of P and Q under standard conditions, then

$$\Delta G^0 = -RT.\ln K \text{ eq}, \tag{1.7}$$

when K eq is expressed in terms of the standard conditions.

The free energy change, ΔG^0, may be expressed in terms of the changes in *heat content* and in *entropy*:

$$\Delta G^0 = \Delta H^0 - T.\Delta S^0, \tag{1.8}$$

where ΔH^0 is the standard heat of reaction and ΔS^0 is the standard entropy of reaction (for an introduction to thermodynamics and for definitions of the thermodynamic terms used here the reader is referred to Everett [1]).

From these equations, it is possible to derive the following relationships which show how the equilibrium constant varies with temperature.

$$\frac{d(\ln K\,\text{eq})}{dT} = \frac{\Delta H^0}{RT^2} \quad \text{or} \quad \frac{d(\ln K\,\text{eq})}{d\left(\frac{1}{T}\right)} = \frac{-\Delta H^0}{R}. \tag{1.9}$$

If the heat of the reaction is independent of temperature then (1.9) gives

$$\ln K\,\text{eq} = \frac{-\Delta H^0}{R}\left(\frac{1}{T}\right) + \text{a constant} \tag{1.10}$$

and thus, from a graph of $\ln K$ eq against $1/T$ one may obtain by experiment a value for the heat of reaction. Since we may use (1.7) to obtain ΔG^0 and (1.8) for ΔS^0, it is possible to find values for all the thermodynamic parameters of the reaction.

The Arrhenius Equation and the Collision Theory

Most chemical reactions proceed far more rapidly when the temperature is raised; very approximately an increase of ten degrees may cause the rate to be doubled [5]. Arrhenius [6] found that the relationship between the rate constant and the temperature could be expressed by the equation

$$\ln k = \ln A - \frac{E}{RT} \quad \text{or} \quad k = A.e^{-E/RT}, \tag{1.11}$$

where A and E are constants. This is known as the Arrhenius equation, and from a graph of $\ln k$ against $1/T$ one may obtain values for A and E.

A simple explanation for this relationship is afforded by the *collision theory*, which may be briefly described in the following way. In order that the reaction (1.1) may take place, a molecule of X must come into contact, or collide, with one of Y. The rate of reaction will therefore depend on the frequency of such collisions. If the reaction occurs in the gas phase the kinetic theory of gases may be used to calculate the collision frequency. Our discussion will concentrate on reactions in solution and the kinetic theory may be extended to apply to these conditions. Among other things the collision frequency depends upon the concentration of each species, according to the order of the collision.

$$\text{Frequency} = \text{constant}.[\text{X}][\text{Y}]. \tag{1.12}$$

The calculated collision frequency turns out to be very much greater than that expected from the reaction rate, and it is clear that only a small proportion of the collisions lead to a reaction. In fact, only collisions involving molecules which possess an exceptional amount of energy will give rise to products. This amount of energy which the molecules must have in order to undergo a fruitful collision is the *energy of activation*. The Maxwell–Boltzman law indicates that the fraction of molecules in a system which possess at least an amount of energy E at temperature T is approximately proportional to the term $e^{-E/RT}$. The frequency of fruitful

collisions, which should be equal to the rate of the reaction, may therefore be expressed

$$\text{Frequency} = \text{rate} = A[\text{X}][\text{Y}].e^{-E/RT} \tag{1.13}$$

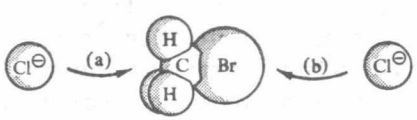
Comparison with (1.2) indicates that the rate constant k is given by

$$k = A.e^{-E/RT}.$$

This is equation (1.11) and from this we infer that in the Arrhenius equation, A must be a factor related to the frequency of collisions (the frequency factor) and E is the energy of activation for the reaction. It has already been said that it is possible for values for A and E to be obtained experimentally.†

It became apparent to early workers that, in many cases, the experimentally-determined values for A did not correspond with the calculated values based on collision frequencies and so a further modifying factor was introduced. For almost all chemical reactions, the molecules which collide must do so with the correct alignment or orientation to each other. The majority of organic reactions are controlled by severe steric requirements in this way. For example, in the case of the reaction between methyl bromide and the chloride ion, which is represented semipictorially below, only a collision involving relative movement in the direction of the arrow (a) will result in reaction. Relative movement in any other direction such as (b) will not be fruitful. In other words the methyl bromide must present to the chloride ion the face opposite the bromine atom. In more complicated reactions, both reacting species must present the correct faces at the moment of collision:

$$\text{Cl}^{\ominus} + \text{CH}_3\text{Br} \rightarrow \text{Cl.CH}_3 + \text{Br}^{\ominus}$$



Because of this effect the number of collisions leading to reaction is further decreased, and the steric factor introduced to allow for it is usually less than one, although in some cases, especially reactions involving ions, the correction factor may be greater than unity. If we define this steric

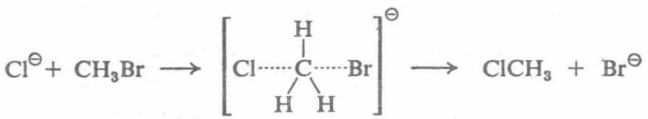
† The graphical method used for the determination of A and E is based on the assumption that A does not vary with temperature, and hence that the plot will be a straight line. In fact, the collision number does vary with temperature, being proportional to $T^{1/2}$. However, over small temperature ranges this variation is not great, especially in comparison with changes in the term $e^{-E/RT}$. For example, over the range 273–303 °K, A will increase by a factor of about 1·05, whereas the term $e^{-E/RT}$ will increase by a factor of about 18 (assuming a value for E of approximately 22 kcal/mole). Early experimentalists also failed to observe a very small change in E with temperature.

factor P as the probability that colliding molecules with sufficient energy will react, the expression for the rate constant becomes

$$k = A.P.e^{-E/RT}. \tag{1.14}$$

The Transition-State Theory

The steric factor P mentioned above is purely empirical and can only be obtained by comparing theoretical with experimental results. For this reason, the collision theory leaves much to be desired. The *transition-state theory*, proposed by Eyring and his colleagues [7], approaches the problem from an entirely different point of view. If we consider in detail the processes which take place during the reaction just mentioned, that is, between methyl bromide and the chloride ion, we may envisage the intermediate situation where the bond between the carbon and chlorine atoms is only partially formed and that between the carbon and bromine atoms is only partially broken. At a certain stage the free energy of the system is at a maximum and this state is known as an activated *complex* or the



transition state. All reactions are pictured as passing through such a transition state, and the transition state is considered to be in equilibrium with the starting materials. If the equilibrium constant for this process is K^* then the rate constant can be shown to be proportional to K^*, the constant of proportionality being almost exactly equal to $\kappa T/h$, where κ is Boltzman's constant, T is the temperature and h is Planck's constant. Thus,

$$k = (\kappa T/h)K^*. \tag{1.15}$$

As before, (1.7), we may relate an equilibrium constant to a free energy change and if ΔG^* is the free energy change in passing from starting materials to activated complex, i.e. the *free energy of activation*, then

$$k = \left(\frac{\kappa T}{h}\right) \exp\left(\frac{-\Delta G^*}{RT}\right). \tag{1.16}$$

Also as before, ΔG^* may be considered in terms of the heat and entropy changes

$$\Delta G^* = \Delta H^* - T\,\Delta S^*. \tag{1.17}$$

From (1.17) we obtain

$$k = \left(\frac{\kappa T}{h}\right) \exp\left(\frac{\Delta S^*}{R}\right) \exp\left(\frac{-\Delta H^*}{RT}\right), \tag{1.18}$$

where ΔH^* is the heat of activation and ΔS^* the entropy of activation.

It may be shown that the following three relationships hold, and these may be used to obtain values for the three activation parameters.

$$\Delta G^* = -RT \ln \frac{kh}{\kappa T}$$

$$\Delta H^* = -R\left[\frac{(d\ln k)}{d(1/T)} + T\right] \tag{1.19}$$

$$\Delta S^* = \frac{\Delta H^* - \Delta G^*}{T} = R\left[\frac{Td \ln k}{dT} + \ln\frac{kh}{\kappa T} + T\right]. \tag{1.20}$$

Inspection of equation (1.19) and the equivalent form of (1.11) shows that the Arrhenius activation energy, obtained from a plot of $\ln k$ against $1/T$ differs from the heat of activation ΔH^* by an amount equal to RT. At normal temperatures this is equal to about 0·6 kcal/mole and this difference is often within the experimental error of the determination. Sometimes this difference is ignored and the terms energy of activation and heat or enthalpy of activation are used to refer to the same quantity. Comparison of equations (1.18) and (1.14) suggests that the steric factor P might be interpreted in terms of the entropy of activation. We have already referred to P as the probability, given that the energy (or better enthalpy) requirement is satisfied, that the collision will result in reaction. Entropy is a measure of the randomness of a system, or a measure of the freedom from restraint of that system. If the transition state for a reaction is more ordered or has fewer degrees of freedom than the starting materials then the entropy of activation will be negative, a decrease in entropy having occurred in the formation of the transition state. Such a situation exists when there are strict steric requirements to be met, i.e. when we might expect P to be low.

At this stage it is worthwhile for us to examine some examples where the entropies of activation have easily discernible effects. These examples help to explain the nature of entropy effects. Reactions which occur by the association of molecules, that is when the total number of species decreases, are accompanied by a negative entropy of activation. Clearly when two molecules coalesce to form an activated complex their freedom of movement is severely restricted and so the transition state possesses a lower entropy than the unassociated starting materials. Therefore the overall order of a reaction, which for a single-step process is a measure of the number of molecular species which become associated with each other, can have a significant effect on the entropy of activation and therefore on the rate. This phenomenon is very important in connection with proximity effects which are discussed on page 52.

If two similar reactions proceed by the same mechanism and in one of them the transition state is highly crowded then this reaction will have the larger negative entropy of activation. This is clearly shown in several