The Development of the Understanding of Enzyme Catalysis in the 20th Century: Contributions from Carbohydrate Enzymes

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Abstract: One hundred years ago, at the beginning of the 20th Century, enzymes were just beginning to be recognized as powerful catalysts that had a high degree of specificity. Michaelis-Menten kinetics indicated that catalysis occurred after an enzyme-substrate complex was formed. Studies on several carbohydrate enzymes, invertase, phosphorylase, dextranucrase, α-amylases, β-amylases, lysozyme, sucrose phosphorylase, cellulose synthase, and starch synthase have contributed a large body of information about subsite binding specificity, the formation of enzyme-substrate complexes, the formation of a covalent enzyme-product-intermediate from an SN2 reaction that undergoes a second reaction to give the final product(s). Mechanisms for several carbohydrate enzymes are presented.

Key words: mechanisms of enzyme catalysis, SN2 reactions, covalent intermediates, carbohydrate enzymes, substrate binding subsites, lock and key hypothesis

In 1894, Emil Fischer,1-3 the famous German carbohydrate chemist, became interested in enzymes, primarily because of their high degree of substrate and product specificities that they displayed. To explain this specificity, he postulated that enzymes were like locks and substrates were like keys that very specifically fit into the locks to give a complex where the specific reaction occurred.

At the beginning of the 20th Century, about 100-years ago, enzymes were just being recognized for their great power in catalyzing reactions with a high degree of specificity and a high rate of reaction. Eventually, it was recognized that most of the reactions that were catalyzed by enzymes would not take place on their own, and it was the function of enzymes to act as biocatalysts for living cells to carry out the various processes of life.

In 1913, Michaelis and Menten,4 in their classic study of the hydrolysis of sucrose by yeast invertase, measured the rate of the reaction (initial velocity, \(v_0\)) versus the substrate concentration \([S]\) and found that the rate was linearly dependent on the concentration of the substrate (sucrose) up to a certain concentration and then the rate started to slow down and eventually became constant, no matter how much substrate was added. This constant rate was the so-called maximum velocity, \(V_{\text{max}}\). From this study, they postulated that a reversible enzyme-substrate complex, the Michaelis Complex or ES complex, was being formed. They proposed that the following reactions were taking place, where E is enzyme, S is substrate, and P is product(s)

\[
E + S \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} ES \overset{k_{+2}}{\rightarrow} E + P
\]

and they then derived the following relationship, the Michaelis-Menten equation, to explain the dependence of \(v_0\) on \([S]\) and the rectangular hyperbolic curve that they observed:

\[
v_0 = \frac{V_{\text{max}}[S]}{K_m + [S]}
\]

where \(K_m\) is the Michaelis constant and equal to \(k_{-1} + k_{+2}/k_{+1}\).

These ideas and postulates were being developed even before the exact chemical nature of enzymes was known. There was preliminary evidence that enzymes might be proteins, but a big controversy developed about whether they were indeed proteins. Starting in 1918, the German chemist, Willstätter, had been engaged in a systematic study of the chemical nature of enzymes and had published extensively on the purification of enzymes by primarily using adsorbents such as cellulose and starch. The yields obtained were very small and the big differences between the measurement of enzyme activity relative to specific chemical tests for proteins and carbohydrates were not appreciated. Many of their most active enzyme preparations did not give a positive test for protein or carbohydrate and sometimes they gave a positive test for carbohydrates but not for proteins. Willstätter and his students concluded that proteins and other colloidal substances were merely nonspecific adsorbents or carriers of the enzyme activity, which was a molecule of unknown chemical composition, belonging neither to the class of proteins, carbohydrates, or lipids.5

The chemical nature of enzymes remained unknown for over 10-years. In 1926, J.B. Sumner reported the crystallization of the first enzyme, urease, from jack beans and showed that it was a protein and catalyzed the breakdown of urea to CO₂ and NH₃.6 Willstätter and his students attacked Sumner’s results and methods. At first, Sumner was ignored and then he was dismissed as a poor experimentalist with a preposterous claim. Willstätter told Sumner that if he digested his urease crystals with trypsin, the protein would be digested away, leaving the enzyme ac-
tivity intact. Sumner said that he had done this, but urease was very resistant to trypsin digestion. Willstätter dismissed this as poor laboratory technique on Sumner’s part. Support for the protein nature of enzymes came later from Northrup, who crystallized pepsin in 1930 and Northrup and Kunitz, who crystallized trypsin, chymotrypsin and their inactive precursors, trypsinogen and chymotrypsinogen between 1931 and 1935. Analyses of these crystalline compounds were shown to be proteins that catalyzed the hydrolysis of peptide bonds and had several hundred times the enzyme activity, on a weight basis, than their starting materials.

**Contributions of important carbohydrate enzymes.**

In 1939, Cori and Cori were studying the action of muscle phosphorylase and found that it catalyzed the breakdown of glycogen in the presence of inorganic phosphate (P) to give d-glucose-1-phosphate (d-Glc-1-P), but in the presence of a-Glc-1-P and absence of P, d-glucose was added to the nonreducing-ends of glycogen, giving the elongation of a glycogen chain. Simultaneously in 1940, Hanes showed the same kinds of reactions for potato phosphorylase, reacting with starch. These were the first reports of enzymic syntheses of polysaccharides. For about 20-years, it was thought that muscle phosphorylase and potato phosphorylase were the enzymes involved in glycogen and starch biosynthesis. In 1960, however, it was shown that these phosphorylases were exclusively degradative enzymes rather than synthetic enzymes and they formed a-Glc-1-P by the reaction of P with the nonreducing-end glucose residues of glycogen and starch. When the phosphorylase reaction was conducted in the synthetic direction by starting with a-Glc-1-P and starch or glycogen, glucose was added to the nonreducing-ends, but the reaction quickly slowed down and stopped after several glucose residues had been added to the glycogen or starch chains. It was shown that the equilibrium ratio of P to a-Glc-1-P at pH 6.8 was 3.6 and the concentration of P in plant and animal tissues was many fold higher than a-Glc-1-P, making the reaction in the synthetic direction very improbable in vivo. Nevertheless, during the period of 1940 to 1960, the requirement for a “primer” for the biosynthesis of glycogen and starch developed. Even though it was shown that the phosphorylases were not the synthetic enzymes for glycogen and starch, the concept of a primer was firmly imbedded in the literature and minds of many investigators, without evidence, as the working hypothesis for all polysaccharide syntheses for over 60-years, from 1940 to the present. In 1953, Leloir and Cabib showed that a, a-trehalose was biosynthesized by yeast, using a new kind of high-energy glucosyl donor, a nucleotide diphospho sugar, UDPGlc, and then Leloir et al. showed that sucrose was also biosynthesized from UDPGlc in plants. In 1961, Leloir et al. reported a new pathway for the biosynthesis of starch in which UDPGlc and ADPGlc were glucosyl donors for starch biosynthesis by starch synthase. The concept of a primer, however, was retained and it was assumed that the glucose was being added to the nonreducing-ends of the primer.

Also in the 1940’s, Hehre was studying the synthesis of dextran from sucrose by *Leuconostoc mesenteroides* dextranucrase. Sucrose was acting as a high-energy donor of d-glucose. The synthesis was assumed, from the beginning, to require a primer and the reaction was adding glucose units to the nonreducing-ends of the primer chains. It was not until 1974 that Robyt et al. showed by pulse and chase studies with 14C-sucrose that a primer was not necessary and that the synthesis was from the reducing-end rather than the nonreducing-end. The lack for the need of a primer in dextran biosynthesis was later confirmed by using a constituent mutant of *L. mesenteroides* B-512F that produced a dextranucrase completely free of any dextran and hence free of any primer.

Several carbohydrate enzymes were crystallized in the period, 1940–1960. Lysozyme, an enzyme that lyses bacteria by hydrolyzing the cell wall, was crystallized from chicken egg-white. Human and porcine pancreatic a-amylases, the bacterial a-amylase from *Bacillus subtilis* (the organism was later reclassified as *B. amyloliquifaciens*), and human salivary a-amylase were crystallized.

**Structure and function of the active-sites of enzymes.**

By the 1960’s, it was pretty well accepted that enzymes formed an enzyme-substrate complex to carry out catalysis. To form this complex, the substrate is bound at a special site in the enzyme structure, the active site, where catalysis takes place to give product(s). To explain both specificity and catalysis, the active site was postulated to have two distinct sites, the binding site and the catalytic site. The binding of the substrate takes place in a cavity or groove or in a pocket of the enzyme structure, where hydrogen bonds and hydrophobic bonds specifically bind the substrate with amino acid side-chain groups. The cavity or pocket, thus, has a stereochemical shape and is the lock into which the substrate very specifically fits, as a key fits into its lock. The active site has an affinity for the substrate that is inversely proportional to the Michaelis constant (1/Km). This specific binding of the substrate into the binding-site gives the specificity that so impressed Fischer.

When bound in the active site, the substrate is specifically oriented with two catalytic groups, a nucleophile or base and an acid or proton donor. These groups then carry out acid-base catalysis to break or make specific bonds in the substrate to give products. The catalytic groups are primarily responsible for the pH dependence of the enzyme. The optimum pH for the action of the enzyme is the pH that gives the maximum amount of nucleophile (base) and the maximum amount of the proton donor (acid).

However, by the middle of the 20th Century, the formation of an ES complex was still a hypothesis as no direct evidence for the formation of the complex had been made. In 1965, an X-ray “picture” of an inhibitor binding to lysozyme was obtained. This picture showed that lysozyme had six binding subsites in which each bound an N-acetyl-D-glucosamine or an N-acetyl-D-muramic acid residue, with the catalytic groups located between subsite 2 and 3, starting from the reducing-end of the substrate.

Three years before the lysozyme report, Robyt and
French\textsuperscript{30} reported studies on the action pattern of \textit{B. subtilis} \(\alpha\)-amylase and proposed nine glucose binding subsites at the active site. The catalytic groups were postulated to be located between the third and fourth subsite from the reducing-end of the substrate, based on the types of products produced from different substrates. The enzyme was proposed to have a \textit{dual-product specificity} in that maltootroise and maltotetraose were the principal products. At the time, an active site composed of nine subsites was considered to be very large. This relatively large active site was confirmed 7-years later by Thoma et al.\textsuperscript{31} from the determination of the Michaelis parameters \((K_m\text{ and } kcat)\) of individual maltodextrin substrates from maltootroise to maltotetraose (G3 to G12) and the determination of the binding energies for each subsite (see Fig. 1C). It should be noted that Robyt and French postulated the formation of a covalent enzyme-product intermediate in the catalytic process.

In 1970, from a similar study of the action pattern of porcine pancreatic \(\alpha\)-amylase, Robyt and French\textsuperscript{30} postulated five glucose binding subsites, with the catalytic groups located between subsite 2 and 3 from the reducing-end of the substrate (see Fig. 1A). This hypothesis was confirmed by X-ray crystallographic studies in 1987\textsuperscript{32} and the determination of the subsite binding energies in 1987\textsuperscript{33} and 1994\textsuperscript{34}. In 1978, Suganuma et al.\textsuperscript{35} determined that \textit{Aspergillus oryzae} \(\alpha\)-amylase (Taka-amylase A) has seven glucose binding subsites, with the catalytic groups located between the third and fourth glucose subsite from the reducing-end (Fig. 1B).

There are two significant, crystalline exo-acting amylases that were studied, \(\beta\)-amylase found in plants (sweet potato, soy beans, barley and wheat) and elaborated by bacteria (\textit{B. polymyxa}, \textit{B. megaterium}, \textit{B. cereus}, and \textit{Pseudomonas sp. BQ6}) and glucoamylase produced by fungi, \textit{Aspergillus niger}, \textit{Rhizopus delemar} and \textit{R. niveus}. Both of these exo-acting amylases give products with anomeric inversion at the hydrolyzed site. \(\beta\)-Amylase gives \(\beta\)-maltose from the nonreducing-ends of starch chains and glucoamylase gives \(\beta\)-glucose from the nonreducing-ends of starch chains. \(\beta\)-Amylase stops when it reaches an \(\alpha\)-1\(\rightarrow\)6 branch linkage, giving a high molecular weight product, a \(\beta\)-limit dextrin. Amylopectin is converted into approximately 50% w/w \(\beta\)-maltose and 50% \(\beta\)-limit dextrin. Glucoamylase, on the other hand, will hydrolyze the \(\alpha\)-1\(\rightarrow\)6 branch linkages of amylpectin, although at a rate of 1/30 of the hydrolysis of the \(\alpha\)-1\(\rightarrow\)4 linkage. Consequently, glucoamylase can completely convert starch to \(\alpha\)-glucose, and it is now used in the industrial conversion of starch to glucose syrups. These glucose syrups in turn can be converted into high fructose syrups by the action of glucose isomerase.

It is interesting to note that the endo-acting enzymes, such as the \(\alpha\)-amylases and lysozyme, catalyze hydrolysis with 100% retention of the configuration at the reducing-ends of the products, and the exo-acting enzymes, such as \(\beta\)-amylase and glucoamylase, catalyze hydrolysis with 100% inversion of the products. The active sites of both of the exo-acting amylases are pockets into which the nonreducing-end of a starch or maltodextrin chain must bind for catalysis to occur. In the case of \(\beta\)-amylase, there are six subsites, with the catalytic groups located between the second and third from the nonreducing-end. In the case of glucoamylase, there are seven subsites, with the catalytic groups located between the first and the second subsite from the nonreducing-end. After the formation of \(\beta\)-maltose by \(\beta\)-amylase, a flap above the pocket opens and the \(\beta\)-maltose product diffuses out of the active site.\textsuperscript{41} The flap then closes and the starch chain moves to fill the empty subsites and another \(\beta\)-maltose product is produced, giving what is known as \textit{multiple attack}.\textsuperscript{42} With glucoamylase, when the \(\beta\)-glucose product is formed, it cannot be released until the chain moves out of the active site. These mechanisms for product release give \(\beta\)-amylase one of the highest turnover numbers known and glucoamylase one of the lowest turnover numbers.

\textbf{Mechanisms of catalysis.}

The question remains that after the enzyme-substrate complex is formed, how does the enzyme carry out the catalytic process? Using information obtained from X-ray crystallographic structure of lysozyme, some kinetic data, and reasoning by analogy from the acid catalyzed hydrolysis of glycosides, Phillips et al.\textsuperscript{43} and Rupley\textsuperscript{44} proposed a mechanism that involves the formation of an oxycarbanion ion. They proposed that an acid group at the active site adds its proton to a reactant oxygen on the N-acetyl-D-glucosamine substrate, giving an acid-catalyzed cleavage of the C–O glycosidic bond and the formation of an oxycarbanion ion; water then adds to the oxycarbanion ion to yield a hemiacetal and the regeneration of the acid catalyst. There, however, is a problem with this mechanism in that acid-catalyzed hydrolysis of glycosides gives both the \(\alpha\)- and \(\beta\)-anomers and lysozyme gives a single anomer with the \(\beta\)-configuration.\textsuperscript{45} Further, the mechanism is a Sn1 (\textit{substitution-nucleophilic-unimolecular}) reaction and very few organic reactions go through this type of reaction mechanism, with the formation of a carbanion intermediate. Only those reactions that produce a highly stabilized carbanion ion, with a high degree of resonance between many resonating atoms will occur by a Sn1 type reaction. The proposed oxycarbanion ion only involves two atoms, the ring oxygen and C1. The major-
ity of organic reactions proceed by an SN2 (substitution-
nucleophilic-bimolecular) reaction, whereby a nucleophile makes an attack on the reactant and displaces a leaving group, forming a covalent bond to the reactant. For lysozyme to give a product that retains 100% of the β-configuration of the substrate, the oxycarbonium ion mechanism is highly improbable. A SN2 type reaction that initially gives an inverted intermediate would be much more probable. This intermediate could then undergo a second reaction that would give a β-product, with retention of the configuration of the original substrate, murein, the bacterial cell wall polysaccharide. The SN2, double displacement reaction would, thus, form a covalent enzyme intermediate. The fact that lysozyme also catalyzes transglycosylation reactions with the exclusive formation of β-glycosidic linkages, further supports a SN2 double displacement reaction and the formation of a covalent intermediate. Although a covalent enzyme intermediate has never been demonstrated for lysozyme, its formation is highly probable.

The first enzyme to be shown to form a covalent intermediate was sucrose phosphorylase. This enzyme catalyzes the cleavage of the glucose-fructose acetal linkage by inorganic phosphate to give α-1-phosphate (α-Glc-1-P). It was recognized that sucrose phosphorylase catalyzes a phosphate-α-Glc-1-P exchange as well as a fructose-sucrose exchange in the absence of the respective cosubstrates as shown in the following reactions:

\[ \text{Glc} - \text{Fru} + ^{14}\text{C-Fru} \xrightarrow{\text{sucrose phosphorylase}} \text{Glc} - ^{14}\text{C-Fru} \]

\[ \alpha\text{-Glc-1-P} + ^{32}\text{P} \xrightarrow{\text{sucrose phosphorylase}} \alpha\text{-Glc-1-P} \]

This exchange reaction was suggestive of the formation of a covalent enzyme intermediate. Two catalytic groups are known to be at the active site, a carboxylate group of Glu35 and a carboxyl group of Asp52. A covalent glucose-enzyme intermediate was isolated by rapidly denaturing a mixture of enzyme and 14C-sucrose. The denatured enzyme contained no fructose but had 14C-glucose covalently linked to the enzyme. This glucosyl enzyme bond was alkali-labile and hydroxide ion released the β-D-glucopyranose intermediate. In the enzyme reaction, the β-D-glucose intermediate is released and inverted by the attack of inorganic phosphate at C1 to give α-Glc-1-P, with overall retention of the glucose configuration of sucrose. The reaction is, thus, a SN2 double displacement reaction that forms a covalent enzyme intermediate. The mechanism for sucrose phosphorylase is shown in Fig. 2.

Another covalent enzyme intermediate was observed for porcine pancreatic α-amylase by using 13C-nuclear magnetic resonance (NMR) at subzero temperatures (−20°C) and a 13C-enriched maltotetraose substrate. Spectral summation and difference techniques revealed a broad resonance peak, whose chemical shift, relative signal intensity, and time-course appearance corresponded to a β-carboxyl-acetal ester covalent enzyme-glucosyl intermediate. This study also indicated a SN2 double displacement, covalent intermediate mechanism for the α-amylase catalyzed hydrolysis of α-1→4 glycosidic bonds. X-ray crystallographic studies of porcine pancreatic α-amylase indicated that the catalytic groups are two aspartic acid carboxyl groups. One of the carboxyl groups is a carboxylate nucleophile and the other is a protonated carboxyl group that donates a proton to the leaving, glycosidic oxygen. Carboxylic acid residues (Asp and Glu) are present as catalytic groups for many different α-amylases, as well as for a cyclomaltodextrin glucanlytransferase, suggesting that these enzymes all catalyze the cleavage of the α-1→4 glycosidic linkages by an identical mechanism. The mechanism for the cleavage of the α-1→4 glycosidic bond by α-amylases is shown in Fig. 3.

A covalent enzyme intermediate was obtained by rapid denaturation and isolation during the reaction of B. macerans cyclomaltodextrin glucanlytransferase with cyclomaltohexaose. The enzyme opens the cyclomaltohexaose ring and forms a covalent enzyme-maltohexaose complex, which was trapped. This complex usually goes on to react with some acceptor such as water or glucose to give maltotetraose or maltoheptaose, respectively.

The lysozyme catalysis of the hydrolysis of the glycosidic linkages of chitin or murein is very similar to the reactions of sucrose phosphorylase, α-amylase, and cyclomaltodextrin glucanlytransferase. The fact that lysozyme cleaves the β-1→4 glycosidic bond with retention of the β-configuration at the newly produced reducing-end and the fact that lysozyme also catalyzes transglycosylation reactions with carbohydrate acceptors strongly indicates that lysozyme forms a covalent enzyme intermediate in cleaving the β-1→4 glycosidic linkage. A proposed
mechanism for the cleavage of the $\beta$-1→4 glycosidic bonds by lysozyme, with retention of the configuration, is shown in Fig. 4.

The mechanism of hydrolysis for the inverting enzymes, $\beta$-amylase and glucoamylase is more problematic in that an SN2 mechanism would require a triple displacement to obtain an inverted product. Yet, protein structure homology and evolutionary theory would suggest that the inverting enzymes would also form a covalent enzyme intermediate, similar to the retaining enzymes. Or put another way, the exo-acting (inverting) enzymes should have a mechanism similar to the endo-acting (retaining) enzymes. Carboxylate base and carboxyl acid groups have been identified at the active sites of both the exo- and endo-amylases by X-ray crystallography and site-directed mutagenesis. A SN2 mechanism in which a covalent enzyme intermediate is formed is also possible for the inverting enzymes without triple displacement reactions and still give inversion of the configuration. The covalent intermediate would be the usual carboxyl-$\beta$-acetal ester, identical to the covalent intermediate formed by $\alpha$-amylases, but in the case of the inverting enzymes, the water attacks the carbonyl-carbon of the acetal-carboxyl ester, instead of the C1-carbon of the glucose. This displaces the C1-O oxygen of the intermediate, giving an inverted, $\beta$-hemiacetal product (see Fig. 5 for the mechanism). The polysaccharide synthesizing enzymes, dextranu-
Fig. 6. Mechanism for the reaction of starch branching enzyme to give an α-1→6 branch linkage by a Sn2 reaction and the formation of a covalent enzyme-intermediate.

**Reaction 1**

**Reaction 2**

In the biosynthesis of bacterial cellulose, a covalent intermediate is formed with a polyisoprenoid lipid pyrophosphate. A phosphotransferase catalyzes the reaction of UDPGlc with bactoprenol phosphate (Bp-P) to give bactoprenol pyrophosphoryl glucose (Bp-P-P-Glc). The α-configuration of the glucose residue in UDPGlc is retained as the phosphate group of Bp-P attacks the phosphate group attached to glucose in UDPGlc, leaving the phosphate attached to the glucose in the α-configuration. Cellulose synthase then catalyzes the reaction of two bactoprenol pyrophosphoryl glucose molecules together in which the C-4-OH of one glucose unit of Bp-P-P-Glc makes a Sn2 attack onto C1 of the other glucose unit in Bp-P-P-Glc to give a β-linkage and bactoprenol pyrophosphoryl cellobiose. The Sn2 reaction gives inversion of the configuration of α-glucose to give a β-1→4 linkage between the two-glucose units. Elongation of the cellulose chain continues with the C-4-OH of another Bp-P-P-Glc attacking C1 of the reducing-end glucose of Bp-P-cellobiose to give Bp-P-cellotriose, and so forth with a C-4-OH of glucose units of several Bp-P-P-Glc attacking C1 of the growing cellulose chain attached to bactoprenol pyrophosphate, as shown in the following reactions:
Similar kinds of reactions take place for other $\beta$-linked heteropolysaccharides of Salmonella O-antigen polysaccharide in which a heterotetrasaccharide is assembled on bactoprenol pyrophosphate and transferred to the reducing-end of a growing O-antigen polysaccharide attached to bactoprenol pyrophosphate. Bacterial cell wall polysaccharide, murein, is also synthesized in a similar manner in which N-acetyl-D-glucosamine (NAG) and N-acetyl-D-muramic acid (NAM) are joined together by a $\beta$-1→4 linkage and attached to bactoprenol pyrophosphate. The NAM-NAG unit is then transferred to the reducing-end of a growing poly-NAM-NAG chain by attack of the C-4-OH of NAM onto C1 of NAG of the growing murein chain. Xanthan a cellulose analogue polysaccharide is also synthesized in the same manner in which a pentasaccharide (D-mannose, D-glucuronic acid and D-mannose trisaccharide) attached to the reducing-end of cellobiose is built up on Bp-P-P and transferred to C1 of a growing xanthan chain attached to Bp-P-P.

Conclusions and summary.

In enzyme catalysis, the formation of covalent enzyme intermediates is indicated when (1) there is retention of an asymmetric configuration, (2) there are exchange reactions observed between reactants and products, (3) the enzyme catalyzes transfer (transglycosylation) reactions, (4) actual intermediates are stable and can be isolated, (5) reactive intermediates can be trapped and/or isolated by rapid denaturation of the enzyme during catalysis, and (6) reactive intermediates can be detected by some physical measurement, such as nuclear magnetic resonance. But, why do enzymes form covalent intermediates in performing catalysis? The majority of chemical substitution reactions occur by $\text{SN}_2$ mechanisms in which a covalent bond is formed with the first substrate reactant, followed by a second reaction with another substrate reactant and the formation of the product. $\text{SN}_1$ chemical reactions only occur when the resulting carbonium ion is highly stabilized by many resonance forms. With the $\text{SN}_2$ reaction and the formation of a covalent enzyme-intermediate, the reaction is divided into two partial reactions, which lowers the activation energy as shown in Fig. 7. The overall enzyme catalyzed reaction requires the formation of a ternary complex in which the enzyme, the first substrate, and the second substrate must all be aligned at one time. The $\text{SN}_2$, double displacement mechanism, however, only requires the alignment of two things at a time. This, thus, gives the $\text{SN}_2$ mechanism an entropic advantage over the $\text{SN}_1$, single displacement mechanism. Further, in a single displacement reaction, after alignment, the enzyme must then somehow induce catalysis or reaction between the substrates to give products. With the double displacement mechanism, the formation of a covalent enzyme-intermediate provides a relatively stable, but high enough energy species (intermediate) that can readily react with the second substrate to give the product.

With carbohydrate enzymes that break or make glycosidic linkages, the formation of an oxycarbonium ion would give a species that is very unstable. This species could not provide for 100% retention or 100% inversion of the anomeric carbon, necessary to give the observed configuration of the product. No known enzyme catalyzed reaction gives racemization of an asymmetric optical center.

In summary, studies in the 20th Century on several different carbohydrate enzymes show that enzymes catalyze reactions by first forming an enzyme-substrate complex. This complex is formed by the interaction of the substrate with specific groups in the binding-site of the active site, as a key would specifically fit into its own lock, thus imparting specificity to the enzyme. After the formation of the ES complex, a catalytic nucleophile at the active site attacks the substrate by an $\text{SN}_2$ reaction, giving a covalent intermediate, simultaneously displacing a group from the substrate that is protonated by a second acidic catalytic group. The covalent intermediate has sufficient energy so that a second substrate reactant can readily displace the nucleophile, completing the reaction, giving a product with retention of the configuration, where an optical, asymmetric center is involved, and regenerating the nucleophile and the acid catalytic groups for the next reaction. Several $\text{SN}_2$-type mechanisms are given in Figs. 2–6 for different carbohydrate enzyme catalyzed reactions.

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Understanding Enzyme Catalysis


