Stereochemistry and the Mechanism of Enzymatic Reactions

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CONTENTS

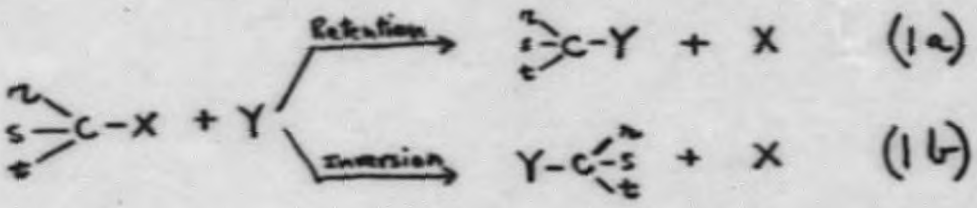
I. INTRODUCTION

The isolation and purification of an enzyme makes it possible to consider the reaction which it catalyzes from the standpoint of organic chemistry, i.e. as a reaction between organic molecules in which the catalyst happens to be a protein. Such a consideration allows the tools of physical organic chemistry to be applied to the study of the enzyme mechanism, and, since a number of enzymes have been purified, to obtain correlations of what might otherwise appear to be unrelated processes. One of the particularly powerful tools used in the elucidation of chemical mechanisms is stereochemistry, and it would be expected that similar conditions might lead to a clarification of enzymatic mechanisms and enzyme-substrate intermediates. In this article, the effect of enzymatic reactions on the configuration of the asymmetric carbon atoms involved in the reaction has been examined and the observed changes described by mechanisms which are compatible with both the chemical and biochemical evidence.

In most cases in which the substrate for an enzymatic reaction contains an asymmetric carbon atom, only one enantiomorph or diastereomer will react. In many of these cases, as in the proteolytic enzymes, the asymmetric carbon atom is one or more bond distances removed from the point of reaction and the configurational requirement is explained by the necessity for a particular geometric "fit" of attracting and repelling groups of the enzyme and substrate. In some cases, an asymmetric carbon atom is formed from previously symmetrical substrate molecules and Ogston (1948) has elegantly demonstrated how such reactions can lead to an understanding of the steric interactions of the enzyme - substrate complex. In other cases, an asymmetric carbon atom is present at the site of bond cleavage in both the substrate and

product molecules and it is these reactions which will provide the desired information about the mechanism of bond cleavage.

The enzymatic reactions considered in the article are all of the types shown in equation 1, where a group, X, at an asymmetric carbon atom is replaced by a group, Y, with either retention (Equation 1a) or inversion (Equation 1b) of stereochemical configuration. The enzymatic reactions in which the configurations of initial substrate and final product are known are summarized and mechanisms which account for the observations are discussed.



(1a)

(1b)

The two fundamentally different mechanisms that arise from these considerations are (a) a single displacement mechanism resulting in inversion, and (b) a double displacement mechanism resulting in retention. While the stereochemical evidence is of necessity limited to substrates containing an asymmetric carbon atom, the fairly wide range of reactions considered indicates that the mechanisms may have a general validity for any enzymatic substitution in which a group X is replaced by a group Y.

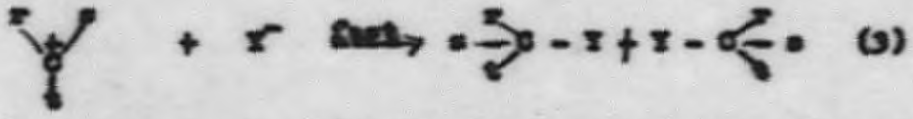
II. MECHANISM OF NON-ENZYMATIC SUBSTITUTION REACTIONS

Before discussing the enzyme mechanisms, it is desirable to review briefly the currently accepted chemical mechanisms for the replacement of a group X by a group Y at a saturated carbon atom. The mechanisms for these replacements have been established with notable success for a wide variety of chemical reactions in which X may be chloride, bromide, iodide, hydroxyl, glucosyl, methoxyl, thiomethyl, etc. and Y may be hydroxide ion, water, ammonia,

hydrosulfide ion, etc. Much remains to be explored and points of controversy still exist in many borderline cases but the mutual consistency of the stereochemical and kinetic evidence and the qualitative quantum mechanical arguments indicate that the basic features of the mechanisms can be accepted with considerable confidence. For detailed surveys of the literature, the reader is referred to the many reviews on the subject (e.g. Hammett, 1940; Winstein, 1951; and Hughes, 1951); in this paper only a simplified picture of the mechanisms will be described.

The most generally observed replacements can in the extreme be separated into two mechanisms which give either a racemic or an inverted product and which follow either "first order" or "second order" kinetics. The first, which pertains in the hydrolysis of phenylethyl chloride, for example, is depicted in equations 2 and 3. The initial and rate determining step in this mechanism is the ionization of rstC-X to give the free carbonium

(2)

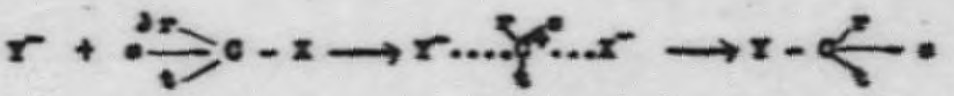


(3)

ion, rstC$^+$, and the anion, X$^-$. The free carbonium ion then reacts rapidly with Y$^-$ to give the final product, rstC-Y. Because the carbonium ion assumes essentially a planar configuration and because Y$^-$ can approach from either side, the final product in this reaction will be racemic even in those cases in which the initial reactant is optically active. In addition, the rate of reaction will be independent of the concentration or electron-sharing

tendency of Y^-. The addition of sodium hydroxide does not accelerate the hydrolysis of phenylethyl chloride in aqueous solution, for example, showing that the rate with the strong base, hydroxide ion, is no greater than with the relatively weak base, water. Since the rate depends only on the concentration of retCX to the first power, the reaction kinetics are first order.

The second extreme is the classic Walden inversion mechanism illustrated in equation 4. In this case, the attack of Y^- on the carbon atom occurs



(4)

in the rate determining step and is essentially simultaneous with the departure of X^-. The overall rate of the reaction depends, therefore, on both the concentration of Y^- and of retCX, i.e. the kinetics are second order. In the simultaneous attack by Y and departure of X, the retC bonds are inverted "like the vanes of an umbrella in a high wind".

Although these two mechanisms are clearly distinguishable in many cases, evidence is accumulating to show that they may represent differences in degree rather than kind. Thus, in the ionization mechanism, the rate depends only on the concentration of retCX in polar solvents, but the rate varies greatly with changes in solvent. Moreover, in a non-polar solvent both the hydrolysis of triphenylmethyl chloride and methyl bromide are third order reactions (Swain, 1948; Swain and Eddy, 1948). Since in polar solvents, the former reacts by the ionization mechanism and the latter by the Walden inversion mechanism, the similarity in kinetics in the non-polar solvent suggests that the two mechanisms may represent limiting cases of a more universal picture. In any case, it appears evident that the replacement of the group X is aided by electron-attracting molecules which have an affinity

for X and by electron-donor molecules which make a "backside" approach to the carbon from which X is departing. The magnitude of the effect that these electron-attracting and electron-donor molecules have on the reaction rate will depend on the conditions and the molecules undergoing reaction.

Whether these are called two limits of the same mechanism or two different mechanisms, correlation of mechanisms with types of compounds is sufficiently advanced so that knowledge of the chemical structure of the compound undergoing reaction and the experimental conditions is in many cases sufficient to make an accurate prediction of the mechanism of reaction. In aqueous solution, for example, tertiary halides such as tertiary butyl chloride, react by the ionization mechanism whereas primary halides such as methyl bromide react by the Walden inversion mechanism.

Another method of replacing a group X at an asymmetric carbon atom involves the formation of a cyclic intermediate and gives a product of retained configuration. This mechanism which has been referred to as an internal nucleophilic substitution reaction (Coudry, Hughes, Ingold, Masterman and Scott, 1937) occurs under restricted conditions, e.g. low temperature with certain reagents such as thionyl chloride or phosphorus pentachloride (cf. equation 5).

(5)

This mechanism occurs with the type of structures which tend to react by the ionization mechanism, e.g. the aryl alkyl carbinols (Dostrovsky, Hughes, and Ingold, 1946).

A glance at the substrates of the enzymatic reactions under consideration here shows that they are all compounds which would be expected to undergo the Walden inversion type mechanism. The ionization mechanism occurs with tertiary carbon atoms or aryl substituted secondary carbon atoms. The internal displacement mechanism occurs with similar compounds with certain reagents under special conditions. The substrates are almost all primary carbon atoms and the reagents could not give cyclic internal intermediates in most, if not all, of these cases. In spite of the fact that no direct analogy between the ionization or internal displacement and the enzymatic reactions seems likely, the close relationship between all the substitution mechanisms indicates that features of these two reaction mechanisms may be pertinent. For example, the favorable geometric arrangement afforded by the cyclic intermediate in the internal displacement may be roughly similar to the role of the enzyme which attracts the I^- molecule to a position favorable to reaction.

III. ENZYMATIC REACTIONS PROCEEDING WITH RETENTION OF CONFIGURATION

In Table 1 are listed the enzymes which catalyze reactions without changing the spatial relationships of the asymmetric carbon. The evidence for the configuration of substrate or product falls into two general categories. (1) If the compounds are stable they may be degraded by classical methods to compounds of known configuration, be tested as substrates with enzymes of known specificity and/or be examined in relation to theoretical rules of rotation. (2) If the compounds are unstable, the enzymatic reaction must be run in a polarimeter and the observed rotations during the course of the reaction compared with independently measured specific rotations of the pure substances. The type of evidence is listed for each substrate and

product.

It may seem at first sight that circular reasoning is involved when the configuration of a compound is established by enzymatic evidence and the stereochemical course of an enzymatic reaction is then based on this configuration. Actually it is not for the kind of reasoning utilized in Table 1. In that table the designation "enzyme specificity" is used to indicate that the particular compound is acted on by an enzyme whose absolute specificity for substrates of a particular configuration has been demonstrated. Thus, sucrose is assigned an alpha glucosidic configuration because it is hydrolyzed by maltase. This follows logically since maltase hydrolyzes only alpha glucosides. Nothing about the mechanism of the reaction is implied nor is a knowledge of the mechanism necessary for such a conclusion.

The erroneous use of enzymatic data arises when knowledge of the mechanism is essential but has not been obtained. For example, the fact that alpha glucose is the initial product of invertase action on sucrose has been cited as evidence that sucrose is an alpha glucoside. This follows only if the mechanism of the enzyme action is known to retain the alpha configuration. Similarly, Armstrong's (1903) assumption that α-glucose would be produced by the action of maltase was only a guess since the stereochemistry of maltase action was unknown. The pitfalls in this kind of evidence are best illustrated by alpha and beta amylase, which act on the same substrate, starch, giving alpha maltose in one case and beta maltose in the other.

Many reactions in addition to those listed undoubtedly proceed with retention but the appropriate data have not been obtained to classify them with certainty. (Some reactions which are closely analogous to those listed have been deliberately omitted to avoid excessive duplication. For example, potato phosphorylase as well as muscle phosphorylase is known to

catalyze reactions with retention of configuration.) It is evident, however, that the phenomenon of retention during enzymatic reactions has been established beyond doubt and that it is observed in a number of widely different enzyme types.

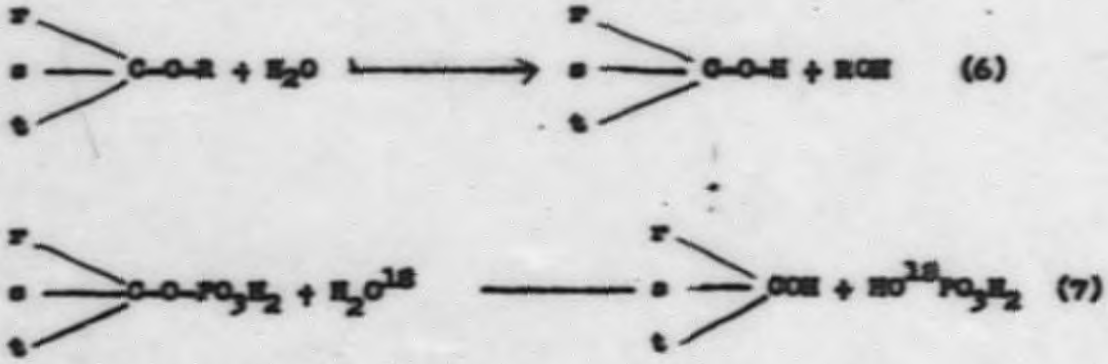
Since we are interested in the reaction mechanism of an individual enzyme, it is important that the reactions be the result of a single enzyme's action and not the net reaction of several separate enzymatic processes. Sufficient information is available from purification procedures to indicate that most of the reactions listed in Table 1 are indeed catalyzed by a single enzyme. A few, dextran sucrase for example, have only been tested in bacterial extracts and the product indicated could conceivably, therefore, be the net reaction obtained by the successive action of two or more enzymes. Because of their similarity to other enzymes whose purity is known, however, it is likely that they are single enzymes and it is desirable to include them in the table provided the qualifications with respect to their purity are remembered.

IV. MECHANISM OF THE REACTION PROCEEDING WITH RETENTION

In Armstrong's classic work on the glucosidases, it was assumed that the enzymatic reactions would proceed with retention of configuration. The correctness of this hypothesis in these cases has led many to assume that certain "steric restraints" are inherent properties of enzymes and that retention of configuration is the natural consequence of these "steric restraints". The fact that a number of enzymatic reactions proceed with inversion clearly indicates the necessity for a revision in our thinking and the desirability of replacing the vague "steric restraint" explanation with concrete and detailed mechanisms.

(1) Mechanism involving only apparent replacement at the asymmetric atom

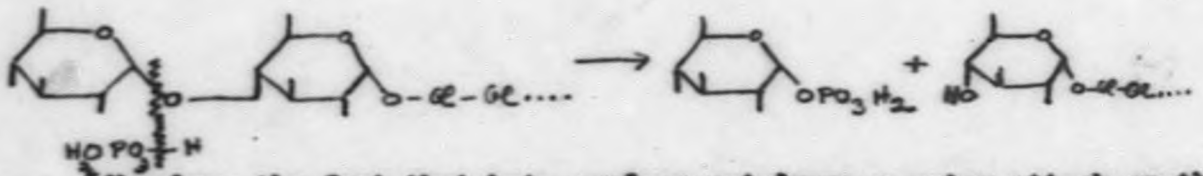
In examining the reactions which proceed by retention it is seen that some of the apparent replacements at the asymmetric carbon may not actually involve a reaction at this atom at all. For example, in hydrolytic reactions in which an -OR group is replaced by an OH group (cf. Equation 6), bond cleavage may actually occur between the O and the R rather than between the O and the C. In this case, no displacement occurs at the asymmetric carbon atom and no inversion would be expected whether or not "steric restraints" are present. A mechanism of this type is observed in the case of alkaline phosphatase where isotopic experiments have clearly shown that P-O bond cleavage rather than C-O cleavage is the enzymatic pathway (cf. Equation 7) (Cohn 1949; Stein and Koshland 1952). No inversion at the C-3 atom of the ribose molecule would occur, therefore, as a result of phosphatase action on adenosine-3-phosphate.



(6)

(7)

The isotopic evidence that phosphorolysis of the terminal glucose group in glycogen by muscle phosphorylase involves cleavage of the glycosyl-carbon ether-oxygen bond (Cohn, 1949) similarly explains the retention of configuration at the C-4 atom of the glucose remaining on the chain (Equation 8).

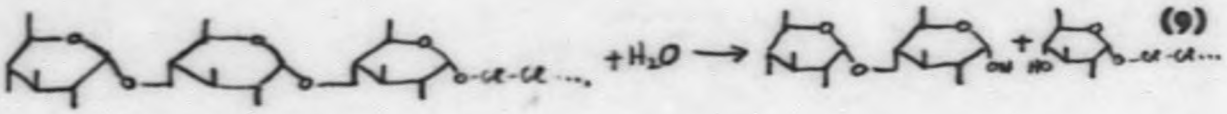


(8)

Likewise, the fact that beta amylase catalyzes a water attack on the C-1 carbon atom (see following section) explains the failure to observe any inversions at the C-4 atom of the glucose remaining on the polysaccharide.

(2) Mechanism involving an enzyme-substrate intermediate

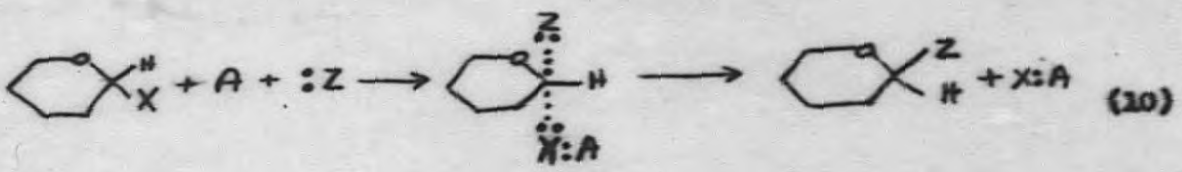
The mechanism discussed in the preceding section, although clearly pertinent in some cases involving retention, will not suffice for all. In the reactions of sucrose phosphorylase and muscle phosphorylase, for example, Cohn (1949) has shown by isotopic experiments that carbon-oxygen splitting occurs at the C-1 atom. Thus, these displacements must occur at the C-1 atom but the configuration of this atom in the final product is still unchanged. Similarly, in the trans-N-glycosidase (Kalckar, McElroy, and Hoff-Jorgensen, 1952) catalyzed reaction, the nature of the products and reactants shows that cleavage must occur between the C-1 atom of the ribose and the nitrogen of the purine and yet the configuration of the C-1 atom is retained. In the hydrolysis of starch catalyzed by alpha amylase, the cleavage point is not known but retention of configuration is observed on both sides of the oxygen atom involved in the split (cf. Equation 9). If it is assumed that cleavage occurs between the C-1 atom and the oxygen, the above described mechanism



(9)

explains the retention at the C-4 atom remaining on the polysaccharide but does not explain the retention at C-1, and vice versa. Another mechanism must, therefore, be devised to clarify the observed retentions at carbon atoms which are directly involved in the substitutions.

To do this is is necessary to consider the behavior of the analogous compounds in the absence of enzymes. A number of the replacement reactions at the anomeric carbon atom of glucose, for example, can be summarized by equation (10).

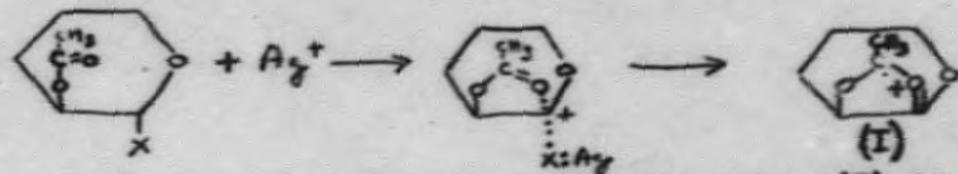


(10)

In this reaction the acidic or electron-seeking reagent, A, attracts the unshared electrons on the departing group, X, weakening the C-X bond. At essentially the same time, the electron-donor group, Z, approaches from the "backside" and ultimately forms a bond with the C-1 atom. Thus, the replacement of X occurs giving a product of inverted configuration. The Koenigs-Knorr reaction of alpha tetraacetyl glucosyl halides would be a typical example of this mechanism. In that case, the electron-seeking group, A, is the silver ion, the X group could be Cl, Br, I or F, and the entering group, Z, could be OMe, OAc, PO_3H_2, SO_3H, or benzyl (Pigman and Goepp, 1948). In acid catalyzed hydrolyses, the electron-seeking group may be the hydrogen ion, the departing X, an ethoxyl group, and the incoming, Z, a water molecule.

If the Koenigs-Knorr reaction is performed on a glycosyl halide having a trans acetoxy group at the C-2 atom, the essential features of the mechanism are similar but the stereochemistry of the product is changed (Frush and Isbell, 1941). In this case, the oxygen atom of the neighboring

acetoxy group is the electron-donor reagent which approaches the backside of the C-1 atom as the halide atom departs (cf. Equation 11). This results



(11)

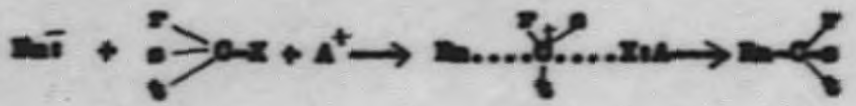
in the formation of a metastable intermediate (I) which then reacts with an electron-donor reagent in the solvent, to give a final product with the group Z on the same side of the ring as the initial group X (cf. Equation 12). The intermediate can resonate between several forms and a full covalent bond

(12)

between the oxygen and the carbon is probably not developed, but the force is sufficiently strong to preserve the configuration of the asymmetric atom and prevent racemization until the reaction with Z: has occurred. Thus, this mechanism essentially involves two successive Walden inversions to give a final product of retained configuration. The neighboring acetoxy group competes successfully with the group Z as the initial electron-donor reagent because of its favorable geometric position and its proximity to the backside of the C-1 atom.

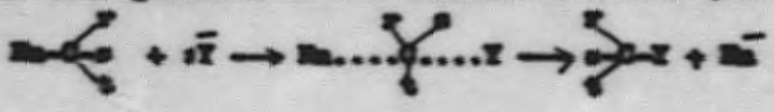
Applying these mechanisms to the enzymatic reactions, it might be expected that a group on the enzyme surface with an unshared pair of electrons would act as the initial backside attacking reagent analogous to Z: in equation (10) or to the oxygen of the neighboring acetoxy group in equation (11). The affinity of the substrate for the enzyme surface will

place the electron-donor group on the enzyme surface in a favourable position for reaction just as in the case of the adjacent acetoxy group. The result will be an enzyme-substrate intermediate of inverted configuration (cf. Equation 13) (Koshland, 1951, 1952). The intermediate can then react, again with inversion, with the second substrate, Y, to form a final product having the



(13)

same configuration as the initial substrate (cf. Equation 14). As in the case



(14)

of the neighbouring acetoxy group, the two successive inversions give an overall retention of stereochemical configuration.

In the above generalised equations, the electron-seeking group has been designated as A without indicating its source and the enzyme substrate intermediate has been written with a heavy line which might indicate a full covalent bond. Actually, A might come either from the solution, e.g. a hydrogen ion, or might be an electron-seeking site, e.g. a proton donor, on the enzyme surface. The heavy line joining the enzyme and the asymmetric carbon atom is used for convenience in this generalised formulation to indicate only that the overall attraction is sufficiently strong to prevent racemization. It is highly likely that the interaction between the carbon atom and the electron-sharing group on the enzyme surface will vary in the different enzymatic reactions. In some a full covalent bond may form. In others, a resonating transition intermediate analogous to the acetoxy neighboring group intermediate may be formed. In all cases, however, the enzyme-substrate interaction keeps the enzyme

on the "backside" of the substrate molecule and allows the approach of Y only from the front. It should be emphasized that the attraction of the enzyme for other parts of the substrate molecule, e.g. the hydroxyl groups on the C-2, C-4, etc. carbon atoms, will also tend to hold the substrate on the enzyme surface and will, therefore, allow a fairly weak interaction between the C-1 atom and the electron-donor group on the enzyme surface to retain the configuration of the intermediate.

The mechanism is probably best illustrated by taking a specific reaction such as the formation of sucrose catalysed by sucrose phosphorylase (Hassid and Doudoroff, 1951). Glucose-1-phosphate has the acid lability and alkali stability typical of acetals. It is, therefore, reasonable to expect an acidic or electron-seeking group in the enzyme-substrate complex to accelerate the departure of the phosphate ion. The specificity of the enzyme indicates the C-2, C-4, and C-6 hydroxyl groups are attracted to the enzyme surface. A schematic picture of the mechanism may then be seen in Figures 1 and 2. In Figure 1, as the bond between the carbon atom and the phosphate group breaks, the electron-donor site, D, starts to share its electrons with the backside of the C-1 atom of the glucose. The intermediate formed may not have a perfect tetrahedral arrangement of the bonds around the C-1 atom, but the relative arrangement of the enzyme-, H, C-, and C-groups is the opposite of the phosphate, H, C-, and C-groups in the substrate, In the second stage of the reaction the fructose molecule makes an electron sharing attack, displaces the enzyme, and forms the product sucrose with the alpha configuration (cf. Figure 2).

(3) Mechanism involving backside attack by a group in the substrate molecule

The above mechanism is consistent with all the chemical and biological evidence, but there remains the question of whether it is unique. Careful

Fig. 1. Formation of Glucose-Enzyme Intermediate from Glucose-1-phosphate.

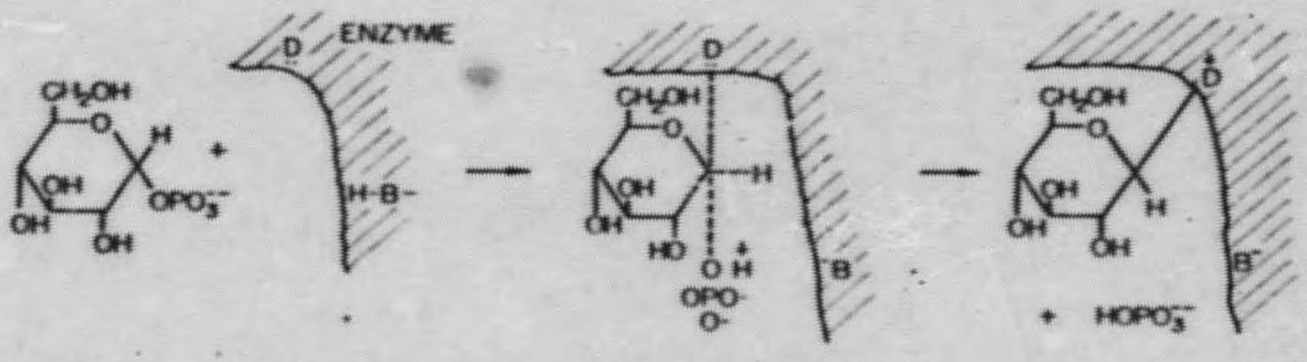
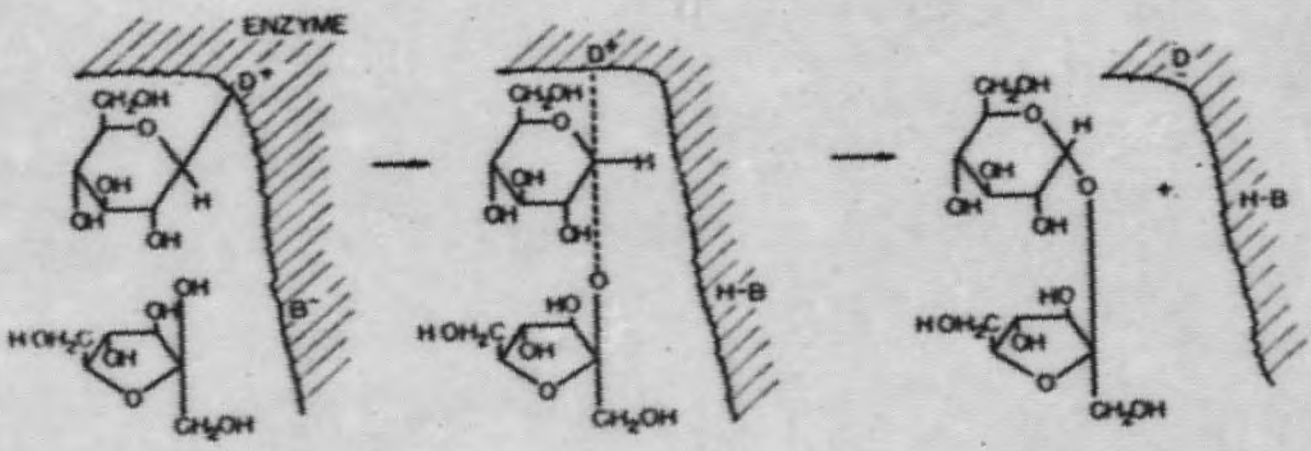

ENZYME
D
H-B⁻
B⁻
HOPO₃⁻

Fig. 2. Formation of sucrose from glucose-enzyme intermediate.


ENZYME
H-B

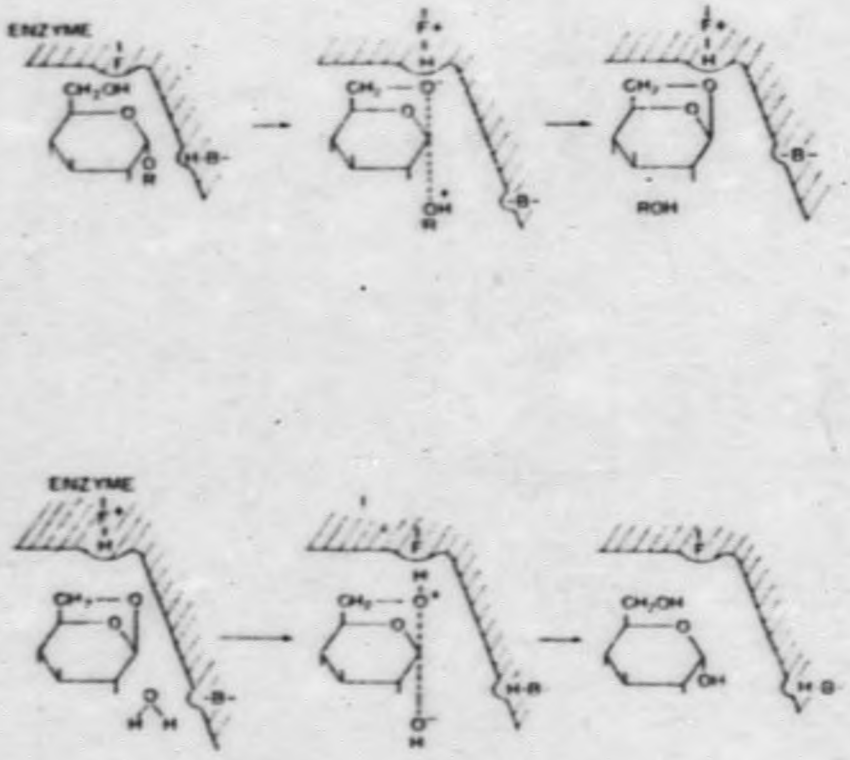
consideration indicates that there is one other mechanism, which, although less likely, cannot be excluded at present. This mechanism is essentially similar to that postulated above except that a group in the substrate molecule itself, rather than one on the enzyme surface, provides the electrons for the initial backside sharing. The backside sharing of electrons by hydroxyl groups with other positions in the same molecule is known and leads both to the formation of anhydro derivatives (Peat, 1946) and to neighboring group effects (Winstein, 1951). A schematic version of this is shown in Figures 3 and 4.

The general requirement for this type of mechanism is that there must always be a nucleophilic group in the substrate molecules in a position to make a backside attack. The enzyme would, therefore, exhibit "absolute specificity" for at least one nucleophilic group in this position, e.g. D-desrhamnose would not be a substrate for alpha glucosidase if the hydroxyl on C-6 is involved as an electron-donor. All the substrates listed in Table 1 have at least one trans hydroxyl group and the specificity studies indicate that not all the trans groups can be replaced or modified without destroying the susceptibility of the substrate to enzyme action. The specificity studies cannot be used, however, as evidence for such a mechanism because the replacement or modification of certain cis hydroxyls, also, destroys enzyme action. It can, at the present time, only be said that a potentially active hydroxyl group is available in those cases in which retention of configuration is known. Some typical data are summarized in Table 2.

There are a number of reasons for favoring the mechanism in which the enzyme provides the electrons for the backside attack. Firstly, coenzyme molecules are undoubtedly active as nucleophilic reagents (Koshland, 1952) and it is logical to expect similar action by groups on the enzyme surface. Secondly,

Fig. 3. Formation of anhydride intermediate from alpha glucoside.

Fig. 4. Formation of glucose from anhydride intermediate.


ENZYME
ROH
ENZYME

levoglucosan has been shown to be inactive in yeast extracts (Gottschalk, 1927) which contain maltase (Fischer, 1895) and other α-glucosidic enzymes (Kiessling, 1939; Schaffner and Specht, 1938). Thirdly, the similarity in specificity properties and the difficulty in separation of many of the carbohydrases suggests a similarity in mechanism that seems more reasonable with the enzyme as electron donor. Thus, beta mannosidase and beta galactosidase have a close resemblance in physical and catalytic properties (Veibel, 1950). The only hydroxy group, in beta mannose which is trans to the C-1 hydroxyl, however, is at C-4, whereas the only trans hydroxyl in beta galactose is at C-2. Fourthly, the appearance of a separate anhydride intermediate is not consistent with the kinetics of some of the purified enzymes. Fifthly, strong neighboring participation effects have been observed only for adjacent groups and the formation of four and five membered anhydro rings proceeds in many cases only if an epoxide intermediate is formed. Since no such adjacent trans group exists, for example, in alpha glucose derivatives reacting at the C-1 atom, and since levoglucosan is inactive, either 1,3 and 1,6 participation effects or a 1,3 anhydro ring must be postulated for the reactions of alpha glucose compounds. None of these seems particularly probable. These reasons are not conclusive, however, and further work with anhydride intermediates and specificity studies will be needed to establish individual mechanisms.

Whatever the source of electrons in the initial stage of the reaction, certain features are apparently common to both detailed mechanisms: (1) two discrete displacements occur, (2) an intermediate of inverted configuration is formed, and (3) the covalent bond of the departing group, X, is broken before the bond to the incoming X is formed. These features should then be common to all those reactions involving replacement with retention at an asymmetric carbon atom.

Other features, such as the relative rates of step 1 and step 2, may vary from enzyme to enzyme. Thus, both the sucrose phosphorylase reaction and muscle-phosphorylase reaction proceed by this mechanism whereas one enzyme catalyzes the exchange of phosphate with glucose-1-phosphate (Doudoroff, Barker, and Hassis, 1947) and the other does not (Cohn and Cori, 1948). Differences of this kind depend on the relative rates of formation and reaction of the inverted intermediate and the nature of the activation by substrate, X, and are readily accommodated within the framework of the proposed mechanism.

V. ENZYMATIC REACTIONS PROCEEDING WITH INVERSION

The number of known enzymatic reactions proceeding with inversion is less than the number in which the configuration of the asymmetric atom is retained. Those that are known are listed in Table 3.

These reactions all involve cleavage of a bond at an asymmetric carbon atom. Whether or not the reaction occurs by a displacement mechanism, however, is certainly ambiguous for the last two enzymatic reactions. In both of these cases, a change in configuration occurs but the same four groups are attached to the asymmetric carbon atom in the product as in the substrate. Reversible dehydrogenation and hydrogenation has been suggested as a possible cause of the observed change in spatial arrangement (Leloir, 1951) and an aldol type cleavage and recondensation is possible for the galactose-glucose transformation.

The first two reactions undoubtedly involve direct displacements on the asymmetric carbon atom. In these cases a group X is replaced by a group Y. In the glucose-galactose transformation where the four groups are the same the facts that the uridinediphosphoglucose is converted to uridinediphospho-galactose (Leloir, 1951), that galactose-1-C^{14} forms glucose-1-C^{14} (Topper and

Stetten, 1951) and that glycogen when synthesized from galactose in D_2O contains no more deuterium than when synthesized from glucose (Stetten and Klein, 1946) indicate that a direct displacement at the C-4 atom is the correct mechanism. The racemase action could be the result of a displacement, by hydride ion for example, but in this case other mechanisms, formation of a Schiff's base for example, seem more likely (Metzler and Snell, 1952; Olivard, Metzler, and Snell, 1952). There remain, then, three cases in which inversions probably occur by displacement in enzymatic reactions.

A mechanism which explains the observed inversion is that the two substrate molecules are attracted to adjacent sites on the enzyme surface and then a direct initial collision between the substrates occurs. Thus, in the type of mechanism illustrated in equation (10), the role of the group Z: which acts as the initial electron-donor molecule is taken by the second substrate Y. The simple observation of a net inversion can be explained by any odd number of displacements at the asymmetric carbon, but a mechanism of 3 or 5 successive displacements on the enzyme surface is highly improbable. Moreover, isotope evidence has been presented for the occurrence of a direct collision displacement reaction by the substrates on the surface of alkaline phosphatase (Stein and Koshland, 1952). It can be assumed, therefore, that those reactions involving a displacement with inversion proceed by a direct single step collision of substrates on the enzyme surface (cf. Figure 5 and discussion by Fitting and Doudoroff, 1952). In this mechanism, as in the mechanism leading to retention, activation by an electron-seeking group near X and a basic group near Y might be expected but these have been omitted from the figure to emphasize the relationship of Y, B, and X.

VI. NOMENCLATURE

In the previous discussion, we have used the terms "one step" and "two step" in discussing the mechanisms resulting in inversion and retention, respectively. This is correct if it is understood that reference is being made to the collision of the substrate molecules in the sense under discussion here. However, ambiguity may easily arise. Thus, the substrate molecules, coenzymes and metal ions are not all likely to be absorbed on the enzyme simultaneously even in a "one step" mechanism. It seemed desirable, therefore, to use a precise nomenclature which would specify clearly what is meant by the different mechanisms.

It is suggested that the mechanism illustrated in Figure 5 be referred to as a single displacement mechanism.

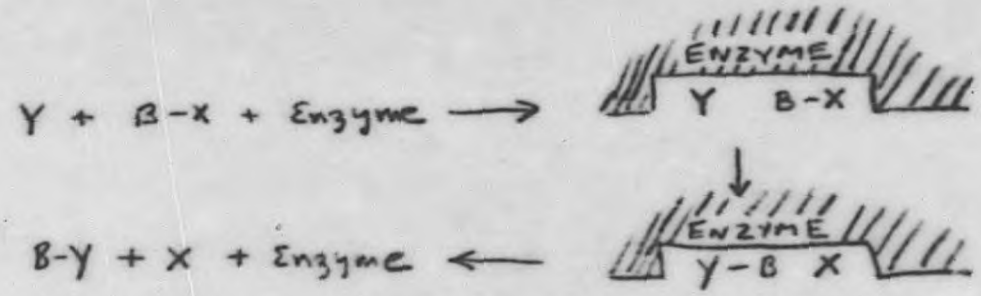


Figure 5

(Y, B and X may refer to atoms or groups of atoms and may be charged or uncharged.) The significant feature is that the covalent bond between B and X is broken simultaneously with or after the nucleophilic attack on B by molecule Y. The single displacement mechanism leads to inversion of configuration when asymmetric carbon atoms are involved.

The mechanism illustrated in equations (15) and (16) is consequently referred to as a double displacement mechanism. In this case it is

$$En + B\text{-}X \rightarrow En\text{-}B + X \qquad (15)$$

$$En\text{-}B + Y \rightarrow B\text{-}Y + En \qquad (16)$$

noted that the B-X bond is broken before the formation of the B-Y bond. The double displacement mechanism leads to retention when the reaction occurs at an asymmetric carbon atom.

VII. RELATIONSHIP TO OTHER ENZYMATIC SUBSTITUTION REACTIONS

As mentioned in the introduction, the diversity of the enzymatic reactions which can be correlated by the single and double displacement mechanisms suggests that the mechanisms may indeed be general for other enzymatic substitution reactions. The analogy between enzymatic behavior and the reactions of substrates in the absence of enzymes leads one to expect displacement mechanisms in many enzymatic reactions. As one example, the ability of acetyl phosphate to undergo displacements on either carbon or phosphorus in biological systems is paralleled by similar behavior in aqueous solutions in the presence of simple catalysts such as hydrogen and hydroxyl ions (Bentley, 1949; Koshland, 1952a). A reversal in mechanism of hydrolysis of this compound from phosphorus-oxygen cleavage to carbon-oxygen cleavage can also be caused at a single pH by a simple catalyst such as pyridine (Koshland, 1952a). It seems likely, therefore, that the enzymatic substitution reactions like their non-enzymatic analogs proceed largely by displacement mechanisms.

In the absence of an asymmetric carbon atom the rigorous proof that a particular reaction is either a single or double displacement is not simple, but there are several possible approaches. Doudoroff, Barker, and Hassid (1947) used the exchange of radioactive phosphate with glucose-1-phosphate in the presence

of sucrose phosphorylase as evidence for a glucose-enzyme intermediate. In this case the overall retention of configuration, i.e. the fact that the exchange is not accompanied by racemization, excludes the possibility that the exchange occurs by a single displacement mechanism. In the case of a symmetrical molecule kinetic and inhibitor data could in some cases be combined with a similar type of exchange as evidence for a double displacement mechanism. Mechaelis and Wilson (1951) have used inhibitor data to support a two step mechanism for acetyl cholinesterase. The powerful analogies between the action of glyceraldehyde phosphate dehydrogenase and the coenzyme A catalyzed transacetylations has led to the postulate of a two step mechanism for the transacetylase action of the dehydrogenase (Harting and Velick, 1952). In hydrolytic reactions involving compounds containing doubly bonded oxygen, the presence of O^{18} in the unhydrolyzed ester can be used as evidence for a single displacement mechanism (Stein and Koshland, 1952). Thus, it appears that other replacements of a group X by a group Y can be placed in the categories suggested by those containing an asymmetric carbon atom, and it is at least an enticing possibility that all enzymatic substitution reactions can be correlated by these mechanisms.

The role of a nucleophilic site on an enzyme surface which forms an enzyme substrate intermediate has interesting implications in relation to the evolution of coenzyme molecules. For example, the glyceraldehyde phosphate dehydrogenase which has been shown to have transacetylase properties (Harting and Velick, 1952) is a sulfhydryl enzyme and coenzyme A which is of such importance in acetyl transfer reactions has been shown to have the sulfhydryl group as the reactive portion (Lynen, Reichert and Rueff, 1951). It is possible that in the evolution of enzyme systems a small fragment containing an active site may have broken away from the larger protein molecule. The utilization

of this fragment as a shuttle may then have increased the efficiency of the metabolic system so that it remained as a coenzyme molecule.

Applying this reasoning in reverse, it might be well to look for groupings on enzyme surfaces analogous to those that are known to be active in coenzymes. For example, the importance of phosphate groups in coenzymes suggests that the phosphate in phosphorylated proteins may in some cases be the site of enzyme activity.

VIII. SUMMARY

In those enzymatic reactions involving the substitution of a group X at an asymmetric carbon atom by a group Y, the product has either the same configuration as the initial substrate or has an inverted configuration. Sixteen examples of the former and four examples of the latter are listed.

Mechanisms which account for the retention are (a) the replacement of the group X does not actually involve cleavage of a bond to the asymmetric carbon atom, and (b) the net replacement occurs by two successive displacements on the asymmetric carbon atom. The initial displacement in the latter case probably involves the backside attack by an electron-sharing site on the enzyme surface to form an enzyme-substrate intermediate of inverted configuration. The second displacement occurs when the group Y reacts, again with inversion, with the intermediate to form a product having the same configuration as the initial substrate. The possibility that the inverted intermediate is formed by backside attack of another functional group in the substrate molecule is considered improbable but not rigorously excluded.

Of the four examples in which the product has a different configuration from the initial substrate, three apparently proceed by direct displacement of the group Y on the asymmetric carbon atom. In this case the electron-repelling and attracting sites on the enzyme surface catalyze the reaction by their

polarizing effects but make no direct attack on the asymmetric atom.

These two mechanisms which are consistent with the biochemical evidence and physical organic theory may apply not only to reactions at asymmetric carbon atoms but to many other enzymatic substitution reactions as well. They correlate the observed analogies between some reactions occurring on holo-enzymes with those which are known to proceed through coenzyme intermediates and suggest a possible mechanism for the evolution of coenzymes. They also give clues in the search for other active groups on the enzyme surface.

IX. REFERENCES

ARMSTRONG, E. F. (1903). Study on enzyme action. I. The correlation of the stereoisomeric and glucosides with the corresponding glucoses. J. Chem. Soc. 83, 1305.

BALLOU, C. E., ROSEMAN, S., & LINK, K. P., (1951). Reductive cleavage of benzyl glycosides for relating anomeric configurations. J. Am. Chem. Soc. 73, 1140.

BATES, C. J. (1942). "Polarimetry, saccharimetry and the sugars", Circular C-440 of the National Bureau of Standards, P. 411.

BENTLEY, R. (1949). Mechanism of hydrolysis of acetyl dihydrogen phosphate J. Am. Chem. Soc. 71, 2765.

BERGMAN, M., SCHOTTE, H. & LECHNISKY, W. (1922). Über 2-desoxyglucose. Ber. 55, 158.

COHN, M. (1949). Mechanisms of cleavage of glucose-1-phosphate. J. Biol. Chem. 180, 771.

COHN, M. & CORI, C. (1948). On the mechanism of action of muscle and potato phosphorylase. J. Biol. Chem. 175, 89.

CORI, C. F., COLOWICK, S. P. & CORI, G. T. (1937). The isolation and synthesis of glucose-1-phosphoric acid. J. Biol. Chem. 121, 470.

CORI, C. F., SCHMIDT, G. & CORI, G. T. (1939). The synthesis of a polysaccharide from glucose-1-phosphate in muscle extract. Science 89, 464.

CORI, G. T., SWANSON, M. A., & CORI, C. F. (1945). Mechanism of formation of starch and glycogen. Federation Proceedings 4, 234.

COWDRY, W. A., HUGHES, E. D., INGOLD, C. K., MASTERMAN, S. & SCOTT, A. D. (1937). Reaction kinetics and the Walden inversion VI. Relation of steric orientation to mechanism in substitutions involving halogen atoms and simple or substituted hydroxyl groups. J. Chem. Soc. 1267.

DAVOLL, J., LYTHGOE, B. & TODD, A. R. (1946). The configuration at the glycosidic centre in natural and synthetic pyrimidine and purine nucleosides. *J. Chem. Soc.* 833.

DAVOLL, J., LYTHGOE, B. & TODD, A. R. (1948). A. Synthesis of adenosine. *J. Chem. Soc.* 967.

DAKER, V. D. & STACEY, M. (1939). The polysaccharide produced from sucrose by betabacterium vermiforme (Ward-Mayer). *J. Chem. Soc.* 985.

DOBTROVSKY, I., HUGHES, E. D. & INGOLD, C. K. (1946). Mechanism of substitution at a saturated carbon atom. The role of steric hindrance. *J. Chem. Soc.* 186.

DOUDOROFF, M., BARKER, H. A. & HASSID, W. Z. (1947). The mechanism of action of sucrose phosphorylase as a glucose-transferring enzyme. *J. Biol. Chem.* 168, 725.

DURSCH, H. R. & REITHEL, F. J. (1952). The hydrolysis of glucose-1-phosphate. *J. Am. Chem. Soc.* 74, 830.

FAIRHEAD, E. C., HUNTER, M. & HIBBERT, H. (1938). The structure of dextran synthesised by Leuconostoc Mesenteroides. *Can. J. Research* B16, 151.

FISCHER, E. (1894). Einfluss der configuration auf die wirkung der enzyme I. *Ber.* 27, 2985.

FISCHER, E. (1894a). Einfluss der configuration auf die wirkung der enzyme II. *Ber.* 27, 3479.

FISCHER, E. (1895). Ueber den einfluss der configuration auf die wirkung der enzyme III. *Ber.* 28, 1429.

FITTING, C. & DOUDOROFF, M. (1952). Phosphorolysis of maltose by enzyme preparations from Neisseria meningitidis. *J. Biol. Chem.* 199, 153.

FREEMAN, G. G. & HOPKINS, R. H. (1936). The mechanism of degradation of starch by amylase. III. Mutarotation of fission products. *Biochem. J.* 30, 451.

FRENCH, D. (1951). Structure of Pan's crystalline trisaccharide, Science 113, 352.

FRENCH, D., PAZUR, J., LEVINE, M. & NORBERG, B. (1948). Reversible action of amoerumamylase. J. Am. Chem. Soc. 70, 3145.

FREUDENBERG, K., BLOMQUIST, G., EWALD, L. & SOFF, K. (1936). Hydrolyse und acetolyse der starke und der schardingerdextrine. Ber. 69, 1258.

FREUDENBERG, K. & FRIEDRICH, K. (1930). Methylierte tri-, und tetrasaccharide aus cellulose und stark. Naturviss 18, 1114.

FREUDENBERG, K. & SOFF, K. (1936). Uber den abbau der starke mit acetylbromid. Ber. 69, 1252.

FRIES, N. L. & ISBELL, H. S. (1941). Sugar acetates, acetylglycosyl halides, and orthoacetates in relation to the Walden inversion. J. Res. National. Bur. Standards 27, 413.

GOTTSCHALK, A. (1927). Garungs-und phosphorylierungversuche an xanthorunhydrin. Z. physiol. chem. 170, 23.

HAMMETT, L. P. (1940). "Physical organic chemistry", McGraw Hill, New York. p. 131.

HARTING, J. & VELICK, S. (1952). Reactions of acetyl phosphate catalyzed by 3-phosphoglyceraldehyde dehydrogenase. Federation Proceedings 11, 226.

HASSID, W. Z. & BARKER, H. A. (1940). The structure of dextran synthesized from sucrose by betacoccus arabinosaceus, orla jensen. J. Biol. Chem. 134, 163.

HASSID, W. Z., CORI, G. T. & MERRADY, R. M. (1943). Constitution of the polysaccharide synthesized by the action of crystalline muscle phosphorylase. J. Biol. Chem. 148, 89.

HASSID, W. Z. & DOUDOROFF, M. (1950). Adv. in Carbohydrate Chem. 5, 29.

HAWORTH, W. N. & PERCIVAL, E. G. (1931). Evidence of continuous chains of α-gluco-pyranose units in starch and glycogen. J. Chem. Soc., 1342.

HERSE, E. J. & HAMILTON, D. M. (1946). Bacterial synthesis of an amylopectin-like polysaccharide from sucrose. J. Biol. Chem. 166, 777.

HERSE, E. J., HAMILTON, D. M., & CARLSON, A. S. (1949). Synthesis of a polysaccharide of the starch-glycogen class from sucrose by a cell-free bacterial enzyme system. J. Biol. Chem. 177, 267.

HELFERICH, B. & GRUNLEF, S. (1927). Die p-Tolualsulfoester des vanillin-β-d-glucosids und ihre spaltbarkeit durch mandelemulsin. XXVIII Mitt. Über Emulsin. J. Prakt. Chem. (2) 148, 107.

HELFERICH, B., KLEIN, W., & SCHAEFER, W. (1926). Zur spezifität der α-glucosidase aus hefe. Ber. 59, 79.

HELFERICH, B. & LANGE, O. (1933). Über emulsin XI., Z. physiol. Chem. 216, 123.

HESTRIN, S. (1949). Action pattern of crystalline muscle phosphorylase. J. Biol. Chem. 179, 943.

HUDSON, C. S. (1908). The inversion of cane sugar by invertase. J. Am. Chem. Soc. 30, 1564.

HUDSON, C. S. (1909). The significance of certain numerical relations in the sugar group. J. Am. Chem. Soc. 31, 66.

HUDSON, C. S. (1909). The inversion of cane sugar by invertase III. J. Am. Chem. Soc. 31, 655.

HUGHES, E. D. (1951). Reaction of halides in solution, Quarterly Reviews (London), 5, 245.

KALCKAR, H. M. (1947). Studies of the enzymes of purine metabolism. J. Biol. Chem. 167, 461.

KALCKAR, H. M., MACNUTT, W. S. & HOFF-JORGENSEN, E. (1952). Trans-N-glycosidase studied with radioactive adenine. Biochem. J. 50, 397.

KIESSLING, W. (1939). Über den das glykogen phosphorylierenden fermentprotein-komplex und eine enzymatische reversible glykogensynthese. Bio. Z. 302, 90.

KOSHLAND, D. E. JR. (1951) The mechanism of the hydrolysis of acetyl phosphate and its relation to some enzymatic reactions. "Phosphorus Metabolism" 1, 536 Edited by W. D. McElroy and Bentley Glass, Johns Hopkins Press, Baltimore.

KOSHLAND, D. E. JR. (1952). Retention of configuration and the mechanism of enzyme action. Federation Proceedings 11, 262.

KOSHLAND, D. E. JR. (1952a) Effect of catalysts on the hydrolysis of acetyl phosphate. Nucleophilic displacements in enzymatic reactions. J. Am. Chem. Soc. 74, 2286.

KUHN, R. (1925). Der wirkungsmechanismus der Amylasen: ein Beitrag zum konfiguration sproblem der starke. Ann. Chem. Justus Liebigs, 443, 1.

LEBOIR, L. F. (1951). The metabolism of hexosephosphates. "Phosphorus metabolism". Edited by McElroy and Glass, Johns Hopkins Press, Baltimore, 1, 77.

LING, A. R. & NANJI, D. R. (1925). The constitution of polymerised amylose, amylopectin, and their derivatives, J. Chem. Soc. 127, 629.

LYNEN, F., REICHERT, E. & RUEFF, L. (1951). Zum biologischen abbau der essigsaure. Article No. VI. Aktivierte essigsaure ihre isolierung aus hefe und ihre chemische natur. Ann. Chem. Justus Liebigs 574, 1.

MEACHER, W. R. & HASSID, W. Z. (1946). Synthesis of maltose-1-phosphate and D-xylose-1-phosphate. J. Am. Chem. Soc. 68, 2135.

METZLER, D. E. & SNELL, E. E. (1952). Deamination of serine. J. Biol. Chem. 198,353.

MEYER, K. H., GURTLER, P. & BERNFELD, P. (1947). Structure of amylopectin, Nature 160, 900.

MEYER, K. H. (1942). Recent developments in starch chemistry. Adv. in Colloid Science 1, 143.

MEYER, K. H., BERTHOUD, V. & STEINHEIL, P. (1940). Recherches sur l'amidon (XI) Sur la nonhomogeneite de l'amidon. Helv. Chim. Acta 23, 845.

MEYER, K. H., HOPFF, H. & MARK, H. (1929). Ein beitrag zur konstitution der starke. Ber 62, 1103.

MONTGOMERY, E. M., WEAKLEY, F. B. & HILBERT, G. E. (1947). Isolation of 6-[α-D-glucopyranosyl]-D-glucose (Isomaltose) from enzymic hydrolysates of starch. J. Am. Chem. Soc. 71, 1682.

NEUBACK, K. & AHLBORG, K. (1940). Uber Grenzdextrine und starke. XXIII mitteilung: spezifitat der amylasen und produkte ihrer wirkung. Biochem. Z. 311, 213.

NEUBACK, K. & AHLBORG, K. (1940). Uber grenzdextrine und starke VIII mitteilung: die konstitution eines starkegrenzdextrins. Nachweis α-glykosidischer 1,6-bindungen in dextrin und starke. Biochem. Z. 307, 69.

NACHMANSOHN, D. & WILSON, I. B. (1951). The enzymic hydrolysis and synthesis of acetylcholine. Adv. in Enzymology, 12, 259.

OSTON, A. G. (1948). Interpretation of experiments on metabolic processes, using isotopic tracer elements. Nature 162, 963.

OLIVARD, J., METZLER, D. E. & SNELL, E. E. (1952). Catalytic racemization of amino acids by pyridoxal and metal salts, J. Biol. Chem. 199, 669.

O'SULLIVAN, C. & TOMPSON, F. W. (1890). Invertase: A contribution to the history of an enzyme of unorganised ferment. J. Chem. Soc. 57, 834.

PEAT, S. (1946). The chemistry of anhydro sugars. Adv. in Carbohydrate Chemistry 2, 38.

PEAT, S., SCHLOTTERER, E. & STACEY, M. (1939). Constitution of the dextran produced from sucrose by Leuconostoc dextranicum (betacoccus arabinosaceus hemolyticus). J. Chem. Soc. 581.

PETERSEN, S. R. (1934). Zur fermentativen spaltung von disaccharidglykosiden. Chem. Zentr. 1934, I, 1825.

PICMAN, W. W. & GOEPP, R. M. JR. (1948). "Chemistry of the Carbohydrates", Academic Press, p. 444.

PICMAN, W. W. & GOEPP, R. M. JR. (1948a) ibid p. 162.

POTTER, A. L. SOWDEN, J. L., HASSID, W. Z. & DOUDOROFF, M. (1948). ∝-L-glucose-1-phosphate. J. Am. Chem. Soc. 70, 1751.

REITHEL, F. J. & CLAUCOMB, C. K. (1949). Synthesis of derivatives of glucose-4-phosphoric acid. J. Am. Chem. Soc. 71, 3669.

SAMEC, M. (1935). Uber die wirkung von β-amylase auf einige starkesubstanzen. (4. mitteilung uber enzymatische amylolyse in der von M. Samec und E. Waldschmidt-Leitz begonnen untersuchungsreihe). Z. Physiol. Chem. 236, 103.

SCHAFFNER, A. & SPECHT, H. (1938). Uber die amylasen der hefe und uber die umsetzungen der glucose-1-phosphorsaure durch hefe extrakte. Naturwiss.26,494.

STEIN, S. S. & KOSHLAND, D. E. JR. (1952). Mechanism of action of alkaline phosphatase. Arch. Biochem. & Biophys. 39:229.

SWAIN, C. G. (1948). Kinetic evidence for a termolecular mechanism in displacement reactions of triphenyl methyl halides in benzene solution. J. Am. Chem. Soc. 70, 1119.

SWAIN, C. G. & EDDY, R. W. (1948). Concerted displacement reactions XI. Termolecular displacement reactions of methyl halides in benzene solution. J.Amer.Chem.Soc.70, 2989.

STEIN, S. S. & KOSHLAND, D. E. JR. (1952a). Mechanism of the phosphatase-catalyzed hydrolysis of phosphate esters. Am. Chem. Soc. Abstracts, 122nd meeting P. 11C.

STETTEN, D. JR. & KLEIN, S. (1946). The origin of the stable hydrogen in glycogen formed from various precursors. J. Biol. Chem. 165, 157.

TOPPER, Y. J., & STETTEN, D. JR. (1951). The biological transformation of galactose into glucose. J. Biol. Chem. 199, 149.

VEIBIL, S. (1950). Beta glucosidase. "The Enzymes", Edited by Sumner and Myrback, Vol. 1. Part 1. P. 621.

WEIDENHAGEN, R. (1928). Zur frage der enzymatischen rohrzuckerspaltung. Naturwiss 16, 654.

WEIDENHAGEN, R. (1928a). Uber die enzymatische rohrzuckerspaltung. Z. ver. deut. Zucker-Ind., 78, 539.

WEINSTEIN, B. (1951). Neighboring groups in displacements and rearrangements. Bull. Soc. Chim. France, 18, 655.

WOLFROM, M. L., SMITH, C. S., FLETCHER, D. E. & BROWN, A. E. (1942). The beta form of the Cori ester (d-glucopyranose-1-phosphate). J. Am. Chem. Soc. 64, 23.

WOLFROM, M. L., SMITH, C. S., & BROWN, A. E. (1943). Application of the mercaptatelation assay to synthetic starch. J. Am. Chem. Soc. 65, 255.

WOLFROM, M. L., THOMPSON, A. & CALKOWSKI, T. T. (1951). 4-α- Isomaltopyranosyl-D-glucose. J. Am. Chem. Soc. 73, 4093.

WOOD, W. A. & GUNSALUS, I. C. (1951). D-alanine formation: a racemase in streptococcus faecalis. J. Biol. Chem. 190, 403.

Table 1.

Enzymatic Substitution Reactions which Proceed with Retention of Configuration

Enzyme	Substrate	Evidence for Substrate Configuration	Product	Evidence for Product Configuration
1. Sucrose phosphorylase	∝-glucose-1 phosphate	Optical rotation[1]	∝-glucose-1-fructose (Sucrose)	Cleaved by maltase[2]
2. Alpha amylase	Starch	Degradation to maltose derivatives[3], optical rotation[4], attacked by ∝-glucosidase[5].	∝-maltose and/or ∝-dextrins	Mutarotates downwards[6].
3. ∝-glucosidase	∝-methyl glucoside	Optical rotation[7] analogy to benzyl compounds[8].	∝-glucose	Mutarotates downwards[9].
4. β-glucosidase	β-methyl glucoside	Optical rotation[7] analogy to benzyl compounds[8].	β-glucose	Mutarotates upwards[9]
5. Muscle phosphorylase	∝-glucose-1-phosphate	(see above)	Amylose-like polysaccharide	Degraded to maltose[10,11]
6. Invertase	Sucrose	(see above)	∝-glucose	Mutarotates downwards[12]
7. Beta amylase[13]	Amylose	(see above)	Amylose polymer of n-2	Degradation to maltose[10]
8. Alkaline phosphatase	Glucose-4-phosphate	Synthesis[14]	Glucose	Chromatographic identification[15]
9. Acid phosphatase	Glucose-4-phosphate	Synthesis[14]	Glucose	Chromatographic identification[15]

Table 1 Continued

	Enzyme	Substrate	Evidence for Substrate Configuration	Product	Evidence for Product Configuration
10.	Trans-N-glycosidase	Inosine	Deamination of adenosine[16]	Adenosine	Synthesis, Metaperiodate degradation[17]
11.	Amylosucrase	Sucrose	(see above)	Glycogenic polysaccharide	Enzyme specificity[18] degraded to maltose[19]
12.	Transglucosidase of A. Oryzae	Maltose	Optical rotation, enzyme specificity[20]	Panose	Degradation to maltose and isomaltose[21]
13.	Dextransucrase	Sucrose	(see above)	Dextran	Optical rotation[22]
14.	Dextrandextrinase	Dextrin	Degraded to maltose	Dextran	Optical rotation[22]
15.	Q enzyme	Amylose	(see above)	Amylopectin	Degradation to α-1,6 disaccharide[23]
16.	Bacillus macerans amylase	Amylose	(see above)	Cyclomaltose	Split ring hydrolyzed by β-amylase[24]

(1) Cori, Colowick, and Cori (1937); Wolfrom, Smith, Fletcher and Brown (1942).

(2) Weidenhagen (1928a and b).

(3) Freudenberg and Soff (1936); Freudenberg and Friedrich (1930); Haworth and Percival (1931).

(4) Freudenberg, Klonquist, Knald, and Soff (1936); Meyer, Hopff and Mark (1929).

(5) Meyer (1942) (1947).

(6) Kuhn (1925); Freeman and Hopkins (1936).

(7) Hudson (1909); Bates and associates (1942).

(8) Ballou, Rosenman, and Link (1951).

(9) Armstrong (1903)

(10) Ling and Nanji (1925); Meyer, Brentano, and Bernfeld (1940); Samec (1935).

(11) Haseid, Cori, and McReady (1943).

(12) O'Sullivan and Tompson (1890); Hudson (1908)(1909).

(13) This evidence demonstrates that beta amylase retains the configuration of the C-4 atom. It causes inversion of the C-1 atom as discussed in the following section.

(14) Reithel and Claycomb (1949).

(15) Dursh and Reithel (1952).

(16) Kalckar (1947).

(17) Davoll, Lythgoe and Todd (1946) (1948).

(18) Mehre and Hamilton (1946); Mestrin (1949).

(19) Mehre, Hamilton and, Carlson (1949).

(20) Pigman and Goepp (1948).

(21) French (1951); Wolfrom, Thompson, Galkowski (1951).

(22) Fairhead, Hunter, and Hibbert (1938); Peat, Schlueterer and Stacey (1939); Haseid and Barker (1940); Baker and Stacey (1939).

(23) Myrbäck and Ahlborg (1941); Myrbäck and Ortenblad (1941); Montgomery, Weakley, and Hilbert (1947).

(24) Cori, Swanson, and Cori (1945).

Table 2

Potentially Active Groups in the Substrate Molecule

Enzyme	Position of Trans Hydroxyl Groups	Position of Groups Whose Replacement or Modification Destroys Enzyme Activity
Sucrose Phosphorylase	C-3, C-6	C-2, C-4, C-6 (1)
Muscle Phosphorylase	C-3, C-6	C-2, C-4, C-6, (1, 2)
α-D-glucosidase	C-3, C-6	C-2, C-4, C-6 (3)
β-D-glucosidase	C-2, C-4	C-2, C-3 (4)

(1) Meagher and Hassid (1946), Potter, Sowden, Hassid, and Doudoroff, (1948).

(2) Cori, Schmidt, and Cori (1939); Wolfrom, Smith, and Brown (1943).

(3) Bergmann, Schotte, and Lechinsky (1922); Fischer, (1894); Peterson (1934); Helferich, Klein, and Schaefer (1926).

(4) Helferich and Gruniler (1937); Helferich and Lange (1933).

Table 3

Enzymatic Reactions which Invert the Configuration of the Asymmetric Atom

Enzyme	Substrate	Evidence for Configuration of Substrate	Product	Evidence for Configuration of Product
Maltose phosphorylase	Maltose	Optical rotation, Enzyme specificity (1)	β-glucose-1-phosphate	Identical with synthetic compound (2)
Beta amylase	Amylose	Degraded to maltose (3)	β-maltose	Mutarotates upwards (4)
Galactosaldehase	Uridine diphosphoglucose	Hydrolysis to glucose (5)	Uridine diphosphogalactose	Hydrolysis to galactose (5)
Alanine racemase	L-alanine	Rotation (6)	D-alanine	Reacts with D-amino acid oxidase (6)

(1) Pigman and Goepp, (1948a).

(2) Fitting and Doudoroff, (1952.)

(3) Ling and Nanji (1925); Meyer, Brentano, and Bernfeld (1940); Samec, (1935).

(4) Hahn (1925); Freeman and Hopkins, (1936).

(5) Leloir (1951).

(6) Wood and Gunsalus (1951).