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Stereochemistry Involved in the Mechanism of Action of Dextransucrase in the Synthesis of Dextran and the Formation of Acceptor Products[1]

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A proposed sequence of events in the synthesis of dextran and in the formation of acceptor products by dextransucrase from *Leuconostoc mesenteroides* B-512F has been developed with molecular models. The following mechanism is postulated: (1) two nucleophiles at the active site displace fructose from two sucrose molecules, giving two β-glucosyl intermediates; (2) these two β-glucosyl units rotate together so that the C_6-hydroxyl of each is apposed to the α-side of C_1 of the other; (3) one glucosyl unit assumes a boat conformation in which the bond to the enzyme is axial; (4) the C_6-hydroxyl oxygen of the other glucosyl unit makes a nucleophilic attack on C_1 of the first, displacing the enzyme nucleophile and making an α-1,6 bond; (5) rotations about the new α-1,6 linkage remove the transferred glucose from the active site. The free enzyme nucleophile attacks another sucrose as in step (1), and then steps (2)–(5) are repeated as the reducing-end glucosyl unit of the growing chain assumes the boat conformation and is attacked by the C_6-hydroxyl of the new glucosyl unit, which displaces the enzyme nucleophile and forms another α-1,6 linkage, about which rotations occur to remove the growing dextran chain from the active site. An additional feature of the mechanism presented here is a pair of enzymic proton-exchange groups, which protonate the glycosidic oxygen of sucrose to facilitate cleavage, and then remove a proton from the attacking C_6 hydroxyl during the polymerization reaction.

Acceptors are polyhydroxy compounds which are capable of nucleophilic attack on enzyme-bound β-glucosyl or dextranosyl units to give α-glucosides or dextranosides. Noting the broad acceptor specificity of the enzyme and the unusual structure of some of the acceptor products, we have proposed that acceptor specificity is determined not by an enzymic binding site per se, but by the formation of hydrogen-bonded complexes between the acceptors and the glucosyl or dextranosyl enzyme intermediates. The acceptor attack on C_1 of the β-glucosyl enzyme is mediated by the same proton-exchange group as that proposed for catalysis of polymerization. It is shown that specific multiple hydrogen bonding to the glucosyl–enzyme intermediate can account for the formation of the observed acceptor products from α-methyl-D-glucoside, D-fructopyranose, isomaltose, maltose, β-D-mannopyranose, β-D-galactofuranose, cellobiose, lactose, β,β-trehalose, α,β-trehalose, and raffinose.

INTRODUCTION

Dextrans are a family of α-1,6-glucans synthesized from sucrose by dextransucrases, elaborated by members of the genera *Leuconostoc* and *Streptococcus*

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(Lactobacillaceae). Many of these polysaccharides, including the commercially important dextran produced by *L. mesenteroides* NRRL B-512F, consist of α-1,6 linked glucose units with about 5% α-1,3 branch linkages.

Using pulse and chase techniques with [^{14}C]sucrose and dextransucrase from *L. mesenteroides* B-512F, which had been covalently attached to Bio-Gel P2 beads, Robyt *et al.* (*1*) found that dextran is synthesized from the reducing end and that during synthesis glucose and dextran are covalently attached to the active site of the enzyme. Robyt *et al.* (*1*) proposed a mechanism (see Fig. 1A) for the synthesis in which two nucleophiles at the active site, X_1 and X_2, attack two bound sucrose molecules to give two glucosyl units covalently linked to the nucleophiles through carbon-1. In subsequent steps, the C_6-hydroxyl (the primary hydroxyl group) of one of the glucose units effects a nucleophilic attack on C_1 of the other glucose unit, forming an α-1,6 glucosidic linkage. Simultaneously this transformation releases the active site nucleophile, which can now attack another sucrose molecule to give a new glucosyl unit attached to the active site. The C_6-hydroxyl of this new glucosyl unit then attacks the C_1 of the isomaltosyl unit, forming another α-1,6 linkage; and in the presence of sufficient sucrose, this process continues with the two catalytic groups, X_1 and X_2, at the active site, alternately forming covalent complexes with glucose and the growing dextran chain. The process is stopped when an acceptor molecule interacts with the active site and one of its hydroxyl groups attacks the C_1 of the enzyme–glucosyl or dextranosyl unit and releases it from the enzyme (see Fig. 1B) (*2*).

The catalytic groups at the active sites of many enzymes involve both a Lewis

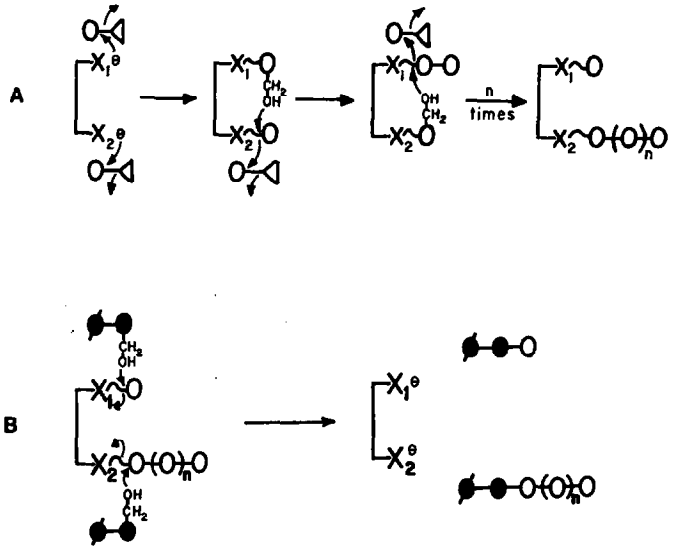

FIG. 1. Schematic presentation of the mechanisms proposed for the action of dextransucrase by Robyt *et al.* (*1, 2*). (A) Mechanism for the polymerization of dextran. (B) Mechanism for some acceptor reactions. X_1 and X_2, catalytic nucleophiles at the active site; O–△, sucrose in which O is a glucosyl unit; △, fructosyl unit; O–O, glucopyranosyl units linked by an α-1,6-glucosidic bond; and ●–●, disaccharide acceptor molecule, in which ● is a reducing monosaccharide unit.

acid and a Lewis base (an electrophile and a nucleophile) (*3*). In the reaction of dextransucrase the nucleophiles (or X groups) are represented in the present study as phosphate groups and the electrophiles or proton donors as protonated amino groups (see Fig. 2 for the mechanism of cleaving sucrose by dextransucrase). We favor phosphate or pyrophosphate groups as the nucleophiles, as they would give covalent intermediates that are relatively stable and yet have sufficient energy for the synthesis of the α-1,6 linkage. A carboxylate ion has also been suggested as a possible nucleophile (*4*). Likewise an imidazolium ion has been suggested (*4, 5*) as being involved and could be the proton donor as well as a protonated amino group. The exact nature of the nucleophile and the proton donor, however, has no real bearing on the mechanistic details considered here.

In the reaction, there is retention of the α-configuration of glucose from sucrose to dextran, and there is the formation of a covalent glucosyl–enzyme intermediate (*1*). These two conditions suggest a double displacement mechanism (*6*) which would require that the glucose be covalently attached to the enzyme by a β-glucosyl linkage.

The proposed mechanism for dextran formation was presented in schematic form (*1*) (see Fig. 1A). In the present study, we have used models to create a three-dimensional picture of dextran synthesis. We show that the glucosyl–enzyme intermediate and the growing dextranosyl chain can be stereochemically oriented to give the formation of the α-1,6 bond as required by the proposed mechanism and that the growing dextran chain can be transferred back and forth between the two nucleophilic groups without excessive movement of the whole chain. We also develop a hypothesis for the specificity and mechanism of the

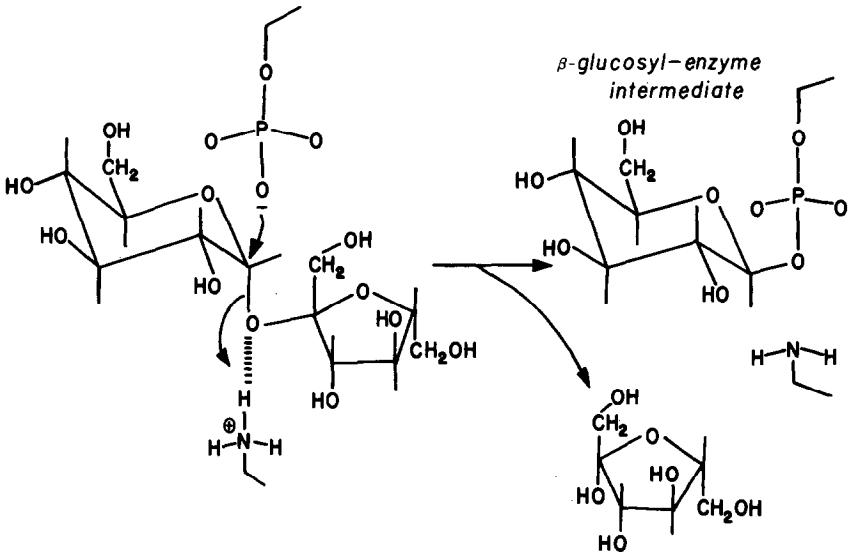

FIG. 2. Reaction mechanism for the cleavage of sucrose by the enzyme catalytic groups and the formation of a covalent glucosyl–enzyme intermediate by dextransucrase.

acceptor reactions, which occur when other carbohydrate molecules are present or are added to the dextransucrase–sucrose digest.

METHODS

The models of Figs. 3 and 5 were constructed from a "Minit" molecular model kit (Cochrane's of Oxford, Leafield, Oxford, U.K.). Models were suspended by white threads above a white background and photographed with a 35-mm camera.

With a scale of 2 cm = 1 Å, the model bond lengths are as follows: C–C and C–O, 1.5 Å; C–H, O–H, and N–H, 1.0 Å; phosphate P–O, 1.8 Å; and N—O and O—O for hydrogen bonds, 3.0 Å. Some of the schemes shown in Figs. 3 and 5 were also reproduced with Ealing CPK space-filling models to ascertain the permissibility of nonbonded interatomic distances.

RESULTS AND DISCUSSION

Mechanism of Dextran Synthesis

Figure 3A shows the model of half of the active site in which the phosphate nucleophile is attacking C_1 of the glucose moiety of sucrose and the protonated amino group is donating a proton to the leaving fructose moiety. In the full active site this reaction occurs with two sucrose molecules and two sets of catalytic groups (Fig. 1A, step I) to give two β-linked enzyme–glucosyl complexes (Fig. 3B). Each glucose unit rotates around the phosphorus–oxygen–carbon bonds so that its C_6-hydroxyl group is positioned opposite C_1 of the other glucose unit (Fig. 3C).

Nucleophilic displacement of the phosphate group requires that the face of C_1 opposite the leaving group be accessible. To place the phosphate leaving group in

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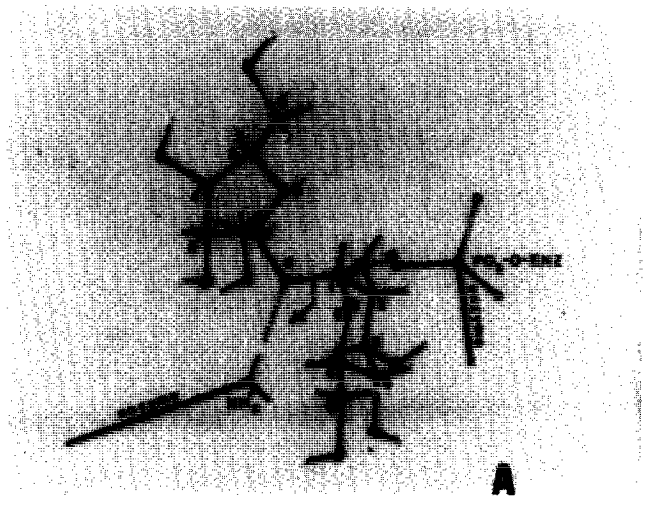
FIG. 3. Mechanistic sequence for the synthesis of dextran by dextransucrase showing stereochemical relationships using models. Hydrogen bonds are shown as striped bonds. Carbon atoms are numbered; ring and acetal oxygens are identified. (A) One-site (half-active site) nucleophilic attack on sucrose by the enzyme catalytic groups, phosphate and protonated amino group, to give a glucosyl–enzyme intermediate. (B) Two glucosyl–enzyme intermediates (full active site). (C) Two glucosyl–enzyme intermediates rotated into position to place each of their C_6-hydroxyl groups opposite C_1 of the other glucosyl unit. (D) Glucosyl unit on the bottom in a boat conformation and the glucosyl unit on top with its C_6-hydroxyl hydrogen bonded to the enzyme amino group and the oxygen atom attacking C_1 of the bottom glucosyl unit. (E) Isomaltosyl–enzyme complex after formation of the α-1,6 glucosidic bond. (F) Rotation around the ψ bond of the newly formed α-1,6 linkage. (G) Rotation around the ϕ and ω bonds of the newly formed α-1,6 linkage to completely move the nonreducing glucose residue of the isomaltosyl unit from the active site. (H) Isomaltosyl unit on one site, and the catalytic groups of the other site attacking another sucrose molecule. (I) Isomaltosyl unit on one site and glucosyl unit on the other site. (J) Rotation of the glucosyl unit into position for its C_6-hydroxyl group to attack C_1 of the isomaltosyl unit. (K) Isomaltotriosyl-enzyme complex after formation of the α-1,6 linkage. (L) Removal of the isomaltotriosyl chain from half of the active site by rotations around ϕ, ψ, and ω bonds of the newly synthesized α-1,6 linkage. (M) A side view of the extended dextranosyl chain and a glucosyl unit in position for its C_6-hydroxyl group to attack C_1 of the chain.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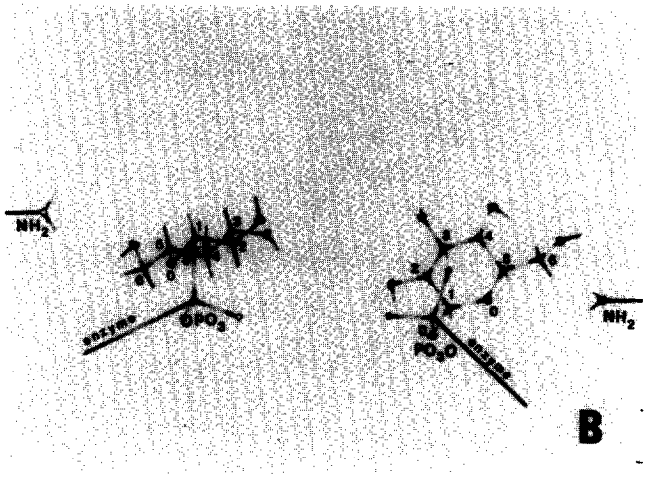
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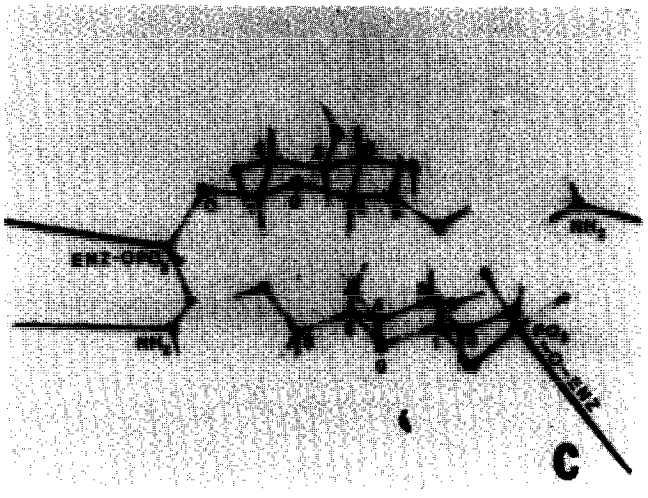
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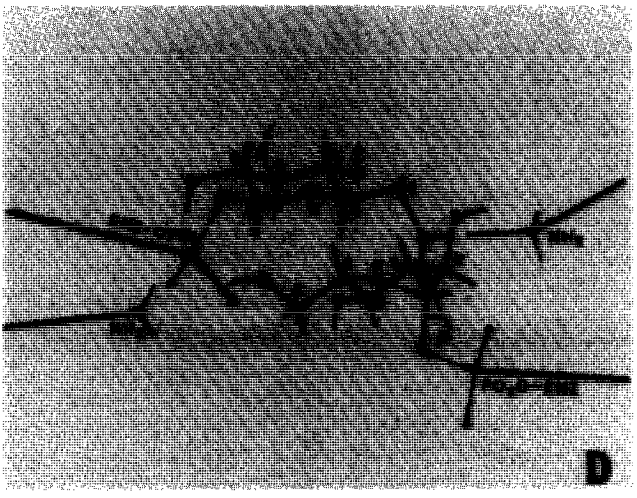
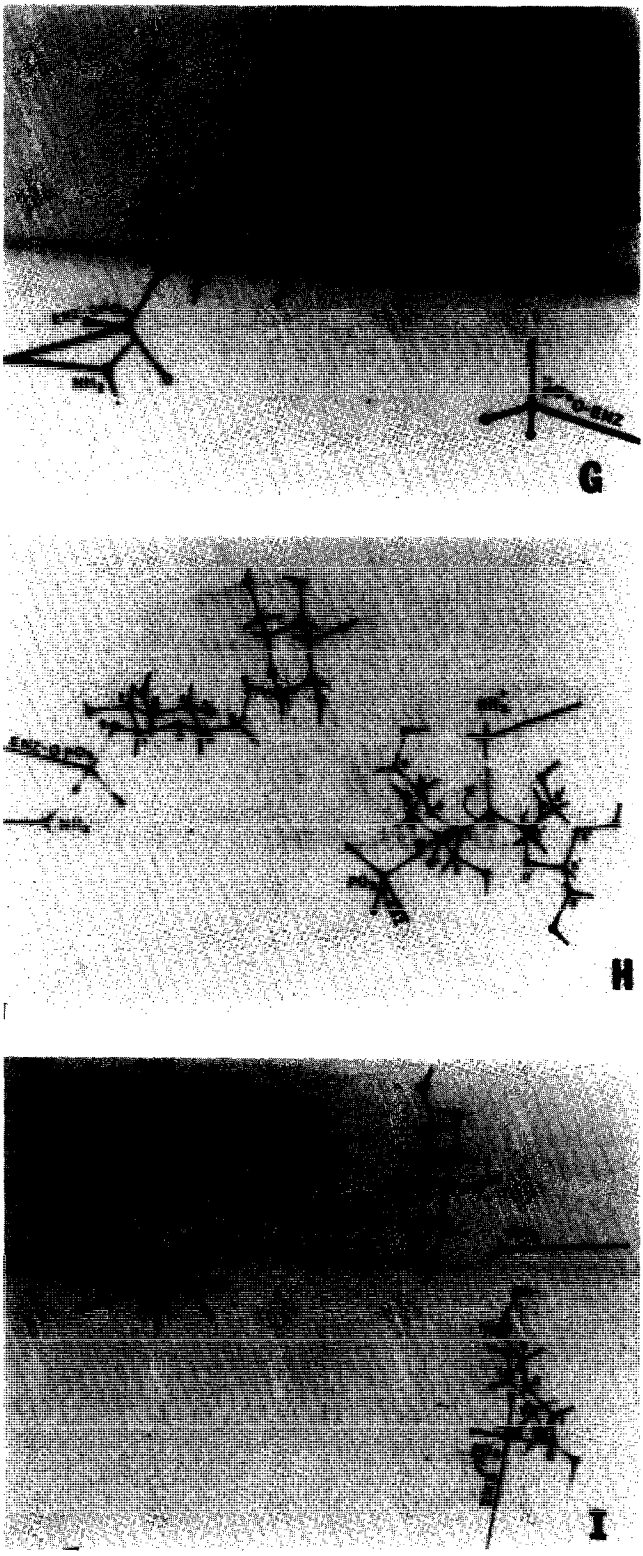


FIG. 3—(*Continued*).



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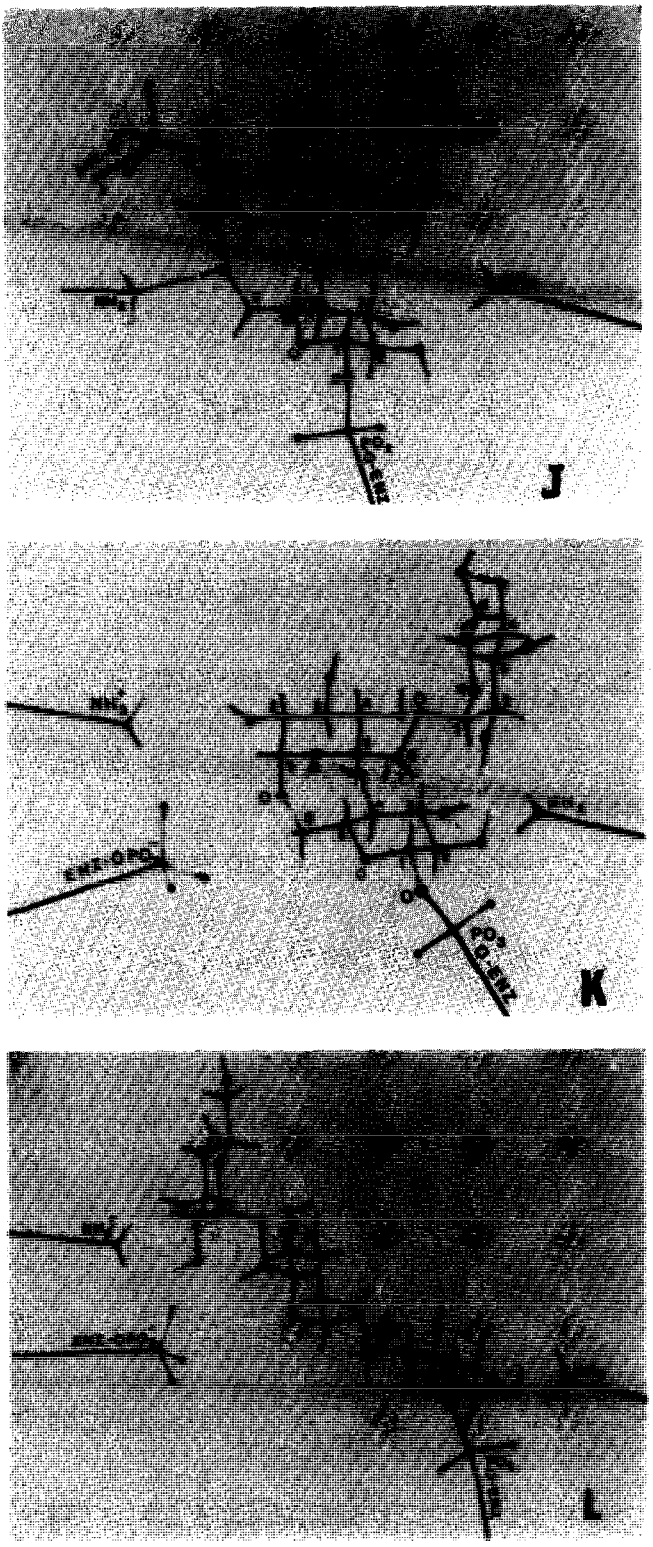

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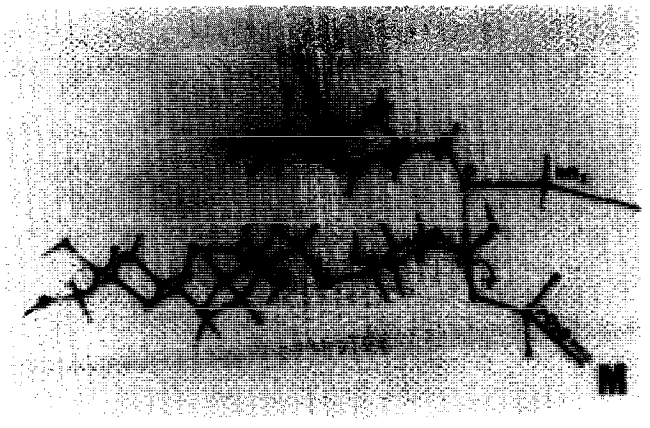


FIG. 3—(*Continued*).

this stereochemical arrangement with respect to the attacking C_6-hydroxyl group, the glucosyl unit must have a boat or twist conformation in which the phosphate group is axial to the glucose ring (Fig. 3D). In addition, a basic group from the enzyme is required to abstract the proton from the C_6-hydroxyl group. The originally protonated amino group, which contributed its proton to the leaving fructose, can now act as this base and abstract the proton from the C_6-OH group. The formation of this hydrogen bond increases the nucleophilic character of the hydroxyl oxygen and facilitates its attack on C_1. During the reaction (most likely proceeding in a concerted manner) the hydrogen is abstracted by the amino group, the phosphate group is displaced, and an α-1,6 glucosidic bond is formed between the two glucose units (Fig. 3E). The glucose unit, that has just been attacked and released from the active site phosphate-group, leaves the site by rotating around first the ψ bond (Fig. 4) of the newly synthesized α-1,6 linkage and then the ϕ and ω bonds (Fig. 3F and G). The α-1,6 glucosidic linkage is extremely flexible because of the three bonds—ϕ, ψ, and ω—that make up the linkage (see Fig. 4). Rotations around these bonds can readily occur (*7*, *8*). These rotations remove the

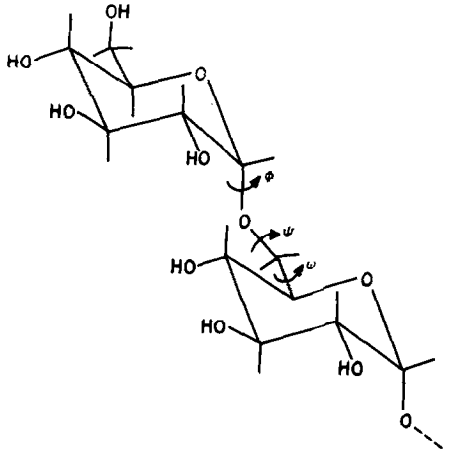


FIG. 4. ϕ, ψ, and ω bonds of the α-1,6 glucosidic linkage.

glucose unit from the active site and in effect produce an extrusion of the growing dextran chain (in this specific example, an isomaltose unit) from the enzyme active site.

The catalytic groups, the released phosphate and the resulting protonated amino group, are now in a form to cleave another sucrose molecule. Figure 3H shows the phosphate group making an attack on C_1 of the glucosyl unit of a third sucrose molecule and the protonated amino group donating a proton to the oxygen atom of the leaving fructose unit. The glucose unit becomes attached to the phosphate by a β-glucosidic bond (Fig. 3I). It now rotates around the phosphorus–oxygen–carbon bonds as before. This places its C_6-OH in position to form a hydrogen bond with the amino group on the other side. The hydroxyl oxygen then makes a nucleophilic attack onto C_1 of the isomaltosyl unit in which the first glucosyl unit is in a boat conformation (Fig. 3J). There is then formed another α-1,6 glucosidic bond which results in an isomaltotriosyl–enzyme complex (Fig. 3K). Rotations around the ϕ, ψ, and ω bonds of this newly synthesized linkage move the glucose unit penultimate from the reducing end out of the active site (Fig. 3L). The polymerization of the chain continues in a similar manner as long as there are sucrose molecules available for donating glucose units and acceptor reactions do not occur to release the dextran from the active site. Figure 3M shows a side view of the growing dextranosyl chain and the glucosyl–enzyme complexes.

The two site model presented explains (a) the retention of the configuration of C_1 of the glucose unit from sucrose to dextran, (b) the covalent attachment of both glucose and dextran to the active site, and (c) the addition of the glucose unit to the reducing end of the growing dextran chain. The covalent attachment of glucose and dextran to the active site preserves the high energy of the sucrose linkage which is necessary for the synthesis of polysaccharide. The mechanism postulates a minimum number of catalytic groups that operate as general acid–base catalysts to promote the cleavage of sucrose and the formation of α-1,6 linkages between glucose units. The present study demonstrates that the two-site model, in which glucose and dextran alternate between the two sites, is quite feasible on a stereochemical and a molecular basis.

A one-site model would fail to account for the simultaneous linkage of glucose and dextran to the active site. Further, a one-site model in which only glucose is covalently attached to the enzyme would not provide the high-energy bond at the reducing end of dextran necessary for the synthesis of a glycosidic linkage.

A model for reducing end addition, entailing two nonequivalent sites of attachment, one for glucose and the other for dextran, would result in the wrong stereochemistry for the glycosidic bond. A glucosyl unit transferred from sucrose to the glucosyl site would have the β-configuration. After the C_6-hydroxyl of the glucosyl unit displaces the dextran from the dextranosyl site, the chain would have to be transferred to the dextranosyl site by displacement by the dextranosyl site group. The linkage of the chain to this group would be α. The displacement of the chain by the C_6-hydroxyl of the glucose unit would thus result in a β-1,6 linkage between the glucose units. The resulting polysaccharide would be a β-glucan instead of the observed α-glucan. Of course, a second glucosyl site could be

postulated so that glucose is transferred twice on the enzyme before it is incorporated into polysaccharide. This could be extended indefinitely to give the correct stereochemistry as long as there were an even number of glucosyl sites. It would seem unnecessary, however, to postulate *n* number of glucosyl sites when the model with two equivalent sites suffices.

Mechanism of Acceptor Reactions

The so-called acceptor reactions occur when carbohydrate molecules, other than sucrose, are present or are added to the dextransucrase–sucrose digest (Fig. 1B). The specificity of the acceptor reaction is very broad (i.e., many different carbohydrate molecules will act as acceptors), in contrast to the narrow specificity

TABLE 1

OBSERVED ACCEPTORS AND THEIR PRODUCTS IN THE REACTION OF DEXTRANSUCRASE AND SUCROSE

Acceptor	Product(s)	Reference
D-Glucose	Isomaltose	17
α-Methyl-D-glucoside	α-Methylisomaltoside	9
D-Fructose	Leucrose and isomaltulose	15, 16
D-Mannose	α-D-Glucopyranosyl-β-D-mannopyranoside	11
D-Galactose	α-D-Glucopyranosyl-β-D-galactofuranoside	11, 12
Isomaltose	Isomaltotriose	13, 17
Maltose	6^2-α-D-Glucopyranosyl maltose (panose)	13, 17, 18, 28
Cellobiose	2^1-α-D-Glucopyranosyl cellobiose	13
β,β-Trehalose	β-Isomaltosyl-β-D-glucopyranoside	13
α,β-Trehalose	β-Isomaltosyl-α-D-glucopyranoside and α-isomaltosyl-β-D-glucopyranoside	13
α,α-Trehalose	None	13
Kojibiose (2-α-D-glucopyranosyl-D-glucose)	2-α-Isomaltosyl-D-glucose	13
Nigerose (3-α-D-glucopyranosyl-D-glucose)	3-α-Isomaltosyl-D-glucose	13
Sophorose (2-β-D-glucopyranosyl-D-glucose)	2-β-Isomaltosyl-D-glucose	13
Laminaribiose (3-β-D-glucopyranosyl-D-glucose)	3-β-Isomaltosyl-D-glucose	13
Gentiobiose (6-β-D-glucopyranosyl-D-glucose)	6-β-Isomaltosyl-D-glucose	13
Leucrose (5-α-D-glucopyranosyl-D-fructopyranose)	5-α-Isomaltosyl-D-fructopyranose	17
Lactose (4-α-D-galactopyranosyl-D-glucose)	2^{Glc}-α-D-Glucopyranosyl lactose	19
Lactulose (4-β-D-galactopyranosyl-D-fructose)	4^{Fru}-β-D-Galactopyranosyl sucrose	20
Isomaltulose (6-α-D-glucopyranosyl-D-fructose)	6-α-Isomaltosyl-D-fructose	21
Melibiose (6-α-D-galactopyranosyl-D-glucose)	Structure not determined	17, 22
3-O-Methyl-D-glucose	6-α-D-Glucopyranosyl-3-O-methyl-D-glucose	23
Theanderose (6^{Glc}-α-D-glucopyranosyl sucrose)	6^{Glc}-α-Isomaltosyl sucrose	24
Raffinose (6^{Glc}-α-D-galactopyranosyl sucrose)	2^{Glc}-α-D-Glucopyranosyl raffinose	22
Riboflavin	5′-D-Riboflavin-α-D-glucopyranoside	25
Glycerol	Structure not determined	26
D-Sorbitol	Structure not determined	26
Dextran	α-1,3-Dextranosyl dextran or α-1,3-glucopyranosyldextran	8, 10, 27, 28

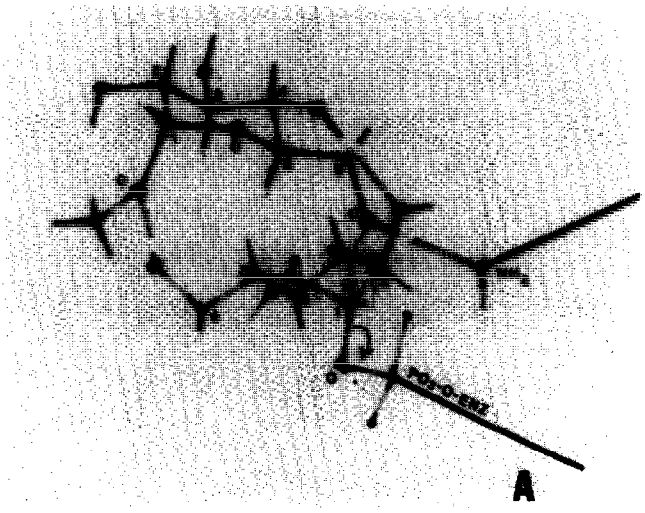
of the substrate sucrose (*8, 9*). There have been at least 30 different acceptor products reported (Table 1). The first acceptor product results from the addition of the glucosyl unit or dextranosyl unit to the acceptor molecule (*2*). If the acceptor is a low-molecular-weight carbohydrate, the displacement of the glucosyl unit results in an oligosaccharide (*2*). The displacement of the dextranosyl chain results in dextran terminated in the reducing end by the acceptor (*2*). Subsequent products may occur with the acceptor product acting as an acceptor. In the case of the oligosaccharide product, a series of homologous oligosaccharides results (*2, 10*). When a dextran chain is the acceptor, the glucosyl or dextranosyl units of the enzyme complex are added to the dextran acceptor through the formation of an α-1,3 glucosidic branch linkage (*11*).

With many of the acceptors, e.g., D-glucose, α-methyl-D-glucoside, isomaltose, maltose, β,β-trehalose, and α,β-trehalose, the product results in the attachment of a glucopyranosyl unit to the acceptor by an α-1,6 glucosidic bond (Table 1). Some of the acceptor molecules, however, give unusual products. For example, D-mannose and D-galactose give nonreducing disaccharides, α-D-glucopyranosyl-β-D-mannopyranoside (*12*) and α-D-glucopyranosyl-β-D-galactofuranoside (*12, 13*). Cellobiose gives an unusual reducing trisaccharide, in which a glucopyranosyl unit is linked to C_2 of the reducing moiety giving 2^1-α-D-glucopyranosyl cellobiose (*14*). α,β-Trehalose gives two trisaccharides depending on whether the α- or β-linked glucose moiety of trehalose furnishes the acceptor hydroxyl (*14*). Two carbohydrates, α,α-trehalose (*14*) and D-xylopyranose (*15, 18*), have been reported not to be acceptors.

By using models of the acceptor molecules to consider the formation of specific acceptor products, we have developed a hypothesis for the mechanism of the acceptor reaction. We postulate that in view of the broad specificity of the acceptor reactions, the acceptor molecules do not bind to a specific acceptor binding site on the enzyme. Rather, the acceptor molecules form hydrogen-bonded complexes with the glucosyl- or dextranosyl–enzyme complexes. The resulting ternary complexes lead to the specific products that are observed. The models show (Fig. 5 and Table 2) that specific hydrogen bonds can be formed between the acceptor and the glucosyl–enzyme complex.

We considered 12 acceptors whose products are known. They are the commonly encountered acceptors, α-methyl-D-glucoside, D-fructose, isomaltose, and maltose, and acceptors that gave unusual products, D-mannose, D-galactose, cellobiose, lactose, the trehaloses, and raffinose. The acceptors, the products, and the specific hydrogen bonds that can be formed to give the observed products are summarized in Table 2. The monosaccharides form three hydrogen bonds, two with the glucosyl unit and one with the catalytic amino group of the enzyme. D-Fructose is an exception; it forms four hydrogen bonds, three with the glucosyl unit and one with the catalytic amino group. As in the mechanism for the synthesis

FIG. 5. Hydrogen bonded complexes of nine acceptor molecules with the covalent glucosyl–enzyme intermediate. (A) α-methyl-D-glucoside, (B) β-D-mannopyranose, (C) β-D-galactofuranose, (D) β,β-trehalose, (E) α,β-trehalose (α-glucosyl unit attacking), (F) α,β-trehalose (β-glucosyl unit attacking), (G) cellobiose, (H) β-isomaltose, (I) α-maltose.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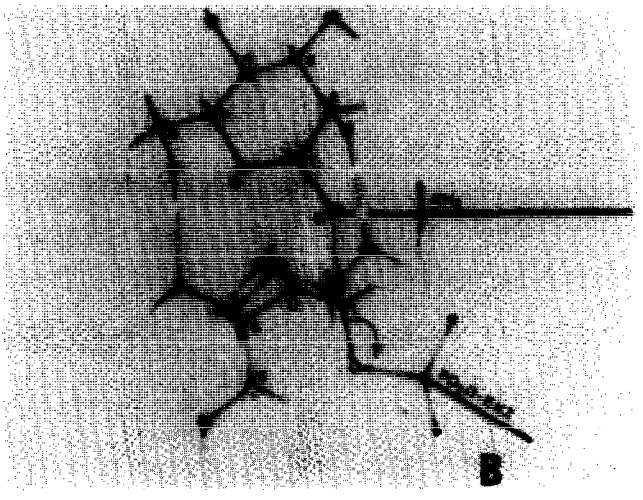
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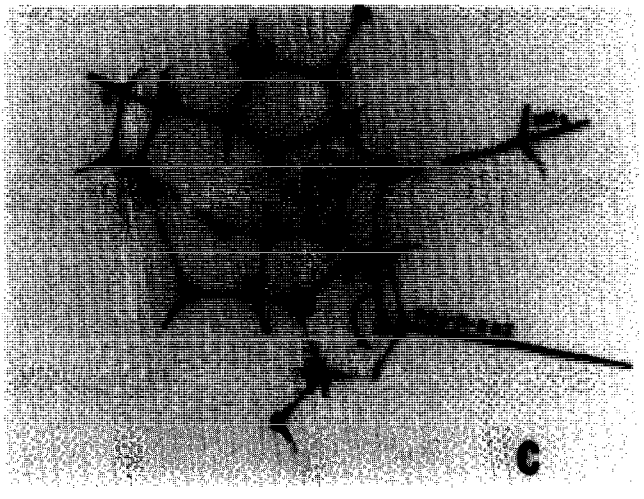
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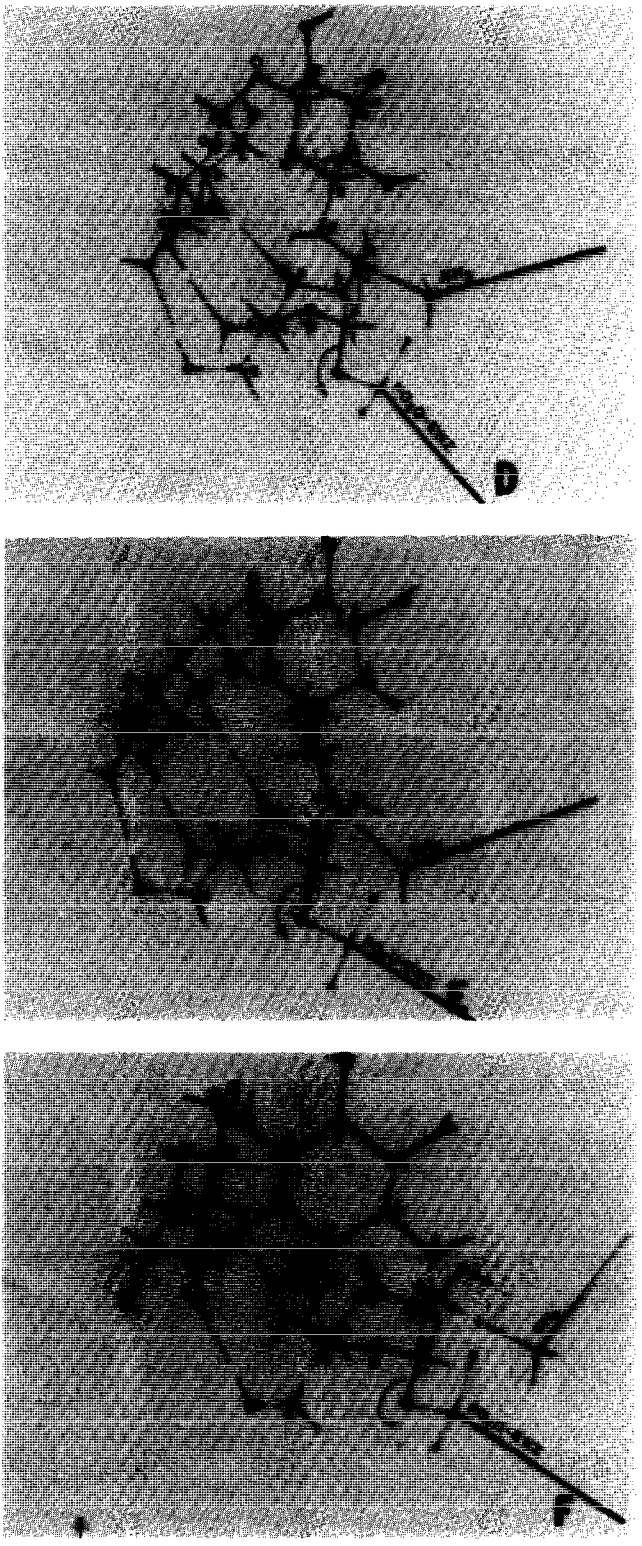

FIG. 5—(*Continued*).

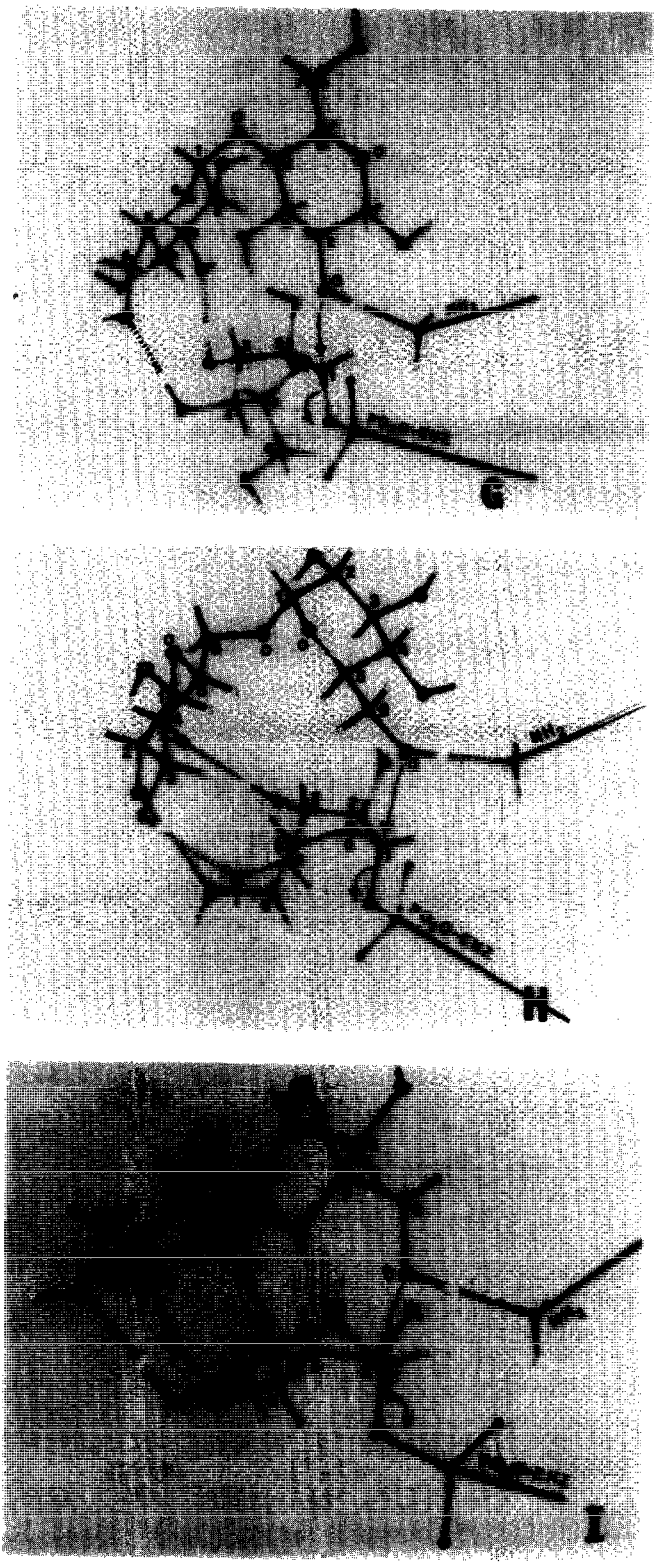

FIG. 5—(*Continued*).

TABLE 2

HYDROGEN-BONDED COMPLEXES OF ACCEPTORS WITH THE GLUCOSYL–ENZYME INTERMEDIATE OF DEXTRANSUCRASE

Acceptor	Product	Attacking hydroxyl	H-Bond oxygen pairs[a] Acceptor	Glucosyl–enzyme
			C	*C*
α-Methyl D-glucopyranoside	α-Methyl isomaltoside	C_6	1——6 4——2	
β-D-Fructopyranose	Leucrose	C_5	1——6 3——4 4——2	
Isomaltose (α or β)	Isomaltotriose	$C_{6'}$	2——6 3——4 4——3	
α-Maltose	6^2-O-α-D-Glucopyranosyl maltose (panose)	$C_{6'}$	1——4 2——3 6——6	
β-D-Mannopyranose	α-D-Glucopyranosyl-β-D-mannopyranoside	C_1	2——2 6——4	
β-D-Galactofuranose	α-D-Glucopyranosyl-β-D-galactofuranoside	C_1	5——4 6——3	
Cellobiose (α or β)	2^1-O-α-D-Glucopyranosyl cellobiose	C_2	3′——3 6′——4	
Lactose (α or β)	2^{Glc}-O-α-D-Glucopyranosyl lactose	C_2	3′——3 6′——4	
β,β-Trehalose	β-Isomaltosyl-β-D-glucopyranoside	C_6	3′——3 4′——4 6′——6	
α,β-Trehalose	α-Isomaltosyl-β-D-glucopyranoside	C_6^α	3^β——3 4^β——4 6^β——6	
	β-Isomaltosyl-α-D-glucopyranoside	C_6^β	3^α——6 4^α——4	
Raffinose	2^{Glc}-O-α-D-Glucopyranosyl raffinose	C_2	2^{Gal}——2 3^{Gal}——3 4^{Gal}——4 6^{Gal}——6	

[a] In all cases, either hydroxyl of the hydrogen-bond pair can act as a hydrogen donor, except the methoxyl group of α-methyl glucoside in which the acetal glycosidic oxygen must be a hydrogen-bond acceptor.

of dextran, the hydrogen bond with the catalytic amino group increases the nucleophilic character of the oxygen atom of the attacking hydroxyl group and orients it to make an attack on C_1 of the glucosyl–enzyme complex. The amino group then abstracts the proton, and a glycosidic bond is formed between the acceptor and the glucosyl unit, which is thereby released from the enzyme complex.

For all disaccharide acceptors considered, three or more hydrogen bonds can be formed, one always being with the catalytic amino group. The trisaccharide, raffinose, can form five hydrogen bonds, four with the glucosyl unit, which is the maximum possible number. No hydrogen-bonded complexes could be formed for the two nonacceptors, α,α-trehalose and D-xylopyranose.

In the reaction forming branch linkages in dextran by acceptor reactions, we postulate a hydrogen-bonded complex between the acceptor dextran and the dextranosyl–enzyme complex that places a C_3-hydroxyl group of a glucose unit in the interior part of the acceptor–dextran chain into position for attack on the C_1 of the reducing end of the dextranosyl–enzyme complex. It is known that dextran molecules associate in solution (*9*, *29*). The specific type of association, however, is not known. Using models, we have been able to form two types of two-chain hydrogen-bonded complexes, a parallel double helix and an antiparallel ribbon helix (models not shown).

The use of the Ealing CPK models showed that in no instances examined were there any problems in the mechanism with nonbonded atoms interfering with each other.

The building of models for molecules undergoing reaction does not in itself *prove* the proposed mechanism for the enzymic catalysis but does demonstrate that the proposed mechanism is feasible. Inability to work out such a model pathway would greatly detract from any proposed mechanism.

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