Influence of the acceptor during transglucosylation by transglucosylamylase of *Candida tropicalis*

L. K. Nakamura

Northern Regional Research Laboratory, Peoria, Illinois 61604

Received February 19, 1970


The nature of glycoside substrates influences the fraction of glucosyl residues transferred to competing acceptors. This influence suggests that the mechanism of transglucosylation reactions catalyzed by transglucosylamylase involves the formation of intermediate ternary enzyme-glycoside acceptor complexes. Evidence suggests that the ternary complex involving phenyl-α-D-glucose (PAG) forms and decomposes by a Random Bi Bi rather than by an Ordered Bi Bi mechanism. Polyhydroxylated compounds appear to compete better with water than do monohydroxylated substances for the acceptance of glucosyl residues that are transferred from PAG. Methanol and glycerol inhibit the breakdown of PAG noncompetitively. The alcohols have a higher affinity for the acceptor sites than does water. However, the slower decomposition rate of the alcohol ternary complex, in comparison to that of the water complex, reduces the overall breakdown rate of PAG.


La nature des substrats glucosidiques influence la fraction des résidus glucosyl transférés aux accepteurs compétitifs. Cette influence semble indiquer que le mécanisme des réactions de transglucosylation catalysées par la transglucosylamylase implique la formation d'intermédiaires, soit des complexes ternaires enzyme-glycoside-accepteur. Il s'avère que le complexe ternaire impliquant le phényl-α-D-glucose (PAG) se forme et se décompose selon un mécanisme de Hasard Bi Bi plutôt qu'un mécanisme Bi Bi Ordonné. Les composés polyhydroxyés, comparativement aux composés monohydroxyés, semblent de meilleurs compétiteurs vis-à-vis de l'eau pour la réception des résidus glucosyl transférés à partir du PAG. Le méthanol et le glycérol inhibent la dégradation de PAG de façon non compétitive. Les alcools ont une plus grande affinité que l'eau pour les sites accepteurs. Cependant, le taux de décomposition du complexe ternaire alcool est plus lent que celui du complexe eau, ce qui réduit le taux de décomposition global du PAG.

**Introduction**

Glycosidases can be considered as "two-substrate" enzymes, which catalyze the transfer of glucosyl residues from donor glycosides to acceptor substrates. The nature of the glycoside may influence the point of substitution on a polyhydroxyic acceptor (1), or affect the ratio of the products when two competing acceptors are present (2). Other evidence for the involvement of ternary complexes is the analysis of the kinetics of dextrose transglycosylation under conditions where sucrose, the glucosyl donor substrate, also acts as an acceptor. The results show that the rate of sucrose decomposition is proportional to the concentration of the enzyme donor-acceptor triad (3). On the basis of thermodynamic studies, von Ebert and Stricker (4) propose the involvement of ternary complexes for inversion, fructosyl sucrose synthesis, and levan formation by levansucrase. These observations suggest that transfer reactions involve the decomposition of ternary complexes where the glycoside and acceptor are simultaneously bound to the enzyme. In other words, the observations are incompatible with the involvement of a binary complex, which hypothesizes a two-step process. Because transfer reactions involve the formation and decomposition of ternary complexes, different acceptors, other than water, may either enhance or inhibit the breakdown rate of the donor glucoside obtained by hydrolysis alone (5-7). Whether enhancement or inhibition will occur depends on the relative rates of formation and decomposition of the ternary complexes involving water or the acceptor.

Jermyn (5-7) developed techniques that enabled him to analyze the effect of acceptors on the rate of formation and decomposition of ternary complexes in glucosyl transfer reactions mediated by β-glucosidase of *Stachybotrys atra*. His technique involved the use of a simple glycoside, such as phenyl-β-D-glucose, and analysis of products formed only during the initial phases of the reaction. The method eliminated
complicating factors, such as hydrolysis of the product glucoside or transfer of glucosyl residues to the donor itself, product-glucose, or glucoside.

Prompted by Jermyn's work, we examined phenyl-\(\alpha\)-D-glucose as a possible substrate for transglucosylamylase of Candida tropicalis, and studied the influence of acceptors on glucosyl transfer reactions. Transglucosylamylase can mediate transfer of glucosyl residues from amylaceous substrates to a variety of mono- and polyhydroxylic compounds (8, 9).

This paper presents evidence indicating the involvement of ternary complexes in the transfer reactions catalyzed by transglucosylamylase. It also examines in detail how an added acceptor affects the transfer reaction.

**Materials and Methods**

**Enzyme Preparations**

Transglucosylamylase present in cell-free extracts of Candida tropicalis NRRL Y-1367 was purified approximately 100-fold by the method described previously (9). Further purification was achieved by chromatography on a Duolite C-10\(^{-}\) column, which was eluted stepwise with McIlvaine buffer at pH 3.4 and 5.4 (10). Transglucosylamylase was eluted with pH 5.4 buffer. Fractions containing the enzyme were pooled and dialyzed against distilled water at 4 C for 24 h. The preparation contained 0.10 unit of enzyme per milliliter, which was approximately 150-fold purified. A unit is the amount of enzyme required to hydrolyze 1.0 \(\mu\) mole of phenyl-\(\alpha\)-D-glucose (hereafter designated as PAG) per minute at 40 C when the substrate concentration is 1.0 mM.

**Disc Gel Electrophoresis**

The homogeneity of the enzyme was examined by polyacrylamide gel electrophoresis at pH 9.1 according to the method of Davis (11) with a 7.5\% acrylamide concentration. Varying amounts of the enzyme preparation were examined. A current of 2.5 mA per column (5.0 \(\times\) 40.0 mm) of gel was applied. Proteins were fixed and stained with 0.5\% Amido Black in 7.0\% acetic acid. Sections of unstained gels, corresponding to protein bands in the stained gels, were excised, crushed, and suspended in 1.5 ml of 1.0 mM PAG in 0.5 M acetate buffer (pH 4.2). After incubation for 1 h at 40 C, 0.5 ml of the reaction mixture was analyzed for the release of phenol. If no hydrolysis occurred at 1 h, the incubation was extended to 3 h and again examined for release of phenol.

**Analysis of Reaction Mixtures**

Soluble starch, dextrin, and amylopectin were determined by Sawai and Hehre's (8) method. Glucose equivalence for a given amount of hydrolysis of any one substrate was calculated by comparison to the amount of glucose obtained through total hydrolysis with 2 N HCl at 100 C for 4 h. The respective amounts of glucose obtained for completely hydrolyzed solutions of 1\% 16 D.E. dextrin and reagent dextrin were 9.5 and 8.9 mg/ml. By comparing the expected with the observed glucose production, one can calculate percentage transglycosylation to either water or added acceptor. Glucose was determined with the Glucostat reagent. It was also determined by the o-toluidine method when \(p\)-nitrophenyl-\(\alpha\)-D-glucose (PNAG) was the substrate (12). Reducing sugar was estimated by Somogyi's (13) procedure. Phenol was measured by the method of Gottlieb and Marsh (14). \(p\)-Nitrophenol was determined by reading absorbance at 400 nm at pH 8.6.

**Estimating \(T_{50}\)**

The mathematics and rationale for the use of \(T_{50}\) are discussed by Jermyn (2, 5). The \(T_{50}\) notation is defined as the concentration of added acceptor at which 50\% of the glucosyl residue is transferred to the added acceptor and 50\% to water (5). If \(t\) is the fraction transferred to the acceptor, \(T_{50}\) is the acceptor concentration where \(t/(1-t) = 1.0\). Determination of \(T_{50}\) involves plotting the logarithm of the acceptor concentration \(c\) against \(\log t/(1-t)\) and extrapolating the line through the point where \(\log t/(1-t) = 0\).

This plot is based on the observation that the relationship between \(t\) and \(c\) takes a generalized sigmoid form. If it were a true sigmoid curve, the equation

\[
\log [t/(1-t)] = K_1 \log c + K_2
\]

would hold (5). When the value of \(t\) ranges between 0.15 and 0.85, the relationship between \(\log [t/(1-t)]\) and \(\log c\) becomes very close to being linear.

**Chemicals**

The chemicals or reagents used were: PAG and PNAG (Sigma Chemical Co., St. Louis. Mo.). Glucostat (Worthington Biochemical Corp., Freehold, N.J.), 16 D.E. dextrin (Corn Products Co., Pekin, Ill.), and reagent dextrin (Merck & Co., Inc., Rahway, N.J.). Glucosyl glycerol was prepared in this laboratory.

**Results**

**Disc Gel Electrophoretic Analysis of Enzyme Preparation**

Polyacrylamide gel electrophoresis of 30 \(\mu\)g of protein of the enzyme preparation revealed two components (Fig. 1a). A third component was noted when protein was increased to 60 or 300 \(\mu\)g (Fig. 1 b and c).

Analysis of sections from unstained gels showed that PAG-hydrolyzing activity could only be associated with one component, i.e. the most intense band. No PAG-hydrolyzing activity could be attributed to the minor bands even after 3 h of incubation.
Glucosyl Transfer with Different Substrates and Added Acceptors

The proportion of glucosyl residues transferred to water was in general affected by the donor substrate when either glycerol or 1,3-propanediol was present (Table I). Percentage transfer to water at different degrees of consumption of the substrates was constant. Since no significant differences in expected and observed glucose occurred when up to 10% of the substrates was consumed in the absence of added acceptor, glucose to product-glucose transfer was probably negligible or nonexistent. Therefore, the reduction of glucosyl transfer to water in the presence of added acceptor reflects a true transfer value of glucose to that acceptor.

Because adequate levels of transfer occurred with PAG as the donor, further studies with glycerol or methanol as acceptor were done with this chemical. Furthermore, the breakdown rate of PAG is amenable to more precise analysis than are complex, high-molecular-weight substances. It is also available in pure form, and preliminary experiments showed that no transfer
### TABLE I
Effect of substrate on transfer fraction

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Percentage substrate consumed in</th>
<th>Glucose observed (mM) in</th>
<th>Percentage transfer to water in</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water only</td>
<td>Glycerol and 1,3-Propanediol</td>
<td>Water only</td>
</tr>
<tr>
<td>Dextrin, 16 D.E.</td>
<td>1.0</td>
<td>1.0</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>2.5</td>
<td>1.07</td>
</tr>
<tr>
<td>Dextrin, reagent</td>
<td>1.0</td>
<td>1.0</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>2.0</td>
<td>1.00</td>
</tr>
<tr>
<td>Phenyl-α-D-glucose*</td>
<td>10.0</td>
<td>1.0</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>21.0</td>
<td>10.0</td>
<td>0.22</td>
</tr>
<tr>
<td>p-Nitrophenyl-α-D-glucose*</td>
<td>10.0</td>
<td>10.0</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>15.0</td>
<td>14.4</td>
<td>0.15</td>
</tr>
</tbody>
</table>

**NOTE:** The reaction mixtures contained 0.125 unit of enzyme, 100.0 mg of the required amylaceous substrate, 250.0 μmoles of acetate buffer at pH 4.2, and the indicated amounts of acceptor other than water in a total volume of 10.0 ml. Reaction mixtures contained 10.0 μmoles phenyl-a-glucose (PAG) or p-nitrophenyl-a-glucose (PNPG) in place of the amylaceous substrate. Reaction mixtures containing no added acceptors were run for each substrate. Incubation temperature was 40 °C. Samples (1 ml) were removed at 5-min intervals, heated at 100 °C for 5 min, and analyzed for residual donor substrate and glucose. Phenol and reducing sugar were determined when PAG was the substrate. p-Nitrophenol and glucose were determined when PNPG was the substrate.

Preliminary experiments showed that transglucosylamylase could not readily transfer glucosyl residues from dextrans to phenol. Therefore, it was assumed that no transfer of glucose to product-phenol would take place when PAG was the donor substrate.

**Effect of Enzyme Concentration on PAG Hydrolysis**

A linear relationship exists between increasing enzyme concentration and PAG hydrolysis where the release of phenol after 10 min ranges from 0.02 to 0.24 μmole/ml (Fig. 2). When the enzyme concentration is approximately 0.013 unit/ml, the phenol released is about 0.10 μmole/ml per 10 min, which is equivalent to 10% hydrolysis of PAG. This enzyme concentration was used in most of the subsequent experiments.

Since analysis showed that throughout the progress of the hydrolysis both phenol and glucose (determined as reducing sugar) were released in essentially equimolar amounts, no transfer took place to acceptors other than water.

**Determination of T50 with Various Acceptors**

The T50 values for a series of mono- and polyhydroxylic alcohols were determined to find, if possible, substances with high, as well as low, acceptor activity. Figure 3 plots log c against log t/(1-t) for glycerol, 1,3-propanediol, and methanol substances with low, intermediate, and high T50 values. Table II contains the T50 values of several different acceptors. The data suggest that the efficiency of transfer to a primary alcohol varies inversely with its chain length as the T50 values of 1.7 and 4.6 M for methanol and n-propanol show. Comparison of T50 values of ethanol (2.2 M) with ethylene glycol (0.22 M) and of n-propanol (4.6 M) with propylene glycol (0.23 M) or glycerol (0.14 M) indicates that acceptor capability improves markedly with poly-
TABLE II

<table>
<thead>
<tr>
<th>Acceptor</th>
<th>T50 (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>1.7</td>
</tr>
<tr>
<td>Ethanol</td>
<td>2.2</td>
</tr>
<tr>
<td>n-Propanol</td>
<td>4.6</td>
</tr>
<tr>
<td>i-Propanol</td>
<td>5.5</td>
</tr>
<tr>
<td>Ethylene glycol</td>
<td>0.22</td>
</tr>
<tr>
<td>1,2-Propylene glycol</td>
<td>0.23</td>
</tr>
<tr>
<td>1,3-Propylene glycol</td>
<td>0.69</td>
</tr>
<tr>
<td>Glycerol</td>
<td>0.14</td>
</tr>
<tr>
<td>Pentaerythritol</td>
<td>0.35</td>
</tr>
</tbody>
</table>

NOTE: Reaction mixture contained 0.05 unit enzyme; 4.0 μmols PAG; 25.0 μmols acetate buffer at pH 4.2; and 0.2, 0.4, 2.0, 4.0, or 8.0 μmols acceptor in a total volume of 4 ml. Incubation was for 10 min at 40°C. Reaction was stopped by heating for 5 min at 100°C.

TABLE III

<table>
<thead>
<tr>
<th>PAG concentration (mM)</th>
<th>Percentage transfer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glycerol</td>
</tr>
<tr>
<td>0.1</td>
<td>44.5</td>
</tr>
<tr>
<td>1.0</td>
<td>45.0</td>
</tr>
<tr>
<td>10.0</td>
<td>43.4</td>
</tr>
</tbody>
</table>

NOTE: Reaction mixture is similar to that described for Table II, except that PAG concentrations were 0.4, 4.0, and 40.0 μM. The respective amounts of glycerol and methanol were 0.2 and 1.0 mM.

Fig. 3. Relation between r and acceptor concentration for glycerol, ○: 1,3-propanediol, ▲: and methanol, ●. Reaction mixture is given in Table II.

TABLE III

Effect of PAG concentration on transfer fraction

<table>
<thead>
<tr>
<th>PAG concentration (mM)</th>
<th>Percentage transfer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glycerol</td>
</tr>
<tr>
<td>0.1</td>
<td>44.5</td>
</tr>
<tr>
<td>1.0</td>
<td>45.0</td>
</tr>
<tr>
<td>10.0</td>
<td>43.4</td>
</tr>
</tbody>
</table>

NOTE: Reaction mixture is similar to that described for Table II, except that PAG concentrations were 0.4, 4.0, and 40.0 μM. The respective amounts of glycerol and methanol were 0.2 and 1.0 mM.

Fig. 3. Relation between r and acceptor concentration for glycerol, ○: 1,3-propanediol, ▲: and methanol, ●. Reaction mixture is given in Table II.

Fig. 3. Relation between r and acceptor concentration for glycerol, ○: 1,3-propanediol, ▲: and methanol, ●. Reaction mixture is given in Table II.

Glycerol and methanol were chosen for further studies because they represent acceptors with large differences of acceptor capability. The kinetics of PAG consumption were observed in the presence of either acceptor and from that were derived inferences about the influence of the acceptor in the reaction.

Constancy of Percentage Transfer with Varying PAG Concentrations

Table III shows that the percentage transfer to the acceptors does not vary significantly over a wide range of concentration of PAG. Since the respective transfer values with glycerol (0.1 M) and with methanol (1.0 M) were 44.3 and 39.2%, transglucosylation is independent of donor substrate concentration.

Relationship Between Transfer Ratio and Acceptor Concentration

The relationship between transfer ratio and acceptor concentration differentiates between a fixed linear and a random sequence mechanism for the transglucosylation reaction (2). If a linear sequence mechanism is involved, the plot of transfer ratio against acceptor concentration produces a straight line.

For a transglucosylamylase-catalyzed reaction, the plot of the ratio of product-glucoside (phenol released minus glucose released) to glucose against either glycerol or methanol concentration shows a nonlinear relationship (Fig. 4). This observation suggests that the ternary complex is not formed by a fixed-sequence mechanism (2).

Effect of Acceptors on Kinetics of PAG Consumption

Lineweaver–Burk plots (Figs. 5 and 6) show the relationship between PAG and reaction velocity at various concentrations of either glycerol or methanol. Both acceptors inhibit consumption of PAG noncompetitively. The respective values for \( K_m \) and \( V_{max} \) in the absence of added acceptor are \( 4.5 \times 10^{-4} \) M and 0.015 μmole/ml per min.

The data were further analyzed in detail according to Jermyn’s (5) procedure. If \( V_{max} \) without acceptor is set at unity, then relative \( V(V_{acceptor}/V_{max}) \) values can be calculated for the different concentrations of the added acceptors. These relative \( V \) values are plotted against log c (Fig. 7a). (Only plots with glycerol are presented, since similar plots were also obtained with methanol.) However, since the reaction is the
Fig. 4. Effect of acceptor concentration on transfer ratio. The conditions for the reaction were the same as described in Table II, except that the amounts of glycerol were 0.16, 0.32, 0.48, 0.80, 2.00, 3.20, and 4.00 mmoles. The amounts of methanol were 0.32, 0.40, 2.00, 6.00, 8.00, and 10.00 mmoles.

Fig. 5. Effect of increasing glycerol concentration on the rate of PAG breakdown. The basic reaction mixture contained 0.013 unit/ml enzyme, 0.1 M acetate buffer at pH 4.2, and 2.0, 5.0, 10.0, or 50.0 × 10⁻⁴ M PAG. The indicated amounts of glycerol were incorporated into the mixtures. Incubation and analytical procedure were as described in Fig. 2.

sum of two reactions, relative $V$ can be partitioned into $V_g$ (for glycerol) and $V_w$ (for water) by reference to the plot depicting the relationship between transfer fraction and log $c$ (Fig. 7a). Thus, if relative $V$ has been halved at a certain acceptor concentration and if transfer fraction is 0.6, then the respective values $V_g$ and $V_w$ will be 0.3 and 0.2. Actual plots of the variance of $V_g$ and $V_w$ with acceptor concentration are seen in Fig. 7b. This procedure is valid only if it can be demonstrated that the transfer fractions are independent of donor concentration (5).

In Fig. 7c, relative $V_g$ is plotted against relative $V_w$ and the line extrapolated to relative $V_w = 0$. Since the plot has been extrapolated to saturation concentration of both acceptors, i.e. water and glycerol, the resulting numerical value, 0.70, represents the ratio of the rate of decomposition of the enzyme-glycerol-PAG complex to that of the enzyme-water-PAG complex.

The kinetic data (Fig. 6) for methanol are treated similarly, and the plot of $V_m$ (for methanol) against $V_w$ gives a value of 0.65 when $V_w = 0$.

Discussion

Jermyn (2) showed that the nature of the donor substrate can affect percentage transfer when a transferring enzyme is acting in the presence of two competing acceptors only if the system involves enzyme-acceptor-donor ternary complexes. In order for the nature of the donor to affect transfer fraction with a binary two-step system, the whole system would have to be reversible. Reversibility may depend on the relative magnitude of the affinity ($K_m$) of the
enzyme for the substrate and product. For example, if the apparent $K_m$ for the product was sufficiently low compared to that of the substrate, the reaction would be reversible even if the concentration of the product formed was low. If the reaction was reversible, the situation would make sense only if the donor and products have concentrations of the same order of magnitude (2). In view of the effect of product concentration, it was necessary that the initial velocities of the reaction be analyzed.

It has been roughly estimated that the molecular weight of dextrins is about 10,000 (15). Based on this molecular weight estimation, the concentration of dextrin used is about 1 mM. The $K_m$ has been determined to be about 0.5 mM for dextrins. From the amount of glucose produced in the presence of glycerol, it is estimated that the highest concentration of glucosyl glycerol produced is 0.7 mM (Table I). Comparison of the product concentration to the substrate level and $K_m$ value of 30.0 mM for glucosyl glycerol indicates that the forward reaction was greatly favored.

When PAG and PNPG at 1.0 mM concentration are the substrates, the highest concentration of transfer product formed with glycerol as acceptor is 0.05 mM. Comparison of this product concentration with $K_m$ of 0.45 mM for PAG, 0.25 mM for PNPG, and 30.0 mM for glucosyl glycerol indicates that the reverse reaction would not be a significant part of the reaction. Assuming that the $K_m$ for glucosyl 1,3-propanediol would be of the same order of magnitude as that for glucosyl glycerol, one can also conclude that the reverse reaction is negligible when 1,3-propanediol is the acceptor with the dextrins of the phenyl glucosides as substrates.

The indicated insignificance of the reverse reaction shows that the different transfer values obtained with the various donor and acceptor substrates is attributable only to a mechanism involving ternary complex formation (Table I). Although the enzyme is capable of transferring glucosyl residues to product glucose when acting on dextrins, the fraction of transfer amounts to less than 1% when 10% or less of either of the dextrins is hydrolyzed. Therefore, the difference in transfer values obtained with the two dextrins appears to be true.

Further evidence for ternary complex formation is the inhibition of transglucosylamylase by the substrate, maltose, at high concentrations (16). The kinetic data are compatible with a mechanism involving the formation of ternary complexes where a molecule of maltose encroaches on the acceptor sites at the expense of water (16).

Transfer to glucose or to itself appears to be very low or nonexistent when PAG served as substrate; this condition makes it useful for more extended studies. The ease with which breakdown and glucosyl transfer can be estimated by determination of phenol and reducing sugar made PAG an ideal donor substrate for studying the effect of added acceptors. The constancy of transfer fraction to glycerol and methanol with variation in PAG concentration, and the relationship between added acceptor concentration and product ratio shown in Fig. 4, suggest the random sequence mechanism (2) shown in Scheme I, where E, D, Q, and P’s are the enzyme, donor substrate, phenol, and glucosidic products.

\[
\begin{align*}
E + A_1 &\rightleftharpoons EA_1 & EA_1 + D &\rightleftharpoons EA_1D &\rightarrow P_1 + Q \\
E + D &\rightleftharpoons ED & ED + A_1 &\rightarrow EA_1D &\rightarrow P_1 + Q \\
E + A_2 &\rightleftharpoons EA_2 & EA_2 + D &\rightleftharpoons EA_2D &\rightarrow P_2 + Q
\end{align*}
\]

Scheme I

$A_1$ and $A_2$ represent the acceptors, the first of which is water. Ternary complexes are represented by EDA's. According to Cleland's (17) nomenclature, the mechanism is Random Bi Bi. Mathematical analysis of the mechanism predicts the following relationship between product ratio and $A_2$ concentration:

\[
\frac{[P_2]}{[P_1]} = \frac{w[A_2]^3 + x[A_2]^2 + y[A_2]}{p[A_2]^2 + q[A_2] + r}
\]

which reduces to

\[
[P_2]/[P_1] = K_1 [A_2] + K_2
\]

at high values of $[A_2]$ and to

\[
[P_2]/[P_1] = 0 \text{ for } [A_2] = 0.
\]

The data appear (Fig. 4) to be compatible with this equation. The Random Bi Bi mechanism is conceptually simple and is the one most likely to be involved with transferring enzymes.
TABLE IV

Summary of data on the transfer of glucosyl residues from PAG to methanol and glycerol

<table>
<thead>
<tr>
<th>Acceptor</th>
<th>Rate of decomposition of enzyme-PAG-alcohol relative to enzyme-PAG-water</th>
<th>T_{50} (M)</th>
<th>Percentage acceptor sites occupied by alcohol at T_{50}</th>
<th>Percentage transfer to alcohol when acceptor sites equally occupied</th>
<th>Molarity at which acceptor sites occupied for alcohol relative to that for water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>0.65</td>
<td>0.65</td>
<td>60.5</td>
<td>39.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Glycerol</td>
<td>0.70</td>
<td>0.14</td>
<td>58.2</td>
<td>41.2</td>
<td>0.08</td>
</tr>
</tbody>
</table>

*Obtained from Fig. 7.
†Calculated: \(0.7(1-x) = 1\) where \(x\) equals the percentage of acceptor sites occupied by the alcohol.
‡Calculated from Fig. 7.

The Random Bi Bi mechanism and the type of relationship \([P_2]/[P_1]\) has to donor and acceptor concentrations are the bases for the \(T_{50}\) determination and partitioning of the kinetic parameter, \(V_{max}\), into its two components.

Kinetic studies permitted the evaluation of the effect of either glycerol or methanol in the PAG consumption reaction. Generally, noncompetitive inhibition is assumed to involve reaction of the inhibitor with a region other than the active center so that combination of the substrate with the substrate site is unaffected but the rate-determining breakdown of the enzyme-substrate complex is prevented. In the present situation where methanol and glycerol noncompetitively inhibit the consumption of PAG by transglucosylamylase, the alcohols compete with water for the acceptor sites and form enzyme-PAG-alcohol complexes which decompose more slowly than the enzyme-PAG-water triad (Table IV). The reduced decomposition rate of the enzyme-PAG-alcohol complexes plus the greater affinity of the alcohols over water for the acceptor sites explain the decrease in PAG consumption rates (Table IV). Glycerol, having a much higher affinity for the acceptor site than methanol, predictably caused a more marked decrease in PAG breakdown rate. On the other hand, the high affinity of glycerol enables the enzyme to accomplish 50% transfer at a concentration of 0.14 \(M\) as compared to 1.7 \(M\) required with methanol.

Jermyn (6) has reported that hexane-1,6-diol bound to the acceptor site of S. atra \(\beta\)-glucosidase much more readily than water and that the resulting complex was more active than the one involving water. The partial relative \(V\) for 1,6-hexanediol, when \(V_e = 0\), was 4.63, and the affinity for the acceptor site was of the same magnitude as glycerol. In contrast to the situation involving glycerol, PAG, and transglucosylamylase, increasing concentrations of 1,6-hexanediol enhance the breakdown of phenyl-\(\beta\)-D-glucose by \(\beta\)-glucosidase. We were not able to find an acceptor that could enhance the breakdown of PAG by transglucosylamylase.

In summary, then, added acceptors compete with water for the acceptor sites of the enzyme during formation of ternary complexes. Consequently, the rate of glucoside donor consumption and the amount transferred to competing acceptors at a given concentration will depend on their relative affinities for the acceptor site and on the subsequent decomposition rate of the ternary complex.