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# AN INVESTIGATION OF THE PROPERTIES OF RABBIT MUSCLE OLIGO-1,4 → 1,4-GLUCANTRANSFERASE

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## SUMMARY

An investigation of the properties of purified rabbit muscle amylo-1,6-glucosidase/oligo-1,4 → 1,4-glucantransferase was conducted utilizing a modification of the iodine-complex spectrum of amylopectin and glycogen phosphorylase limit dextrin. A rapid and convenient method of assaying for the transferase activity was developed to study its activity.

The pH optimum of the transferase differs from that of amylo-1,6-glucosidase, which is associated with it throughout purification. Tris, which both inhibits and shifts the pH optimum of the glucosidase, has little or no effect on the transferase. $MoO_4^{2-}$, on the other hand, inhibits the transferase but not the glucosidase.

The action of the transferase on amylopectin is not dependent on the glucosidase. With glycogen phosphorylase limit dextrin, however, the modification in iodine color is a product of both glucosidase and transferase action.

Assay of tissue extracts utilizing the modification of the iodine-complex spectrum of phosphorylase limit dextrin was found to be specific for glucosidase–transferase activity.

The differences in properties of the glucosidase and the transferase suggest that they have different active sites even though they are apparently associated with the same protein molecule.

## INTRODUCTION

Oligo-1,4 → 1,4-glucantransferase ($\alpha$-1,4-glucan:$\alpha$-1,4-glucan 4-oligoglucantransferase, EC 2.4.1.14) and amylo-1,6-glucosidase (dextrin:6-glucohydrolase, EC 3.2.1.33) constitute the mammalian debranching system of glycogen phosphorylase limit dextrin[1]. Both enzymatic activities are associated with each other throughout

Abbreviation: EGTA, ethyleneglycol-bis-($\beta$-aminoethyl ether) *N,N'*-tetraacetic acid.

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purification and even in highly purified preparations appear as a homogeneous protein entity[1,2]. They are usually assayed by their combined action on glycogen phosphorylase limit dextrin[1,4]. In recent years the glucosidase has also been assayed independent of transferase, by the use of either glucosidase specific substrates such as 6[3]-$\alpha$-glucosyl-maltotetraose, "fast $B_5$", or $\alpha$-glucosyl Schardinger dextrin[1,5] or by utilizing the ability of the glucosidase to reincorporate glucosyl moieties[6-8]. The transferase activity has been, on the other hand, chiefly assayed by the process of determining the extent of its action on amylodextrins[1,9]. These substrates are difficult to obtain and the analytical procedures are time consuming. Other methods of determining transferase activity, independent of glucosidase, have utilized the ability of the enzyme to alter polysaccharide structure but suffer from the disadvantage that they are not sufficiently quantitative[6].

The action of the transferase on amylopectin or glycogen has been examined by noting the change in the iodine-complex spectrum of the polysaccharide[6,10-12]. These studies have indicated that the enzyme can attenuate the outer branches of these polysaccharides to produce longer chains which take on an increasing degree of amylose-like character in terms of their iodine-complex spectrum. These results have been largely inconclusive due to contaminants such as $\alpha$-amylase ($\alpha$-1,4-glucan 4-glucanohydrolase, EC 3.2.1.1), which counteracted the results of chain elongation[10].

It became of interest to reexamine the change in iodine-complex spectrum with a highly purified preparation of the glucosidase–transferase[2] which did not contain these contaminating enzymatic activities in order to quantitatively assay the transferase. The method developed has been used to study the properties of the transferase.

## EXPERIMENTAL

### *Methods*

*Assay of oligo-1,4 → 1,4-glucantransferase by modification of the iodine-complex spectrum of amylopectin.* The standard assay solution contained 0.10 ml 1% amylopectin, 0.020 ml 0.5% gelatin, 0.020 ml 0.005 M EDTA (pH 7.0) at 25°, 0.020 ml 0.50 M sodium maleate (pH 5.8) at 25°, 0.020 ml water and/or sample addition (resultant pH 6.0 at 25°). The assay solution was incubated at 30° and the reaction started by the addition of 0.020 ml enzyme solution. The reaction was stopped by the addition of 0.20 ml 0.20 M HCl (resultant pH approx. 1.8). To this were added 2.6 ml $I_2$–KI reagent (total volume 3.0 ml, pH approx. 2.6). Method blanks (zero time controls) were prepared by adding HCl before enzyme addition. The absorbance at 620 nm was recorded after 20 min. The enzyme was diluted with a solution containing 0.05 M sodium maleate, 0.05% gelatin, 0.01 M 2-mercaptoethanol, and 0.005 M EDTA (pH 6.0) at 25°. Several precautions need to be observed. Addition of the 0.20 M HCl is critical since the development of the iodine color is sensitive to slight changes in pH. The method of addition of the iodine reagent is also important. Excess aeration should be avoided. This is overcome if the reagent is introduced with a syringe or automatic pipette with the tip placed at the bottom of the tube, so that addition of the reagent causes mixing. Upon addition of the iodine reagent, a film-like deposit develops on the walls of the incubation tube. This necessitates decanting the solution into cuvettes at the time of absorbance measurement. The development of the iodine color takes

about 20 min. After this interval, there is a slow change, which is constant between sample and blank (for a period of at least 90 min) but which requires readjustment of the blank (approx. every 5–10 min). Although 0.01 M 2-mercaptoethanol is used in the enzyme diluent[2], the presence of this concentration of 2-mercaptoethanol in the assay solution has an adverse effect on the development of the iodine color.

The iodine reagent was prepared according to KRISMAN[13] by first dissolving 0.26 g of $I_2$ and 2.6 g KI in 10 ml of water. This solution is stable when stored in the cold in a brown glass bottle. The $I_2$–KI solution (1.0 ml) is added to 260 ml of saturated $CaCl_2$ solution to make the iodine reagent (sufficient for 100 assays). The reagent should be prepared daily and kept in a brown glass bottle. Erratic results are obtained using $CaCl_2$ contaminated with sufficient $Ca(OH)_2$ to give an alkaline solution (*cf.* ref. 13). The saturated solution of $CaCl_2$ was prepared by dissolving 130 g of $CaCl_2 \cdot 2H_2O$ (J. T. Baker and Co., No. 1332) in 100 ml of hot water and filtering through a glass fiber ultra filter paper (No. 984-H, Reeve Angle and Co., Clifton, N.J.) while still warm (pH at 1 to 10 dilution 5.6–6.2).

*Other analytical methods.* Absorption spectra were obtained with a Beckman Model DK-2 ratio recording spectrophotometer. Other spectrophotometric determinations were made using a Zeiss Model PMQ spectrophotometer or a Coleman Model 6/20 spectrophotometer.

Determination of glucosidase–transferase activity by glucose release from glycogen phosphorylase limit dextrin was done spectrophotometrically using a coupled NADPH generating system as described previously[2]. Enzyme units reported in this paper were determined by this procedure unless otherwise specified. Liberation of glucose from glucosyl Schardinger dextrin was determined by the same method as that employed for glucose release from limit dextrin[2]. Determination of glucosidase activity alone by reincorporation of [$^{14}$C]glucose into glycogen was done as described previously[7,8]. Protein and carbohydrate concentration determinations were done as described previously[2]. All pH determinations were made with a Radiometer Model 25 pH meter, standardized at the temperature of usage. The pH values given are those of the buffer at the concentration employed.

## *Materials*

*Purified glucosidase–transferase preparations.* The enzyme used in these experiments was a highly purified preparation (specific activity approx. 7 units/mg) prepared according to the method described previously[2] and kept at a concentration of 5–6 units/ml. The preparation was dialyzed at 4° against 0.05 M sodium maleate, 0.01 M 2-mercaptoethanol, 0.005 M EDTA (pH 6.0) at 25° before use.

*Tissue extracts.* Tissue extracts were prepared as described previously[8] by using 2.5 ml of 0.01% $KHCO_3$ + 0.004 M EDTA (pH approx. 7.5) at 25° per 1 g wet weight muscle. Extracts were prepared fresh daily.

*Substrates.* Glycogen phosphorylase limit dextrin, Type III glycogen storage disease glycogen, rabbit liver glycogen, glucosyl Schardinger dextrin and uniformly labeled [$^{14}$C]glucose were prepared or obtained as described previously[2,8,14]. The amylopectin employed in these experiments was a preparation of genetically pure waxy maize starch consisting of approx. 98% amylopectin (average exterior chain length 14 residues). The preparation was a gift of Dr. B. A. Lewis, University of Minnesota, St. Paul, Minn.

*Miscellaneous materials.* All other reagents and materials were of the usual commercially available reagent grade unless otherwise specified. All buffers were in either the $Na^+$ or the $Cl^-$ counter-ion form unless otherwise specified. Tris solutions were adjusted to pH 6.0 at 25° at the concentration employed unless otherwise specified.

## RESULTS

### *Modification of the iodine spectrum of amylopectin by oligo-1,4 → 1,4-glucantransferase and assay of the enzyme*

The ability of the transferase to modify the iodine-complex spectrum of amylopectin was investigated. The standard glucosidase–transferase incubation mixture described previously[2] was used with 0.5% amylopectin in place of glycogen phosphoryl-

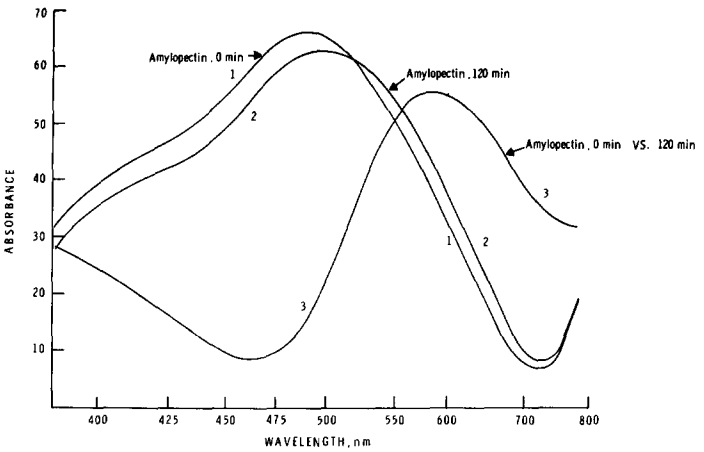
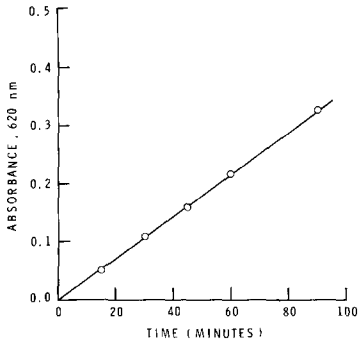



Fig. 1. Absorption spectra and absorbance difference spectrum of amylopectin in the presence of iodine before and after action of oligo-1,4 → 1,4-glucantransferase. The standard amylopectin assay was used as described in the text. The incubation mixture (0.20 ml) contained 0.53 unit of enzyme per ml. Curve 1 is the spectrum at 0 time. Curve 2 is the spectrum after 120 min incubation. Curve 3 is the difference spectrum between 0 and 120 min. Curves 1 and 2 were obtained on the 0.0–0.1 absorbance scale after a 10-fold dilution of the polysaccharide–iodine solution with additional iodine reagent. Curve 3 was obtained on the −0.3–0.7 absorbance scale after a 2-fold dilution of the polysaccharide–iodine solution with additional iodine reagent.

ase limit dextrin as a substrate. The iodine spectra were determined using the method of KRISMAN[13]. As shown in Fig. 1, there is a shift to longer wavelengths indicating the formation of longer outer chains having more amylose-like character. The difference spectrum shows a maximum at 590 nm. That this change is due to transferase action alone was verified by the fact that no glucose production could be detected throughout the course of the change (measured with the coupled hexokinase glucose-6-phosphate dehydrogenase NADPH producing method[2]). Because of high absorbance of the blank (amylopectin zero time) 620 nm was used for the standard amylopectin assay. Although the iodine color varied with time, the difference between the blank and sample remained constant and was compensated for by (approx. 5–10 min) readjusting the



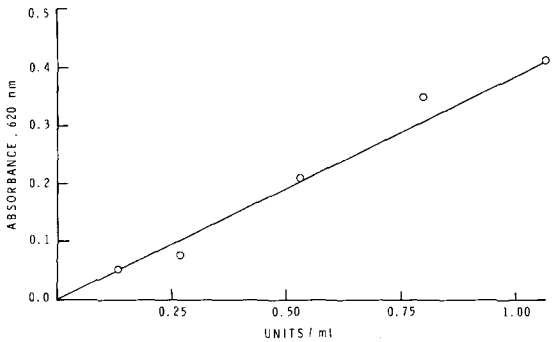




Fig. 2. Iodine color development as a function of time in the presence of oligo-1,4 → 1,4-glucantransferase. The standard amylopectin assay was used as described in the text. The incubation mixture contained 0.53 unit of enzyme per ml. The absorbance represents the difference between 0 min and the time of incubation shown.

Fig. 3. Iodine color development as a function of enzyme concentration. The standard amylopectin assay was used as described in the text. The total incubation volume was 0.20 ml and the time of incubation was 60 min. The enzyme solution used contained 5.30 units/ml.

blank. The increase in absorbance as a function of time of incubation with transferase is linear throughout the interval measured (Fig. 2) and is proportional to enzyme concentration (Fig. 3).

*Properties of oligo-1,4 → 1,4-glucantransferase*

The pH optimum of the enzyme was investigated. It is apparent (Fig. 4) that the pH optimum of the transferase differs from that of the two enzymes measured together[2], or that of the glucosidase alone[8]. The transferase has a more acidic optimum (approx. 6.0) in all of the buffers tested *versus* that of 6.6 for the combined activities or the glucosidase alone. In addition, there was no shift in pH optimum or inhibition by Tris (*cf.* refs. 2, 8). The transferase assay at pH 6.0 (*cf.* EXPERIMENTAL) is linear through an absorbance range of 0.0–0.4 at 620 nm (> 60 min). An oligo-1,4 → 1,4-

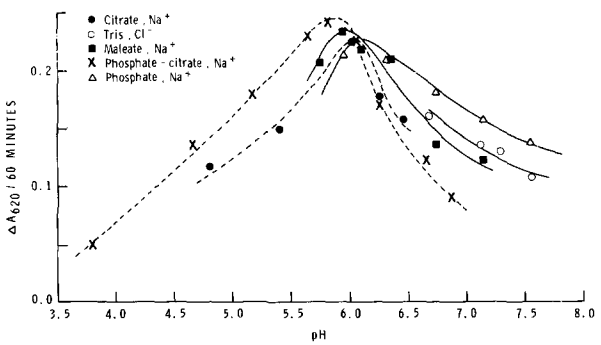



Fig. 4. Activity of oligo-1,4 → 1,4-glucantransferase as a function of pH in various buffers. The standard amylopectin assay was used as described in the text. Each incubation solution (0.20 ml) contained 0.53 unit of enzyme per ml. All buffers were at 0.01 M concentration. The time of incubation was 60 min.

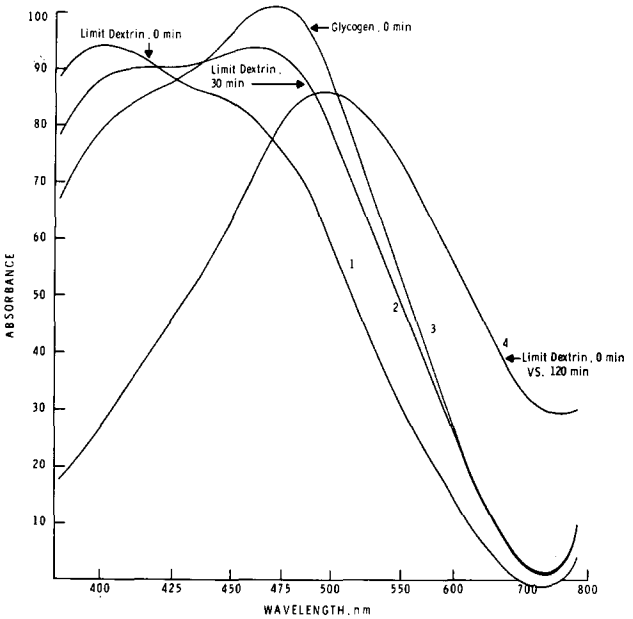



Fig. 5. Absorption spectra and absorption difference spectrum of glycogen phosphorylase limit dextrin in the presence of iodine before and after enzymatic action contrasted to the absorption spectrum of glycogen. The standard assay was used as described in the text except that limit dextrin was substituted for amylopectin at the same concentration (Curves 1 and 2). The incubation mixture (0.20 ml) contained 0.013 unit of enzyme per ml. Curve 1 is the limit dextrin spectrum at 0 time. Curve 2 is the limit dextrin spectrum after 30 min incubation. Curve 3 is the spectrum of glycogen at 0 time where glycogen was substituted for limit dextrin in the incubation mixture. Curve 4 is the limit dextrin difference spectrum between 0 and 120 min. Curves 1–3 were obtained on the 0.0–0.1 absorbance scale after 5-fold dilution of the polysaccharide–iodine solution with additional iodine reagent. Curve 4 was obtained on the −0.3–0.7 absorbance scale after a 2-fold dilution of the polysaccharide–iodine solution with additional iodine reagent.

glucantransferase unit is defined as that activity catalyzing an increase in absorbance of 0.001/min at pH 6 under the assay conditions described.

The action of the transferase on glycogen phosphorylase limit dextrin was examined as shown in Fig. 5. The difference spectrum had a maximum at approx. 500 nm. As seen, the absorption maximum shifts from that characteristic of limit dextrin to one resembling glycogen. This would be anticipated if both oligotransferase and amylo-1,6-glucosidase were acting on the structure. The combined action of both enzymes on limit dextrin would generate a structure whose outer tiers resemble glycogen[1,12]. The formation of a glycogen-like spectrum is in agreement with the finding of HERS *et al.*[6] that prolonged incubation of phosphorylase limit dextrin with glucosidase-transferase produces an iodine spectrum identical to glycogen.

The enhancement of the iodine spectrum of limit dextrin by the glucosidase is understandable on the basis of what is known of the structure of limit dextrin and the respective actions of glucosidase and transferase[1,12]. The transferase elongates the outer main nonreducing terminal chain (four residues) of the symmetrical limit dextrin structure producing an asymmetric structure whose longer main chains (7 residues) have more amylose-like character. This is enhanced by glucosidase removal

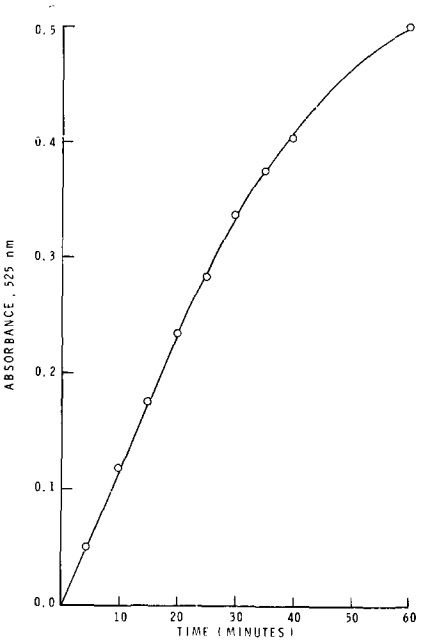



Fig. 6. Modification in the iodine spectrum of glycogen phosphorylase limit dextrin as a function of time in the presence of enzyme. The standard assay was used substituting limit dextrin for amylopectin. The incubation mixture (0.20 ml) contained 0.013 unit of enzyme per ml. The absorbance represents the difference between 0 min and the time of incubation shown.

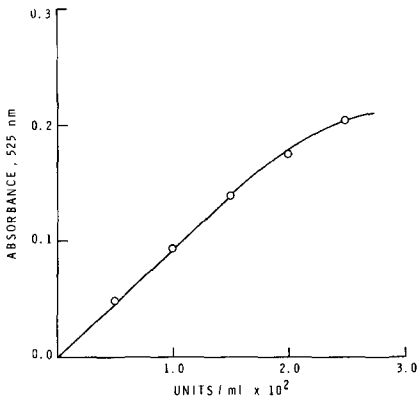



Fig. 7. Modification in the iodine spectrum of glycogen phosphorylase limit dextrin as a function of enzyme concentration. The standard assay was used substituting limit dextrin for amylopectin. The incubation volume was 0.20 ml. The enzyme solution used contained 0.26 unit/ml. The incubation time was 10 min.

of the branch point residue exposing a linear segment down to the next branch point (approx. 9–10 residues).

The modification of the iodine spectrum with time and with enzyme concentration is shown in Figs. 6 and 7. These results are similar to those shown in Figs. 2 and 3 using amylopectin as a substrate. It is of interest to note that the shift produced using limit dextrin as a substrate compared with amylopectin (compare with Fig. 2) is about 150-fold greater at equivalent enzyme concentrations.

Glycogen was also used as substrate for the transferase. The transferase was able to produce a modification in the iodine-complex spectrum with a difference spectrum maximum occurring at 550 nm; however, the magnitude of the change was only approx. 13% relative to that found with amylopectin under equivalent conditions. This explains why glycogen, as compared with phosphorylase limit dextrin, was a poor substrate for the glucosidase–transferase system when glucose production was determined[2].

The ability of the transferase to modify the iodine-complex spectrum of glycogen isolated from the liver of a patient with Type III glycogen storage disease[14] was also investigated. This type of glycogen has been reported to have properties similar to limit dextrin[15] due to a genetic lack of the glucosidase–transferase system. The results

using it as a substrate in the iodine spectrum assay indicate that this is so since they were identical to those found using limit dextrin (*cf.* Fig. 5).

*The effect of Tris on inhibition of the glucosidase–transferase system*

Tris markedly inhibits the glucosidase–transferase system, the extent depending on the method of assay[2,8]. When the combined glucosidase–transferase system is measured by liberation of glucose from phosphorylase limit dextrin, the inhibition is the least; when the action of the glucosidase alone is measured by action on glucosyl Schardinger dextrin, inhibition is more marked; and when the glucosidase is measured by reincorporation of glucose, inhibition is the most pronounced[2,8]. The effect of Tris on the transferase alone has not been previously determined. From the results seen in Fig. 4, when amylopectin is employed as a substrate, it is apparent that Tris neither inhibits the transferase nor causes a shift in pH optimum as it does with the glucosidase. To more clearly define this observation, the effect of Tris on the action of the glucosidase and/or transferase was investigated by several methods. The results are shown

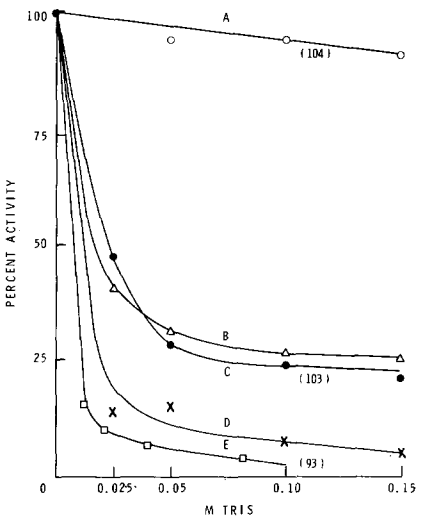



Fig. 8. The effect of Tris on the action of oligo-1,4 → 1,4-glucantransferase and amylo-1,6-glucosidase as measured by different methods. The iodine-spectrum method of assay is that described in the text. Glucose release was measured by the coupled NADPH producing system referred to in the text. Incorporation of [$^{14}$C]glucose into glycogen was determined by the method referred to in the text. Curve A measures oligo-1,4 → 1,4-glucantransferase alone by the standard amylopectin iodine-complex method. The incubation time was 8 h and 0.053 unit of enzyme per ml was used. Curve B measures the combined action of oligo-1,4 → 1,4-glucantransferase and amylo-1,6-glucosidase by modification of the iodine spectra of glycogen phosphorylase limit dextrin. The incubation period was 20 min and 0.013 unit of enzyme per ml was employed. Curve C measures the combined action of oligo-1,4 → 1,4-glucantransferase and amylo-1,6-glucosidase by liberation of glucose from glycogen phosphorylase limit dextrin. The incubation time was 20 min and 0.003 unit of enzyme per ml was employed. Curve D measures amylo-1,6-glucosidase alone by glucose release from glucosyl Schardinger dextrin under the same conditions as in Curve C. Curve E measures amylo-1,6-glucosidase by incorporation of [$^{14}$C]glucose into glycogen. The incubation time was 20 min and 0.026 unit of enzyme was employed . The numbers in parentheses are the percent activities obtained under equivalent conditions when 0.2 M Tris was added after the enzymatic reaction had been stopped. The reaction conditions were chosen to give optimum values in the absence of Tris.

in Fig. 8. As can be seen (Curve A), at 0.05 M Tris there is only slight inhibition (< 10%) of the iodine-complex modification of amylopectin by transferase. At the same concentration of Tris, however, there is a pronounced (> 85%) inhibition of the glucosidase (Curves D and E) when glucosidase-specific substrates were employed. Inhibition by Tris of the combined glucosidase–transferase action on phosphorylase limit dextrin (Curves B and C) shows that combined action on limit dextrin is greatly inhibited by Tris. The inhibition monitored by modification in the iodine spectrum (Curve B) closely parallels the inhibition of glucose release from limit dextrin (Curve C). This further indicates that the glucosidase plays a role in modification of the iodine spectrum when limit dextrin is used as a substrate. Thus, when amylopectin, which is not acted on by glucosidase as measured previously, was used as a transferase substrate (Curve A), little inhibition was noted.

*The effect of $MoO_4^{2-}$ as an inhibitor of transferase action*

It has been reported that $MoO_4^{2-}$ prevents partially purified preparations of glucosidase–transferase from increasing the iodine stain of amylopectin[10]. This observation was verified using the present highly purified glucosidase–transferase preparation and the standard iodine-complex assay with amylopectin as a substrate. At 0.001 M $(NH_4)_2MoO_4$ concentration there was no detectable inhibition of the transferase, whereas at 0.01 M concentration there was 72% inhibition. Control experiments were done to preclude the effect of $MoO_4^{2-}$ itself in causing a shift in the iodine-complex spectrum. $NH_4^+$ causes no significant inhibition at the concentrations employed[2]. Parallel experiments were also run using the [$^{14}$C]glucose reincorporation assay for glucosidase activity[8] with the same concentrations of $MoO_4^{2-}$. Setting the activity of the glucosidase at 100% in the absence of molybdate ion, the activity in the presence of 0.01 M $MoO_4^{2-}$ was found to be nearly identical (116%). The activity determination was not affected by the addition of $MoO_4^{2-}$ after the termination of enzymatic action in the absence of the ion. Thus, no inhibition of glucosidase activity was found. This indicates that the effect of $MoO_4^{2-}$ is on the transferase and not the glucosidase.

*Measurement of oligotransferase activity in tissue extracts*

Transferase activity in rabbit muscle extracts was tested using the iodine assay with amylopectin as a substrate. From the ratio of glucosidase units (Table I) to transferase units determined for the purified enzyme and the number of glucosidase units in the tissue extract, an absorbance (approx. 0.125) was estimated for the transferase assay in a 3-h incubation period using undiluted extract. However, no significant change in iodine color was found under these conditions. Because of the possible interference from the α-amylase found in muscle extracts[10] the assay was rerun in the presence of 0.01 M ethyleneglycol-bis(β-aminoethyl ether) *N,N'*-tetra-acetic acid (EGTA). EGTA has been reported to inhibit calcium-containing enzymes such as α-amylase[16]. Under these conditions, there was still no significant production of iodine color. This may indicate that the lack of production of iodine color is not due primarily to interference by α-amylase. It is possible that any transferase activity on amylopectin in the extracts was negated by the presence of branching enzyme (amylo-1,4 → 1,6-transglucosylase, EC 2.4.1.18, α-1,4-glucan:6-glycosyl transferase). Since branching enzyme acts on exterior chains having an average of 11–20 glycosyl residues[17], the outer chains of amylopectin which have an average length of 12–17

TABLE I

RATIO OF PURIFIED ENZYME ACTIVITY *versus* TISSUE EXTRACT ACTIVITY DETERMINED BY DIFFERENT METHODS

The procedures used for Methods I and II are referred to in the text. The procedure used for Method III is that described in the text. The units used in Method I are based on glucose release from glycogen phosphorylase limit dextrin. The units used in Method II are based on [$^{14}$C]glucose incorporation into glycogen. The units used in Method III are those defined in the text based in this case on a change in the absorbance of the iodine-complex difference spectrum of glycogen phosphorylase limit dextrin at 525 nm. The purified enzyme solution employed in Method II contained 3.20 limit dextrin units/ml. The purified enzyme solution employed in Method III contained 5.3 limit dextrin units/ml.

| *Method* | *Purified enzyme (units/ml)* | *Tissue extract (units/g wet wt.)* | *Ratio** | *% Variation*** |
|---|---|---|---|---|
| I. Liberation of glucose from glycogen phosphorylase limit dextrin | 3.20 | 4.03 | 0.795 | 0.0 |
| II. Incorporation of [$^{14}$C]glucose into glycogen | 4.16 | 5.24 | 0.780 | 2.0 |
| III. Modification in the iodine spectrum of glycogen phosphorylase limit dextrin | $2.33 \cdot 10^4$ | $2.84 \cdot 10^4$ | 0.821 | 3.3 |
| Modification in the iodine spectrum of glycogen phosphorylase limit dextrin in the presence of 0.10 M Tris | $7.16 \cdot 10^3$ | $8.41 \cdot 10^3$ | 0.850 | 6.9 |

* Ratio of purified enzyme units to tissue extract units.

** Percent variation of ratio from that determined by Method I which was arbitrarily set at 0.0.

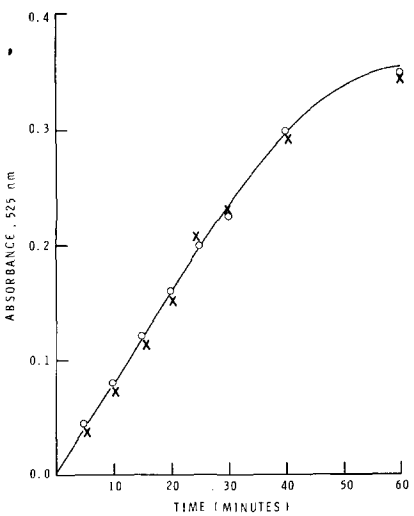



Fig. 9. Modification in the iodine-complex spectrum of glycogen phosphorylase limit dextrin in the presence of muscle extract. The standard iodine assay method described in the text was used except that limit dextrin was substituted for amylopectin. The tissue extract was prepared as a 1 to 20 (w/v) solution by the procedure referred to in the text and was further diluted 10-fold in the incubation solution. The dots (○) show the course of the reaction in the absence of EGTA. The crosses (×) show the course of the reaction in the presence of 0.01 M EGTA.

residues[18] would form an ideal substrate, especially upon elongation by transferase. For this reason phosphorylase limit dextrin was utilized as a substrate since the average chain length of a transferase-elongated chain would be 7 glucosyl residues, short of the length required for branching enzyme action. The results of this experiment are shown in Fig. 9. It should be noted that EGTA has no effect on the iodine color assay, indicating that α-amylase may not interfere under these conditions. With added 0.1 M Tris, the enzyme activity was reduced by 66%. This differed by only approx. 4% (Table I) from the effect of 0.1 M Tris on the development of iodine color using the purified glucosidase–transferase in place of the extract. From the values of glucosidase and transferase activity measured for the purified preparation and the tissue extract, the ratio of glucosidase units to transferase units in the purified preparation was compared to that found in the tissue extract. The results of various methods of measurement of glucosidase and/or transferase ars shown in Table I. As seen, the change in iodine color produced by the tissue extract is due to the glucosidase–transferase activity present. This is in line with evidence that the ratio of the glucosidase and transferase activities does not change throughout purification[1] and correlates with the finding that the ratio of limit dextrin units (measured by glucose production) and [$^{14}$C]glucose reincorporation units is the same in both the purified preparation and in tissue extracts[8]. The present results thus indicate that the iodine color assay for transferase using limit dextrin as a substrate is specific for the glucosidase–transferase system in tissue extracts.

## DISCUSSION AND CONCLUSIONS

The ability of oligo-1,4 → 1,4-glucantransferase to modify the iodine-complex spectrum of amylopectin serves as a convenient means of studying the action of the enzyme. The assay developed provides a rapid and simple means of quantitating transferase activity using readily available materials. The use of amylopectin as a substrate is specific for the transferase and does not require the combined action of both glucosidase and transferase as do methods which use glycogen phosphorylase limit dextrin as a substrate. This indicates that the transferase can act independent of subsequent action by the glucosidase.

Modification of the iodine-complex spectrum of glycogen phosphorylase limit dextrin by the glucosidase and the transferase indicates that the two activities act in a concerted manner with each contributing to the spectral change produced. The modification appears to be quite specific in tissue extracts as well as in purified preparations.

The apparent inability in tissue extracts of transferase to modify the iodine spectrum of amylopectin is probably due to the low levels of activity present, the possible interference of α-amylase, and to the action of branching enzyme which would tend to negate any chain elongation by the transferase[19]. This also explains why extensive iodine color formation was observed (Fig. 9) when limit dextrin was substituted for amylopectin, since even glucosidase debranched limit dextrin would be a poor substrate for branching enzyme. Apparently α-amylase is not significantly involved using limit dextrin as a substrate, since there was no appreciable variation in iodine color formation with the tissue extracts compared to the purified enzyme on an equivalent unit basis (Table I).

The difference in pH optimum between the transferase and the glucosidase (*cf.* Fig. 4), the inability of Tris either to shift the pH optimum of the transferase or to cause pronounced inhibition as it does with the glucosidase (*cf.* Fig. 8 and ref. 2, 8), and the inhibition shown by $MoO_4^{2-}$ of the transferase alone are of considerable interest since they suggest, aside from an apparent difference in substrate specificity, that the glucosidase and the transferase activities may have different active sites.

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