THE ACTION OF THE GLYCOGEN DEBRANCHING ENZYME SYSTEM IN A MUSCLE PROTEIN PARTICLE

T. E. Nelson, R. C. White and T. E. Watts

Department of Biochemistry
Baylor College of Medicine
Texas Medical Center
Houston, Texas 77025

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SUMMARY

Recently a protein-glycogen particle has been isolated from rabbit muscle and shown to contain phosphorylase as well as phosphorylase kinase and phosphorylase phosphatase activity (1). We have found that the glycogen phosphorylase limit dextrin debranching enzyme system (amylo-1,6-glucosidase/oligo-1,4→1,4-glucantransferase) is also present in the complex. A study of the action of the debranching enzyme system related to that of phosphorylase in the complex indicates that the debrancher participates in glycogen degradation as a function of the glycogen concentration. At high glycogen concentration the attack of phosphorylase was found to be essentially multi-chain (random) and independent of the debrancher, whereas at lower glycogen concentrations degradation proceeded with concomitant participation of the debranching system. It has been proposed that the protein-glycogen particle is a structural as well as a functional unit of the cell (1). The present results indicate that the debranching system is an essential component of the particle, and suggest that it is a multi-enzyme complex designed for the complete degradation of glycogen. The activity of the debranching enzyme system is an order of magnitude less in the complex, indicating that some type of constraint is imposed on it by the organizational structure.

The debranching enzyme system of rabbit muscle acts on the glycogen limit dextrin produced by phosphorylase to remove the branch chain linkage. This then allows further degradation of the structure by phosphorylase (2-5). Although it is well-known that total degradation of glycogen by phosphorylase requires participation of the debranching enzyme system amylo-1,6-glucosidase/oligo-1,4→1,4-glucantransferase, the degree to which the debrancher participates in normal *in vivo* glycogen degradation has not been clear (5,6).

Recently a protein-glycogen particle has been isolated from rabbit muscle and shown to contain phosphorylase as well as phosphorylase kinase and phosphorylase phosphatase activity (1). It has been suggested that the protein-glycogen particle is a structural as well as a functional unit of the cell (1).

There are indications that the particle may contain glycogen synthetase as well (7). The present results indicate that the debranching enzyme system is also a component of the particle and plays a concomitant role in the degradation of glycogen by phosphorylase.

The 80,000 x g protein-glycogen particle was prepared as described by Fischer and co-workers (procedure B) (1) and was found to contain ca. the same ratio of phosphorylase a to b and ca. the same yield of total phosphorylase per initial gm wet weight tissue as reported. The presence of the glucosidase-transferase in the particle was classically demonstrated by the release of glucose using glycogen phosphorylase limit dextrin as a substrate (2,5). Limit dextrin has been demonstrated to be specific for glucosidase-transferase in muscle extracts under the conditions employed (2,5,8,9). In addition, the production of glucose from limit dextrin by the particle was inhibited by Tris. Tris is known to inhibit the glucosidase-transferase (5,8,9). The fact that at high glycogen concentrations (see below) no glucose was produced indicates that glucose production from limit dextrin is due to the debranching system and not to contaminating glucosidase activities which would work on glycogen as readily as on limit dextrin. The ratio of phosphorylase units to glucosidase-transferase units in the particle was found to be approximately the same as reported previously for muscle extracts (5-9). This suggests that the relationship of the glucosidase-transferase to the other enzymes in the protein-glycogen particle is similar to that observed in the initial extract, and that these activities may exist in a fixed ratio to one another in the particle.

The extent to which the debrancher participates in glycogen degradation by phosphorylase in the protein-glycogen particle was investigated. Participation of the glucosidase-transferase is required (2-5) for phosphorylase to proceed beyond the formation of a limit dextrin structure in its degradation of glycogen. Since Tris is known to inhibit the glucosidase-transferase (5,8,9), its effect on glycogen degradation by phosphorylase was investigated

as a means of isolating the action of the debrancher during degradation. The action of phosphorylase was measured in the degradative direction in the presence of AMP (1 mM) using a method similar to those previously employed (10,11) by determining glucose 1-phosphate via a coupled reaction employing phosphoglucomutase glucose 6-phosphate dehydrogenase and production of NADPH. Tris was found to have no effect on purified phosphorylase under any of the conditions employed. The concentration of glycogen contributed by the particle in the assay mixture was ca. 0.15% and was not included in the substrate concentrations shown. The amount of glycogen isolated with the particle represents ca. 20% of the usual amount present in muscle. The effect of Tris on glucose 1-phosphate production at 1% glycogen concentration is shown in Figure 1. There was no detectable formation of glucose at this substrate concentration. Glucose is a product of glucosidase-transferase action on glycogen phosphorylase limit dextrin in the absence of Tris (2-5). This indicates that under these conditions the debranching system is not acting. Apparently, no substrate for it (limit dextrin) is being formed by phosphorylase and, accordingly, there is no effect of Tris on the system.

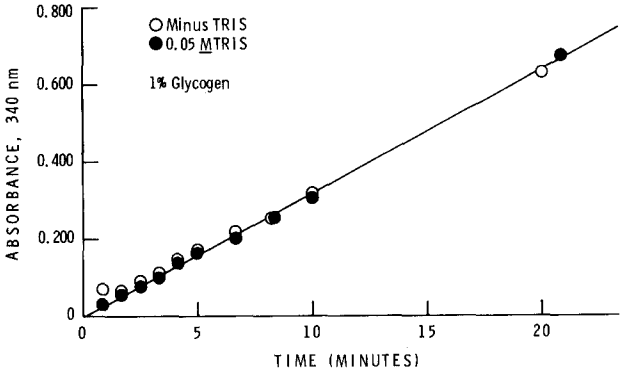

FIG. 1 - The action of phosphorylase in the protein particle at 1% glycogen concentration in the presence and absence of Tris. The extent of phosphorylase action at constant enzyme concentration was determined at the intervals shown. The production of glucose 1-phosphate was determined as described in the text. The protein-glycogen complex was used at a five-fold dilution of the 80,000 x g pellet. The incubation mixture contained 100 microliters of the enzyme solution per ml in 0.075 M sodium phosphate, 1% glycogen, 0.001 M AMP, 0.02 M 2-mercaptoethanol, pH 6.5.

The fact that under substrate saturating conditions for phosphorylase glucose is not produced by the glucosidase-transferase and inhibition of it has no effect suggests that the action of phosphorylase on any particular substrate molecule never reaches the stage of a limit dextrin structure. Since the removal of an average of four glucosyl residues per branch produces the limit dextrin structure, it appears that under these circumstances phosphorylase always removes less than four residues per branch. This suggests that the action of phosphorylase is probably multi-chain (random), i.e., the removal of only a single residue per each chain encounter (cf. ref. 12); this is in agreement with an earlier indication of multi-chain action (13).

The K_m of phosphorylase in the particle for glycogen was determined in the degradative direction in the presence and absence of Tris. The results are shown in Figure 2. They indicate that the action of phosphorylase is inhibited by Tris at substrate concentrations below 1% (0.5% to 0.05%).

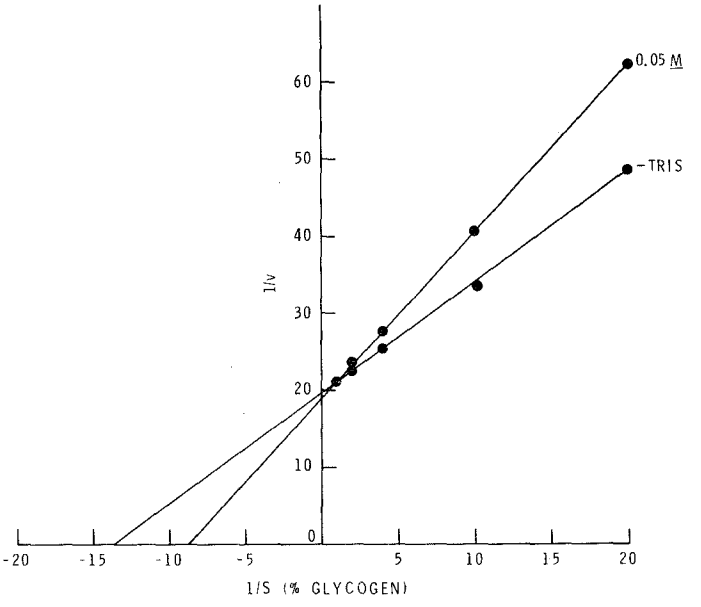

FIG. 2 - Inhibition by Tris of the action of protein particle phosphorylase on glycogen. The extent of phosphorylase action at constant enzyme concentration and various substrate concentrations was determined as described in Figure 1 at intervals of 2, 4, 8, 12 and 16 minutes. Initial velocities were extrapolated. The intersection of the two curves occurs at 1% glycogen concentration. The K_m in the presence of 0.05 M Tris was found to be 0.114%, the K_m in the absence of Tris was 0.073%.

Glucose was produced at the lower glycogen concentrations in the absence of Tris. The formation of glucose indicates that the debranching system is operating under these conditions. This signifies that the limit dextrin stage was reached. Inhibition by Tris of the debranching enzyme system would thus prevent action by phosphorylase beyond this point. The double reciprocal plot (Figure 2) suggests that the effect of Tris is essentially competitive; it inhibits the debranching enzyme system from providing additional substrate to phosphorylase. In effect, this reduces the number of productive phosphorylase substrate encounters and is essentially the type of situation which occurs during competitive inhibition.

These findings suggest that the glucosidase-transferase appears to be a concomitant participant in glycogen degradation within the usual physiological range of glycogen concentrations found in muscle, ca. 0.5% (14).

These studies also indicate that the rate at which the enzymes act in the particle are different from the rate at which they act either as purified enzymes or when the particle is dissociated by dilution. The action in the particle state is significantly slower. Dilutions of the complex by ca. 1000-fold (causing its dissociation) have been reported to increase the rate of phosphorylase b, phosphorylase kinase and phosphorylase phosphatase by an order of magnitude (1,15,16). The rate of the glucosidase-transferase is also increased under the same conditions by approximately 12-fold. This suggests that the organizational structure of the protein-glycogen complex imposes at least an order of magnitude of constraint on the action of the enzymes involved. This may constitute an additional form of regulation imposed upon glycogen degradation.

The K_m for glycogen of phosphorylase in the complex was determined in the degradative direction (1 mM AMP) to be 0.073%. This is somewhat higher than the value determined in the degradative direction (1 mM AMP) for the purified enzyme of 0.02% (11). There is no apparent explanation for this except that the properties of the enzymes in the complex appear to be signif-

icantly different from those of the purified enzymes.

The presence of the glucosidase-transferase and the role that it appears to play suggest that the muscle protein-glycogen particle is not an aggregate of phosphorylase and its affiliated control enzymes, but is an interrelated multi-enzyme complex designed for the overall degradation of glycogen.

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