SUBUNIT STRUCTURE OF YEAST AND RABBIT-MUSCLE GLUCOSIDASE-TRANSFERASES

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1. Introduction

Amylo-1,6-glucoside/1,4-α-glucan 4-α-glucan 4-α-glycosyltransferase (EC 3.2.1.33; EC 2.4.1.25) is the two component glycogen debranching enzyme system which is present in mammalian tissues and in yeast [1]. The extensively studied examples are those of rabbit muscle [2, 3] and of yeast [4, 5]. The enzyme systems from both sources have been purified to homogeneity [2, 3, 5], and in both cases the two enzymic activities which are essential to the debranching process are associated with a single protein species. The subunit structures of these two proteins are therefore of some interest. In this work we report a study of the subunit structures of the yeast and rabbit muscle glucosidase-transferase by the technique of sodium dodecyl sulfate [SDS] gel electrophoresis.

2. Materials and methods

Rabbit muscle glucoside-transferase was prepared by a procedure based on that described by Brown and Brown [2]. The DEAE-cellulose (Whatman DE 52) chromatography step was repeated twice, in an attempt to remove phosphorylase *b* which is the major contaminant of the preparation [2, 3] at this stage. Phosphorylase *b* activity was found to be present even after this treatment, and the preparation was therefore treated with rabbit muscle phosphorylase *b* kinase [6] to convert the phosphorylase *b* into the *a* form, which is more strongly retained on DEAE-cellulose than the *b* form [2]. The preparation was then chromatographed twice on Sephadex G-200. The final preparation had a specific activity of 6.6 IU/mg, and was homogeneous on disc gel electrophoresis.

Yeast glucosidase-transferase was prepared as described by the method of Carter and Lee [7], with the modification that the preparation was finally rechromatographed three times on Sephadex G-200. The preparation had a specific activity of 9.4 IU/mg and was apparently homogeneous by disc gel electrophoresis.

Disc gel electrophoresis in the presence of SDS was carried out as described by Weber and Osborn [8] including their procedure of sample preparation. An acrylamide:bisacrylamide ratio of 77:1 was used. Final acrylamide concentrations in the gels were 10%. Carboxymethylation of the glucosidase-transferase with iodoacetate was carried out as described by Craven et al. [9] after the protein had been incubated in 9 M urea, 1 M-mercaptoethanol for 20 hr at 37°. The material was then dialyzed against 0.01 M sodium phosphate pH 7.0, 0.1% SDS, 0.1% mercaptoethanol prior to electrophoresis on SDS gels.

Gel electrophoresis in the presence of 9 M urea [Mann Research Corp. ultrapure grade], was carried out as described by Parish and Marchalonis [10]. Samples were prepared by incubation for 18 hr at 30° in 9 M urea, 1 M mercaptoethanol. Gels were usually run with 5–10 μg protein, and were stained for protein with Coomaasie Blue.

3. Results

In the first series of experiments the subunit structures of the yeast and rabbit muscle glucosidase-transferase were investigated by SDS gel electrophoresis. In each experiment the marker proteins were run in the same experiment, and a calibration curve was derived

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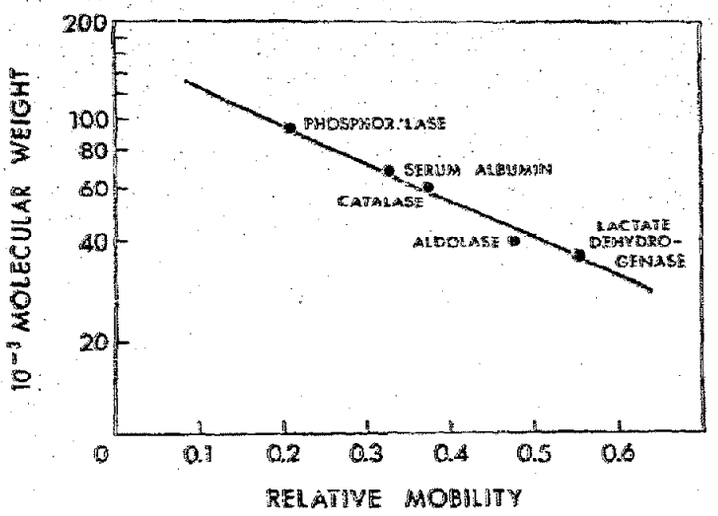

Fig. 1. Calibration of the SDS gel electrophoresis system. The standards used were rabbit muscle phosphorylase *b*, bovine serum albumin, beef liver catalase, rabbit muscle aldolase and lactate dehydrogenase.

for each experiment, as shown in the example in fig. 1. The yeast glucosidase-transferase showed the presence of three bands, of molecular weight 118,000, 83,000 and 66,000, respectively. The rabbit muscle glucosidase-transferase showed a major band of 118,000, and a minor band of 94,000 molecular weight (fig. 2A).

In a second series of experiments the proteins were subjected to carboxymethylation prior to SDS gel electrophoresis, in order to ensure the complete dissociation of the proteins. The yeast glucosidase-transferase again showed three subunits, of molecular weights 122,000, 85,000 and 70,000. The rabbit muscle glucosidase-transferase showed a major band of 120,000 molecular weight and a minor band of 92,000 molecular weight (fig. 2B).

In a third series of experiments the rabbit muscle and yeast glucosidase-transferases were examined by gel electrophoresis in the presence of urea. The yeast glucosidase again showed the presence of three subunits, while the rabbit muscle glucosidase-transferase showed the presence of one major band and a faster migrating minor band (fig. 2C).

4. Discussion

In an earlier investigation of the properties of yeast glucosidase-transferase, Lee et al. [5] had reported a molecular weight of about 280,000 by high-speed sedimentation equilibrium experiments in the analytical ultracentrifuge; their experiments also indicated that the enzyme system dissociated at low protein concentrations. Lee et al. [5] also reported that SDS electrophoresis is dissociated yeast glucosidase transferase into three subunits, but their preliminary data of the

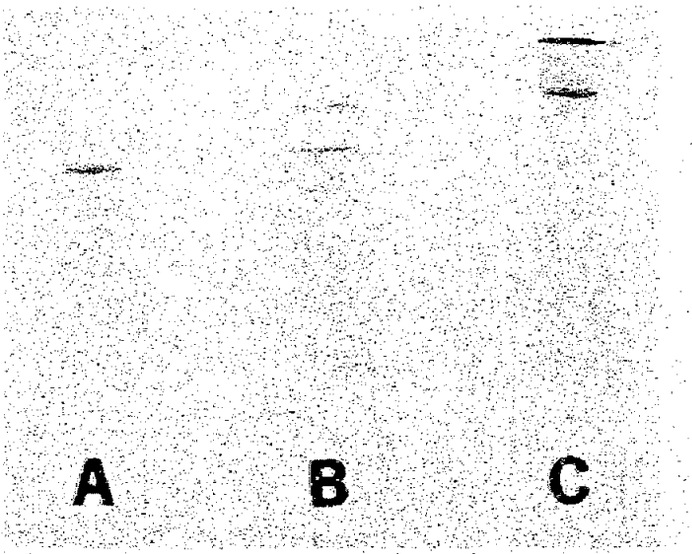

Fig. 2. SDS and urea gel electrophoresis of rabbit muscle glucosidase-transferase. A) SDS gel electrophoresis of rabbit muscle glucosidase-transferase. B) SDS gel electrophoresis after carboxymethylation. C) Gel electrophoresis in 9 M urea.

molecular weights (175,000, 94,000 and 74,000) were inconsistent with the molecular weight of the undissociated enzyme. Our results confirm the presence of three subunits, and our new data of molecular weights of these subunits show these to be about 120,000, 85,000 and 70,000, respectively. These molecular weights are consistent with the molecular weight of 280,000 determined for the undissociated enzyme [5], if it is assumed that the enzyme consists of three basic subunits. Carboxymethylation of the proteins before SDS gel electrophoresis confirmed that the slowest migrating band did not represent undissociated material. Gel electrophoresis in the presence of urea also confirmed that only three subunits were present. The urea experiments were done since resolution on SDS gels is a function of size only, while the resolution in normal gel electrophoresis is a function of both size and charge.

The rabbit muscle glucosidase-transferase always showed one major band, and one minor band. The major band had a molecular weight of 120,000. On the basis of a reported molecular weight of 270,000 for the rabbit muscle enzyme [1, 13], it was considered that the system consisted of two subunits of similar molecular weight [1]. However, Brown and Brown [12] have recently reinvestigated the molecular weight of rabbit muscle glucosidase-transferase, and have obtained a value of 170,000. It should be noted that the similarity of molecular weight of the glucosidase-transferase with that of rabbit muscle phosphorylase (185,000, [13]) as well as the similarity of their behaviour on ion exchange chromatography [2], makes it very possible that phosphorylase *b* may be a contaminant of our rabbit muscle glucosidase-transferase preparation. Although our preparation was free of phosphorylase *b* activity, it is possible that inactive phosphorylase protein was still present. This point is emphasized in view of the presence of the minor band of molecular weight 92–94,000. It seems likely that this represents dissociated phosphorylase *b* protein, since this enzyme has two identical subunits of 92,500 [13]. At the present stage it can only be said that there is evidence for a subunit of 120,000 molecular weight for the rabbit muscle glucosidase-transferase. This is difficult to explain, in view of the reported molecular weight of 170,000 for the enzyme system [12]. However, it may be noted that Brown and Brown, in a recent report on the subunit structure of glucosidase-transferase, have also observed a major subunit species of 120,000 by SDS gel electrophoresis and a number of smaller, but minor bands [12].

Acknowledgement

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