Glycogen-Associated Proteins of Rat Liver[1] (37430)

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A number of enzymes of glycogen metabolism have been found attached to glycogen macromolecules in cells. Such enzymes, associated with the storage polymer, form a unit of function for glycogen metabolism which has been called the glycosome (1).

Glycosomes have been separated from rat liver homogenates by precipitation with the bean lectin concanavalin A and shown to contain a number of enzymes of glycogen metabolism in an active form (2). In addition, this product has been utilized as the basis of a cell-free system for glycogen metabolism, in which both degradation and synthesis occur *in vitro* (3).

Utilizing such a preparation of glycosomes, the glycogen may be degraded by activation of phosphorylase which leaves the attached protein in solution. These various proteins, normally attached to intracellular glycogen, may then be characterized electrophoretically. This report shows the first such preparation utilizing this system, and demonstrating a reproducible electrophoretic pattern of glycogen-associated protein of rat liver.

Materials and Methods. Rat liver was homogenized and treated as previously described (3). The homogenate was spun at 8000*g* and the supernatant, which contains most of the glycogen, treated with 1 mg/ml concanavalin A. The glycosome–concanavalin aggregates were collected at 4000*g* and washed 3 times in Tris–HCl at pH 8. The pellet was finally resuspended in medium containing 0.05 *M* Na_2HPO_4, 0.001 *M* EDTA, 0.001 *M* AMP, 0.1 *M* Tris–HCl, pH 6.8. The resuspended pellet was placed in a dialysis bag and dialyzed overnight against 1 liter of the same buffer at room temperature. Phosphorylase was activated under these conditions and glycogen breakdown was complete. Aliquots of the remaining protein-containing solution were then placed on 7.5% acrylamide gels, and subjected to electrophoresis at pH 8.3 for 1 hr at 3 mA/gel. The gels were fixed in 7% acetic acid and stained with Amido Schwartz.

Electron microscopy was performed on concanavalin aggregates by fixation in buffered Osmium tetroxide and glutaraldehyde as previously described (1).

Results and Discussion. The concanavalin precipitate of glycogen-associated protein included 30.9% of the glycogen in the original homogenate but only 7.3% of the total protein of the homogenate and only 11.4% of the total material absorbing at 260 nm. Electron micrographs showed intact glycogen rosettes of varying sizes as normally seen in liver. In addition, there were some coprecipitating membrane fragments. It is known from previous work that such concanavalin precipitates contain activities of glycogen synthetase, phosphorylase, UDPG pyrophosphorylase, and branching enzymes (2, 3). Other enzyme activities have not been looked for in these preparations, but one would expect to find other enzymes of glycogen metabolism normally attached to the particle.

When precipitated glycogen was dialyzed against the glycogenolysis medium overnight at room temperature, phosphorylase was activated and glycogen totally depleted. The glycogen-associated proteins were then released in solution in the dialysis bag. This material in the dialysis bag was then applied to acrylamide gels for electrophoresis. Membrane fragments and some concanavalin

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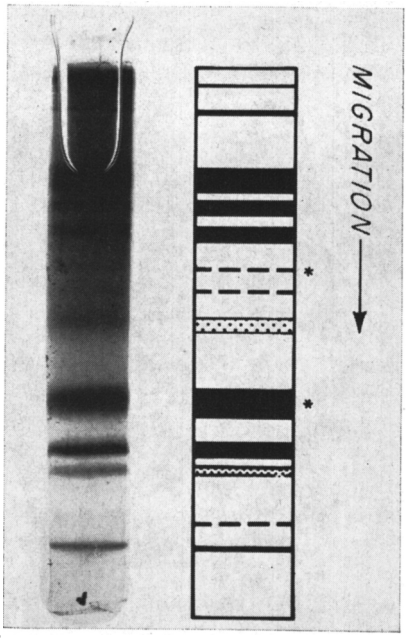

FIG. 1. Acrylamide gel electrophoresis of glycogen-associated protein of rat liver. Proteins were solubilized by degrading glycogen with which they were associated. Two bands (starred) are due to concanavalin used to precipitate glycogen–enzyme complexes.

A (which tends to autoaggregate) did not enter the pores of the gel whereas the soluble proteins migrate easily. The resulting readily reproducible pattern is illustrated in Fig. 1. The adjacent drawing shows the relative intensity of the bands. The two bands indicated by an asterisk were identified in control preparations with identical mobility of two fractions of concanavalin. These electrophoretic patterns show that a large number of proteins are glycogen-associated. The enzyme activities associated with these bands and their specific role in the architecture of the glycogen macromolecule remain to be established. The demonstration of such variety in these proteins, provides the basis for further study of the normal architecture of the glycogen macromolecule.

Summary. The glycogen-metabolizing organelle of rat liver was separated by precipitation with the bean lectin concanavalin A. Attached to these particles are enzymes involved in glycogen synthesis and degradation. By activation of phosphorylase *in vitro*, the glycogen polymer was totally degraded leaving in solution the glycogen-associated proteins. These were separated by electrophoresis in acrylamide gels which showed the variety of protein found attached to liver glycogen in the cell.

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