

The "Glycogen Granule" Revisited

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There is a gap between biochemical findings and ultrastructural interpretation of "glycogen granules". Biochemists have recognized that glycogen contains covalently bound proteins. These include enzymes involved in glycogen metabolism: glycogenin (protein primer responsible for initiation of glycogen synthesis), glycogen synthase and phosphorylase, and presumably other regulatory enzymes¹⁻³. The structures formed by the association of glycogen and protein have been called protein-glycogen complexes¹, considered as proteoglycans³, or as dynamic cellular organelles, **glycosomes**⁴.

The question arises as to why the biochemical recognition of a protein component in glycosomes has not been acknowledged in electron microscopy (EM)? This protein is visible in every section stained by uranium (U) and lead (Pb) salts where it appears as 20-30 nm granules⁵ (Fig. 1). However, these granules are commonly interpreted as glycogen, despite the fact that glycogen does not react with ionic compounds and therefore cannot be stained by U-Pb. Glycogen stained histochemically by periodic acid-thiosemicarbazide-silver proteinate (PA-TSC-SP)⁶ appears as 2-3 nm particles that form aggregates corresponding in size to the U-Pb stained protein granules (Fig. 2). It has been demonstrated that glycogen stained histochemically represents material different from protein stained by U-Pb⁵. When both components are dissociated by low pH, which occurs in tissue treated *en bloc* with uranyl acetate, protein solubilizes and is washed from the tissue, whereas 2-3 nm glycogen particles, which are not fixed, float within the cell and aggregate into large irregular clumps. These clumps are visible after histochemical staining, (Fig. 3) but in sections treated with U-Pb they remain as white unstained spots (Fig. 4).

The historical reason for the confusion in the ultrastructural interpretation of glycosomes is that early (1950/60's) EM studies found that the 20-30 nm particles stained by U-Pb disappear after digestion of tissue by amylase. Since enzymatic digestion was an ultimate proof in those days, it was concluded by several researchers that the granules represent glycogen (see 5) and this conclusion entered the textbooks. Subsequent findings that these granules also disappear after proteolytic digestion⁷ did not change the established opinion. In the light of present biochemical knowledge it is evident that the enzymes bound to glycogen solubilize after their carrier, glycogen, is digested by amylase which results in the disappearance of protein granules. Unfortunately, the traditional, misleading interpretation still remains in the textbooks. Although a drawing of a glycosome composed of glycogen and protein is presented in Fig. 14-12 of "The Molecular Biology of the Cell"⁸, there is no indication that both of these components can be distinguished with EM.

It seems that the differential identification of glycogen and protein using EM comprises an area for the collaboration between electron microscopists and biochemists. Numerous EM observations need further elucidation. For example, commonly observed differences in the electron density of glycosomal enzymes in the same section suggest differences in their structure, composition, or activity in particular glycosomes (Fig. 1). The recognition that glycosomes attached to other cellular structures (membranes and filaments) are resistant to high acidity⁹ suggests that they may correspond to the controversial desmoglycogen recognized in early biochemical studies (see 3). The molecular weight of glycogen also seems to remain controversial. Does a single molecular of glycogen form the 20-30 nm aggregate of particles (glycosome) observed in EM or the 2-3 nm particle visible within the glycosome (Fig. 2)? The latter would correspond to the small molecules suggested by Cori¹⁰. Consequently, is the glycogenin molecule located as a core of a glycosome or does every 2-3 nm particle within the glycosome contain its own glycogenin core?

The first microscopic observation that glycogen in the tissue is always associated with another material was that of Paul Ehrlich in 1883¹¹. Perhaps after more than one hundred years, we should revisit the morphology of glycosomes with the modern microscopic and molecular biology techniques. ■

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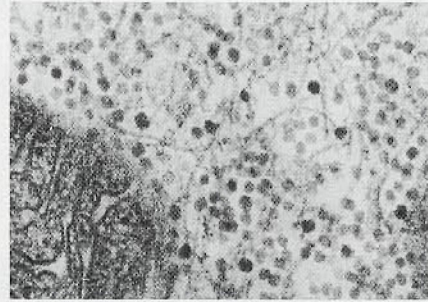


Fig. 1. Protein component of glycosomes; U-Pb; x 120,000

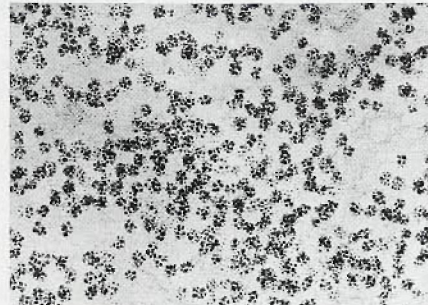
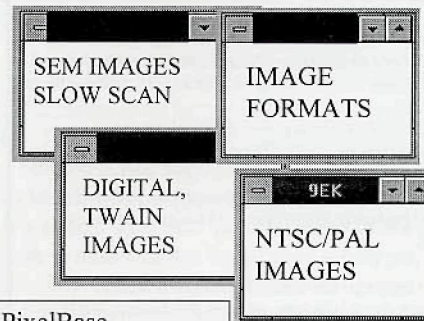


Fig. 2. Glycogen in glycosomes; PA-TCS-SP; x 120,000

Figure 3 & 4 on next page

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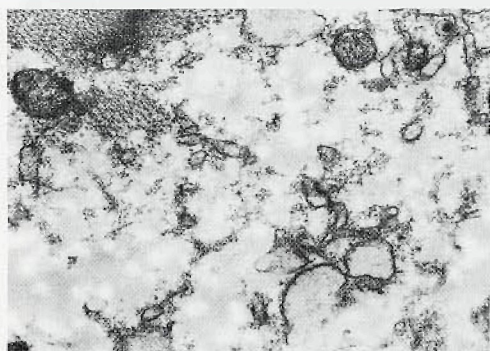


Fig. 3. Tissue treated *en bloc* with uranyl acetate, stained by PA-TSC-SP; glycogen particles form large, irregular clumps; x 28,500

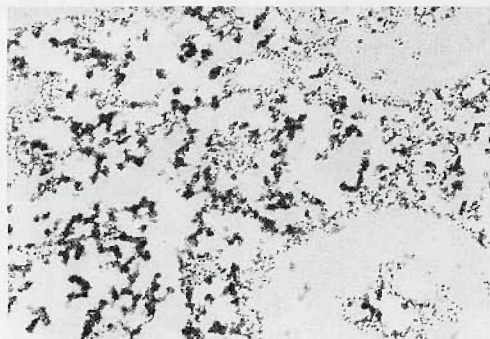


Fig. 4. Sections from the same block as in Fig. 3 stained by U-Pb; protein granules disappear and unstained spots corresponding to the clumps of glycogen particles in Fig. 3 are visible; x 28,000

Tricks of the Trade

When a sample is limited in amount, such as one or a very few particles, and a variety of tests may be necessary before identification is confirmed, the sample can be crushed between 2 half-slides pressed by thumb and finger with a sliding motion of the slides. Tiny individual particles can then be picked out for microchemical, hotstage, XRD or optical crystallographic tests. These techniques are described in "The Particle Atlas"; for the ultramicrominiaturization of microchemical tests, see *The Microscope* 19, 235-241 (1971). Gary Valaskovic
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