The "Glycogen Granule" Revisited

Krystyna Rybicka, SUNY at Buffalo

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 enzymes[1-3]. The structures formed by the association of glycogen and protein have been called protein-glycogen complexes[1], considered as proteoglycans[3], 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 granules[5] (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-Pb[5]. 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 disgestion 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 digestion[7] 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 acidity[9] 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 Cori[10]. 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 1883[11]! Perhaps after more than one hundred years, we should revisit the morphology of glycosomes with the modern microscopic and molecular biology techniques. ■

1. Meyer, F. et al. 1970. J. Biol. Chem. 245, 6642-8
2. Smythe, C. & Cohen, P. 1991. Eur. J. Biochem. 200, 625-31.
3. Whelan, W.J. 1986. BioEssays. 5, 136-40.
4. Scott, R.B. & Still, W.J.S. 1968. J. Clin. Invest. 47, 353-9
5. Rybicka, K. 1979. Virchows, Arch. B. Cell Path. 30, 355-47.
6. Thiery, J.P. 1967. J. Microscopie. 6, 987-1018.
7. Rosati, G. 1967. J. Ultrastruct. Res. 18, 444-55.
8. Alberts, B. et al. 1994. Molecular Biology of the Cell, Garland Publ. Inc., New York.
9. Rybicka, K. 1981. J. Histochem. Cytochem. 29, 553-60.
10. Orell,S.A.et al. 1964, Control of Glycogen Metabolism, J.A.Churchill, London, pp.29-52.
11. Ehrlich, P. 1883. Zeitschr. Klinische Medizine, 6, 33-53

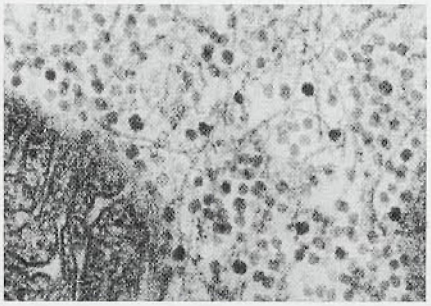

Fig. 1. Protein component of glycosomes; U-Pb; x 120,000

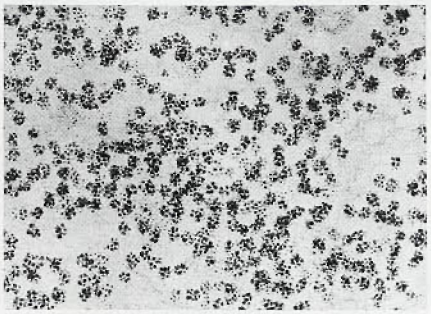

Fig. 2. Glycogen in glycosomes; PA-TCS-SP; x 120,000

Figure 3 & 4 on next page

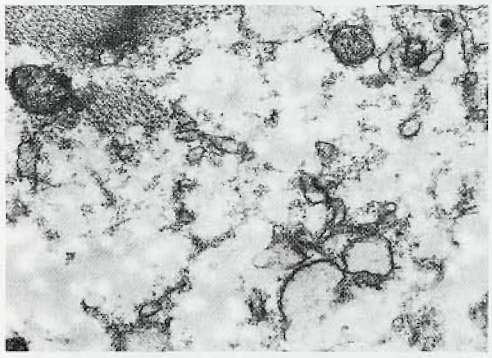

Fig. 3. Tissue treated *en bloc* with uranyl acetate, stained by PA-TSC-SP; glycogen particles form large, irregular clumbs; x 28,500

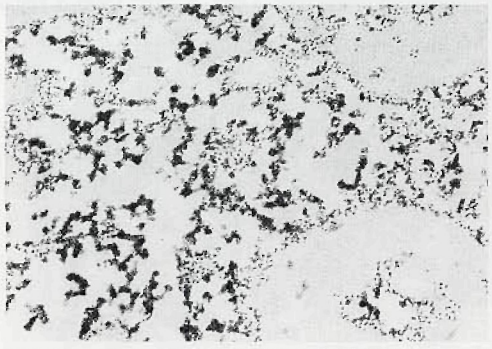

Fig. 4. Sections from the same block as in Fig. 3 stained by U-Pb; protein granules disappear and unstained spots corresponding to the clumbs of glycogen particles in Fig. 3 are visible; x 28,000

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*

*Reprinted with permission from The Microscope, **31**, 333 (1983)*

McCrone Research Institute
MICROSCOPY COURSES

McCrone Research Institute offers a variety of 5-day courses that emphasize polarized light microscopy. All courses offer a strong foundation in both theory and application. Here are descriptions of some of our more popular courses.

Courses with General Focus

Applied Polarized Light Microscopy - A survey course on using microscopy for particle identification, microchemical tests, crystal characterization, dispersion staining, hot-stage (fusion methods), and photomicrography. This course may serve as prerequisite for advanced courses.

Forensic Microscopy - This course is very similar to course 201. Materials examined emphasize trace evidence; that is, glass, soils, hair, fibers, paint, explosives, drugs, etc. You are more likely to have classmates from forensic laboratories in this course, but you will leave with the same skills as course 201. This course also serves as a prerequisite for advanced courses.

Microscopy for Art Conservators – This course is intended for conservators of art objects, especially paintings, but also textiles and paper. Methods of sampling, characterization and identification of pigments, grounds, media and fibers by polarized light microscopy will be covered.

Pharmaceutical Microscopy – Identification of particulate contaminants and examination of the solid state (e.g., polymorphism, solvation and particle size).

Specialized Courses - Methodology

Computer Assisted Microscopy - Learn the many ways computers can assist a microscopist. Start with basic principles, followed by hands-on tutorial sessions where you use spreadsheets, databases, image editing and image processing. Students proceed working with the microscope and computer. A basic knowledge of microscopy is needed but no previous computer experience required

Crystal Morphology & Optics - A basic polarized light microscopy course emphasizing petrographic methods. Applicable especially to study of minerals, explosives, drugs, and paint pigments.

Particle Identification and Manipulation - A major problem in the identification of small particles is the manipulation of single-particle samples. Often less than 10 μm in diameter, the particle must be collected, isolated, cleaned and mounted for a specific analysis such as XRD, PLM, SEM, TEM, Auger, SIMS, EMA, IMA, MOLE etc. Many of the necessary particle handling techniques have been developed in the McCrone Associates laboratory.

Photomicrography - A hands-on course covering the theory and practice of light microscopy to ensure the best visual and photomicrographic images. Taught by John G. Delly, author of the Kodak book, *Photography Through the Microscope* and co-author of the *Particle Atlas*.

Sample Preparation Methods - This is a special course offering emphasizing the isolation and manipulation of microscopical samples for Polarized Light Microscopy, microchemical testing, FTIR microscopy and instrumental analysis.

Special Methods in Light Microscopy-A - The classical methods of light microscopy will be discussed, demonstrated and practiced. Techniques and instruments to be covered include: holographic microscopy, infrared microscopy, infrared absorption/microscopy, fluorescence, inverted microscopes, x-ray microscopy , schlieren microscopy, Zernike phase contrast, Nomarski differential interference contrast, Hoffman modulation contrast, anoptral contrast, interference microscopy, video projection, hotstage methods, subnanogram microchemical tests, DTA on a microscope slide, particle handling, sample preparation and others.

Specialized Courses - Materials

Identification of Small Particles – A course in the collection, fractionation, manipulation, characterization and identification of single particles in the subnanogram range.

Mineral Identification - A course in optical mineralogy for those interested in microscopical characterization, identification and comparison of more common minerals. Recognition of crushed and detrital mineral grains by polarized light microscopy, microchemical tests and other micro physical tests (e.g.,, density, magnetism).

Microchemical Analysis - This course is intended for microscopists who wish to obtain chemical information directly from micro samples. Systematic application of microchemical tests for organic and inorganic samples. Particle handling techniques and practical problem-solving.

Advanced Microchemical Analysis - A continuation of the course *Microchemical Analysis* for analytical microscopists who wish to improve their ability to identify small particles or obtain chemical information from small quantities of material. Students are encouraged to bring practical problems to the course as examples.

Polymer, Fiber & Film Microscopy - Chemical, optical and physical characterization of polymers. The optical properties are determined by crystallographic procedures. These permit identification of polymers (bulk, fiber and film) and allow a better understanding of their physical properties, orientation and crystallinity.

Specialized Courses - Asbestos Identification

Microscopical Identification of Asbestos – An introductory course that stresses the proper microscopical alignment, procedure and technique for fiber identification. Discussion includes both serpentine and amphibole asbestos types: their crystallographic properties, the various substances likely to occur (and often confused) with asbestos and the procedures for identification.

Advanced Asbestos Identification – This course is for the experienced analyst. Discussion includes the identification and differentiation of all asbestos fibers and fibrous substitutes. Various immersion techniques for n_D measurement are emphasized, including the construction and interpretation of dispersion staining graphs. Sample preparation techniques of altered samples, floor tiles and other complex matrices are covered. Addressed are quantitation methods, NVLAP requirements and QA/QC in the laboratory.

Asbestos Fiber Counting (NIOSH 582) – This is the NIOSH 582 course: "Sampling and Quantitating Airborne Asbestos Dust". Asbestos minerals and other fibrous material are covered so that discrimination can be made within the limits of phase contrast microscopy. Statistics and sampling-related mathematics are presented; the proper calibration of air sampling equipment is covered. At the conclusion of the course, the student will be able to properly use the sampling and microscopical equipment and be able to apply the procedures for the collection and quantitation of ambient dust samples.

Quantitative Asbestos Analysis – This class is devoted entirely to the various techniques for determining the amount of asbestos in bulk samples. Weight percent standards are prepared and evaluated for QA/QC. Topics include: sample preparation; point counting using several graticules and protocols; percent determination by area, volume and gravimetric methods; inter/intra laboratory training programs. Interactive computer programs will be used, and made available for educational purposes in your laboratory.

For a Course Catalog, Dates and Registration Information

Contact Nancy Daerr, McCrone Research Institute, 2820 S. Michigan Ave., Chicago, IL 60616-3292
Phone: 312/842-7100 or Fax: 312/842-1078