Reprinted from:
"33rd Ann. Proc. Electron Microscopy Soc. Amer."
Las Vegas, Nevada, 1975. G. W. Bailey (ed.).

PHOTOELECTRON MICROSCOPY OF BIOMEMBRANES: OBSERVATION OF EXTERNAL PHOTOEMISSION FROM SPINACH CHLOROPLASTS

Rudy J. Dam, George H. Lesch, David W. Deamer* and O. Hayes Griffith

Institute of Molecular Biology and Department of Chemistry, University of Oregon, Eugene, Oregon 97403 and *Department of Zoology, University of California, Davis, California 95616

Understanding the detailed structure and function in biological membranes is a current problem in molecular biology. Ultrastructural studies, *based on the ability to discriminate chemically different sites on the membrane surface*, would provide crucial data relevant to a number of questions. This paper describes a promising microscopic technique, based on the photoelectric effect, which has this capacity. A sample is irradiated with UV light above the photoelectric threshold, and the resulting photoelectrons are accelerated and focused by conventional electron optics. Figure 1 illustrates schematically the technique of photoelectron microscopy (PEM) in comparison with two more familiar techniques, fluorescence microscopy and transmission electron microscopy (TEM). Efforts to utilize the photoelectric effect to study biological surfaces were begun in this laboratory in 1968 and the initial images of organic surfaces and a mammalian tissue are described by Griffith *et al.* (1-4). We report here the first photoelectron image of plant organelles. The samples, spinach chloroplasts, were chosen for this preliminary study because the preparations are well characterized and their size permits observation in our prototype low magnification instrument.

In order to examine spinach chloroplasts, a suspension of isolated chloroplasts, prepared by the method of Whitehouse *et al.* (5) and placed in a medium containing 5% gelatin, was confined between the ends of two sample rods and brought to liquid nitrogen temperature. The rods were then separated, leaving fractured chloroplasts imbedded in frozen medium on both sample rod surfaces. This "freeze-squeeze" method is a type of freeze fracture sample preparation. One of the rods was then immediately inserted into the photoelectron microscope. Immediately upon switching on the UV lamp with the monochromator set at 200 nm, bright spots appeared against a black field. Typical results are shown in Fig. 2a. These images were deflected by magnetic fields and disappeared when the incident light was switched off. There is no doubt that these images are formed by electrons photoejected from the sample by UV light. A control sample containing the gelatin medium but no chloroplasts appeared dark under these conditions. Figure 2b is a visible reflected-light micrograph at about the same magnification (although not the same field) of the chloroplast sample after removal from the photoelectron microscope. The chloroplasts are clearly visible as dark disc-like features against a bright field in the optical micrograph. In comparing Figures 2a and 2b, the photoelectron images appear larger because of the lower resolution of our prototype PEM, and possibly chloroplast aggregation or chlorophyll surface migration. From their number and general distribution, we conclude that the bright spots in the PEM image are produced by chloroplasts. Fig. 2a is, to our knowledge, the first photoelectron micrograph of plant organelles.

What is the source of contrast in Fig. 2a? In order to answer this question micrographs were recorded as a function of wavelength of incident light. The intensity of the spots decreases with increasing wavelength, and the spots disappear above 240 nm. This parallels the behavior of photoelectron images of chlorophyll isolated from these chloroplasts. The presence of chlorophyll in the sample shown in Figures 2a and 2b was verified by observation of its characteristic fluorescence at 680 nm; the chlorophyll appeared to be localized within the chloroplasts. On the basis of this

Reprinted from:
"33rd Ann. Proc. Electron Microscopy Soc. Amer."
Las Vegas, Nevada, 1975. G. W. Bailey (ed.).

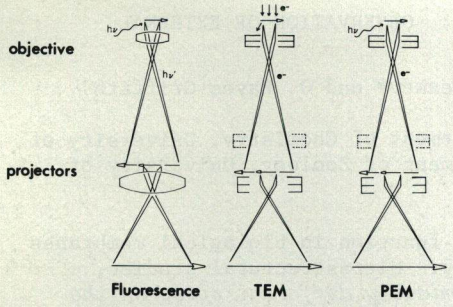

Fig. 1 The techniques of fluorescence microscopy, TEM and PEM compared. PEM shares with fluorescence the use of incident exciting light, and with TEM the advantages of electron image formation. PEM is a form of emission microscopy, and except for certain design considerations, the approach is related to the imaging of photoelectrons in metallurgy (6).

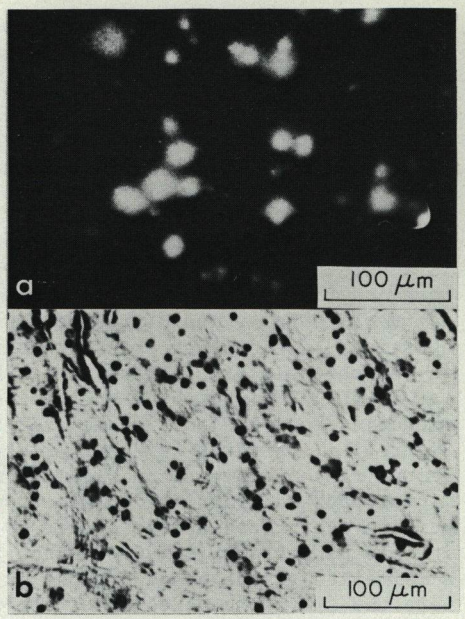

Fig. 2a Low magnification photoelectron micrograph of a freeze-cleaved chloroplast preparation, using 200 nm incident light.

Fig. 2b Reflected light micrograph of the same sample. The chloroplasts are visible as small black spots against a bright field.

evidence, we tentatively conclude that chlorophyll is the source of contrast in the photoelectron images of Fig. 2a, and that the bright spots are chloroplasts that have been cleaved so as to expose chlorophyll.

We wish to thank Dr. Howard T. Bonnet and Keith F. Kongslie for useful discussions. The photoelectron microscope was funded by U.S. Public Health Service grant CA11695 from the National Cancer Institute. This particular series of experiments was supported by National Science Foundation grant no. GB-32808.

1. Griffith, O.H., G.H. Lesch, G.F. Rempfer, G.B. Birrell, C.A. Burke, D.W. Schlosser, M.H. Mallon, G.B. Lee, R.G. Stafford, P.C. Jost and T.B. Marriott, Proc. Natl. Acad. Sci. U.S.A. 69, 561 (1972).
2. Dam, R.J., K.F. Kongslie, and O.H. Griffith, Biophys. J. 14, 933 (1974).
3. Birrell, G.B., C. Burke, P. Dehlinger and O.H. Griffith, Biophys. J. 13, 462 (1973).
4. Burke, C.A., G.B. Birrell, G.H. Lesch and O.H. Griffith, Photochem. Photobiol. 19, 29 (1974).
5. Whitehouse, D.G., L.J. Ludwig and D.A. Walker, J. Exp. Botany 22, 772 (1971).
6. Wegmann, L., J. Microscopy 96 (1), 1 (1972).