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PHOTOELECTRON MICROSCOPY AND QUANTUM YIELDS OF MEMBRANE PHOSPHOLIPIDS.

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Contrast in photoelectron microscopy (PEM) depends on differences in the absolute photoelectron quantum yield defined as the number of electrons released divided by the number of incident photons. Recently, the photoelectron quantum yields of the amino acids and homopolymers were measured (1). These data define the range of quantum yields expected from membrane proteins. The other major membrane component is lipid and we report here the photoelectron quantum yields of the common phospholipids.

Synthetic L-α-dipalmitoylphosphatidylcholine (DPPC), L-α-dipalmitoylphosphatidylethanolamine (DPPE), L-α-dipalmitoylphosphatidylserine (DPPS), dipalmitoylphosphatidylglycerol (DPPG), and bovine heart diphosphatidylglycerol (DPG, or cardiolipin) were obtained commercially. DPPS, DPPC and DPPG were estimated to be at least 90% free of organic impurities by TLC analysis and DPPE and DPG migrated as single spots. Commercial, 99+% pure cholesterol was recrystallized from a chloroform:methanol (2:1 v/v) solution. Thin phospholipid films were prepared in two ways. One method, used for all phospholipids, was to place several drops of 5 to 10 mg/ml solution in chloroform:methanol on the end of a stainless steel sample rod and to allow the solvent to evaporate at room temperature. In the second method (used for all phospholipids except DPG), several drops of an aqueous dispersion of phospholipid vesicles, dialized against distilled water to remove salt and buffers from the aqueous phase, were placed on a nitrocellulose-carbon covered sample rod and allowed to dry. These two methods gave identical yield values within experimental error. Cholesterol samples were prepared by atomizing a 10 mg/ml solution in chloroform:methanol until a smooth, opaque coating formed on the sample rod.

The quantum yield curves of three phospholipids found in mammalian membranes (DPPE, DPPC and DPPS) are plotted vs. wavelength in Fig. 1. Since the acyl chains of these phospholipids are identical, the differences in quantum yield are due to differences in photoemission from the head groups (X). Preliminary quantum yield curves of the recrystallized isolated head groups phosphoryl ethanolamine, phosphoryl choline and phosphoryl serine are higher but exhibit the same relative order as the corresponding phospholipid curves, in qualitative agreement with this conclusion. The curve for DPPE lies a factor of 4-10 above the other phospholipids, suggesting the possibility of distinguishing clusters or domains of DPPE in phospholipid mixtures by photoelectron microscopy.

Fig. 2 shows the quantum yield data of two phospholipids without amino groups (DPG and DPPG) and of cholesterol. These data resemble the curves for PS and PC, so that only PE stands out in this series. For comparison, the shaded band defining the range of yield curves for 19 amino acids is reproduced in Fig. 2. The photoelectric effect is approximately additive, so that this band defines the range of quantum yield curves expected for all membrane proteins without prosthetic groups. It is clear that the quantum yield curves of the lipids lie within or below the yield curves of proteins. One interesting consequence of these low yields is the high contrast expected between dye molecules such as phthalocyanine (Pc) and membrane surfaces. From Fig. 2 there are at least three orders of magnitude difference between the quantum yield curves of Pc and the lipids. Image brightness in photoelectron microscopy is proportional to quantum yield, and Fig. 3 illustrates the large contrast between Pc and a background of the phos-

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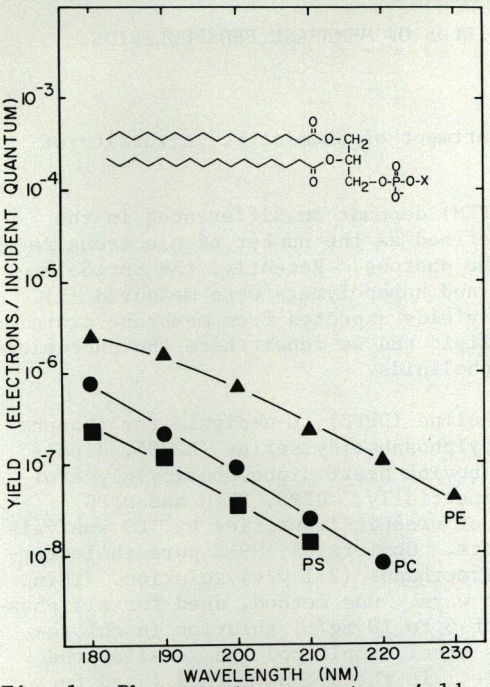

Fig. 1. Photoelectron quantum yield curves of

DPPE (triangles, $X=CH_2CH_2\overset{+}{N}H_3$)

DPPC (circles, $X=CH_2CH_2\overset{+}{N}(CH_3)_3$)

DPPS (squares, $X=CH_2CH(\overset{+}{N}H_3)CO_2^-$)

The yield data are accurate to plus or minus a factor of 2 or 3.

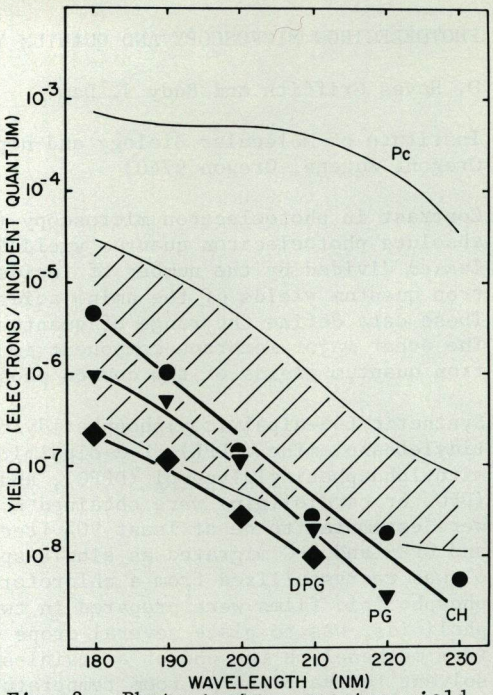

Fig. 2. Photoelectron quantum yield curves of DPG (diamonds), DPPG (triangles, $X=CH_2CHOHCH_2OH$), and cholesterol (circles). The shaded band contains the yield curves of 19 amino acids and Pc is the yield curve for metal-free phthalocyanine. All measurements were done at room temperature.

pholipid DPPS. The photoelectron quantum yield curves of Fig.1 and 2 explain quantitatively the earlier report (2) of high contrast between several possible photoelectron labels and DPPC at about 200-230 nm.

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2. Birrell, G.B., C.A. Burke, P. Dehlinger and O.H. Griffith, Biophys. J. 13, 462 (1973).

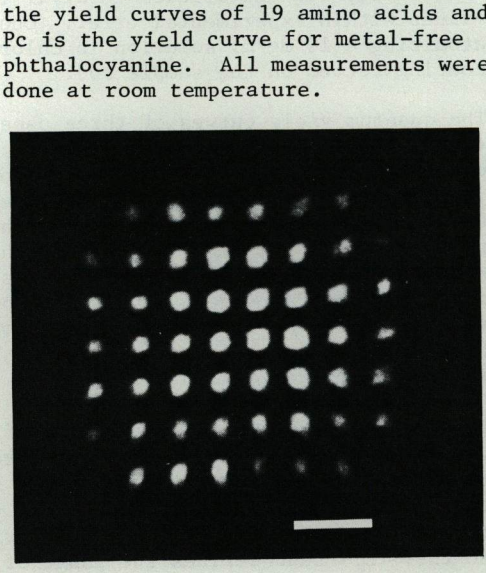

Fig. 3. Low-magnification photoelectron micrograph of a grid pattern of phthalocyanine evaporated onto a uniform film of DPPS. The exposure time was 30 sec on Polaroid type 52 film and the incident wavelength was 200 nm. Bar = 100 μ.