PHOTOELECTRON QUANTUM YIELDS OF THE AMINO ACIDS

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ABSTRACT The photoelectron quantum yields of 21 common amino acids and 15 polyamino acids were measured in the 180–240 nm wavelength region. On the average, the quantum yields of these two groups exhibit quite similar wavelength dependence. For $\lambda > 220$ nm all amino acid and polyamino acid quantum yields are $\leq 10^{-7}$ electrons/(incident) photon. The mean yields increase to about 5×10^{-7} electrons/photon at 200 nm and 5×10^{-6} electrons/photon at 180 nm. L-tryptophan, L-tyrosine, and poly-L-tryptophan exhibit above average yields between 180 and 200 nm. Comparison with the dye phthalocyanine indicates that the quantum yield of the dye is two orders of magnitude greater than that of the amino acids from 200 to 240 nm, suggesting the feasibility of photoelectron labeling studies of biological surfaces.

INTRODUCTION

Determining the relative positions and numbers of specific binding sites on cell surfaces is one of the challenging problems in membrane biophysics. The photoelectric effect has recently been shown to have promise as a new method of mapping the positions of biological surface components (Griffith et al., 1972; Birrell et al., 1973; Burke et al., 1974). This approach, photoelectron microscopy, relies on differences in the photoelectron quantum yields of naturally occurring surface components and of possible label molecules.

The most fundamental of the data needed in photoelectron microscopy are electron quantum yields of the amino acids. These data would also be of interest in certain photobiological studies in the UV region (Setlow, 1960; Berger, 1969; Wirths and Jung, 1972). To our knowledge no electron quantum yield measurements on any amino acids have appeared in the literature. Accordingly, we have measured and report here the essential features of the absolute photoelectron quantum yield curves of the common amino acids and homopolymers over the wavelength range 240–180 nm.

MATERIALS AND METHODS

The following L-α-amino acids were examined in this study: (1) aspartic acid, (2) arginine, (3) glycine, (4) lysine, (5) tryptophan, (6) hydroxyproline, (7) proline, (8) alanine, (9) valine, (10) phenylalanine, (11) histidine, (12) leucine, (13) methionine, (14) glutamic acid, (15) tyrosine, (16) asparagine, (17) threonine, (18) methionine, (19) cystine, (20) serine, (21) cysteine. Amino acids 2–5, 7, 9, and 11–15 were obtained from California Biochemical Co., La Jolla, Calif.; nos. 6, 10, and 20 from Sigma Chemical Co., St. Louis, Mo.; 1 and 16 from National Biochemical Co., Chicago, Ill.; 8, 17, and 18 from Cyclo Chemical Corp., Los Angeles, Calif.; and 19 from Difco Laboratories, Detroit, Mich. All amino acids were purified by recrystallization from distilled water before use.

Homopolymers (molecular weight ranging from 5,000 to 100,000) of amino acids 1–14 were obtained from Sigma Chemical Co.; 15 from Miles Laboratories, Elkhart, Ind. Water was used to solubilize polymers 1, 2, 4, 6 and 14. Polymers 7 and 11 dissolved in slightly acidic water solution (5 drops 0.7 M acetic acid per 1 ml solution). Other solvents for the polymers were as follows: dichloroacetic acid-H_2O, 1:1 (3), dimethylformamide (5), trifluoroacetic acid (8), glacial acetic acid (9, 10, 12), chloroform (13), and methanol (15). Polymers 1, 2, 4, 6, 7, 11 and 14 were purified by dialysis against water; poly-L-tyrosine was recrystallized out of methanol. In all cases the quantum yield curves remained, within experimental error, unchanged with respect to those obtained from the original samples. The remaining polymers were used without further purification.

The sample is supported on the end of a 6.35 mm diameter stainless steel sample rod. In order to suppress photoemission from stainless steel the end of the rod is covered with a thin film of Formvar (Polyvinyl Formal 15/95, Polysciences, Inc., Rydal, Pa.). This is accomplished by dipping the rod briefly in a 1% solution of Formvar in chloroform and allowing the film to dry in air.

The amino acids tend to form irregular crystalline coatings instead of uniform films if the drying conditions are not controlled. Fairly uniform crystalline films can be prepared using the following procedure. Approximately 5 ml of aqueous amino acid solution (50–100 mg/ml) is placed in an atomizer (Brinkmann Instruments, Burlingame, Calif.; SGA Scientific, Inc., Bloomfield, N. J.) which is connected to a variable-pressure nitrogen source. The spraying speed and droplet size can be adjusted by changing the gas pressure; we found 5 lb/in^2 to be optimal. It is of advantage to obtain the smallest possible droplet size. By alternately spraying and forcing warm air from a heat gun (Master Heat Guns, Racine, Wisc.) over the sample rod a very uniform coating of amino acid can be formed. All measurements were made with freshly prepared samples.

The procedure for preparing the polyamino acid films varied slightly according to the solubility of the compound being prepared. For most polymers a 1 mg/ml solution was made, usually requiring 5–10 min bath sonication. Several of the polymers (9, 10, 12) were very nearly insoluble at room temperature. Saturated solutions of these were prepared by heating in glacial acetic acid at 100°C for 1 h and subsequently filtering out the undissolved component through a coarse sintered glass filter (Fasman et al., 1965). Several drops of the resulting solutions were placed on the end of a Formvar-coated sample rod and allowed to dry. It was found that the drying conditions affected different polymers in different ways. Therefore, a number of samples of each compound were made, half of which dried at room temperature and pressure and half at reduced pressure (0.1–1 torr) in a vacuum dessicator. The sample films which appeared most uniform under a stereomicroscope were subsequently used for the quantum yield measurements.

The standard used in this study is metal-free phthalocyanine (Eastman Kodak Co., Rochester, N. Y.), purified by vacuum zonal sublimation as described elsewhere (Burke et al.,

1974). This compound was chosen for its stability and because consistently uniform samples are relatively easy to prepare by vacuum sublimation. The electron quantum yield of phthalocyanine has been measured by Schechtman (1968). A fresh standard sample was prepared prior to each run in order to correct for small daily variations in lamp intensity, phototube gain settings, and optical alignment. All measurements were made in the photoelectron microscope described previously (Burke et al., 1974), by replacing the camera with a photomultiplier tube at the image intensifier output stage. The instrument is an oil-free ultrahigh vacuum system constructed from stainless steel components and copper sealed (Varian Conflat; Varian Associates, Palo Alto, Calif.) flanges, so that surface contamination problems are minimal.

RESULTS AND DISCUSSION

Electron Quantum Yield Curve of Naphthacene

The absolute quantum yield, as used here, is defined as the number of electrons photoejected per incident quantum. In the present apparatus it is not a simple matter to measure the absolute intensity of the light incident on the sample. Therefore, we have devised an approach which involves the measurement of the image brightness of the sample relative to that of a reference compound at the same instrument magnification. Since the image intensifier-phototube system is linear (Burke et al., 1974), the image brightness is proportional to (*a*) the intensity $I(\lambda)$ of UV light incident on the sample and (*b*) the photoelectric quantum yield $Y(\lambda)$ (electrons per incident quantum). Therefore at any given wavelength, different samples will appear relatively brighter or fainter in proportion to their $Y(\lambda)$ values according to the equation

$$Y(\lambda) = \frac{B(\lambda)}{B_o(\lambda)} \cdot Y_o(\lambda), \qquad (1)$$

where $Y(\lambda)$ is the electron quantum yield of the sample, $Y_o(\lambda)$ is the quantum yield of the standard, $B(\lambda)$ is the image brightness of the sample, and $B_o(\lambda)$ is the image brightness of the standard. Neither the incident light intensity nor the resulting electron current are measured. Only relative photometer readings are required.

To test the validity of this technique, we have measured the electron quantum yield curve of naphthacene over the 180–240 nm spectral region. The electron quantum yield curve of naphthacene has been measured in a conventional apparatus by Harada and Inokuchi (1966) and more recently by Hirooka and Harada (private communication). Fig. 1 compares the data of Hirooka and Harada (solid line) and the photoelectron microscope data (triangles). The agreement is very good considering the inherent difficulties in absolute quantum yield measurements. The average data points agree within a factor of 2 to 3 over the entire wavelength range of Fig. 1.

The electric field strength between the sample and the anode is 10^4 V/cm in the present photoelectron microscope, whereas it is only $10–10^2$ V/cm in a conventional apparatus. The data of Schechtman were measured in a conventional apparatus,

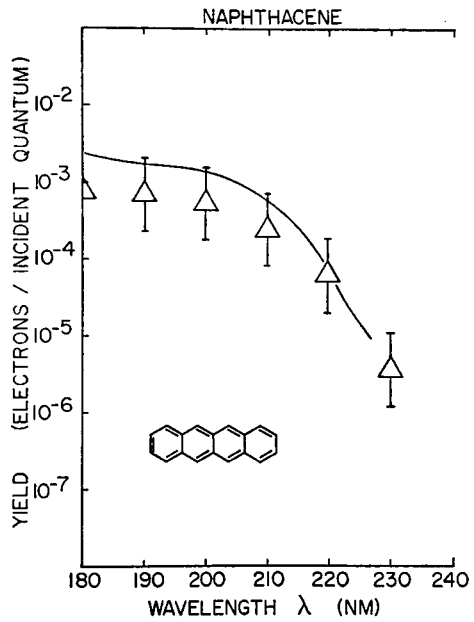

FIGURE 1 Absolute electron quantum yield of naphthacene (tetracene) as a function of the wavelength of incident light. Triangles are data obtained in this study and the solid lines are data of Hirooka and Harada (private communication).

so the use of Eq. 1 assumes that the metal-free phthalocyanine quantum yield data are independent of the electric field strength (up to at least 10^4 V/cm). This assumption has been tested by varying the electric field strengths over the range 20 V/cm to 10^4 V/cm. The number of electrons emitted per second, as measured by the cathode-anode current at fixed incident light intensity, varies by less than a factor of 30% over this range of electric field strengths.

Electron Quantum Yield Curves for the Amino Acids and Homopolymers

The absolute quantum yield of 21 L-α-amino acids were measured at 10-nm intervals from 180 to 240 nm using the above approach. Since there is considerable overlap of the yield curves in this wavelength range, and scatter reveals a typical uncertainty of plus or minus a factor of 3 to 5 for any given data point, presentation of the individual curves is inappropriate. However, the data can be conveniently summarized by presenting the range of quantum yields as a function of wavelength. Of the 21 amino acids, 19 have yield curves which lie within the shaded area shown in Fig. 2. The two exceptions, tryptophan and tyrosine, lie above this band at wavelengths below approximately 200 nm, and are plotted as circles and triangles, respectively. The quantum yield of phthalocyanine (Pc), the photoelectric standard used in this study, is also shown in Fig. 2.

Similarly, the quantum yield curves for the 15 polyamino acids are summarized

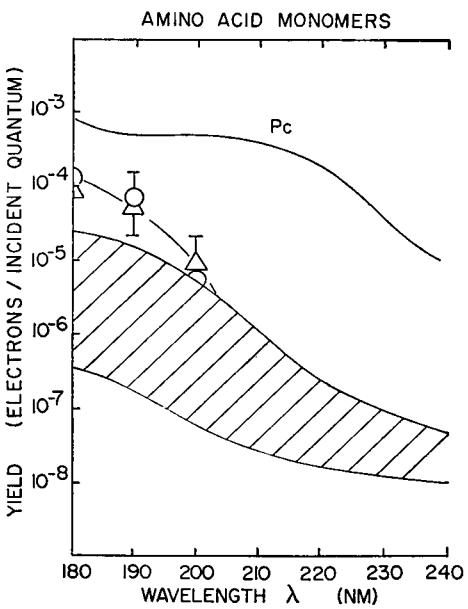

FIGURE 2

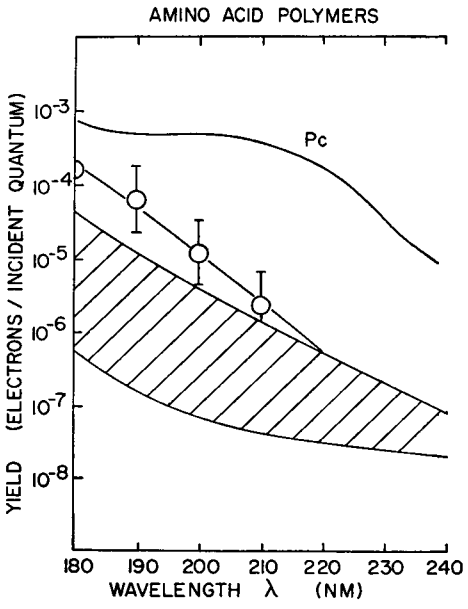

FIGURE 3

FIGURE 2 Spectral dependence of the quantum yields of the amino acids. All amino acids fall into the shaded region except L-tryptophan (○) and L-tyrosine (△). The upper line is the quantum yield curve of metal-free phthalocyanine (Pc) reported by Schechtman (1968). The amino acid data are measured relative to this standard curve.

FIGURE 3 Spectral dependence of the quantum yields of the polyamino acids (shaded region). Poly-L-tryptophan (○) exhibited the highest quantum yields. The upper curve (Pc) is the metal-free phthalocyanine standard curve of Fig. 2.

in Fig. 3. 14 of these are accounted for, within experimental error, by the shaded band. Poly-L-tryptophan, plotted as circles, is again an exception, lying above this region at shorter wavelengths (poly-L-tyrosine is within the upper part of the band).

These results indicate that the quantum yield characteristics of the amino acids and their homopolymers are quite similar. At wavelengths longer than 220 nm all of the amino acids and polymers are poor photoemitters, with quantum yields of 10^{-7} electrons/photon or less. At 200 nm the mean yield for both groups increases to approximately 5×10^{-7} electrons/photon. Finally, at 180 nm the mean yield increases to approximately 5×10^{-6} electrons/photon. At this wavelength poly-tryptophan, tryptophan, and tyrosine have yields on the order of 10^{-4} electrons/photon, well above the other amino acids and polymers.

In summary, the amino acids exhibit relatively small quantum yields at wavelengths longer than 180 nm. This has important implications to possible labeling of biological surfaces. For example, it has been suggested that photoelectron labels could be selectively bound to surface components to provide information about the distribution of specific binding sites (Griffith et al., 1972). Photoelectron labeling

shares with fluorescence labeling the requirement that in some wavelength region, the labels must be visible against the background of the sample. The electron quantum yield of the photoelectron label must, in some wavelength region, greatly exceed the electron quantum yields of typical biological surface components. From this standpoint Figs. 2 and 3 provide encouraging results, since the reference molecule, phthalocyanine, can be viewed as a prototype label. At long wavelengths (>240 nm) neither phthalocyanine nor the amino acids emit a significant number of electrons. At the other extreme of short wavelengths, both phthalocyanine and some of the amino acids have comparably higher yields. Hence in this region there would be little contrast between the photoelectron images of Pc and the amino acids. However, between these limits there exists a window (i.e. 200–240 nm) in which the electron quantum yield of phthalocyanine is two orders of magnitude greater than the amino acids, suggesting good contrast in this region.

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