[CONTRIBUTION FROM THE BIOPHYSICAL LABORATORY OF THE DEPARTMENT OF PHYSICS, UNIVERSITY OF MISSOURI]

Quantum Yield as a Function of Wave Length for the Inactivation of Urease

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Attempts to explain the specificity of enzymes and to elucidate their structure have been numerous. It therefore seemed important to investigate the photochemical behavior of an enzyme with a view to obtaining additional information. For instance, the magnitude of the quantum yield for the inactivation of urease might be expected to give a clue as to whether its property of specificity resides in a unique region of the molecule or is characteristic of the molecule in its entirety. It is known that the urease molecule is large (mol. wt. 483,000) and that its absorption must be distributed among numerous chromophores. Hence, a quantum yield approaching unity would indicate that inactivation may result from an alteration occurring almost anywhere.

Kubowitz and Haas[1] assume a constant quantum yield for the inactivation of urease in the ultraviolet region of the spectrum used. By so doing their data show a direct correspondence between the inactivation and absorption spectra. In fact, the agreement is exceedingly good at each wave length tried except at 2540 Å. where the value from inactivation is twice that obtained from direct absorption measurement. It is surprising that the value at this wave length should disagree in view of the otherwise excellent results. For the inactivation of pepsin Gates[2] states there is a direct correspondence between the inactivation spectrum and the absorption spectrum. This actually seems contrary to the data presented. He gives a misleading value of 1 for the quantum yield when by simple calculation lower values are obtained which are not constant with wave length. Therefore, since the data from the inactivation of the enzymes, urease and pepsin, are not in agreement, additional data should corroborate one or the other. It seemed particularly advisable to check the value at 2540 Å. for urease.

Experimental

Preliminary experiments were done with a Hanovia type Sc-2537 low-pressure mercury discharge tube, which emits primarily the 2537 Å. mercury line. This discharge tube was used without a monochromator. Later data obtained at 2537 Å. by using a monochromator seemed inconsistent with these preliminary results in that the discharge tube proved more efficient than the monochromatic radiation. A spectrogram showed that other mercury lines of shorter wave length were emitted from the discharge tube, in particular the resonance line at 1849 Å. In an attempt to interpret the data from the discharge tube, it was decided to include some inactivation experiments at 1860 Å. as well as at the other wave lengths.

The crystal quartz monochromator used in this experiment had a Young type mounting. The two 30 degree prisms had a height of 65 mm. and the short side of the prisms facing the lenses were 65 mm. wide. The lenses were 70 mm. in diameter and had a focal length of 30 cm. at 5893 Å. A capillary mercury vapor lamp operating at atmospheric pressure under water served as a source from the visible to 2300 Å. in the ultraviolet. A spark between a rotating disk and one stationary electrode served as a source below 2300 Å., zinc emission lines being used at 2100 and 2068 Å. and aluminum at 1989 and 1860 Å. A 20,000 volt 2.5 kva. transformer with a capacitance of about 0.03 microfarad connected across the secondary fed the spark. When using the aluminum electrodes, the monochromator was filled with nitrogen to reduce the absorption of the radiation by the air in the monochromator. At wave length 1860 Å. the introduction of the nitrogen increased the intensity by a factor of six.

The intensity of the radiation was measured by an Eppley surface-type vacuum thermopile, which was calibrated against standard lamp C-241, obtained from the U. S. Bureau of Standards. A galvanometer scale deflection of 1 cm. represented 17.3 ergs/sec. Intensities employed are given in Table I. The fused quartz cell used for irradiation was 4 mm. wide, 10.9 mm. deep and 40 mm. high. The cell was held just in front of the thermopile and was removed when measuring the intensity of the radiation. A fused quartz stirring rod, turned by a small motor, kept the solution in continual motion.

The urease was obtained from Arlco jack bean meal. It was crystallized and reprecipitated by the method of Sumner.[3] The activity of the five solutions prepared deviated less than 10% from 130 units, the value given by Sumner for his best preparations. The concentration of the stock solutions, as determined by a micro-Kjeldahl method, was about 2 mg./cc. They were stored in an ice chest. Dilutions from the stock solutions were buffered by a pyrophosphate buffer as prescribed by Kubowitz and Haas.[1] Two concentrations for the buffer were used: 0.001 molar for the longer wave lengths and 0.000064 molar for the shorter wave lengths. The *p*H of the buffer was 6.5. The urea solution which was used for testing the activity of the urease had the following composition: 3% urea, 5.4% Na_2HPO_4, 4.2% KH_2PO_4, and a drop of toluene.

A portion of the stock solution was diluted with buffer to a concentration of 0.0414×10^{-6} mole/liter for a series

(1) Kubowitz and Haas, *Biochem. Z.*, **257**, 337 (1933).

(2) Gates, *J. Gen. Physiol.*, **18**, 265 (1934).

(3) Sumner, *J. Biol. Chem.*, **69**, 435 (1926); **70**, 97 (1926).

of trials. The activity of the diluted solution was measured by the ammonia produced in its decomposition of the urea solution. A volume of 0.4 cc. of the diluted solution was added to 1 cc. of the urea solution and allowed to react for five minutes at 20°. The reaction was stopped by adding 1 cc. of normal hydrochloric acid. The ammonia produced by the reaction was measured by comparing the color when Nessler reagent was added with the color produced in standard solutions. The color was compared with a colorimeter. The contents of the reaction tube was diluted to 90 cc. before adding Nessler reagent. The activity of the control solution, tested a number of times during a series, remained constant. One-cc. portions were irradiated at selected wave lengths and the activity was measured immediately after each irradiation. The radiant flux absorbed by the layer of solution 10.9 mm. thick was calculated from absorption measurements described later.

Absorption Measurements.—The extinction coefficients of a urease solution, buffered by pyrophosphate, were measured at selected wave lengths. The extinction coefficient is defined by the equation

$$\epsilon = \frac{1}{cd} \log_{10} \frac{I_0}{I}$$

where c is the concentration in moles/liter, d is the thickness of the solution in cm., I_0 is the incident intensity and I is the intensity of the radiation after passing through the solution. Measurements were made on each of the five solutions prepared and it was found that the absorption curves were essentially the same for all. A Spekker photometer with a medium Hilger quartz spectrograph and a photoelectric method were both used. The values shown in Fig. 1 were obtained with a photocell-direct current amplifier for a pyrophosphate buffered solution. Matched cells were used, one containing the solution and the other containing the solvent. In the spectral region from 4043 to 2260 Å. a solution 1 cm. thick was used and at shorter wave lengths the solution was 0.155 cm. thick. A concentration of 1.29×10^{-6} mole/liter was used from 4043 to 2378 Å. and the solution was more dilute for measurements at shorter wave lengths. The logarithm of the extinction coefficient is plotted as a function of the wave length in Fig. 1. At wave lengths shorter than 2400 Å. the present data are in agreement with those of Kubowitz and Haas[1] (shown by squares in Fig. 1), at longer wave lengths the present values are somewhat higher. Kubowitz and Haas found the maximum selective absorption at 2800 Å. Others[4,5] find the maximum near 2650 Å., and from the present data it appears to be about 2700 Å.

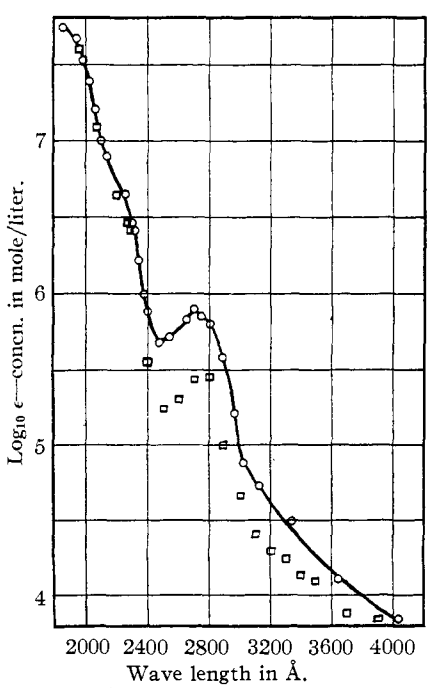

Fig. 1.—Absorption of urease.

Results

The quantum yield for the inactivation of urease by ultraviolet radiation has been calculated as a ratio of the molecules inactivated to the quanta absorbed. The number of molecules inactivated was given by $NV(c_0 - c)$, where N is Avogadro's number, V is the irradiated volume in liters, c_0 is the original and c the final concentration of the active urease in moles/liter. The quanta absorbed were found by the expression

$$\left(\frac{I_0 t}{hC/\lambda}\right) T \frac{c_a}{\epsilon c_0 + k_B} \left[1 - 10^{-(\epsilon c_0 + k_B)d}\right]$$

where I_0 is the incident radiant flux in ergs/sec., t is the time in seconds, h is Planck's constant, C is the velocity of light in cm./sec., λ is the wave length in cm. of the radiation used, T is the transmission of the window of the quartz cell, c_a is the average concentration of the active urease (found by integrating over the value of the dose), ϵ is the molecular extinction coefficient of the urease, k_B is the extinction coefficient of the buffer solution and d is the thickness of the solution in cm. The term $c_a/(\epsilon c_0 + k_B)$ gave the fraction of the total radiant flux absorbed by the active urease throughout a particular trial. Since the absorption coefficient of the urease did not change until more than 0.85 had been inactivated, the original concentration c_0 was used for calculating the total absorbed energy. The calculated values of the quantum yield for the inactivation of urease are given in Table I.

In preliminary experiments the total radiation from the low pressure mercury discharge tube was incident upon the cell. Only the incident radiation below 3000 Å. was measured in these trials. Calculating the quantum yield, as described above, these trials gave the value 0.00382 molecule/quan-

(4) Stern and Salomon, *Enzymologia*, **2**, 96 (1937).
(5) Ito, *J. Biochem. (Japan)*, **24**, 279 (1936).

TABLE I

QUANTUM YIELD FOR THE INACTIVATION OF UREASE

Wave length, Å.	Radiation incident on cell in ergs/sec.		Number of trials	Quantum yield (standard error given)	
1860	320	(slit 2 mm. × 10 mm.)	5	0.00938	±0.00075
1989	130		6	.00400	± .00061
2068	1,000		5	.00399	± .00045
2100	1,200		4	.00225	± .00017
2300	500	(slit 1 mm. × 10 mm.)	6	.00181	± .00021
2390[a]	1,000		9	.00212	± .00010
2537	3,100		15	.000926	± .000056
2650	2,400		8	.000784	± .000108
2804	1,500		10	.000880	± .000071
2967	2,200		1	.000606	.....
3022	7,500		5	.000707	± .000094
3130	8,500		1	.000816	.....
Discharge tube alone[b]			10	.00382	± .00031
Discharge tube + HAc filter[b]			9	.000909	± .000076

[a] Due to the wide slit used for this wave length, which is a combination of 2378 and 2399 Å., other shorter wave lengths came in as impurities. This is probably the reason for the high value at this wave length. [b] Radiation incident on cell is 35 ergs/sq. mm./sec., the whole surface of the cell being exposed.

tum, assuming that all the radiation was wave length 2537 Å. Other trials were made with a 2% aqueous solution of acetic acid between the discharge tube and the quartz cell. The acetic acid absorbed all radiation shorter than 2350 Å. These trials gave for the quantum yield the value 0.000909 molecule/quantum.

Discussion

Kubowitz and Haas[1] did not calculate the quantum yield for the inactivation of urease as the molecular weight was unknown at that time. In 1938, however, the molecular weight of urease was determined by Sumner, *et al.*,[6] by the centrifuge method. This method gave the value 483,000 for the molecular weight. Using this value of the molecular weight and the data of Kubowitz and Haas a quantum yield of 0.003 molecule/quantum has been calculated, the value being constant throughout the spectral region used except at 2540 Å. The present data indicates that the quantum yield is fairly constant within the wave length region from 3130 to 2537 Å., the average value being about 0.0008. At wave lengths shorter than 2537 Å. the quantum yield calculated from the present data increases, reaching a value of 0.00938 at 1860 Å.

The results of Kubowitz and Haas and the author differ in two respects. First, according to the author the quantum yield for the inactivation of urease depends on the wave length at which it was inactivated, while Kubowitz and Haas found that the quantum yield was constant. Second, the average value at the longer wave lengths, found by the author, is only one-fourth the value given by the data of Kubowitz and Haas. It is difficult to explain why two sets of results for the inactivation of urease should disagree in this manner. Since the experimental procedures were different, this may in part explain the disagreement. Therefore, the major differences in procedures will be described. Kubowitz and Haas irradiated a dilute solution which absorbed only a fraction of 1% of the radiant flux at the longer wave lengths and 1–5% at the shorter wave lengths and they used a thin layer of solution, possibly 1–2 mm. thick. The author irradiated a solution 80 times more concentrated than used by Kubowitz and Haas and a layer of solution 10.9 mm. thick. The activity of the urease was also measured by different methods. Kubowitz and Haas measured the activity of their urease solution by the rate of carbon dioxide evolution after adding the urea solution. This rate was measured by a manometer attached to the flask. The alternative method, used by the author, was to measure the ammonia released during the reaction, keeping it in solution and determining the amount with Nessler reagent.

The value of the quantum yield, 0.000926, obtained using monochromatic radiation of wave length 2537 Å., agrees with the value 0.000909, obtained using radiation of wave lengths longer than 2350 Å. from the discharge tube. Assuming that all the effective radiation from the dis-

(6) Sumner, *et al.*, *J. Biol. Chem.*, **125**, 37 (1938).

charge tube was in the two resonance lines, 2537 and 1849 Å., a simple calculation shows that 3–4% of its radiation must be of the shorter wave engths in order to account for the results obtained. This is a plausible explanation for a photograph of the spectrum from the discharge tube showed the following lines below 2537 Å. were present: 2483, 2399, 2378, 2352, 2260, 2100, 2025, 1970, 1942 and 1849 Å. That the mercury line, 1849 Å., is present to an appreciable percentage in a low-pressure discharge tube also has been mentioned by Cline and Forbes,[7] who report about 2%, and by Rössler and Schönherr,[8] who report a maximum of 10% under certain conditions.

The quantum yield for the inactivation of pepsin may be calculated from the data of Gates.[2] From his data the quantum yields at wave lengths 2357, 2509, 2719 Å. are, respectively, 0.0014, 0.00034, 0.00045, and at 2930 Å. the quantum yield is very low, almost zero. These data indicate an increase in quantum yield with decreasing wave length. This is in agreement with the results of the present experiment.

A similarity in the reactions of both the urease and pepsin to ultraviolet radiation is worth mentioning. The loss of enzymatic activity seems to be more sensitive to effective radiant energy than a second observable change, indicated by increased molecular extinction coefficients in the absorbing region. A large amount of the enzymatic activity is actually destroyed before an appreciable change in absorption occurs.

(7) Cline and Forbes, THIS JOURNAL, **61**, 716 (1939).
(8) Rössler and Schönherr, *Z. tech. Physik*, **19**, 588 (1938).

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Summary

1. The absorption spectrum of urease has been measured in a pyrophosphate buffer solution at *p*H 5.6. A maximum molecular extinction coefficient of 0.798×10^6 occurs around 2700 Å., assuming a molecular weight of 483,000, and a minimum occurs at about 2480 Å.

2. The quantum yield for urease inactivation has been determined for a number of wave lengths. From 3130 to 2537 Å. the quantum yield seems to be fairly constant with the value of about 0.0008 molecule/quantum; increasing at shorter wave lengths, it reaches a value of 0.00938 at 1860 Å.

3. The direct radiation from a low-pressure mercury discharge tube (Hanovia Sc-2537) was more efficient in inactivating urease than monochromatic radiation of 2537 Å.; this is shown to be due to radiation of wave length shorter than 2350 Å., probably the mercury line at 1849 Å.

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Molecular Rotation and Polymorphism in the Methyl Chloromethanes

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The almost spherical symmetry of the *t*-butyl chloride molecule and its low heat of fusion led to an investigation of the dielectric constants of the *t*-butyl halides[1] which showed rotational freedom in the solid for some distance below the melting points comparable to that in the liquid state. As rotation was made possible by the approximate similarity in size and shape of the halogen atoms and the methyl groups, it appeared highly probable that molecules containing not only one, but also two and three chlorines attached to the central carbon with methyl groups occupying the remaining positions possessed similar rotational freedom. Transitions have been found in the compound with four methyls around the central carbon, neopentane,[2] and in the compound with four chlorines, carbon tetrachloride,[3] which are to be interpreted as indicating the setting in of molecular rotation.[4] As the molecules of these two substances possess no dipole moments, they cannot be investigated by dielectric constant measurements, but the intermediate compounds have been studied by this method with the aim of enlarging our knowledge of the effect of molecular structure upon the rotational freedom of the molecule in the crystalline state.

The dielectric constant measurements were made with a capacity bridge coupled to a variable oscillator in the general fashion previously de-

(1) Baker and Smyth, THIS JOURNAL, **61**, 2798 (1939).
(2) Aston and Messerly, *ibid.*, **58**, 2354 (1936).
(3) Johnston and Long, *ibid.*, **56**, 31 (1934).
(4) Baker and Smyth, *ibid.*, **61**, 1695 (1939).