INACTIVATION OF PEPSIN BY HIGH INTENSITY ULTRAVIOLET LIGHT AND THE RECIPROCITY LAW

by

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The fundamental rate law for the inactivation of an enzyme E by ultraviolet light[1] for a one-hit process, is

$$\Phi = \frac{E_0 \ln (E_0/E)}{I_{abs}\, t} = \frac{K_\lambda E_0}{I_{abs}} \qquad (1)$$

It follows that to inactivate a given fraction of enzyme the reciprocity law should be obeyed; *i.e.*, $I_1 t_1 = I_2 t_2$ for a given initial active enzyme concentration, E_0. Here Φ is the effective quantum yield for inactivation and I_1, I_2 and t_1, t_2 are intensities and times respectively. With ordinary laboratory intensities the one-hit process is obeyed, perhaps primarily because of the low quantum incidence compared to the number of absorbing molecules in solution. The relatively low intensities utilized do not permit a molecule to absorb two or more quanta before undergoing reactions. It seemed worthwhile to test the reciprocity relationship with a protein having several chromophores per molecule, at much higher intensities than customarily used.

It is theoretically possible for a pepsin molecule to absorb several quanta nearly "simultaneously" prior to the inactivation step, providing sufficiently high intensities of ultraviolet light are employed. Recently apparatus which produces very high intensities, of the order of 10^{21} quanta/flash, has been described by PORTER[2]. We have used a scaled down version of this type of equipment to achieve moderately high intensities. In particular, PORTER's equipment delivered single flashes in the neighborhood of 100 to 500 μF capacity whereas ours were of the order of 20 μF capacity.

With a Hanovia Sc-2537 mercury resonance lamp, illuminated with single bursts of *ca.* 0.5 milli-second duration, the intensity of actinometric radiation measured by a uranyl oxalate actinometer solution was around $2 \cdot 10^{-7}$ einsteins per burst per ml solution. Since our pepsin solution contained *ca.* $5 \cdot 10^{-8}$ moles per ml, it may be seen that the incidence of quanta reaching the solution per burst was comparable to the number of molecules of pepsin per ml. It was found that the pseudo first order rate law, equation (1), was obeyed even at these high intensities.

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EXPERIMENTAL

Irradiation equipment for high intensity

The lamp used was a specially built, straight Hanovia Sc-2537 mercury resonance lamp with tungsten lead wires brought out through graded electrode seals. It contained the usual argon and mercury at low pressures. The distance between electrodes was *ca.* 13.5 cm and the diameter was *ca* 9.5 mm. O.D. The lamps were quickly discolored and had to be cleaned and refilled by the manufacturer after a few hundred bursts.

The energy source for the lamp was made up from banks of condensers with a power supply, Fig. 1. The banks contained 10 Sprague Vitamin Q condensers rated at 2·0.15 μF, 8000 volts. Ten condensers were connected in parallel and the equivalent capacitance of such a bank was 3 μF. The unit consisted of seven banks with a total capacity of 21 μFarads. The connecting leads were $\frac{1}{2}$ by $\frac{1}{16}$ inch copper strips. Two half-wave mercury-vapor rectifiers, RCA 866/866-A supplied DC to the condensers. The circuit was a single-phase full-wave rectifier. The filament transformer was a Stancor P 6133 (2.5 volt center tap, 5 ampere, insulated for 7500 volts). The main voltage was supplied through a powerstat (0–110 volts AC) to a transformer (100 volts to 4000 volts step up). To prevent premature firing of the lamp a special hand-operated spark-gap type switch was installed. This enabled the operator to fire the lamp at the desired voltage and at the desired rate.

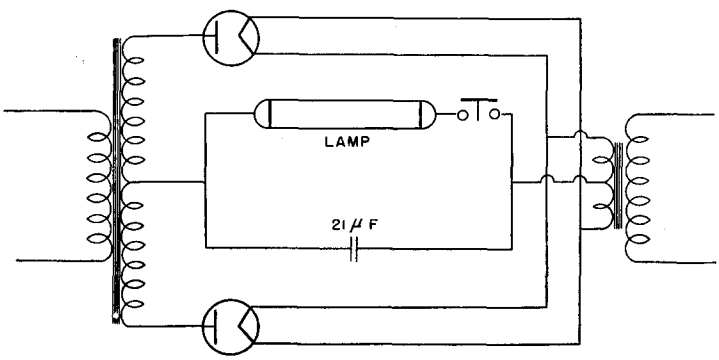

Fig. 1. Power supply for ultraviolet lamp. See Text.

The bank of condensers at a potential of 4,000 volts was discharged through the lamp. PORTER reports that the maximum output of the lamp is reached very rapidly under similar conditions. This is followed by fairly constant emission, the duration of which is proportional to the capacity, and finally an exponential decay. By means of an ultraviolet sensitive phototube and an oscilloscope-camera arrangement it was found that the duration of bursts (flashes) of our equipment was *ca.* 0.5 milli-seconds.

Irradiation procedure with high intensity

For irradiation of the enzyme a plastic trough, 1 cm wide, 8.2 cm long and 1.5 cm deep and separated into two equal sections 4 cm in length by means of a partition, was placed directly under the lamp about 0.5 cm away. In one side of the cell 4 ml of crystalline pepsin* solution was placed. In the other side 4 ml of uranyl oxalate solution[3] (0.75 g uranyl oxalate and 1.26 g oxalic acid per litre) was placed for a simultaneous measurement of the dosage of ultraviolet light (as einsteins per ml per number of bursts used). The quantum efficiency of the actinometer is rather independent of wave-length between 4,350 and 2,540 A. The dosage per burst was governed by the age of the tube and by the number of condensers used. It varied from $1.86 \cdot 10^{-7}$ to $2.70 \cdot 10^{-7}$ einsteins per burst per ml. The condensers were allowed a fixed time to recharge between bursts, usually from 10 to 20 seconds. The solution was stirred after every ten bursts.

The concentration of the crystalline pepsin irradiated was *ca.* 3.9 mg per ml of 0.1 *M* sodium acetate buffer, pH 5.0.

The temperature rise during the irradiation was of the order of 3 or 4° C. The final temperature was never above 25° C. Enzyme activity measurements were performed as previously described[4]. The results of three sets of observations are plotted in Fig. 2. Beside the points are recorded the number of bursts required to give the degree of inactivation found.

* Pepsin from Plaut Research Laboratories[4].

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RESULTS AND DISCUSSION

From Fig. 2 it will be seen that a plot of the logarithms of the per cents of remaining activities versus dosage in einsteins per ml is a straight line. This indicates that the reciprocity law holds even at the high intensities used. In one pair of runs, two series of 40 bursts of unequal intensities gave points falling on or near the same line.

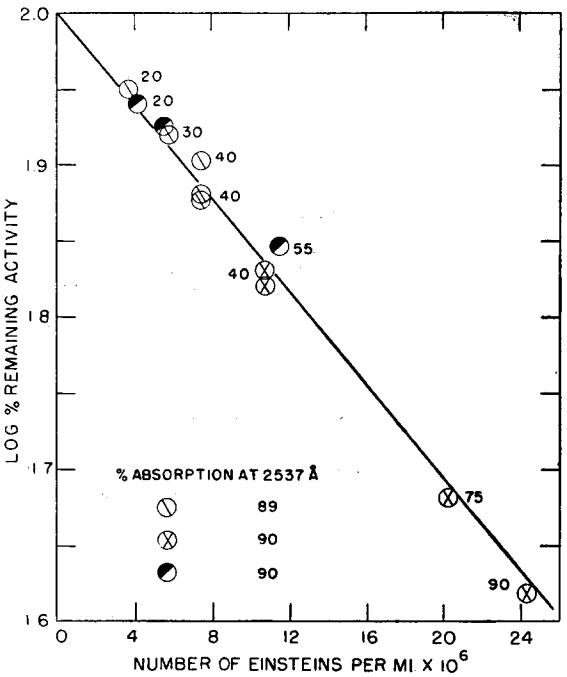

Fig. 2. Activity of pepsin as a function of energy absorbed.

Since an Sc-2537 lamp was employed, most of the intensity of emission was doubtless at 2537 A. Under conditions of fifty times the capacity used here, PORTER found appreciable intensity at the 2652 A mercury line. The applicability of an equation of the form of equation (1) is, however, also usually observed with polychromatic light. Writing equation (1) in differential form, for monochromatic light, we have

$$-\mathrm{d}\,E/\mathrm{d}t \cong K_\lambda E \tag{2}$$

and for polychromatic light

$$\begin{aligned} -\mathrm{d}\,E/\mathrm{d}t &\cong K_{\lambda_1}E + K_{\lambda_2}E + K_{\lambda_3}E + \ldots\ldots \\ &\cong K\,E \end{aligned} \tag{3}$$

If equation (3) holds it means that any changes in adsorption at some wave length λ_1, due to irradiation at λ_2, λ_3, etc., does not appreciably alter the rate of inactivation by light of wave length λ_1 during irradiation. A theoretical calculation with optical density data of PEARSON[4], for the inactivation of pepsin at normal intensity of 2537 A light, performed as described elsewhere[1], indicates that up to 46% inactivation, K_{2652} would be changed only by about 12%. Actually equation (3) is obeyed experimentally

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with both monochromatic (2537 A) and polychromatic ultraviolet light, with pepsin at ordinary laboratory intensities[4], as well as with the high intensities used here.

The quantum yield for the inactivation of pepsin[4] does not vary by more than a factor of *ca.* 3 in the wave length region of 2537 A to greater than 2719 A. It is nearly zero at 2930 A. Wave lengths longer than 3000 A are absorbed to only a small or negligible extent. We can make a rough calculation of the average quantum yield for the high intensity experiment as follows. With a molecular weight of 35,500 for pepsin the concentration of pepsin used was $5.0 \cdot 10^{-8}$ moles per ml. From Fig. 2, at a dose of $10 \cdot 10^{-6}$ einsteins per ml the remaining activity was 71%. Substitution into equation (1) gives an "average" quantum yield of

$$\frac{5 \cdot 10^{-8} \cdot 2.3 \cdot \log \frac{100}{71}}{10 \cdot 10^{-6} \cdot 0.9} = 1.9 \cdot 10^{-3}$$

for a light absorption taken as 90% at 2537 A.

This average quantum yield is virtually identical with that found at normal intensities, namely $2.1 \cdot 10^{-3}$ at pH 5.04. The intensities* used here were about 10^5 times that used previously[4].

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SUMMARY

A test of the reciprocity law for the inactivation of pepsin was performed and found to hold with intensities in the neighborhood of 100,000 times those customarily employed. An apparatus for obtaining such intensities is described.

RÉSUMÉ

Les auteurs ont trouvé que l'inactivation de la pepsine par la lumière ultraviolette suit la loi de réciprocité même lorsque des intensités environ 100,000 fois plus élevées que d'habitude sont employées. Ils décrivent un appareil permettant d'atteindre de telles intensités.

ZUSAMMENFASSUNG

Die Verfasser haben festgestellt, dass die Pepsin-Inaktivierung durch ultraviolettes Licht den sogenannten "reciprocity law" folgt, und zwar auch bei Intensitäten, welche ca. 100,000 mal höher liegen als gewöhnlich. Ein Apparat, mit dessen Hilfe solche Intensitäten erreicht werden können, wird beschrieben.

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* The percent absorption of the pepsin solution was measured in a Beckman D U Quartz spectrophotometer. If experiments are ever carried out at intensities greater than that required to excite nearly all the molecules in a solution simultaneously, BEER's law would expectedly break down.

The calculation of an "average" quantum yield assumes that the quantum efficiency of the uranyl oxalate actinometer is independent of intensity.