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EFFECTS OF ROENTGENRAYS ON THE ENZYME-SUBSTRATE (LUCIFERASE-LUCIFERIN) SYSTEM OF THE CRUSTACEAN CYPRIDINA

by

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Both the site of the primary cellular lesion and the earliest physiological changes produced in animals by ionizing radiation have been investigated for several years. Most of the recent studies deal with alterations in the membrane permeability of certain intracellular structures (KAPLAN 1955). The increase in the activity of several enzyme systems, observed after irradiation, may be due to changes in membrane permeability. BACQ & ALEXANDER (1961) have postulated that enzymes are released from membranes and intracellular surfaces by radiation. Physiological changes then would be produced by the liberated enzymes. DNA, however, is very radiosensitive while DNAase cannot be liberated by small doses of radiation (OKADA et coll. 1957).

Other possible explanations for the increase in enzyme activity observed after irradiation might be: (1) a substrate is rendered more susceptible to enzyme action, or (2) substrates are partly destroyed leaving unused enzymes to accumulate.

To obtain some information about the relative radiosensitivities of an enzyme and its substrate, we have done in vitro studies on the effect of radiation on the

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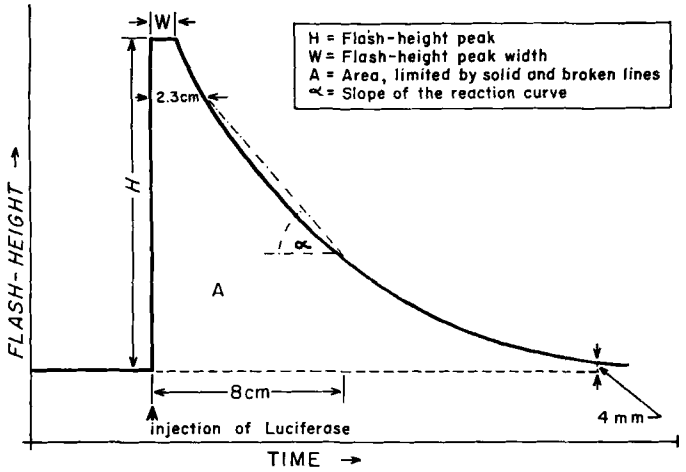

Fig. 1. Schematic figure for explaining the abbreviations used in text and figures.

enzyme-substrate (luciferase-luciferin) system of the small ostracod Cypridina. This system is an excellent one because both the enzyme and the substrate are in highly purified states and can be irradiated separately.

Material and Methods. In these experiments an RCA type 6217 phototube with a range of highest response from 3 500 to 6 000 Å and a Moseley X-Y chart recorder were used. The negative voltage to the phototube was obtained from a Baird-atomic model 312A super-stable high voltage supply. The phototube as well as a test tube with fixed geometry relative to the phototube were in a light-tight box.

All irradiations were done with a beryllium-window roentgen tube (Philips Electronics Instruments, Mt. Vernon, N. Y.) operated at 100 kV and 12 mA, and producing a dose rate of 9×10^4 r/min.

Four hundred micrograms of highly purified luciferin were dissolved in 4 ml of methyl alcohol. In this solution pure luciferin is relatively stable. The luciferase solution consisted of 400 μg of enzyme dissolved in 4 ml of 0.1 M phosphate buffer. Twenty-five lambda samples of the luciferase solution were pipetted into a small lucite container and irradiated with different doses of roentgen rays (3 000 to 4.5×10^5 r).

After irradiation, the sample was diluted to 10 ml with 0.1 M phosphate buffer at pH 7.0, making a final concentration of 0.25 μg/ml. Also, 25 λ of the methanolic luciferin solution were diluted to 10 ml with 0.1 M phosphate buffer producing a final concentration of 0.25 μg luciferin/ml.

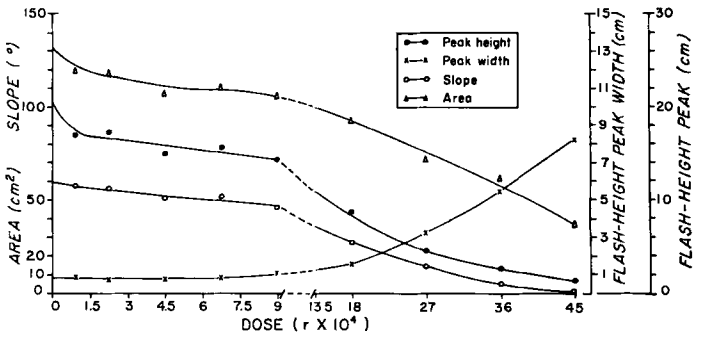

Fig. 2. Flash-height peak, area, slope, and peak width of the *Cypridina* luciferase-luciferin system as a function of the roentgen dose given to luciferase.

First, 10 ml of the buffered luciferin solution were poured into the test tube inside the light-tight box. The reaction was then initiated by injecting rapidly, with a hypodermic syringe, 10 ml of the 0.25 μg/ml buffered, irradiated luciferase solution.

To demonstrate the effect of the radiation on luciferin, 25 λ of the 0.1 mg/ml methanolic luciferin solution were irradiated under the same conditions as the luciferase. After diluting to 10 ml with 0.1 M phosphate buffer, the solution was poured into the test tube and mixed with unirradiated luciferase solution (10 ml), using the method mentioned above. The total light emitted (area under the curve), the slope of the reaction curve, the flash-height peak, and the flash-height peak width, as illustrated in Fig. 1, were determined immediately after mixing the enzyme and the substrate.

Unirradiated control samples were used with each particular experiment. Autoxidation could not be observed during the time measurements were made. All measurements were made at room temperature.

Results and Discussion

The distribution of the values of flash-height peak, area, slope, and peak width for luciferase as a function of the roentgen dose is shown in Fig. 2. Each individual point represents the mean of several experiments. As can be seen, the enzyme is very radioresistant. There is only a slight change in the activity for doses up to about 10^5 r. For higher doses the peak height and the slope decrease simultaneously. The area, however, decreases at a much slower rate. The total light emitted is reduced by only about 50 % after irradiation with 3.6×10^5 r. This is probably not due to an influence of the concentration of the enzyme, as will be shown later on. Related to this might be an increase in the peak width of about 800 % after irradiation with 3.6×10^5 r.

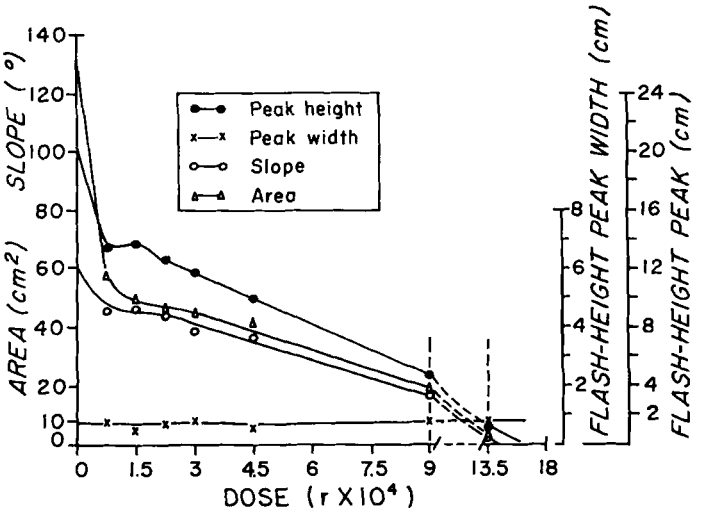

Fig. 3. Flash-height peak, area, slope, and peak width of the *Cypridina* luciferase-luciferin system as a function of the roentgen dose given to luciferin.

The results obtained by the irradiation of luciferin are shown in Fig. 3. In this case, the area, the slope, and the peak height decrease almost at a constant rate within experimental error; but the peak width remains constant. A dose of about 13.5×10^4 r destroys almost completely the substrate.

In Figs 4 and 5 the flash-height peaks and the areas, as obtained after the irradiation of luciferase and luciferin, are compared. The curves were normalized in the control values. As can be seen, the enzyme is much more radioresistant than the substrate.

To demonstrate the effect of the concentration of either enzyme or substrate on the peak height, the slope, and the area, different amounts (5 to 40 λ) of unirradiated luciferase or luciferin solution were mixed together in the test tube. The results are shown in Fig. 6. The peak heights decrease proportionally with decreasing total amounts of either enzyme or substrate. The experimental results are fitted by a straight line, and can be represented by the equation $y = 1.17\ x$. By using half the initial amount (20 λ) only, the peak height is reduced by half. A straight line could also be obtained for the area in case of adding different amounts of luciferin to 40 λ of luciferase. There was, however, no linear decrease in the area with decreasing volumes of luciferase and a constant amount of luciferin (40 λ). A reduction of about 50 % in the amount of the enzyme changed only slightly the total light emitted. The observation can account for the effect mentioned earlier and shown in Fig. 2. Irradiation of luciferase required 3.6×10^5 r to reduce the total light emitted by 50 %. The

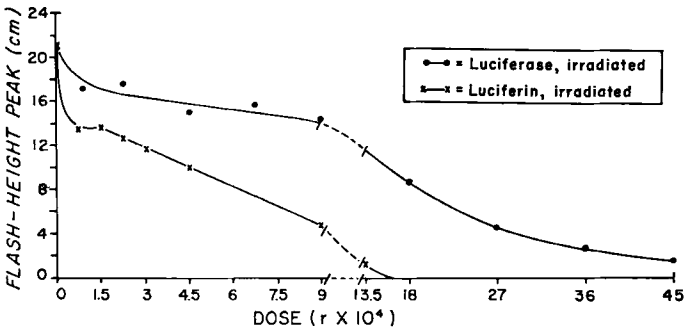

Fig. 4. Flash-height peaks of the *Cypridina* luciferase-luciferin system as a function of the roentgen dose.

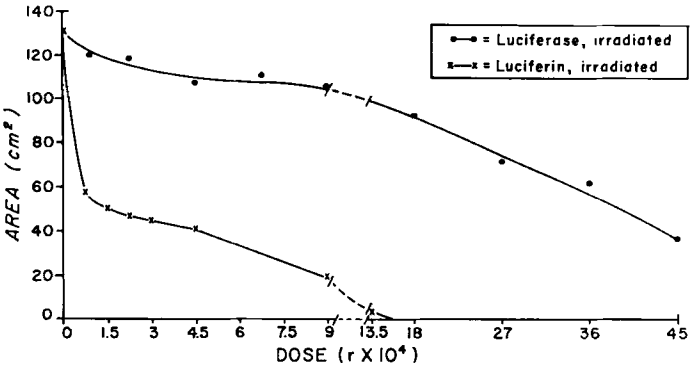

Fig. 5. Area (totally emitted light) of the *Cypridina* luciferase-luciferin system as a function of the roentgen dose.

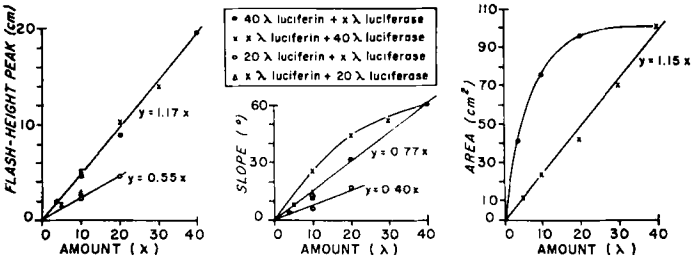

Fig. 6. Flash-height peak, area, and slope of the *Cypridina* luciferase-luciferin system as a function of the quantity of each constituent.

decrease in total light emitted is not proportional to a decrease in enzyme concentration. However, the decrease in the flash-height peak is proportional to the reduction in enzyme concentration and can be used as a measure of radiation effect on the enzyme.

The substrate, luciferin, appears to be more radiosensitive than the enzyme, luciferase. A 50 % reduction in the total light emitted is produced by less than 1×10^4 r when given to luciferin. However, the same amount of reduction in total light emitted, produced by irradiation of luciferase, requires about 30×10^4 r.

A reduction in luciferase concentration of about 75 % as measured by total light output, is required to decrease total light output by 25 % using non-irradiated samples. Thus, a large amount of luciferase would have to be destroyed by irradiation before significant loss of activity could be found, using the area-determination.

The flash-height peak is a linear function of the quantity of luciferase present. If the flash-height peaks are used as criteria for radiosensitivity, luciferase is 3 to 4 times more radioresistant than luciferin, as determined by the 50 % reduction in flash-height peaks of the reaction curves.

Acknowledgements

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SUMMARY

Twenty-five lambda of practically pure luciferase solution (0.1 mg/ml of 0.1 M phosphate buffer) or 25 λ of highly purified luciferin solution (0.1 mg/ml of methyl alcohol) were irradiated with different doses of roentgen rays (3 000 to 4.5×10^5 r). The initial flash-height peak, the slope of the reaction curve, and the quantity of light emitted after combination of enzyme and substrate were determined, using photocell equipment. The results of these in vitro experiments show that the substrate is much more radiosensitive than the enzyme.

ZUSAMMENFASSUNG

Fünfundzwanzig lambda von praktisch reiner Luciferase-Lösung (0,1 mg/ml einer 0,1 M Phosphatpuffer-Lösung) oder 25 λ einer hochgereinigten Luciferin-Lösung (0,1 mg/ml Methylalkohol) wurden mit verschiedenen Röntgendosen bestrahlt (3 000 bis $4,5 \times 10^5$ r). Das anfängliche Maximum, der Abfall der Reaktionskurve, und die nach Kombination von Enzym und Substrat freiwerdende Lichtmenge, wurden mittels einer Photozelle bestimmt. Die Ergebnisse dieser Versuche in vitro zeigen, dass das Substrat wesentlich strahlenempfindlicher als das Enzym ist.

RÉSUMÉ

Vingt-cinq lambda d'une solution pratiquement pure de luciférase (0,1 mg/ml de 0,1 M phosphate tampon) ou 25 lambda de solution de luciférine très purifiée (0,1 mg/ml d'alccool méthylique) ont été irradiés par différentes doses de rayons roentgen (3 000 à $4,5 \times 10^5$ r). La hauteur de l'éclair initial, la pente de la courbe de réaction et la quantité de lumière émise après la combinaison de l'enzyme avec le substrat ont été measurées avec un équipment à cellule photo-électrique. Les résultats de ces expériences in vitro montrent que le substrat est beaucoup plus radiosensible que l'enzyme.

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