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INACTIVATION OF ENZYME FORMATION BY ULTRAVIOLET LIGHT

I. ACTION SPECTRA AND SIZE OF THE SENSITIVE UNIT

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SUMMARY

The action spectra for loss of ability to form the inducible enzymes β-galactosidase and tryptophanase by *Escherichia coli*, and also for colony formation, resemble the absorption spectra of nucleic acids. A minimum size of the sensitive unit for enzyme formation of 300,000 molecular weight units is computed. This suggests that the sensitive unit is either high molecular weight RNA or DNA and not soluble RNA. On the basis of previously reported quantum yields, the size of the target is estimated to be somewhat larger than the minimum, perhaps of mass 700,000. This result is in fair agreement with present estimates of the size of a functional gene or of the RNA of a ribonucleoprotein particle. These data do not permit a choice between RNA and DNA as target materials.

Abbreviations: RNA, ribonucleic acid; DNA, deoxyribonucleic acid.

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INTRODUCTION

An action spectrum is the representation of the relative efficiency of radiation of different wave lengths in bringing about an effect in an irradiated system[1,2]. These action spectra are displayed as a plot of the reciprocal of the amount of light required to produce a given effect as a function of wave length. Close similarity of the action spectrum and the absorption spectrum of some component of the system is taken as evidence that the initial effect of the light is on this component[3].

In the present communication the effect of u.v. light on the formation of bacterial enzymes is considered. An effect on such a phenotypic property, virtually instantaneous and measurable immediately after irradiation, would seem susceptible of more ready interpretation than the commonly investigated effect on viability which represents a complex of many independently affected processes. Action spectra for enzyme formation have been reported for the galactozymase of yeast[4] and the lysine decarboxylase of *Bacillus cadaveris*[5]. In each case, the action spectrum was reported to resemble the absorption spectrum of nucleic acid; the results provided support for the often stated contention that nucleic acids are involved in enzyme synthesis.

The present communication extends the data to include two other enzyme forming systems, those of β-galactosidase and tryptophanase of *Escherichia coli*. The former enzyme is of particular interest because of the extensive investigations carried out on the mechanism of its induction[6]. Both enzymes can be induced in readily determined amounts in a fraction of an hour. In the present experiments, the range of wave lengths used was extended to below 240 mμ where the difference between the absorption spectra of protein and nucleic acid are most evident. The data also permit a calculation of the size of the photosensitive unit.

MATERIALS AND METHODS

The organisms used, a radiation resistant strain of *E. coli*, B/r, or *E. coli* K12 were grown aerobically by swirling at 37° in a synthetic medium, M63, containing per liter 2 g glycerol and the following salts: 13.6 g KH_2PO_4, 2.0 g $(NH_4)_2SO_4$, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.5 mg $FeSO_4 \cdot 7H_2O$. The pH was adjusted to 7.0 with KOH. The bacteria were harvested in the exponential phase of growth, and were irradiated with an apparatus (made available by Dr. C. A. KNIGHT) having as its principal components an AH6 General Electric lamp operating at 500 V and a Bausch and Lomb diffraction grating to obtain the action spectrum. Details of the technique have been described elsewhere[7]. The samples were vigorously stirred during the irradiation period of from 2.5 to 30 min. The actinometry[8] necessary to measure light intensities was performed immediately following the irradiation of each sample and under the same conditions.

In the preliminary experiments a u.v. sterilizing lamp (15 W Sylvania Germicidal-A) with principal output at 2537 Å was used and intensity was determined by a u.v.-sensitive photoelectric meter.

β-galactosidase was induced with $5 \cdot 10^{-4}$ *M* isopropyl-β-D-galactoside and assayed by the *o*-nitrophenyl-β-galactoside procedure[9]. Tryptophanase was induced with $3 \cdot 10^{-3}$ *M* tryptophan and determined by indole production, as measured with Ehrlich's reagent[10]. Viability of the bacteria was measured by plating on broth-agar, using suitable precautions to avoid photoreactivation.

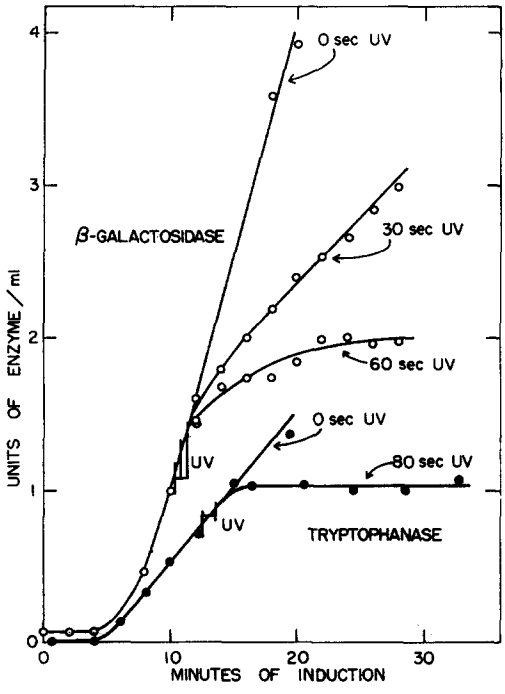

Fig. 1

Fig. 1. Kinetics of inactivation of enzyme formation. The bacteria, *E. coli* K12, were grown and induced at 37° for tryptophanase or at 24° for β-galactosidase. In either case, a portion of the culture was irradiated at an intensity of 27 ergs/mm²/sec u.v. light (2537 Å) at the times and for the durations indicated on the Figure. Irradiation was performed in the growth medium. Aliquots for enzyme assay were taken before irradiation, and from both irradiated and unirradiated samples after irradiations. The data are plotted as enzyme activity (mμ-moles of indole or *o*-nitrophenol produced/min/ml of culture) against time of sampling.

Fig. 2. Enzyme inactivation as a function of dose. *E. coli* K12 were grown and then irradiated with 27 ergs/mm²/sec u.v. light (2537 Å) as described under METHODS. The rate of enzyme formation in the first 40 min after induction is plotted against u.v. dose.

Fig. 3. Action spectra. Reciprocals of the doses required to reduce viability to 5%, β-galactosidase formation to 20%, or tryptophanase formation to 10% of their unirradiated-control rates, divided by the comparable values obtained at 2650 Å, are plotted as a function of wave length. For comparison, an action spectrum for inactivation of aldolase and an absorption spectrum for nucleic acid are shown.

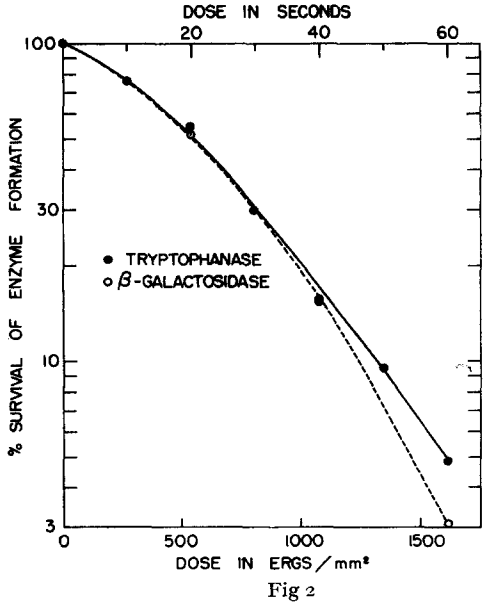

Fig 2

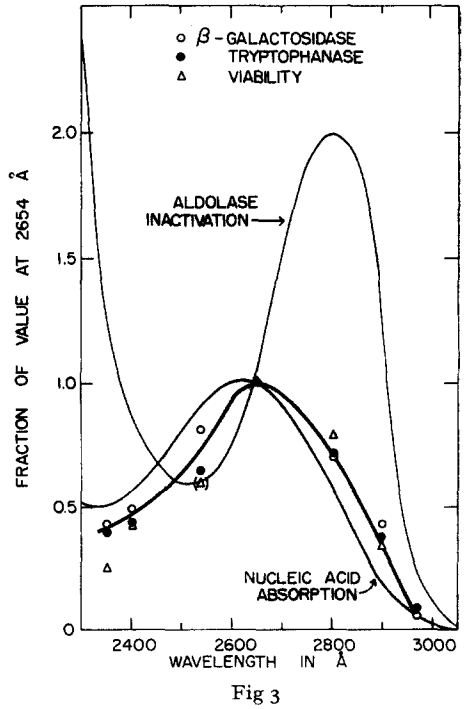

Fig 3

RESULTS

Formation of tryptophanase or β-galactosidase was arrested almost at once following irradiation (Fig. 1). This result suggests that u.v. light has a rather direct effect on enzyme formation, and that there has not been much opportunity for repair processes and secondary effects of irradiation to confuse the interpretation. No evidence for reactivation, by visible light or nutrients, of enzyme forming ability has been found under the present conditions. Other experiments showed that the reciprocity law[1] applied, since the same effect was observed if the total dose was kept constant although applied over different lengths of time (Table I). Irradiation of *E. coli* with different doses of u.v. light resulted in a nonlinear decline of ability to form the enzymes as shown in Fig. 2. Interpretation of these curves in terms of metabolism and multiplicity of targets will be reserved for a later paper.

TABLE I

EFFECT ON β-GALACTOSIDASE FORMATION OF CONSTANT U.V. DOSE GIVEN OVER VARIABLE TIMES

E. coli B/r suspended in medium lacking a carbon source at 0° were irradiated for the times and at the intensities shown. Intensity was varied by altering the lamp-to-sample distance. The samples were then induced at 37° with $5 \cdot 10^{-4}$ *M* IPTG, after addition of glycerol. Rates of β-galactosidase formation in the 40 min following irradiation are shown in the last column as % of control.

Expt.	*Dose ergs/mm²*	*Intensity ergs/mm²/sec*	*Irradiation time (min)*	*Enzyme formation, % remaining*
I	1270	11.2	1.9	12
I	1270	4.4	4.8	14
I	1270	1.4	15.0	11
I	2110	11.2	3.1	0.6
I	2110	4.4	8.00	0.8
I	2110	1.4	25.0	0.6
II	700	75	0.16	64
II	700	23	0.50	72
II	700	12.5	0.93	67
II	1200	75	0.27	32
II	1200	23	0.83	22
II	1200	12.5	1.57	29

To obtain the action spectra, aliquots of the bacterial suspension were irradiated for two different times at each of six wave lengths and the ability of the bacteria to produce enzymes or form colonies were compared with corresponding abilities of unirradiated samples (Table II). Whereas the ability to form β-galactosidase was well retained after storage at 0°, that for tryptophanase was lost in part. These data were plotted as in Fig. 2 to permit estimation of the quantities of light required to produce a given per cent inactivation and the reciprocal of these doses were plotted against the corresponding wave length (Fig. 3). The absorption spectra for nucleic acid[11] and for inactivation of the enzyme aldolase[12] (normalized to unity at 2654 Å) are also shown in the Figure. The data for all three properties are similar to the curve for nucleic acid absorption, and are not like that for protein inactivation.

TABLE II

INACTIVATIONS BY U.V. LIGHT

The experiment was performed as described under METHODS. The bacteria were washed and resuspended at 0° in medium lacking glycerol prior to irradiation. Aliquots were irradiated for the times indicated at approx. 12°, and then were stored at 0° until the irradiations were finished. Inductions and assays were then performed on various samples to which glycerol was added. Values at 2967 Å from a separate experiment.

Wave length Å	*O.D.*	*Photons/ml/min* $\times 10^{-15}$	*Irradiation (min)*	*Per cent survival*		
				Viable bacteria	*β-galactosidase*	*Tryptophanase*
—	—	—	0	(100)	(100)	(100)
2350	0.167	0.81	15	43	62	27
2350	0.167	0.81	30	15	24	9.3
2399	0.148	1.19	10	24	53	21
2399	0.148	1.19	20	2.3	20	8.2
2537	0.163	1.48	10	(5)	17	7.1
2537	0.163	1.48	20	< 0.1	0.5	2.2
2654	0.163	4.03	2.5	3.6	25	5.5
2654	0.163	4.03	5	0.2	4.8	3.8
2803	0.131	3.94	2.5	5.7	33	12
2803	0.131	3.94	5	1.0	15	7.7
2894	0.104	4.10	5	8.2	33	14
2894	0.104	4.10	10	0.4	2.8	2.2
2967	0.131	8.45	6	26	97	57
2967	0.131	8.45	10	4	49	34

DISCUSSION

That an action spectrum should have the same shape as the absorption spectrum of the light-sensitive material can be seen from the following argument. It is assumed that a standard biological effect is produced by the same number of altered molecules, irrespective of the wave length of irradiation[1]. If I_0 is the intensity of incident irradiation, t is the duration of irradiation, and D is the total O.D. of the sample, the amount of light absorbed is $I_0t(1-10^{-D})$. The fraction of this light absorbed by the sensitive substance is A/D, where A is the O.D. of the sensitive substance. The amount of light absorbed by the latter times the quantum yield (ϕ, equal to inactivating events per quantum absorbed) equals the number, N, of damaged molecules:

$$N = \phi A I_0 t (1-10^{-D})/D.$$

As the O.D. approaches zero, $(1-10^{-D})/D$ approaches unity, and for a constant amount of biological damage the reciprocal of the dose (I_0t) becomes proportional to the O.D. of the sensitive material (A). If ϕ is assumed constant at all wave lengths, this reciprocal of the dose for the given effect should be proportional to A at all wave lengths. For comparison of action spectra with absorption spectra, one also has to make the assumption that the light absorption by the sensitive material in the cell is proportional at all wave lengths to its extinction coefficient in the free state.

For a valid comparison to be made between action spectra and absorption spectra, various experimental requirements must be fulfilled[1]. These include low O.D. of the suspension and adherence to the reciprocity law. Also it would seem desirable to extend the measurements over a wave length range that distinguishes between

alternative spectra, corresponding to inactivations of nucleic acids or proteins or required for free radical formation and to use methods of measurement of the biological phenomena that are little subject to complications of secondary effects of the irradiation. Since these precautions were taken into account, the present results are in agreement with earlier results in stating that microbial viability[1] and enzyme forming ability[4,5] are inactivated by u.v. light as a result of damage to nucleic acid.

If it is assumed from the action spectrum that the u.v. sensitive unit is nucleic acid, the data permit an estimation of its size. In the region of Fig. 2, at about 10 % survival, where log of enzyme forming ability is decreasing approximately linearly with dose, an average of one effective hit per unit decreases the rate of enzyme formation by 1/e. At the maximum effective wave length, 2654 Å, $1.2 \cdot 10^{15}$ photons were absorbed per ml to bring about such a reduction of β-galactosidase formation (Table II). (A correction of 25 % for light scatter was made in computing the amount of light absorbed. The correction was estimated by comparing the O.D. of intact and sonically disrupted bacteria.) This amounts to $8 \cdot 10^{6}$ photons absorbed per bacterium for enzyme inactivation to 1/e (compared to $5 \cdot 10^{6}$/bacterium, earlier reported for a lethal effect[13]). If the sensitive unit must absorb $(1/\phi)$ photons on the average for every inactivating hit, then $(1/\phi)/1.2 \cdot 10^{15}$ equals the ratio of the photons absorbed by each sensitive unit to those absorbed by the total material in 1 ml. The ratio of light absorbance of sensitive material and nucleic acid are assumed to be proportional to their relative masses, a reasonable assumption since nucleic acid absorbs virtually all the light at this wave length. Then the sensitive unit must have a mass of $(1/\phi)/1.2 \cdot 10^{15}$ of that of the nucleic acid in one ml. There are approx. $5 \cdot 10^{-6}$ g of nucleic acid/ml, as determined[11] from the O.D. of 0.12. Therefore the size of the sensitive unit equals 2500 $(1/\phi)$ molecular weight units.

Although $(1/\phi)$ is not known for this system, a minimum value can be estimated. Uracil and cytosine nucleotides are altered by u.v. light with a $(1/\phi)$ of about 50, much more efficiently than are purine nucleotides or thymidine[14]; similar values are reported for alteration of pyrimidines in nucleic acids[15]. If, as estimated from base ratios, about 40 % of the light is absorbed by the sensitive pyrimidines in RNA, or 25 % in DNA, 120 photons would be absorbed by the nucleic acid per chemical alteration of the RNA, or 200 for DNA. Then assuming $(1/\phi) = 120$ or 200, the sensitive unit would have a mass of 300,000 or 500,000, respectively. If the former is taken as a minimum estimate, it appears that the unit is not a low molecular weight RNA such as soluble RNA. The reasonable possibilities for the identity of the sensitive material are ribonucleoprotein RNA or DNA.

The use of a value of 120 for $(1/\phi)$ is questionable in these experiments because there is no certainty that every photochemical event which modified a pyrimidine nucleotide *in vitro* must cause loss of activity of a sensitive unit in nucleic acid. An estimate of $(1/\phi)$ for biological effects places the value somewhat higher. For inactivation of infectivity of RNA of tobacco mosaic virus, as determined by the same techniques used in this study[7], $(1/\phi)$ was equal to 280, and higher values have been reported (see ref. 7). Also, an estimate of $(1/\phi)$ for transformation of optochin resistance by transforming principle gave virtually the same value[16]. By use of this figure, the sensitive unit for enzyme formation would have a mass of 700,000. The mass of the sensitive unit is reasonably similar to estimates of the size of DNA in a functional gene[16,17] or to the RNA in a ribonucleoprotein particle[18]. The data

do not permit decision whether it is more likely DNA or RNA that is the sensitive substance.

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