# THE ACTION SPECTRUM FOR THE ULTRAVIOLET INDUCTION OF LYSIS IN *ESCHERICHIA COLI* K-12

by

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Viruses, the smallest particles which possess the properties of living matter, sometimes appear to arise de novo from uninfected cells. A model system for studying this event is to be found in the lysogenic bacteria which carry a potential virus that may destroy them. Lysogenic bacteria hereditarily transmit a non-infectious form of bacteriophage, the provirus, which can transform spontaneously to active virus and multiply, finally lysing the host cell[1]. This transformation can also be brought about artificially under the action of many physical and chemical agents and is then termed induction[2,3]. Ultraviolet light is an inducing agent and it would be of interest to know the wavelength dependence of the process since biological macromolecules (proteins and nucleic acids) have characteristic absorption spectra in the ultraviolet.

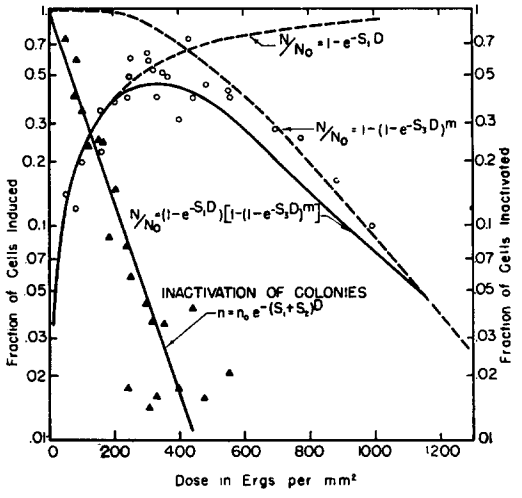


Fig. 1. Dose effect curves for induction and cell inactivation at $\lambda = 2537$ A. The various parameters which the theoretical curves fit at this wavelength are $s_1 = 2.4 \cdot 10^{-3}$ mm²/erg, $s_2 = 5.8 \cdot 10^{-3}$ mm²/erg, $s_3 = 4.3 \cdot 10^{-3}$ mm²/erg, $m = 6$.

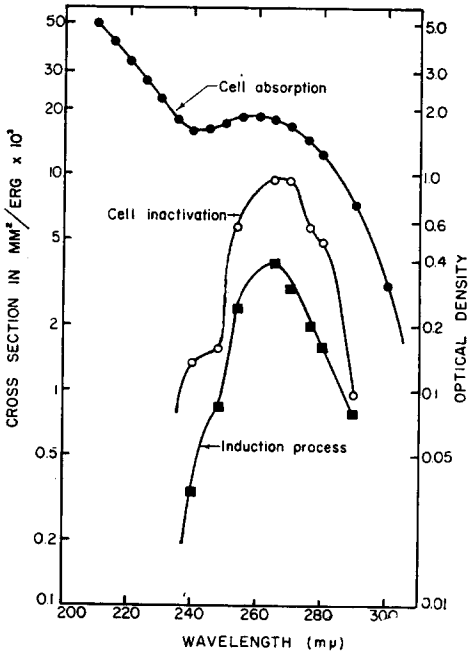


Fig. 2. Action spectra for induction ($s_1$) and cell inactivation ($s_2$) plotted on a semi-logarithmic graph. Also shown is the absorption spectrum of the cells. For details see text.

When the logarithm of the percent induction (the percent of cells which form plaques after U.V. action) is plotted against dose, the resulting curve rises rapidly to a maximum, then drops off and approaches a straight line portion of descending slope (Fig. 1)[4,5]. JACOB[5] has already pointed out that there are several processes occurring simultaneously which may account for this curve. The initial process is the activation of cell lysis which fits the curve $N = N_0(1-e^{-s_1 D})$, where $N$ is the number of induced cells, $N_0$ the total number of cells, $D$ the dose in ergs/mm², and $s_1$ the cross-section for induction in mm²/erg. The cross-section represents the relative efficiency of a given wavelength for effecting a particular biological change. This curve may be interpreted as meaning that a single quantum event in any one inducible unit is sufficient for induction.

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The second process is the inactivation of provirus[4] and fits the curve $1-(1-e^{-s_3 D})^m$,[6] where $s_3$ is the cross-section for proviral inactivation and $m$ is the multiplicity of hit. It is important to note that this multi-hit curve does not necessarily indicate that there is more than one provirus. Not enough data has been collected as yet to determine the wavelength dependence of $s_3$. In summary the overall dose effect curve is $N = N_0(1-e^{-s_1 D})\,[1-(1-e^{-s_3 D})^m]$. Fortunately the two portions of the curve can be analyzed separately to a fair approximation (cf. Fig. 1).

The graph of the logarithm of bacterial survivors versus dose also gives a straight line owing to the direct inactivation of haploid K-12 cells by a single "hit" process plus the disappearance of cells due to lysis, i.e. $n = n_0 e^{-(s_1+s_2)D}$, where $n$ is the number of bacterial survivors and $s_2$ is the cross-section for cell inactivation (Fig. 1).

*E. coli* K-12 $Lp_1^+ Lp_2^s$ [7] was grown in a peptone medium to a titer of $2 \cdot 10^9$ cells/ml, washed twice in phosphate buffer (pH 6.9) and diluted by a factor of $10^4$ before irradiation with monochromatic light of known intensity. At various times aliquots were diluted in broth and incubated with shaking at 37° C for 45 minutes. Platings were then made according to the techniques of WEIGLE AND DELBRUCK[4]. The cell absorption shown (Fig. 2) is that of a titer of $8.1 \cdot 10^8$ cells/ml harvested from peptone broth in the log phase and resuspended in buffer (pH 6.9) after four washings, and is corrected for scattering.

The induction spectrum has a sharp peak at 265 mμ, falling off rapidly on either side, whereas the cell inactivation peak is broader with a slight shoulder at 280 mμ (Fig. 2). Thus both curves indicate nucleic acid chromophores, while the cell inactivation curve may indicate some protein involvement also (cf. LOOFBOUROW[8]). Neither action spectrum follows cell absorption. The rapid drop in cross-section below 240 mμ, with virtually no cell inactivation at 237.5 mμ, indicates heavy absorption in peripheral regions which are not involved with induction or cell inactivation. The fact that more energy is needed to induce than inactivate may indicate that the inactivated cells can still support phage growth. This has already been demonstrated for *E. coli* C invaded by λ phage[9]. It also shows that the inducible substance is not identical with all the units concerned with cell inactivation. Thus induction may involve the activation or inactivation of a particular enzyme forming system, or the direct activation of provirus.

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# THE OCCURRENCE OF FREE THIAMINE PYROPHOSPHATE IN THE SOLUBLE FRACTION OF RAT LIVER HOMOGENATE*

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In a previous note[1] it was reported that centrifugally prepared mitochondrial and soluble fractions of rat-liver homogenate[2] contained practically equal proportions of the cellular thiamine pyrophosphate (TPP). The nuclear fractions and the microsomes were essentially free of TPP. The virtual absence of TPP from rat-liver nuclei was confirmed by applying a recently published method

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