LOCALIZATION OF β-GALACTOSIDASE IN CELLS OF *Escherichia coli* BY LOW VOLTAGE ELECTRON BOMBARDMENT

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ABSTRACT By using low voltage electrons to bombard dried cells of *Escherichia coli*, the inactivation of the enzyme β-galactosidase as a function of depth of electron penetration has been studied. There is little inactivation for a penetration of 100 A, but considerable for a penetration of 300 A. An analysis of the data for six initial electron energies shows that there exist outer and inner bounds of the enzyme region which are approximately 300 and 700 A below the cell surface, respectively.

INTRODUCTION

The enzymatic action of β-galactosidase is destroyed by ionizing radiation (1). In the dry state, for such destruction to take place it is necessary that at least one ionization occur within the molecular unit of the enzyme. In fact, the molecular weight of 330,000, calculated on this basis (1) agrees, within a factor of about two with the molecular weight found by Hu, Wolfe, and Reithel (2) by more usual physicochemical means. Since it proved feasible to recover this activity from dried cells, an attempt to localize the intracellular enzyme was made, using the method previously employed to localize invertase (3) and catalase (4) in yeast cells.

The technique consists of: (*a*) drying cells on a surface in such a way that, on the average, the surface density is far less than one cell per unit cell area; followed by (*b*) bombardment with electrons of low voltage and hence limited penetration. If the enzyme is deep within the cell, heavy bombardment at low voltages will not attenuate activity, since all the ionization takes place in upper layers devoid of enzyme molecules. If the molecules are on the surface, the electrons of lowest energy will produce appreciable loss of assayable activity. An intermediate depth will show as between the two. Penetration to the far side will also give an increase in the observed destruction, if the enzyme is not centrally located.

In the experiments to be described we find evidence based on electron ranges that β-galactosidase is in a layer just beneath the cell wall. In addition, we utilize

the fact that electrons of a given energy do not have the same penetration, but are scattered, or "straggle" in a known way. The inactivation reflects the presence of this effect.

EXPERIMENTAL METHOD

E. coli grown in a minimal glucose liquid medium were transferred to a minimal lactose-containing medium. At the end of the log phase of growth (nearly 16 hours) they were washed three times by centrifugation, and resuspended in distilled water. About 0.03 ml of this suspension was dried on each of about 180 to 200 ½ inch stainless steel disks so that the surface density on the disks was no more than about 0.4×10^7 cells/cm^2, a density safely below one cell per unit cell area. These disks were placed in rows on arcs of circles over which electron guns rotated in high vacuum. The electron beam current could be measured and the accelerating voltage could be set at different known values. The device which makes possible this differential irradiation of individual cells has been described in a previous report (3). The electron range and dosimetry considerations have been discussed in connection with that work. About 35 disks had to be pooled to obtain one experimental point. Control points were made up from disks containing cells subjected to all environmental conditions except irradiation. These disks were in the vacuum chamber but outside the beam areas. On two occasions controls were placed in irradiated areas and subjected to any effects which might have been produced by the photons from the hot tungsten filaments of the electron guns, in the absence of an accelerating field. No significant changes in enzymatic activity could be detected, even when the time for this treatment was more than double that for any involving accelerated electrons. Evidently there are (*a*) too few high energy quanta at this filament temperature, and (*b*) no space charge effects.

After irradiation, 0.02 ml of distilled water was pipetted onto each disk; and this drop was spread and agitated with a glass rod. All the disks making up one experimental point were then dropped into a small Petri dish, which with the disk suspensions contained 6 ml of H_2O. After further agitation this fluid was put through a French pressure cell. 3 ml of what remained was assayed for enzymic activity after a 3 hour incubation with an excess of the substrate, orthodinitrophenol β-D-galactopyranoside. The color product OD was measured at a wavelength of maximum color product absorption, 625 mμ.

RESULTS

Fig. 1 shows the enzymatic activity survival percentage as a function of dose for electrons accelerated by 500, 1000, 1500, 2000, 3000, and 5000 volt fields. The scatter of the points is in some measure due to handling of the material in the procedure including using the high pressure (6000 pounds per square inch) French cell. The plots are linear and not logarithmic so the scatter is, at high survival percentages, more than usually apparent. The 500 volt electrons (range about 100 A in proteinaceous material (4, 5)) can just barely reach an enzyme-containing region. The activity is steadily reduced as the electron energy is increased until at 5000 volts (range 5700 A), it is virtually absent. Although the diameter of an *E. coli*

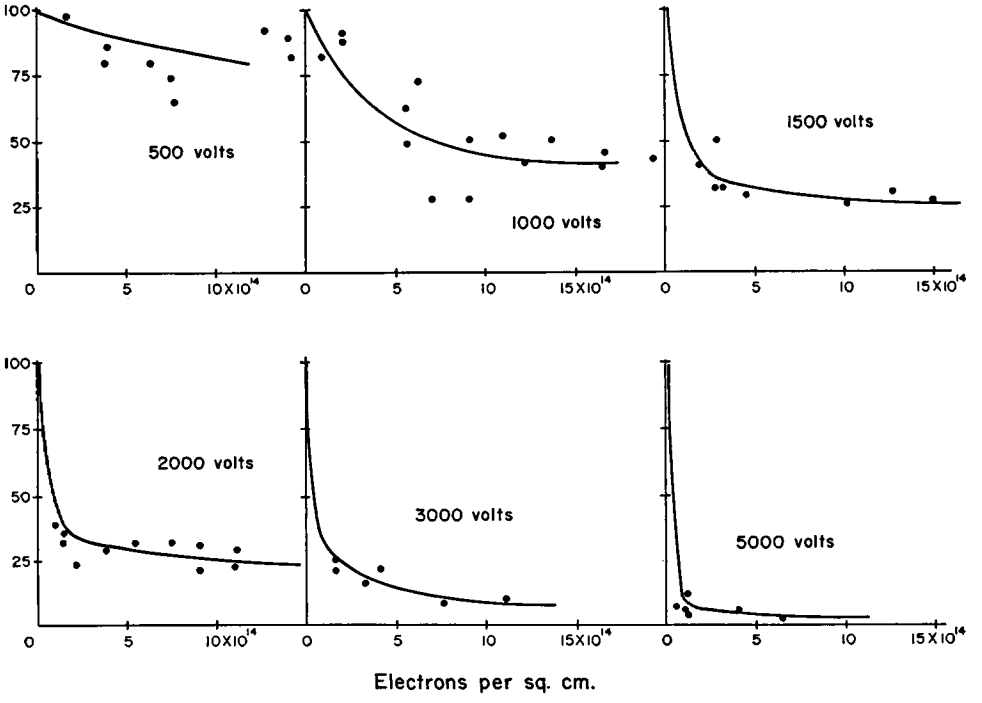

FIGURE 1 A series of "survival" curves for the amount of enzymatic activity remaining after bombardment by varying numbers of electrons per cm^2 at six different energies, and hence depths of penetration of electrons. The loss of activity suggests that β-galactosidase is located just beneath the cell wall, extending from 300 to 700 A from the outer surface of the cell.

cell is 8000 A, the dried cell loses enough water to represent much less than this distance in solid dry protein.

DISCUSSION

The very slight, but definite inactivation obtained with electrons of energy as low as 500 electron volts (range 100 A in protein-like material), shows that the enzyme must be near the surface. This is not an inactivation of enzyme molecules which have "leaked" through the cell surface, for supernatants of resuspended dried cell centrifugations never contained activity. This was true both for irradiated and unirradiated cells.

The zone boundaries can be established by utilizing the fact that electron-straggling beyond calculated ranges in dried protein material will not be noticeable unless the straggling is into an enzyme-rich region of the dried cell. Straggling (6) is the phenomenon which results from all electrons not having zero energy at the end of

the calculated range. A finite number suffer fewer collisions and deflections, so that they can get beyond the average range. We can make some analysis of the data of Fig. 1 as follows:—

Suppose that k is the fraction of enzyme present in the bacterium which can be reached by the electrons and be inactivated by the exponential relationship known to apply to radiation on this enzyme (7). This relation is $E/E_0 = e^{-\alpha D}$ where E is the amount of enzyme still active, E_0 the amount originally present, α is the enzyme inactivation parameter (in general a function of electron energy), and D is the dose in electrons/cm^2. $1-k$ represents the inaccessible fraction. Then

$$E/E_0 = (1 - k + ke^{-\alpha D})$$

This equation, which is useful as a first approximation, does not contain any provision for the amount of effect produced by straggling. Our 3000 and 5000 volt data show that the $(1-k)$ level is very nearly reached with a dose of less than 2×10^{13} electrons/cm^2. This inactivation is the steep, initial part of the curves. If any further inactivation occurs with higher doses, it must be due to straggling, and is only evident because we are assaying the damage produced by stragglers. One method of estimating the portion of this damage is to compare survival at 2×10^{13} with that at 10^{14} electrons/cm^2. If these are the same, straggling is into an enzyme-deficient region, and the surviving activity is due to that enzyme beyond the electron range, protected by this same region. If we make a comparison table the enzyme-rich region is revealed.

TABLE I

Electron energy	Range A	Survival 2×10^{13} e/cm^2	Survival 10^{14} e/cm^2	Difference in survival	Character. Straggling is:
		per cent	*per cent*	*per cent*	
500	100	95	85	10	Approaching enzyme region
1000	300	85	50	35	Into enzyme-rich region below cell wall
1500	700	45	30	15	Leaving enzyme-rich region
2000	1200	32	27	5	Into enzyme-poor region, center of cell
3000	2400	22	7	15	Into enzyme-rich region, lower cell wall
5000	5700	7	0	7	Out of enzyme-rich region, beyond cell

Table I shows that 500 volt electrons only inactivate by straggling into a nearby enzyme-rich region. 1000 volt electrons (range 300 A) reach part of the region and straggle into an enzyme-rich region beyond. 1500 and 2000 volt electrons straggle into an enzyme-deficient region which protects that reached through both

mechanisms (on the other side of the cell) by 3000 volt electrons (range 2400 A in dry proteinaceous material). The high inactivation and low straggling damage produced by the intermediate voltage (1500 and 2000 volt) electrons indicate that (*a*) the damage occurs on the peripheral edge of the beam front (near the cell surface) (*b*) there exists intracellular material not containing enzyme which protects the far side of the cell. Only the 3000 volt electrons can straggle into this latter enzyme-rich region, and produce higher peripheral damage. Therefore, the enzyme in the dried cell is very close to the cell wall material, the outer zone bound being no deeper than 300 A. An estimate of 700 A can be made for the distance of the inner bound from the cell surface.

This qualitative analysis bases its conclusions on the fact that a "plateau" for the value of $1-k$ *exists* at some *intermediate* electron energy. It is not concerned with the magnitude of the survival percentage at this plateau, which is 30 per cent rather than 50 per cent. Either something happens to the bacteria upon drying which produces this asymmetry, or there is a radiation effect we have not detected with larger cells (3, 4). There is no consistent change in enzymatic activity upon drying which could account for it.

It is possible to look at the data in a rather different way, which, however, still ignores asymmetry.

The above equation assumes that a dose D of electrons is always applied to a fraction k of accessible molecules. But the parameter α can only *decrease* as the energy of the incident electrons is *increased,* because higher velocity electrons produce less ionization per unit volume. Contrary behavior means that the other exponential factor D is responsible. It is lower in some regions of the cell than incident beam measurements would indicate; *i.e.,* we have straggling, and we are not measuring a true α.

The slope of the equation at zero dose is $-k\alpha$. If we measure initial slopes in Fig. 1 we obtain the apparent values of α shown in Table II.

Let us first consider α. The variation seen can only be explained on the basis that in the lower energy values all the electrons do not reach the enzyme; for an *increase*

TABLE II

VALUES OF INACCESSIBLE FRACTION, $(1-k)$, AND INACTIVATION PARAMETER α

Voltage	Range A	$(1-k)$	α
			cm^2
500	100	0.8	2.0×10^{-15}
1000	300	0.45	0.4×10^{-15}
1500	700	0.30	6.8×10^{-15}
2000	1200	0.30	11.5×10^{-15}
3000	2400	0.06	11.5×10^{-15}
5000	5700	0.00	11.0×10^{-15}

of electron energy causes an *increase* in its value. It increases up to a range of 1200 A and thereafter remains constant. Therefore we conclude that up to 700 A range a fraction of the incident beam electrons fail to reach the enzyme; *i.e.*, the enzyme zone extends to a greater depth. By the same token, a range of 1200 A is sufficient to guarantee that all incident electrons reach the fraction of enzyme which is accessible.

Turning to the values of $1-k$, the inaccessible fraction, it is seen to be less than unity for the very short electron range of 100 A. This immediately shows that the enzyme must be very close to the bacterial surface. The low value of α, however, suggests that only a small fraction of electrons actually reach the enzyme and therefore that a depth of somewhat more than 100 A for the upper surface of the enzyme is reasonable. However, this upper surface cannot be more than 300 A deep, because the inaccessible fraction is less than one-half at that point, and α is rising in value. As the electron energy increases, the value of the inaccessible fraction remains constant, while α reaches its full value. It is reasonable to suppose that the upper layer has been fully traversed at this energy. As the energy increases further, the inaccessible fraction drops sharply, indicating the lower layer is being reached. The straggling at this energy is rather large, and clear conclusions regarding the lower layer are hard to draw.

There are few reasons to suppose that β-galactosidase migrates to its observed position in the dried cell during the drying process, leaving behind other material to fill the core. Yeast catalase does not migrate to the region containing invertase when yeasts are dried (4). Thus it is reasonable to suppose that β-galactosidase is also located just within the cell wall where its lactose-hydrolyzing function is best utilized. The fact that electrons of range 5700 A pass completely through the cell means that in the dried condition the cell is either flattened or of low internal density. For this reason there is not much effect of enzyme at the ends and sides, and the cell acts mostly as if it were two layers. An attempt to represent the location in the cell is made in Fig. 2.

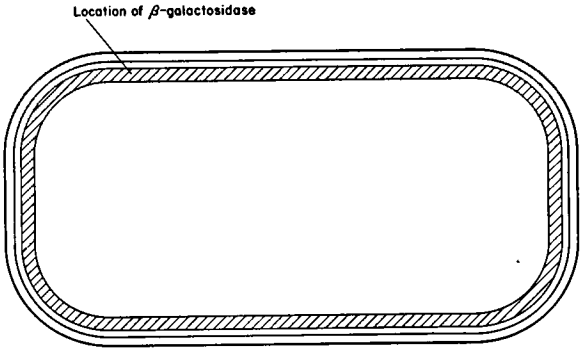

FIGURE 2 An attempt to represent the location of the enzyme β-galactosidase in the cell. It is in a layer about 400 A in extent, probably right at the protoplast membrane.

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REFERENCES

1. POLLARD, E. C., and BARRETT, N., *Radiation Research,* 1959, **11,** 781.
2. HU, A. S. L., WOLFE, R. G., and REITHEL, F. J., *Arch. Biochem. and Biophysics,* 1959, **81,** 500.
3. PREISS, J. W., *Arch. Biochem. and Biophysics,* 1958, **75,** 186.
4. PREISS, J. W., *Arch. Biochem. and Biophysics,* 1959, **79,** 261.
5. LEA, D. E., Actions of Radiations on Living Cells, Cambridge University Press, 1947.
6. WILLIAMS, E. J., *Proc. Roy. Soc. London, Series A,* 1930, **130,** 310.
7. POLLARD, E. C., *Rev. Mod. Physics,* 1959, **31,** 273.