*CORRELATION OF γ-RAY INACTIVATION AND STRAND SCISSION IN THE REPLICATIVE FORM OF φX174 BACTERIOPHAGE DNA**

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An important feature of the inactivation of double-stranded DNA viruses is that one radiochemical lesion induced in the DNA by ionizing radiation, radioactive decay of P^{32} atoms, or ultraviolet light can alone inactivate their reproductive ability, but its efficiency in causing this end point is much less than one.[1] Thus, these viruses can sustain numerous sublethal hits. For the case of X-rays and γ-rays, it has been reported for two systems[2, 3] that double-strand scission is the lesion correlating with lethality, the reason for the low killing efficiency being that this lesion occurs at a lower frequency than other types of lesions. It may be assumed that this explanation applies generally. However, the work reported earlier[4] on double-stranded RF-DNA of φX174 bacteriophage, in which it was observed that the killing efficiency of X- or γ-rays is 0.25 compared to 0.05 for T4 phage,[5] casts doubt on its application to this system. The fivefold disparity in efficiency is difficult to understand, since we would expect that X-rays should without distinction cause double-strand scissions or any other specific lesions in all DNA with the same frequency. Moreover, it was shown that heat-depurination 'hits' also inactivated the RF-DNA with a frequency of about 0.25 at neutral pH. An alternative hypothesis which explained the lethality of this DNA was that a certain 'critical target' must be hit, and this target occupied 0.25 of the viral genome.[4]

This communication deals with the test of the two hypotheses for the RF-DNA of φX174; the distinctive physical properties of the DNA serve well for this purpose. The results obtained indicate that single-strand lesions alone can inactivate this DNA.

Materials and Methods.—Sinsheimer, Burton, and co-workers,[6–10] Hayashi *et al.*,[11, 12] and Jansz and Pouwels[13, 14] have shown (by comparison with the work on polyoma DNA[15, 16]) that preparations of RF-DNA contain two components. Component I consists of circular molecules with separately continuous strands and possesses elements of structure giving rise to an abnormally high sedimentation coefficient (21*S*). Component II sediments at 17*S* and consists of open circles produced from I by a single-strand break in either strand. Furthermore, linear DNA of molecular weight of 3.4×10^6 daltons sediments at about 15*S*.[11] Therefore, when RF-DNA is irradiated, single-strand breaks convert the 21*S* unbroken molecules to the 17*S* form and double-strand breaks produce 15*S* linear molecules (component III). Analytical and sucrose-gradient centrifugation was therefore used to measure the quantity and infectivity of each component as a function of radiation dose, and the number and inactivation efficiency of each kind of break calculated.

The RF-DNA was prepared by the method of Sinsheimer[6] as modified by Carusi[17] except that sucrose-gradient purification was used in place of a Mandel-Hershey column. The infectivity of this DNA was measured using a protoplast assay system described in reference 6.

Samples of RF-DNA of 120 μl in tris buffer, pH 8.0, were frozen rapidly in liquid nitrogen, irradiated at 4.5 kr/min with Co^{60} γ-rays to a series of doses while immersed in liquid nitrogen, and allowed to warm to room temperature. We have found that at -196°C the radioresistance of RF-DNA is independent of the need of broth for protection against 'indirect' effects of radiation. Small samples were used for band centrifugation and sucrose-gradient centrifugation as described below. The concentration of DNA was 50–100 μg/ml. The solution also contained residual RNA resistant to RNase treatment.

Results.—Figure 1 shows a series of densitometer traces of UV photographs of band centrifugation runs at increasing γ-ray doses. A transfer of material from the leading 21*S* (I) component to the 17*S* (II) component is apparent; a shoulder corresponding to 15*S* (III) material appears at higher doses.

Figure 2 shows the infectivities of the bands separated on sucrose gradients for a series of increasing doses. A transfer of infectivity from band I to band II is seen. No infectious material corresponding to 15*S* linear molecules appears, from which we conclude that double-strand breaks are always lethal.

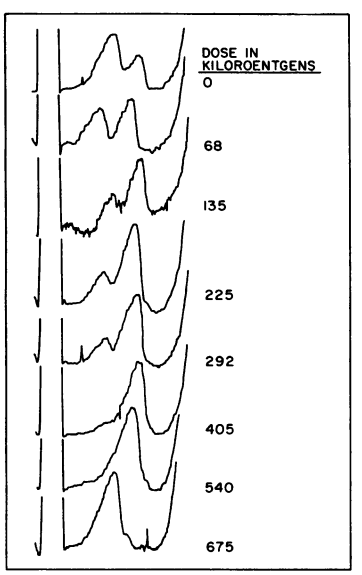

FIG. 1

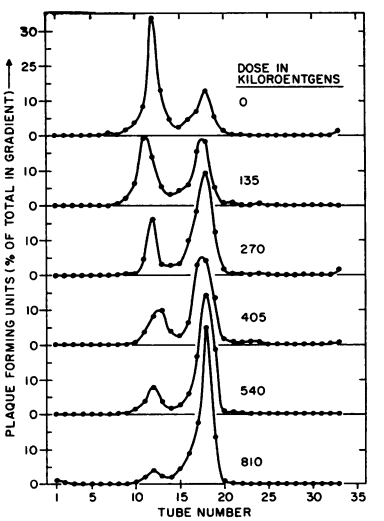

FIG. 2

FIG. 1.—Analytical sedimentation of irradiated RF-DNA. The densitometer traces of the bands observed are shown at approximately the same time after reaching full speed. The corresponding radiation doses are shown at the right of each tracing. The RF-DNA in 0.01 *M* Tris/HCl buffer, pH 8.0, was irradiated frozen in liquid nitrogen. After thawing, 20 μl were placed in the sample well of a 12-mm Kel-F band centerpiece.[22] Sedimentation was performed at 20°C and 42,040 rpm using 1 *M* NaCl as the bulk solution.

FIG. 2.—Sucrose-gradient sedimentation of irradiated RF-DNA. The results are shown for single gradients at a series of doses. The infectivity is expressed as per cent of total plaque-forming units, (PFU), in the gradient. 25 μl of solution, irradiated in liquid nitrogen and thawed, was layered onto 4.6 cc of 5–20% sucrose gradient and sedimented at 32,500 rpm in an SW39 swinging-bucket rotor at 6°C for 8 hr. Ten-drop fractions were collected and assayed with the protoplast assay system using *E. coli* C bacteria.

The fraction of absorbing material (abs) surviving single-strand breaks, and therefore remaining in band I, is given by

$$\left(\frac{I}{I+II}\right)_{abs} = e^{-k_1 D} \tag{1}$$

where D is the dose in megaroentgens (MR), and k_1 is the rate of production of single-strand breaks (breaks/molecule/MR). The fraction of absorbing material surviving double-strand breaks, and therefore remaining in bands I and II, is given by

$$\left(\frac{I+II}{I+II+III}\right)_{abs} = e^{-k_2 D} \tag{2}$$

where k_2 is the rate of production of double-strand breaks (breaks/molecule/MR).

Infectious material is transferred from band I to band II by those single-strand breaks which do not inactivate. The fraction of infectious (inf) material surviving in band I is therefore given by

$$\left(\frac{I}{I+II}\right)_{inf} = e^{-k_1(1-\alpha_1)D} \tag{3}$$

where α_1 is the efficiency of inactivation by single-strand breaks.

Figure 3 shows the results of analytical centrifugation experiments plotted

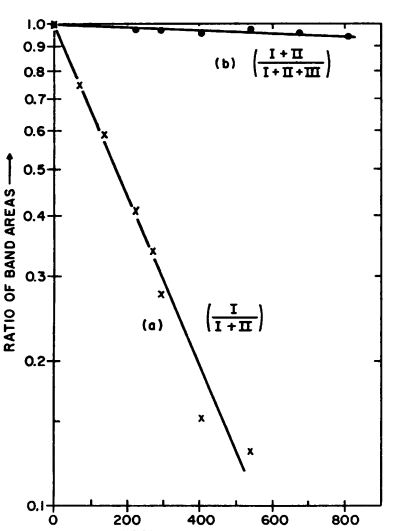

FIG. 3.—Fraction of molecules surviving (*a*) single- and (*b*) double-strand breaks. The slopes of these lines are (*a*) k_1 = 4.0 breaks/molecule/MR, and (*b*) k_2 = 0.076 breaks/molecule/MR.

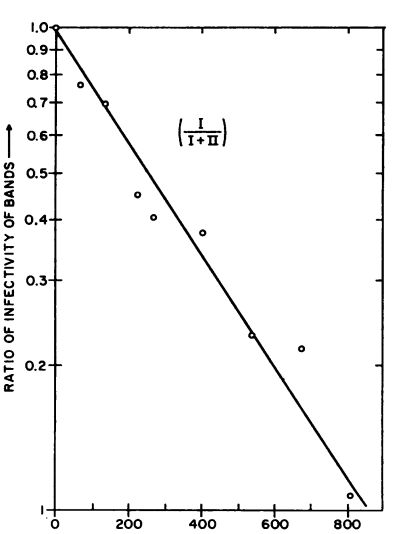

FIG. 4.—Fraction of biological activity surviving in band I. The slope of this line gives the rate of production of nonlethal single-strand breaks, $k_1(1 - \alpha_1)$ = 3.0 breaks/molecule/MR.

according to equations (1) and (2), and Figure 4 shows the results of sucrose-gradient analyses plotted according to equation (3).

From the slopes of these lines we calculate the following: $k_1 = 4.0$ breaks/molecule/MR, $k_2 = 0.076$ breaks/molecule/MR, and $\alpha_1 = 0.35 \pm 0.1$.

Discussion.—Our analysis divides the radiation lesions into lethal and nonlethal double-strand and single-strand breaks and other damage to the bases or sugars which does not involve strand breaks. It has been shown that strand breaks can follow damage to the pentose sugar moeity.[18] In view of the uncertain nature of these lesions, in what follows we have called them base or other damage.

With the rates of double- and single-strand breaks calculated above, at 0.76 MR (D_{37}), RF-DNA has received three single-strand breaks and 0.058 double-strand breaks/molecule. This shows that the inactivation is mainly due to single-strand breaks and base or other damage. Double-strand breaks account for only a small fraction of the inactivation. Assuming 9×10^{11} primary ionizations per gram per roentgen,[4] there are four primary ionizations in the DNA molecule at this dose. Three of these give rise to single-strand breaks and the remaining one to base or other damage. Within the limits of experimental error two alternative interpretations are possible. A value of $\alpha_1 = 0.33$ corresponds to inactivation entirely by single-strand breaks and not at all by base or other damage, whereas $\alpha_1 = 0.25$ corresponds to inactivation by single-strand breaks and base or other damage with equal efficiency. We have chosen the latter more reasonable conclusion.

The value of α_1, 0.25, represents either the relative size of a critical target or the efficiency of a repair system. However, a larger D_{37}, implying greater radiation resistance, is found when host cells known to possess repair systems are used. We therefore conclude that a critical target of 0.25 of the RF-DNA exists. The significance of this value is not yet clear.

The values of k_1 and k_2 are between one and two orders of magnitude less than those measured in free buffer at room temperature.[2, 3, 19–21] These lower values are due mainly to a reduction of the indirect effects of radiation. The ratio $k_1 : k_2$ is about 53, which is larger than previous estimates[2, 3] of the ratio of single:double-strand breaks. The small size and shear insensitivity of the circular RF-DNA reduces the possibility of accidentally converting single- into double-strand breaks. Our measurement of single-strand breaks in RF-DNA does not involve alkaline denaturation so that actual breaks rather than alkali-labile bonds are measured. It has been shown previously[20, 21] that the number of double-strand breaks increases with the square of the dose, implying that they are caused by coincident single-strand breaks. The number of double-strand breaks in the biological dose range used here is too small to distinguish between linear and quadratic behavior.

Summary.—The γ-ray inactivation of ϕX174 RF-DNA is caused mainly by single-strand scissions, base or other damage, and to a very small extent by double-strand scissions. The efficiency of inactivation by single-strand breaks and base or other damage is 0.25.

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