Contribution of Strand Breakage to Inactivation of γ-Irradiated Dry ΦX174-DNA

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By the occurrence of one strand break, the circular and single-stranded DNA of the bacteriophage ΦX 174 is converted to a linear molecule of identical molecular weight, a fact making the separation, by centrifugation, of broken and unbroken molecules difficult. Therefore, in the earlier investigations the number of broken molecules could not be determined directly but was calculated from the amount of smaller fragments originating from circular ΦX-DNA after the occurrence of two and more strand breaks. However, the accuracy of this indirect method is not wholly satisfying.

After testing and improving the methods to separate circular and linear ΦX 174-DNA molecules of identical molecular weight (Lücke-Huhle and Jung [1]) we reinvestigated the contribution of strand breakage to inactivation of ΦX 174-DNA irradiated in the dry state. The DNA of bacteriophage ΦX 174 am3 (Hutchinson and Sinsheimer [2]) was labelled with ^{3}H and extracted using the hot phenol method (Guthrie and Sinsheimer [3]). After purification, the DNA was freeze-dried on microscope cover slips. Several cover slips were placed between the windings of a wire coil into glass ampoules and sealed with a flame evacuating to 10^{-3} Torr for several hours. Irradiation was performed in a ^{60}Co-γ source at a dose-rate of 680 krads/hour. The DNA was dissolved off the cover slips either in 0.5 ml tris buffer to determine plaque-forming ability (Jung and Kürzinger [4]) or in 0.6 ml of an alkaline buffer (pH 12.0, containing 5 per cent sucrose, 1.5 mM Na-citrate, 0.1 mM EDTA, and 4 mM NaOH) for sedimentation analysis.

Fig. 1 shows an example of the distribution of radioactivity from ^{3}H-labelled ΦX 174-DNA after centrifugation in an alkaline sucrose gradient. When the DNA molecules have sedimented nearly to the bottom of the centrifuge tube, the broken molecules are well separated from the undamaged circular molecules. Already in the control samples, a certain portion of the molecules are broken; some

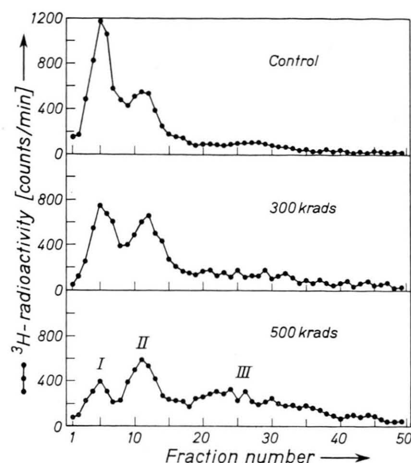

Fig. 1. Sedimentation pattern of ^{3}H-labelled ΦX 174-DNA in an alkaline sucrose gradient after irradiation with ^{60}Co-γ radiation in the dry state. Direction of sedimentation was from right to left. Conditions of centrifugation: 34,000 rpm, 5 °C, 18 hours; 5 to 20 per cent sucrose gradient (pH 12.0) buffered by 5 mM Na-citrate, 1 mM EDTA, and 4 mM NaOH; SW 40 swinging bucket rotor, Spinco L2-50 B ultracentrifuge; 50 fractions collected. I: Unbroken circular ΦX 174-DNA. II: Linear DNA molecules originating from component I by the occurrence of one strand break. III: Linear DNA molecules of reduced molecular weight originating from component II by the occurrence of one or more additional strand breaks.

breaks result from nuclear disintegrations in the radioactively-labelled molecules, some are caused during isolation and freeze-drying of the DNA

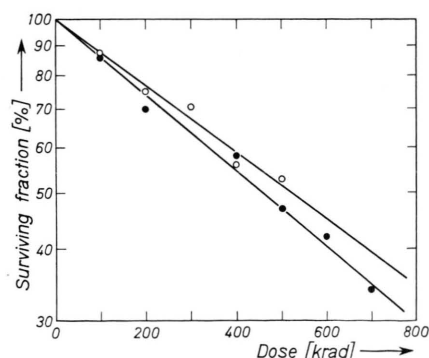

Fig. 2. Inactivation and strand breakage in ΦX 174-DNA after γ-irradiation in the dry state. ● Loss of plaque-forming ability as tested in a spheroplast system. ○ Fraction of unbroken molecules as determined by sedimentation in an alkaline sucrose gradient.

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(Lücke-Huhle and Jung [5]). With increasing dose the amount of circular molecules decreases and that of the broken molecules increases. Especially at higher doses, the appearance of molecules of reduced molecular weight (having suffered two or more breaks) is obvious.

Fig. 2 shows the percentage of infectious ΦX 174-DNA as well as the percentage of unbroken molecules at various γ-ray doses. The data plotted are average values from several experiments, the straight lines represent least square fits. The D_{37} values calculated from the slopes of the dose-effect curves amount to (658 ± 46) krads for the loss of plaque-forming ability and (749 ± 81) krads for the occurrence of strand breaks indicating that under the experimental conditions applied 88 ± 16 per cent of the inactivated ΦX 174-DNA molecules carry at least one strand break.

Since separation of circular and linear ΦX-DNA molecules without addition of formaldehyde is possible only at high pH and low ionic strength, sedimentation could not be performed in neutral gradients. Thus, the breaks determined in this work derive from breaks formed directly by irradiation and from 'alkali-labile bonds' converted to strand breaks by alkaline treatment. In γ-irradiated double-stranded ΦX 174 RF-DNA 22 per cent of the single-strand breaks measured under alkaline conditions are due to alkali-labile bonds (Lücke-Huhle *et al.* [6]). Since the fraction of alkali-labile bonds in γ-irradiated single- and in double-stranded DNA was found to be about the same (Bopp and Hagen [7], Kessler *et al.* [8]) we can assume 22 per cent of the strand breaks determined in the present study also to be due to alkali-labile bonds and 78 ± 7 per cent to be 'real' breaks. Thus, we come to the result that 69 ± 19 per cent of the ΦX 174-DNA molecules irradiated in the dry state are inactivated by strand breakage and only one third by other types of damage.

The value of 69 per cent obtained in this study is substantially higher than the figure of 26 per cent reported by Lytle and Ginoza [9]. The fact that these authors irradiated ΦX-DNA in frozen suspension cannot account for the difference under discussion, since the number of alkali-labile lesions does not depend on whether the DNA is irradiated in frozen solution or in the dry state (Lücke-Huhle *et al.* [6]). The discrepancy may be due to the fact that our method of separation of broken and unbroken molecules substantially increases the accuracy of the experimental techniques applied.

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