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Temperature Dependence of the Ultraviolet Radiation Sensitivity of Clean, Dry T1 Bacteriophage: Relation to a Host-Repairable Component of Ionizing Radiation Direct Action

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This paper involves the excitational component accompanying any ionizing radiation exposure. It is somewhat unusual to discuss biological effects of such photobiological accompaniment of ionizing radiation, primarily by reason of quantum efficiency differences. Although the excitational energy transfer from ionizing particle tracks may run 40%, or more, of the total energy transfer, photobiological quantum efficiencies for damage run typically 10^{-2} to 10^{-4} , while the ionization events tend toward a quantum efficiency of unity. For this reason the element of photobiology within radiation biology is usually negligible.

However, there is at least one system where the excitational component seems to be demonstrable in terms of biological effect. T1 bacteriophage dried from broth and irradiated in vacuo with--in this case--fast electrons shows appreciable plaque survival difference on bacterial host strains E. coli B and E. coli B_{S1}. The most notable difference between these bacterial strains, one a mutant of the other, involves a repair system for excitational damage to DNA. It is not unreasonable to infer that the damage unrepaired by fast electrons for T1 phage assayed for plaque survival on E. coli B_{S1} may be excitational. The difference component is almost negligible for the phage irradiated in nutrient broth suspension, where the overall sensitivity is much higher. Assuming the excitational component to be inherently by direct action only, a moderate indirect action for the ionizing component could dilute it to that extent.

I first studied this fast electron host strain difference in direct action some years ago, in relation to irradiation temperature. At that time there had been a suggestion that the temperature-dependent part of the ionizing direct action represented excitational damage. I played some part in disposing of that notion, but was still interested to have a look at the temperature dependence of the repairable component, which does have the excitational credentials indicated above. That result is shown in the first slide (Figure 1). Although previously reported on, it is necessary background for present discussion.

Plaque survival sensitivity, as an electron cross-section, is plotted against reciprocal absolute temperature. Note that sensitivity increases with temperature and that it is larger for plaque survival on B_{S1} than on B. The difference component, i.e., the presumptive excitational component, runs about 35% of the sensitivity as scored on E. coli B, and shows a dependence upon irradiation temperature quite typical of direct action. It was this finding which assisted toward the conclusion that the temperature dependent part of ionizing radiation direct action was not essentially excitational. Here was a likely excitational component that clearly did not tend to zero at low temperature. The curves, incidentally, are fitted by expressions involving sums of terms with different activation energies, but that understanding need not be developed further here for appreciation of the current point.

I have continued an interest in this problem by wondering what the dependence upon irradiation temperature would be for a bona fide excitational irradiation, such as in the ultraviolet. That test has involved cleaning up the dry T1 preparation so that

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we could get UV through it properly, and also a choice of easy-to-get 254 nm radiation in place of harder-to-get (and indeed, hard to determine in terms of actual absorber chemistry) vacuum UV. Slow electrons (300 ev) in water show a major loss component at 7-8 ev, for example (C. E. Klotz, p 34 in Ausloos' "Fundamental Processes in Radiation Chemistry"), rather than the 5 ev of the Hg resonance line. There are also peaks around 10 and 11 ev. In phage DNA the pattern of excitational energies should be different still, but probably mostly higher than the 5 ev easily available.

Cleaning up the irradiation prep was a particular problem, since T₁ phage doesn't ordinarily dry stably from water alone. In the course of trying sublimable salt additives, however, an old trick for getting clean, dry T₁, I found the water controls suddenly drying out even more stably, and quite acceptably. Without stopping to find out why--perhaps the particular time course of drying, or the fresh lysate I was using--I went ahead with the experiment. The phage was dried in groups of spots, on 5-mil Mylar supports, and irradiated in the same apparatus used previously for fast electrons. A silica window allowed alternate samples to be irradiated by electrons and by the 254-nm radiation of a 4-Watt Hg Mineralight. The samples were removed, resuspended, and scored for plaque survival on the two host strains. Only two irradiation temperatures were used, ambient and that of liquid nitrogen, partly because the plaque survival data were quite noisy and required some accumulation of results. The noise was associated with the drying from distilled water.

The resulting plaque survival data are now shown twice for direct contrast in host strain and in irradiation temperature. The first of these slides (Figure 2) shows the host contrast at both of the temperatures, 78°K and 300°K, the plaque survival curves for UV show some repairable damage. Those for phage scored on B_{S1} lie under those scored on E. coli B. The noise is quite evident, so all data are shown rather than trying to represent by statistical limits on any derived parameters. I do not know why the upturns at low temperature, if real, and have not pursued them.

The same data are shown in temperature contrast on the next slide (Figure 3). The top section involves survival on E. coli B, and the lower on B_{S1}. Within the obvious limit of noise, there is little if any temperature dependence. Indeed, the low temperature irradiation curves lie if anything under those for ambient temperature. Certainly there is nothing like the approximate three-fold dependence in the other direction seen for the fast electron host-related difference component of the broth-dried preparations.

However, the whole system was retested with electrons, as a clean system. The next slide (Figure 4) shows the host-related contrast. Plaque survival on B is mostly above that on B_{S1}, for irradiation at ambient temperature. Within narrower limits the same is true for the low temperature irradiation, where the curves show some perhaps consistent shape features that I don't understand. As the next slide (Figure 5) shows, however, the general temperature dependence is much different than for work with the broth-dried preparations. Taking the broth out has done more than let the UV in; it has changed the system itself, qualitatively and to a considerable degree.

Venturing, after all, to characterize the clean-prep electron curves by average parameters, the last slide (Figure 6) shows the relation of the clean and broth-dried preps. At ambient temperature the clean prep (circles) gave results quite reasonable close to the prior, broth-dried work (for which only the curves are carried over). The cor-

response should not be taken too closely, since a recheck of broth-dry preps with the new lysate, etc., showed some significant changes (20 to 35% less sensitivity at ambient temperature, both strains, and around 10% less at low temperature). The host related difference in sensitivity and its temperature dependence both decreased, in spite of the third from last sentence in the abstract.

However, in spite of these differences in broth-dried preparations, the overall result is that temperature dependence on the clean preparation was much less than when broth-dried. The ranges depend on interpretation of sigmoid curves. The temperature dependence of the difference component is very great.

Curiously, the clean preps are almost consistent with a notion of direct action temperature effect as being excitational, and not as evident in a strain that can repair excitational damage as in one less capable of such repair.

In the course of this work one additional evidence of a photobiological character was seen, in what amounted initially to a house-keeping experiment. In the earlier fast electron work with broth-dried preparations not much attention had been given the possibility of photoreactivation. It occurred to me that the ultraviolet-irradiated preparations should clearly be scored under circumstances safeguarded against photoreactivation. A test of samples from one complete run, involving both temperatures, both radiations, and the differential drying from broth or water, showed that there was no significant photoreactivation under the routine circumstances of plaque assay. However, plaque assays on plates incubated under high fluorescent illumination did show some significant instances of photoreactivation. All UV-irradiated samples showed marked photoreactivation, on both hosts, a finding which could surprise only those remembering early difficulties in photoreactivating T₁ phage UV-irradiated dry. Of more immediate interest, however, is a moderate degree of photoreactivation seen for T₁ phage irradiated with fast electrons. It occurs only to the extent of 10 or 12%, for assay on B_s1 where dark repair would provide less interference. Photoreactivation may also occur marginally for electron-irradiated T₁ phage assayed on E. coli B, perhaps with some effect of irradiation temperature. While these results are still preliminary, the finding of such photoreactivation for electron-irradiated material is additional indication that detectable photobiology can accompany ionizing radiation damage.

TABLE 1

Photoreactivation (PR) of T₁ bacteriophage irradiated in the dry state

Irradiation temperature	Kind of radiation	<u>E. coli</u> host strain	<u>PR sensitivity</u> <u>non-PR sensitivity</u>
78°K, clean	UV	B	0.64
		B _s 1	0.57
78°K, clean	electrons	B	1.05
		B _s 1	0.91
78°K, broth	electrons	B	0.01
		B _s 1	1.00
298°K, clean	UV	B	0.33
		B _s 1	0.51
298°K, clean	electrons	B	0.94
		B _s 1	0.88
298°K, broth	electrons	B	0.97
		B _s 1	0.96

Figure 1 T₁ phage radiation sensitivity as a function of reciprocal absolute temperature, for assay on *E. coli* B and B_{sl}.

Figure 2 Comparison of ultraviolet survival curves for T₁ phage, at two irradiation temperatures, according to host cell strain.

Figure 3 Comparison of ultraviolet survival curves for T₁ phage, for two host strains, according to irradiation temperature.

Figure 4 Comparison of fast electron survival curves for clean T₁ phage, at two irradiation temperatures, according to host cell strain.

Figure 5 Comparison of fast electron survival curves for clean T_1 phage, for two host strains, according to irradiation temperature.

Figure 6 Relation of T_1 phage fast electron sensitivity and irradiation temperature for clean (points) and broth-dried (curves) preparations.