Acta virol. **34** : 401—409, 1990

COMPARISON OF THE ACTION OF IONIZING RADIATION AND UV-LIGHT ON LAMBDA PHAGE. INFLUENCE ON PHAGE ADSORPTION, DNA INJECTION, REPLICATION, AND DNA REPAIR

Z. HRADEČNÁ*, L. KITTLER**

*Institute of Biophysics, Czechoslovak Academy of Sciences, Královopolská 135, 61265 Brno, Czechoslovakia, and **Central Institute of Microbiology and Experimental Therapy, Academy of Sciences of the G.D.R., Beutenbergstrasse 11, DDR-6900 Jena, G.D.R.

Received May 5, 1989; revised November 21, 1989

Summary. — The influence of gamma radiation, X-rays, UV-light at 254 nm and 365 nm, the latter combined with furocoumarin sensitizers has been studied on plaque forming ability, phage adsorption, DNA injection, and replication processes. UV-light (365 nm) plus furocoumarin treatment of phage particles gave rise to two types of DNA crosslinks. Type I crosslink corresponded to furocoumarin mediated covalent linkage between adjacent sites in opposite strands of the double helix. Crosslink type II (hairpin crosslink) required a highly condensed DNA and corresponded to the covalent linking of adjacent sites in double helical segments of a folded DNA molecule. The relationships of the type I crosslinks to inhibition of DNA replication and of the type II crosslinks to suppression of the DNA injection process are discussed. Pronounced deviations in phage inactivation have been obtained by X-ray radiation alone compared with UV-light (254 nm) pretreated and subsequently X-ray irradiated probes. The observed protective effect of the latter was described in terms of an inducible repair mechanism. The same protection has been observed by combination of gamma radiation with a sublethal UV-light (254 nm) dose.

Key words: *phage lambda*; *DNA photoproducts*; *adsorption*; *injection*; *replication*; *inducible repair*

Introduction

Light is an ubiquitous environmental factor. Therefore, interaction studies of electromagnetic waves of varying energy with biological material have been frequently performed (Héléne *et al.*, 1982). In living systems the main

* present address: McArdle Laboratory for Cancer Research, Dept. Oncology, Medical School University of Wisconsin, Madison, U.S.A.
** to whom correspondence should be directed.

target of radiation damages is the genetic material (Smith, 1977). Thus far, DNA under *in vitro* and *in vivo* conditions has been preferentially taken into account when studying the effects of radiation on living systems (Song and Tapley, 1979). Light of various energies, i.e. X-rays or UV-light, causes different damages to the DNA. While ionizing radiation mostly produces single and double strand breaks (Freifelder, 1966), UV-light at 254 nm is mainly responsible for base modifications (Kittler and Löber, 1977). Lower energy light adjacent to UV (365 nm, UVA-light) and in the visible region is capable of modifying the DNA via sensitization mechanisms (Löber and Kittler, 1977; Song and Tapley, 1979; Kittler and Löber, 1988). In this paper we present our results obtained with gamma radiation, X-rays, UV-light at 254 nm, and UVA-light at 365 nm plus furocoumarin derivatives as sensitizers in the phage lambda system. Along this line we have focused our interest on the consequences of the DNA modifications mentioned above on distinct multiplication steps of the phage, especially on adsorption, injection, and replication processes. Finally, repair mechanisms involved in the elimination of these damages will be briefly discussed.

Materials and Methods

Phages and bacteria. Investigations were performed with phage lambda cb_2 and the host bacteria *E. coli* C 600, *E. coli* SR 20 and repair-deficient mutants of these strains which were kindly provided by Dr. M. Sedliaková, Bratislava, Czechoslovakia.

Radiation sources. Thermax X-ray tube operating at 80 kV and 12 mA and ^{60}Co aus gamma radiation source were used. For UV-radiation experiments at 254 nm and 365 nm a low-pressure Westinghouse type WL 782(30) lamp (Philips) and a high pressure mercury lamp (Type HBO 500, VEB Narva, Berlin, G.D.R.) were employed. The latter was supplemented with a glass filter cut off light below 300 nm.

Radioactive labelling of phage DNA. For measurements of phage adsorption and DNA injection the phage lambda DNA was ^{32}P-labelled (Kittler *et al.*, 1977). The phage DNA replication was monitored using ^{3}H-thymidine incorporated by the pulse-labelling technique (Young and Sinsheimer, 1967).

Furocoumarin derivatives. The linear furocoumarin derivative xanthotoxin (8-methoxypsoralen, 8-MOP) and the angular derivative angelicin were used. Both were generous gifts from Prof. G. Rodighiero, Padova, Italy. In order to ensure penetration of these substances into the phage particles, 8-MOP or angelicin (50 μg/ml) were added to the phage suspension 30 min before irradiation.

Sucrose gradient technique. The DNA crosslink formation caused by reaction of photochemically excited furocoumarins was followed by alkaline centrifugation (Cole and Zusman, 1970).

Results

The first studies were designed to distinguish between damages caused by adsorption of the phage particles on the surface of bacteria and phage DNA injection into the host due to X-ray treatment. Fig. 1 shows inactivation of phage lambda and changes in adsorption and DNA injection caused by X-ray radiation in the presence of tryptone or tryptone supplemented with 0.1 mol/l cystamine in nitrogen atmosphere. Phage adsorption and DNA injection decreased with increasing X-ray dose. The inhibition of both corresponds to about 20% of total inactivation. Radiation in 0.1 mol/l cystamine

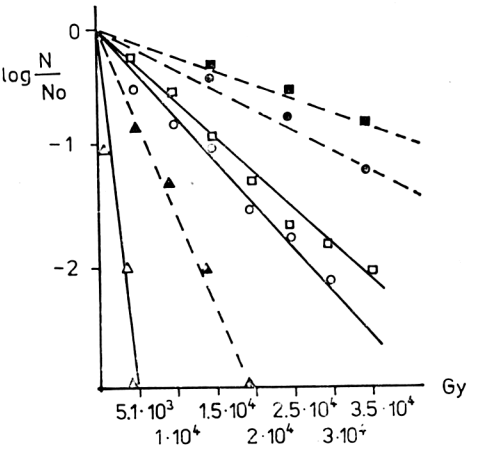
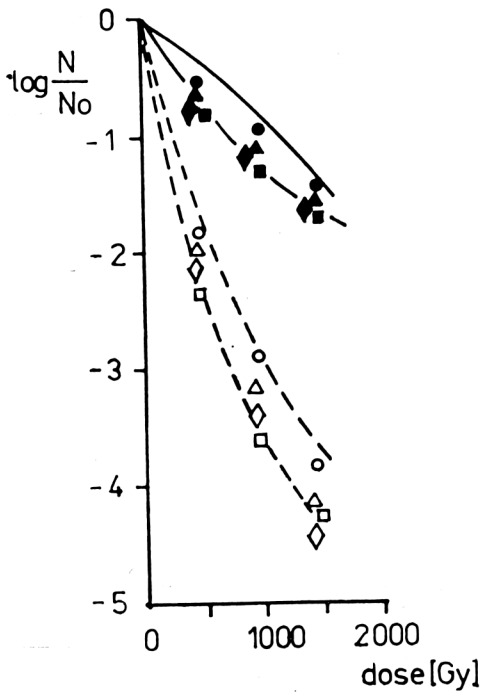


Fig. 1
Effect of X-ray treatment on phage lambda. Inactivation (triangles), adsorption (squares), injection (circles)
Light symbols: irradiated in tryptone
Full symbols: irradiated in tryptone supplemented with 0.1 M cystamine.

solution diminished phage inactivation, the ratio of adsorption and injection to the whole damages (20%) remained, however, unchanged. When looking for reactivation process the only phenomenon observed after X-ray treatment in the phage lambda occurred through genetic recombination as experimentally proved by marker rescue. The effectiveness of repair was markedly en-



Survival rate of phage lambda after gamma irradiation solely and gamma irradiated probes pretreated with a sublethal UV-light (254 nm) dose
Empty symbols: gamma radiation only, full symbols: pretreated with UV-light and subsequent gamma radiation.
● SR 20 (uvr⁺ rec⁺)
▲ SR 22 (uvr⁻ rec⁺)
◆ SR 73 (uvr⁻ rec⁻)
■ SR 74 (uvr⁺ rec⁻)

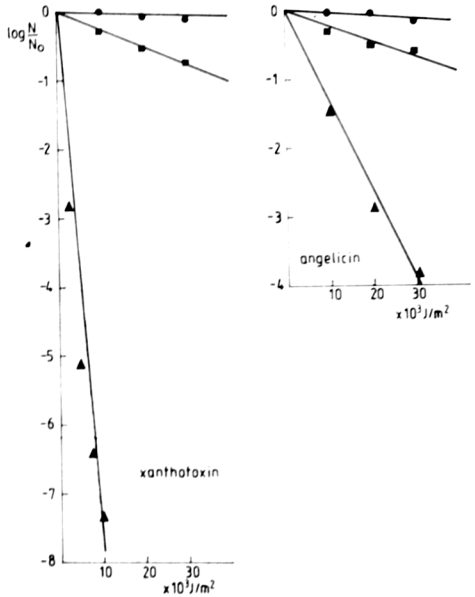


Fig. 3
Influence of furocoumarins plus nea UVA-light (365 nm) on adsorptio (circles), injection (squares), and plaqu forming ability (triangles) of phag lambda cb_2

hanced when phages were pretreated with sublethal doses of UV-light (25 nm) and subsequently irradiated with gamma rays as shown in Fig. 2.

Inactivation studies have been extended also to the near UV-light wave lengths (365 nm) with furocoumarins as sensitizers. Both derivatives, 8-MO and angelicin, reduced the effectiveness of plaque forming ability, phage ad sorption and DNA injection. 8-MOP, however, had a more pronounced actio than the latter (Fig. 3). Phage DNA injection into the host cell as well a phage DNA replication remained practically unaffected when UVA-ligh (365 nm) or furocoumarin derivatives alone were used, while a combinatio of both, however, showed a strong synergistic effect. The DNA-crosslink for

Table 1. Repair of DNA photodamages induced by the combined action of furocoumarins plus UVA-light (365 nm) in the system phage lambda-E. coli

E. coli strains	Plaque forming ability (%)*	
	8—MOP	Angelicin
SR 20 uvr⁺rec⁺	20	90—95
SR 74 uvr⁺rec⁻	8	85
SR 22 uvr⁻rec⁺	0.7	50
SR 73 uvr⁻rec⁻	0.25	28

* related to untreated control

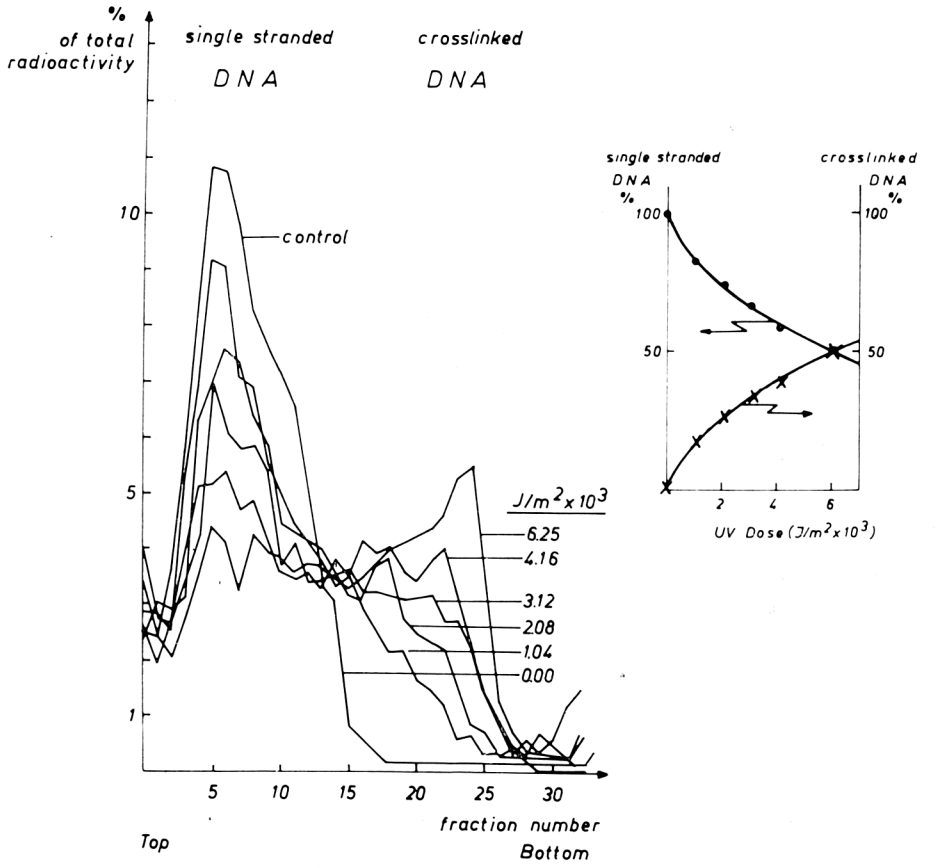


Fig. 4
Distribution of isolated phage lambda ^{3}H-DNA on alkaline sucrose gradient after UVA-light (365 nm) plus xanthotoxin treatment (UV-doses are up to 6.25×10^3 J/m^2)

Table 2. Influence of X-rays and UV-light on phage lambda multiplication

Radiation sources	Inactivation	Adsorption	Injection	Replication
X-rays	+	+	+	+
UV-light (254 nm)[1]	+	−	−	+
UVA-light (365 nm)	.(+)	−	−	−
UVA-light (365 nm) plus furocoumarins	+	−	+	+

[1] Kellenberger *et al.*, 1959
+ = inhibited
− = unaffected

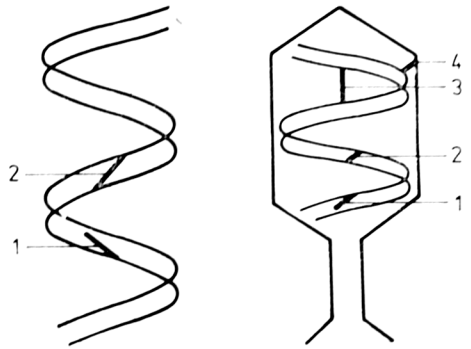


Fig. 5
Schematic representation of possible DNA-furocoumarin crosslinks
Left: in dilute aqueous solution
Right: in phage head
1 monoaddition product
2 crosslink type I
3 crosslink type II
4 nucleic acid — protein crosslink

mation in intact phage particles as a consequence of 8-MOP plus UVA-light (365 nm) treatment was monitored on alkaline sucrose gradient (Fig. 4), where the photochemically crosslinked DNA showed a distinctly different sedimentation profile as compared with single stranded material. In Table 1 the repair of DNA photodamages induced by combined action of furocoumarins plus UVA-light (365 nm) in the system of phage lambda *E. coli* varying in repair capacity is summarized. It is obvious that DNA modifications caused by angelicin can be eliminated with higher efficiency than those generated by 8-MOP.

Discussion

The sensitivity of phages to X-rays depends on the additives in the nutrition medium. For this reason some radical scavangers like cysteine, cystamine or ascorbic acid were supplemented. The observed protective effect (Fig. 1) is strongly indicative that radicals as intermediates are involved in the DNA fragmentation process. Such damaged phage DNA is partially incapable of being injected into the host (Hradečná, 1966). In addition, the adsorption of phage particles on the host is diminished (Fig. 1). Adsorption has been described in terms of protein-protein interactions between the phage tail and the cell surface. The reduced phage adsorption on the cells after X-ray treatment should therefore be viewed as modification of the protein recognition pattern. Our experiments have shown that after X-ray inactivation of the phage lambda a lesser degree of reactivation occurs than for UV-light (254 nm) irradiated suspensions. The phages do not seem to carry a measurable amount of DNA lesions resembling to UV-light (254 nm) damages. If thymine dimers are predominantly responsible for the death of UV-light (254 nm) irradiated phages, then such dimers should not play a dominant role in the inactivation due to X-ray treatment.

UV-light (254 nm) is mainly absorbed by the purine and pyrimidine bases. The effects of X-rays on DNA are less specific. The only noticeable reactivation phenomenon observed with X-ray inactivated lambda phages is a certain marker rescue. That means, undamaged parts of irradiated phage genome are

incorporated to a measurable extent into the non-irradiated phages which simultaneously infect the host. If one corrects for the lack of adsorption and injection, the rescue is nearly as extensive as in the case of UV-light (254 nm) irradiated phages (Hradečná, 1966). It has been shown (Hradečná and Holonová, 1980) that subsequent treatment by two different kinds of radiation such as UV-light and gamma rays increases the resistance of phage if compared to single gamma ray treatment (Fig. 2). The plaque forming ability of irradiated phages was determined with different host strains varying in repair capacity.

The absence of any repair capacity in *E. coli* host cells which render the cells more UV-light sensitive had no influence on the repair of gamma radiation damaged phage particles. The mechanism of this repair does not depend on *uvr*- and *rec*-genes. It could be suggested that the irradiated phage DNA is a signal for some host SOS genes which release the repressed *rec*A protein involved in the repair mechanism. The induction of the SOS repair system can occur upon the introduction of an UV-light damaged DNA molecule (Walker, 1984). The same inducible repair effect was found by the combination of UVA-light (365 nm) and UV-light (254 nm) as well as by visible light and UVA-light (365 nm) (unpublished results).

UVA-light (365 nm) induces the covalent binding of furocoumarins to DNA. Monoaddition products and crosslinks were observed which are both believed to produce mutagenic and lethal events in various types of viruses, bacteria, and eukaryotic cells (Scott *et al.*, 1976). Crosslink formation is strongly influenced by the sterical arrangements of the reactants, i.e. the furocoumarin molecules attached to the thymine residues of the DNA. Thus, the conditions present in dilute aqueous solution are quite different from those in biological systems, e.g. inside the phage head. While in the former case the most important determinant of DNA is its secondary structure, in the latter case the high local concentration of DNA forms a tertiary structure which has a higher degree of compactness. In aqueous DNA solution and in living systems 8-MOP is well-known for its ability to form crosslinks while these photoproducts were not detectable for angelicin. Therefore, we treated phage lambda DNA *in situ* with 365 nm UVA-light in the presence of either angelicin or 8-MOP, which had hardly any effect on the adsorption of phages at the host, while the injection of phage DNA into the bacteria was strongly affected. Both derivatives inhibited injection, but 8-MOP about twice as much as angelicin (Fig. 3).

This deviation is not caused by differences in the penetration properties of furocoumarin derivatives through the cell wall, as could be shown by the close correlation between the reaction efficiencies with isolated DNA and DNA *in situ* of various types of mammalian cells and viruses. The variation in the composition of the cell walls does not indicate significant differences in penetration properties of the drug.

Therefore, we conclude that furocoumarin plus near UV-light treated phage DNA is strongly prevented to leave the phage through the tail for cell infection, and those molecules which are able to penetrate into the host cells are significantly inhibited in their replication ability. These data suggest

the following model (Fig. 5). DNA in diluted aqueous solution and photochemically excited xanthotoxin generate monoaddition products and crosslinks between the complementary strands of the DNA duplex (type I crosslink). For steric reasons, angelicin reacts under those experimental conditions only monofunctionally. Inside the phage heads, however, both drugs forms type II crosslinks which correspond to the bifunctional covalent binding between adjacent sites in the folded structure of the double helix (hairpin crosslinks). Theoretical considerations (Kittler *et al.*, 1980), physicochemical measurements (Kittler and Zimmer, 1976), and electronmicroscopic studies (Ebert *et al.*, 1983) agree well with our prediction concerning the existence of type II DNA crosslinks inside the phage heads and the ability of angelicin to photo-crosslink.

Studies on the repair of DNA damages caused by UV-light (365 nm) plus furocoumarins were performed with different host strains of *E. coli* varying in their repair capacity. The results in Table 1 provide evidence that monoaddition products will be repaired more readily than crosslink and *uvr*-genes display a higher repair capacity than *rec*-genes. For repair of crosslinks both genes are necessary, while monoaddition products can be eliminated by either of the genes alone. Using strain *E. coli* SR 74 (*uvr*$^-$, *rec*$^-$) as a host, the plaque forming ability for phages treated with near UV-light (365 nm) plus 8-MOP is one order of magnitude smaller than for angelicin, meaning that the crosslink monoaddition photoproduct ratio is about 1 : 10.

Finally, the effectiveness of X-rays and UV-light on phage multiplication steps is compiled in Table 2. For all radiation methods used, plaque forming ability of phage lambda was diminished. More differentiated is the behaviour for the distinct multiplication steps of phage adsorption, phage DNA injection and replication. The initial adsorption step is only disturbed by X-ray treatment while UV-light irradiation shows no measurable effect. As expected, X-ray damages on the protein recognition pattern were more pronounced than those due to UV-light which is essential for phage adsorption onto the surface of the host. The inhibition of injection appears to occur due to DNA fragmentation and type II crosslink formation upon X-ray or combined UVA-light (365 nm)plus furocoumarin treatments. Double strand breaks of DNA, DNA base photoproducts, and type I crosslinks produced by treatment with X-rays, UV-light at 254 nm, and UVA-light (365 nm) plus furocoumarins, respectively, are responsible for the reduced replication.

Acknowledgements. The critical reading of the manuscript by Drs. G. Löber (Jena) and W. Guschlbauer (Saclay) is highly appreciated.

References

Cole, R. S., and Zusman (1970): Sedimentation properties of phage DNA molecules containing light-induced psoralen cross-links. *Biochim. Biophys. Acta* (*Amst.*) **224**, 660—662.

Ebert, B., Smotryaeva, M. A., Platzer, M., Kruglyakova, K. E., and Kittler, L. (1983): Dynamic behaviour of crosslinked DNA studied by ESR and electron microscopy. *Studia Biophys.* **97**, 75—79.

Freifelder, D. (1966): Lethal changes in bacteriophage DNA produced by X-rays. *Rad. Res. Suppl.* **6**, 80—96.

Héléne, C., Charlier, M., Montenay-Garestier, T., and Laustriat, G. (Eds) (1982): "*Research in Photobiology*", Plenum Press, New York and London.

Hradečná, Z. (1966): X-ray inactivation of lambda phages. I. Damage on adsorption and injection. *Int. J. Rad. Biol.* **10**, 443—449.

Hradečná, Z., and Holanová, Z. (1980): Induced Repair by Phage Lambda in DNA: Recombination, Interactions and Repair, pp. 515—518. In S. Zadrazil and J. Sponar (Eds): Pergamon Press, Oxford.

Hradečná, Z., and Kittler, L. (1982): Photobiology of furocoumarins. Various types of crosslinking with DNA and their interference with the development of phage lambda. *Acta virol.* **26**, 305—311.

Kellenberger, G., Arber, W., and Kellenberger, E. (1959): Eigenschaften UV-bestrahlter λ-Phagen. *Z. Naturforsch.* **14b**, 615—629.

Kittler, L., and Zimmer, Ch. (1976): Conformational changes of nucleic acids and poly d(A-T). d(A-T) caused by photoaddition of furocoumarins. *Nucl. Acids Res.* **3**, 191—203.

Kittler, L., and Löber, G. (1977): Photochemistry of the Nucleic Acids in Photochemical and Photobiological Reviews, pp. 39—131. In K. C. Smith (Ed.): Plenum Press, New York.

Kittler, L., and Löber, G. (1988): Photoreaction of furocoumarins with nucleic acids, proteins, membranes, and their subunits. *Studia Biophys.* **2—3**, 97—114.

Kittler, L., Hradečná, Z., and Löber, G. (1977): Photochemically induced binding of furocoumarins with lambda phage DNA *in situ*. *Studia Biophys.* **66**, 237—241.

Kittler, L., Hradečná, Z., and Sühnel, J. (1980): Crosslink formation of phage lambda DNA *in situ* photochemically induced by the furocoumarin derivative angelicin. *Biochim. Biophys. Acta (Amst.)* **60**, 215—220.

Löber, G., and Kittler, L. (1977): Selected topics in photochemistry of nucleic acids. Recent results and perspectives. *Photochem. Photobiol.* **25**, 215—233.

Scott, B. R., Pathak, M. A., and Mohn, G. R. (1976): Molecular and genetic basis of furocoumarin reactions. *Mutation Res.* **39**, 29—74.

Smith, K. C. (Ed.) (1977): "*The Science of Photobiology*", Plenum Press, New York and London.

Song, P. S., and Tapley, K. J. (1979): Photochemistry and photobiology of psoralens. *Photochem. Photobiol.* **29**, 1177—1197.

Walker, G. C. (1984): Mutagenesis and inducible responses to deoxyribonucleic acid damage in *E. coli*. *Microbiol. Rev.* **48**, 60—93.

Young, E. T., and Sinsheimer, R. L. (1967): Vegetative bacteriophage λ DNA: II. Physical characterization and replication. *J. mol. Biol.* **30**, 165—200.