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PHOTOBIOLOGY OF FUROCOUMARINS. VARIOUS TYPES OF CRSSOLINKING WITH DNA AND THEIR INTERFERENCE WITH THE DEVELOPMENT OF LAMBDA PHAGE

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Summary. — It was shown that the multiplication of phage lambda was strongly suppressed by furocoumarins after irradiation with near ultraviolet light of 365 nm wavelength. Using xanthotoxin or angelicin there was a marked inhibition of the phage DNA injection and replication but adsorption was unaffected. This inhibition was attributed to various types of DNA crosslinking produced in the phage heads. Type I crosslink corresponded to covalent binding between adjacent sites in opposite strands of the double helix. Crosslink type II (hairpin crosslink) required a highly condensed DNA and corresponded to covalent binding between adjacent sites on double-helical segments of a folded DNA molecule. The relationships of the type I crosslinks to the DNA replication and of the type II crosslinks to DNA injection are being discussed. Like type II crosslinks, the nucleic acid — protein crosslinks hinder injection.

Key words: phage lambda; furocoumarins; crosslinks; adsorption; injection; replication

Introduction

Furocoumarins are increasingly used in molecular biology (Scott *et al.*, 1976; Song and Tapley 1979) since excitation can be performed at wavelengths (365 nm) where in general no other molecular cell constituents absorb. In addition, furocoumarins react preferentially with nucleic acids. As a result of this reaction covalent bonds are produced between furocoumarins and nucleic acid bases (Musajo and Rodighiero, 1970). Both monoaddition and crosslinking were observed which produce mutagenic and lethal events in various types of viruses, bacteria, and eukaryotic cells (Ashwood-Smith and Grant, 1977). Monoadducts are preferentially responsible for mutations and corsslinks for lethal events (Seiki *et al.*, 1978). At present, the photoproducts of furocoumarins with nucleic acids are developing into a valuable tool for

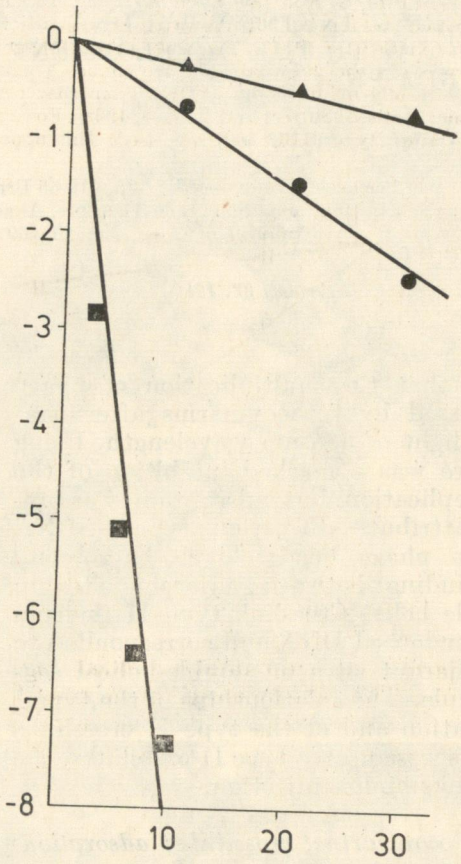

Fig. 1.
Inactivation of phage lambda cb_2 by the combined action of furocoumarins and near ultraviolet light (365 nm)
■——■ xanthotoxin, ●——● angelicin, ▲——▲ control, near ultraviolet light alone.
Abscissa: UV dose ($\times 10^3$ J/m^2); ordinate: log PFU (inactivation)

studying distinct biological processes in situ, e.g. increase of the recombination frequencies of phage lambda (Lin *et al.*, 1977), influence on adsorption, injection and replication of phage lambda (Hradečná *et al.*, 1978), inhibition of polymerase template activity (Ou *et al.*, 1978) and aminoacyl-tRNA synthetase activity (Ou and Song, 1978).

In this contribution we discuss the consequences of furocoumarin-photomodified DNA in the system phage lambda cb_2/E. coli C 600 on adsorption, injection and replication. While injection and replication are inhibited, adsorption is unaffected. Different photoproducts, type I and type II crosslinks, are responsible for interference in replication and injection, respectively.

Materials and Methods

Phages and bacteria. Investigations have been performed with phage lambda cb_2 and bacteria E. coli C 600 as the host. In order to ensure penetration into phage particles furocoumarins (xanthotoxin or angelicin; 50 μg/ml) were added to the page suspension 30 min before irradiation.

Xanthotoxin and angelicin were generous gifts of Prof. G. Rodighiero, Padua, Italy. To measure the dependence of the application of furocoumarin plus light of 365 nm wavelength on adsorption and injection, the phage DNA was ^{32}P-labelled (Kittler *et al.*, 1977). The replication of phage DNA was monitored using ^{3}H-thymidine incorporated by the pulse labelling technique (Young and Sinsheimer, 1967). The separation of photochemically modified phage DNA from coat proteins was performed according to the modified phenol method (Sarfert and Venner, 1962). For irradiation experiments a high—pressure mercury lamp (type HBO 500) was used. This apparatus was described in details elsewhere (Kittler *et al.*, 1977).

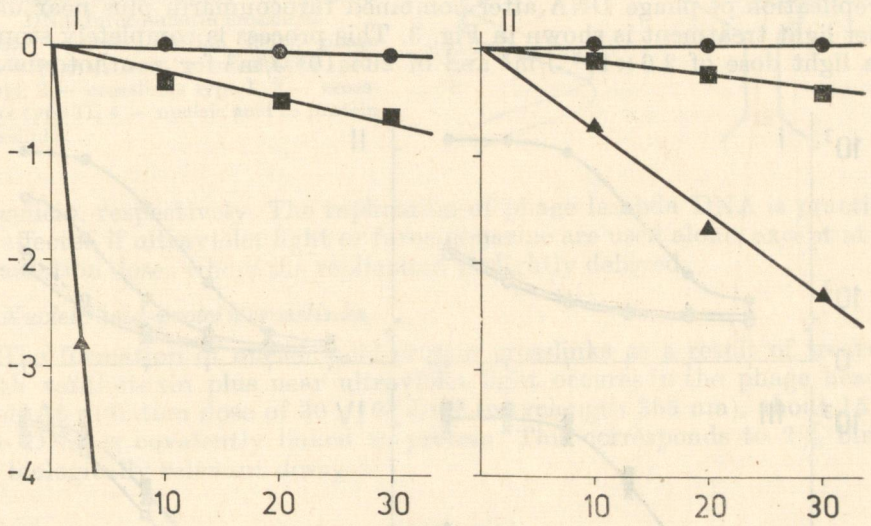

Fig. 2.
Influence of furocoumarins plus near ultraviolet light (365 nm) on adsorption, injection and plaque-forming ability of phage lambda cb_2
●——● adsorption, ■——■ injection, ▲——▲ plaque-forming ability.
I — xanthotoxin
II — angelicin
Abscissa and ordinate as in Fig. 1

Results

Action of furocoumarins

The action of ultraviolet light plus xanthotoxin on microorganisms in general can be described as a function of survivors or plaque forming units (PFU) to the dose. Fig. 1 represents such a dependence for phage lambda treated with furocoumarins and near ultraviolet light. It is obvious thet the flat and linear derivative xanthotoxin acts in a more pronounced way than does the flat and angular derivative angelicin. Light alone only slightly affects the plaque-forming ability.

Adsorption, injection and replication

To obtain more information on adsorption, DNA injection and multiplication of the phage lambda upon furocoumarin treatment, its DNA was

labelled with ^{32}P. Fig. 2 clearly shows that the adsorption of phage particles on bacteria is not affected by photoreaction. However, injection of phage DNA into the host is suppressed. At excitation light dose of 30×10^3 J/m^2 the inhibitory effect of xanthotoxin is about twofold of angelicin, i.e. the inhibition is of 80% and 40% respectively. Xanthotoxin at 4×10^3 J/m^2 produces four orders of magnitude fewer PFU, while angelicin at 25×10^3 J/m^2 diminished the number of plaques by two orders of magnitude. The influence of replication of phage DNA after combined furocoumarin plus near ultraviolet light treatment is shown in Fig. 3. This process is completely stopped at a light dose of 2.6×10^3 J/m^2 and of 20×10^3 J/m^2 for xanthotoxin and

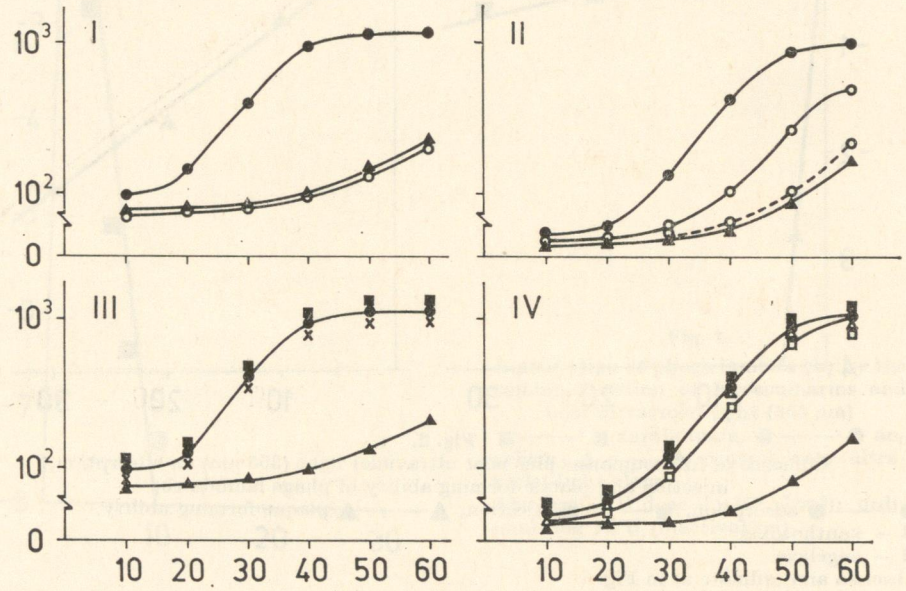

Fig. 3.
Dependence of phage DNA replication on furocoumarin plus ultraviolet light (365 nm) treatment

Measurements of phage DNA replication were started 10 min after infection (for experimental details see Materials and Methods).

I—III: xanthotoxin; ●———● phage DNA replication unaffected, ▲———▲ control of bacterial DNA replication after sublethal ultraviolet light irradiation (254 nm, 80 J/m^2), ○———○ phage DNA replication affected by near ultraviolet light (365 nm, 2.5×10^3 J/m^2) and xanthotoxin, ■———■ phage DNA replication after 365 nm irradiation (2.5×10^3 J/m^2) in the absence of xanthotoxin, ×———× influence of xanthotoxin only on phage DNA replication.

II—IV: angelicin; ●———● phage DNA replication unaffected, ▲———▲ control of bacterial DNA replication after sublethal ultraviolet light irradiation (254 nm, 80 J/m^2). Phage DNA replication after combined treatment with near ultraviolet light (365 nm) plus angelicin ○———○ 10×10^3 J/m^2, ○·····○ 20×10^3 J/m^2; ■———■ influence of angelicin only on phage DNA replication; phage DNA replication after irradiation with near UV light only: △———△ 10×10^3 J/m^2, □———□ 20×10^3 J/m^2.

Abscissae: ^3H-radioactivity counts (ml/min); ordinates: time (in min).

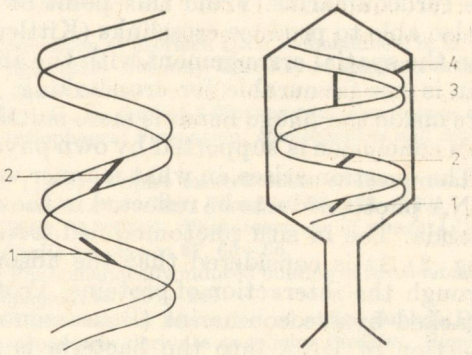

Fig. 4.
Schematic representation of possible DNA-furocoumarin crosslinks
Dilute aqueous solution (left); phage heads (right); 1 — monoaddition products, 2 — crosslinks type I, 3 — crosslinks type II, 4 — nucleic acid to protein crosslinks.

angelicin, respectively. The replication of phage lambda DNA is practically unaffected if ultraviolet light or furocoumarine are used alone, except at high irradiation doses where the replication is slightly delayed.

Nucleic acid-protein crosslinks

The formation of nucleic acid-protein crosslinks as a result of treatment with xanthotoxin plus near ultraviolet light occures in the phage heads in situ. At radiation dose of 30×10^3 J/m^2 (wavelength 365 nm), about 15% of the DNA is covalently linked to protein. This corresponds to 2% binding at biologically relevant dosages.

Discussion

Photochemically induced covalent binding between furocoumarins and nucleic acids will be strongly influenced by the spatial arrangements of the two reactants in the dark. Thus the conditions in dilute aqueous solution are quite different from those inside the phage (Kittler *et al.*, 1980). While in the former case the most important feature of DNA is its secondary structure, in the latter case the highly local concentration of DNA forces it to form a tertiary structure which has a higher degree of compactness. Based on these considerations a scheme for possible furocoumarin-DNA photoproducts has been drawn (Fig. 4). In dilute aqueous solution only monoaddition products and crosslinks between the complementary strands of the DNA duplex (type I crosslinks) are produced. Under such conditions, for steric reasons, the angular molecule, angelicin, acts monofunoctionally and the linear molecule, xanthotoxin, forms both monoaddition products and type I crosslinks.

In addition to the photoproducts type II crosslinks, which correspond to the covalent binding between adjacent sites in the folded structure of the double helix, nucleic acid-protein crosslinks are possibly also formed inside of the phage lambda head. Type II crosslinks need DNA in an extremely compact structure, such as occurs inside the phage head. A prerequisite for photochemically induced crosslink formation is a suitable arrangement of the reactants and also a suitable electron distribution in the excited states of

the furocoumarins. From this point of view it would appear that angelicin is also able to produce crosslinks (Kittler *et al.*, 1980). In dilute aqueous solution the spatial arrangement with the angular furocoumarin derivative angelicin is less favourable for crosslinking. However, the compact DNA structure inside the phage heads is more suitable for angelicin to act bifunctionally. This conclusion is supported by own physico-chemical studies.

The question arises, in what manner will the various types of furocoumarin--DNA photoproducts be reflected in the distinct multiplication steps of phage lambda. The in situ photomodified DNA did not affect the adsorption step (Fig. 2). It is considered that the adsorption of phages on the host occurs through the interaction of proteins. Proteins, however, are not preferentially attacked by furocoumarins (Bensasson *et al.*, 1978). On the other hand, the injection of DNA into the bacteria is strongly altered after furocoumarin treatment. Both xanthotoxin and angelicin cause an inhibition of injection, xanthotoxin suppressing the injection twice as strongly as angelicin (Fig. 2). The same is true in principle for psoralen and bergapten (unpublished). This means that such photomodified DNA is unable to leave the phage through the tail to reach the host. The tail is 7 nm in diameter, while double-stranded DNA is 2 nm. Therefore DNA which possesses covalently linked loops (type II crosslinks) prevents the macromolecule from passing through the phage tail; thus type II crosslinks are responsible for the suppression of DNA injection. The conclusion that adsorption is not affected and injection is suppressed after furocoumarin and ultraviolet light treatment is based on the distribution of radioactive phage DNA. By this technique it is only possible to obtain information as to whether the DNA remains in the phage head or is injected into the host; it does not explain its biological activity. The latter was proved by plaque forming ability. If inhibition of injection is the unique interference in phage multiplication, then both curves in Fig. 2 should decline to the same extent. However, this was not observed, and plaque formation was a few orders of magnitude more suppressed than injection. Thus, besides injection, at least one additional multiplication step must be affected. This is true for replication (Fig. 3). DNA crosslinked by type I can be injected, while DNA with type II crosslink is unable to pass through the phage tail. Crosslinks of type I represent no steric hindrance for DNA injection. Taking this into account, type I crosslinks are rather involved in the inhibition of replication.

In the phage heads monoaddition products will also be generated by combined furocoumarin and ultraviolet light treatment. Belogurov *et al.*, (1976) have shown, in a system similar to ours, that the photoproducts are removed within minutes by repair processes. They are therefore not affected on multiplication. Otherwise, at biologically relevant dosages 2 % of the DNA becomes crosslinked with proteins of the phage shell via furocoumarins. Such modified DNA is also unable to leave the phage head, like DNA with type II crosslinks.

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