Tris Buffer Protects DNA Backbone against Breakage upon Irradiation with Ultraviolet Light

E. KEJNOVSKÝ and J. KYPR

Institute of Biophysics, Czech Academy of Sciences, 61265 Brno, Czech Republic

Abstract. We show that Tris molecules protect DNA against nicking upon irradiation with ultraviolet light. However, the protective effect only concerns DNA backbone but not bases and it is observed in aqueous solution but not in formamide. Changes of pH or ionic strength due to Tris have no effect on the protection. The present observation has a practical importance for photofootprinting studies of DNA and its complexes with proteins but it can also serve as a basis for a development of a novel method reflecting DNA hydration and conformation.

Key words: DNA — Ultraviolet light — Irradiation — Backbone breakage — Photofootprinting

Introduction

Interactions of DNA with ultraviolet light are important in both the basic and applied sciences. Ultraviolet light is a natural component of our environment whose importance has dramatically increased in the past few years due to the Earth ozone layer depletion. It damages DNA, and cells have developed sophisticated enzyme machineries to repair the ultraviolet light-induced lesions. This general knowledge originates from extensive studies of many laboratories done mostly in early sixties but it has only recently been shown that the arrangement of bases in DNA and their flexibility is a factor to which the photodamage is extremely sensitive (Becker and Wang 1989b). The sensitivity has led to a development of photofootprinting methods to monitor DNA conformation (Becker and Wang 1989a; Lyamichev et al. 1990) and DNA-protein interactions (Becker and Wang 1984) both *in vitro* and *in vivo*. Our laboratory also develops photofootprinting methods. During the studies, we have encountered the phenomenon reported in this paper.

Materials and Methods

DNAs

pUC19 DNA was isolated according to Birnboim and Doly (1979) using the *E. coli* JM109 strain transformed with pUC19 plasmid. The DNA was linearised with the restriction endonuclease *Pst*I (ÚSOL Prague). Lambda phage DNA was isolated from the lysogene *E. coli* JRS154 strain by an extraction with phenol and chloroform. After precipitation with ethanol, the DNAs were dissolved in sterile redestiled water or formamide.

DNA irradiation with ultraviolet light

Samples of lambda or pUC19 DNA (20 μl), on a thin plastic plate, were placed onto an ice-water bath and irradiated with a 15 W germicidal bulb (Philips), at an incident fluence rate of 12.7 $Jm^{-2}s^{-1}$, as determined by an PT 100 germicidal photometer (International Light, Inc., Newburyport, MA). The samples were irradiated for different periods of time to get the doses given in figure captions. The distance of the samples from the bulb was constant.

Photoproduct detection

Resistance of DNA to restriction endonuclease cutting (Cleaver 1983) was used to detect base photoproducts. 1 μg of DNA was, immediately after irradiation, digested with 3 units of *Dra*I (Boehringer Mannheim) for 2 hours at 37 °C. The digestion was stopped by the addition of the STOP buffer (6x concentrated: 0.25% bromphenol blue, 30% glycerol, 50 mmol/l EDTA, pH 8.0). Glycerol was added, up to the final 5% concentration, to the uncleaved samples before loading onto the gel.

Electrophoresis

Samples were electrophoresed in horizontal 1.5% (w/v) agarose gels for 2 hours at 100V in 89 mmol/l Tris-borate, 80 mmol/l EDTA, pH 8.0. Gels were stained with 1 μg/ml ethidium bromide, photographed using an orange filter and quantified by densitometry of photographic negatives using the Beckman densitometer, Model R-112.

Results

After precipitation with ethanol, lambda phage DNA was dissolved in redestiled water and irradiated with ultraviolet light (12.7 $Jm^{-2}s^{-1}$ at 254 nm) at doses ranging from 5000 Jm^{-2} to 25,000 Jm^{-2}. The ultraviolet light doses of 5000 Jm^{-2} and 10,000 Jm^{-2} induced no changes in the DNA native gels. However intensity of the original DNA fragment band substantially decreased at higher doses (Fig. 1*a*). Fig. 1*b* shows that Tris-HCl (pH 8.0) strongly inhibits the strand breaking induced by the ultraviolet light irradiation. Dependences of the proportion of undamaged lambda phage DNA molecules on ultraviolet light dose in the presence and absence of Tris-HCl are shown in Fig. 1*c*.

The protective effect was investigated at Tris-HCl concentrations ranging from 0.2 to 20 mmol/l. Even 1 mmol/l Tris-HCl protected the major part of DNA

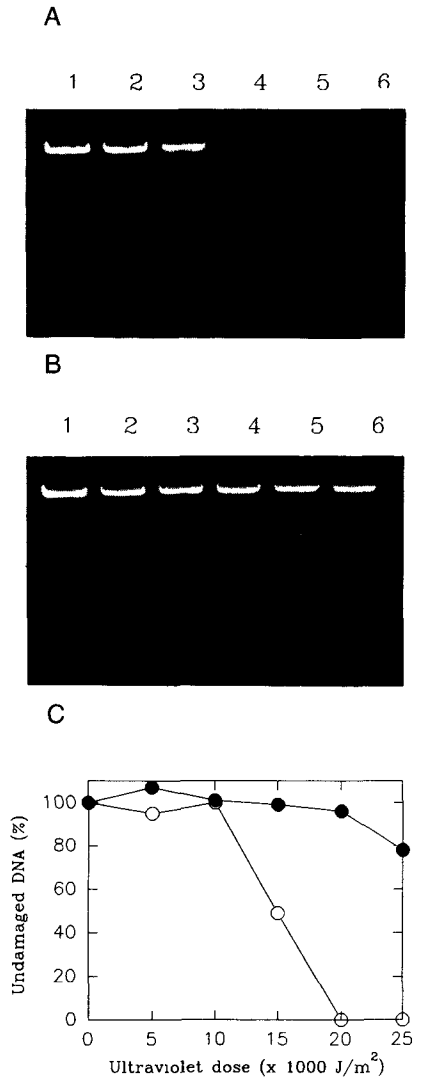

Figure 1. Phage lambda DNA irradiated with an ultraviolet light dose of 0 Jm^{-2}, 5000 Jm^{-2}, 10,000 Jm^{-2}, 15,000 Jm^{-2}, 20,000 Jm^{-2} and 25,000 Jm^{-2} (lanes 1–6, respectively) in water (*A*) and in 20 mmol/l Tris-HCl (pH 8.0) (*B*). (*C*) Dependence of the proportion of lambda phage DNA undamaged by double-stranded breaks on the ultraviolet light dose in the absence (○, open) and presence of 20 mmol/l Tris-HCl (●, closed). Unirradiated DNA represents 100 per cent.

molecules against degradation by ultraviolet light-induced breaks (Fig. 2). The protective effect of Tris-HCl was not due to its effect on the solution ionic strength because addition of 20 mmol/l NaCl exerted no effect as shown in Fig. 2. Changes of pH also had no effect on the protection because the same results were obtained for the solutions containing Tris-HCl whose pH ranged from 4.0 to 8.0 (Fig. 3).

Tris-HCl could have an indirect influence by means of decreasing the effective dose of DNA irradiation. Therefore we examined the effect of Tris-HCl on ultravio-

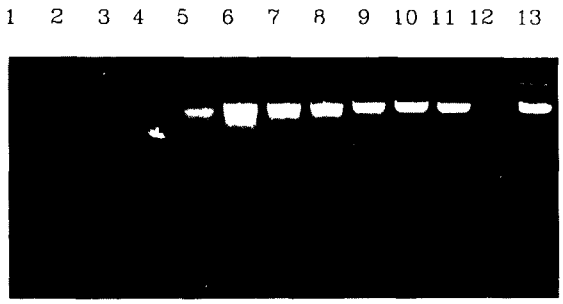

Figure 2. Lambda phage DNA ultraviolet light irradiated with 20,000 Jm^{-2} in the presence of varying concentrations of Tris-HCl (pH 8.0): Lanes 1–11, 0, 0.2, 0.4, 0.6, 0.8, 1.0, 2.0, 4.0, 7.0, 10.0 and 20.0 mmol/l. Lane 12, DNA irradiated in 20.0 mmol/l NaCl; lane 13, unirradiated control DNA.

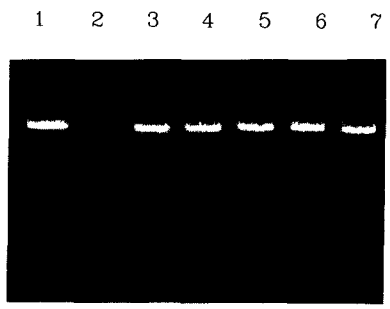

Figure 3. Role of pH on the protective effect of Tris-HCl. Lane 1, unirradiated lambda DNA; lane 2: lambda DNA irradiated with 20,000 Jm^{-2} in the absence of Tris-HCl; lanes 3–7: lambda DNA irradiated with 20,000 Jm^{-2} in the presence of 20 mmol/l Tris-HCl at pH 4.0, 5.0, 6.0, 7.0 and 8.0.

let light damage of nucleotide bases, taking advantage of the well-known fact that a presence of photoproducts in DNA inhibits its cleavage by restriction enzymes (Cleaver 1983). Restriction endonuclease *Dra*I was used in our experiments because its recognition site (TTTAAA) is highly sensitive to ultraviolet light damage. Plasmid pUC19 DNA linearised with *Pst*I was irradiated by ultraviolet light both in the presence and absence of Tris-HCl prior to the digestion with *Dra*I (Fig. 4*a* and 4*b*, respectively). However a similar increase was observed in the amount of partially digested DNA molecules in both cases due to the photodamage of the restrictase target site. This implies that Tris-HCl does not significantly influence the ultraviolet light damage of nucleotide bases. It also directly demonstrates that the protective effect of Tris-HCl is not due to a quenching of the dose, attacking the DNA backbone.

The protective effect of Tris-HCl was also examined in aqueous formamide solutions. We observed that the protective effect of Tris persisted up to 60% formamide but then decreased sharply to vanish in pure formamide (Fig. 5).

Discussion

In aqueous solution, DNA is known to be mostly nicked indirectly by water radicals arising as a result of gamma or UV irradiation (von Sonntag 1987). Tris

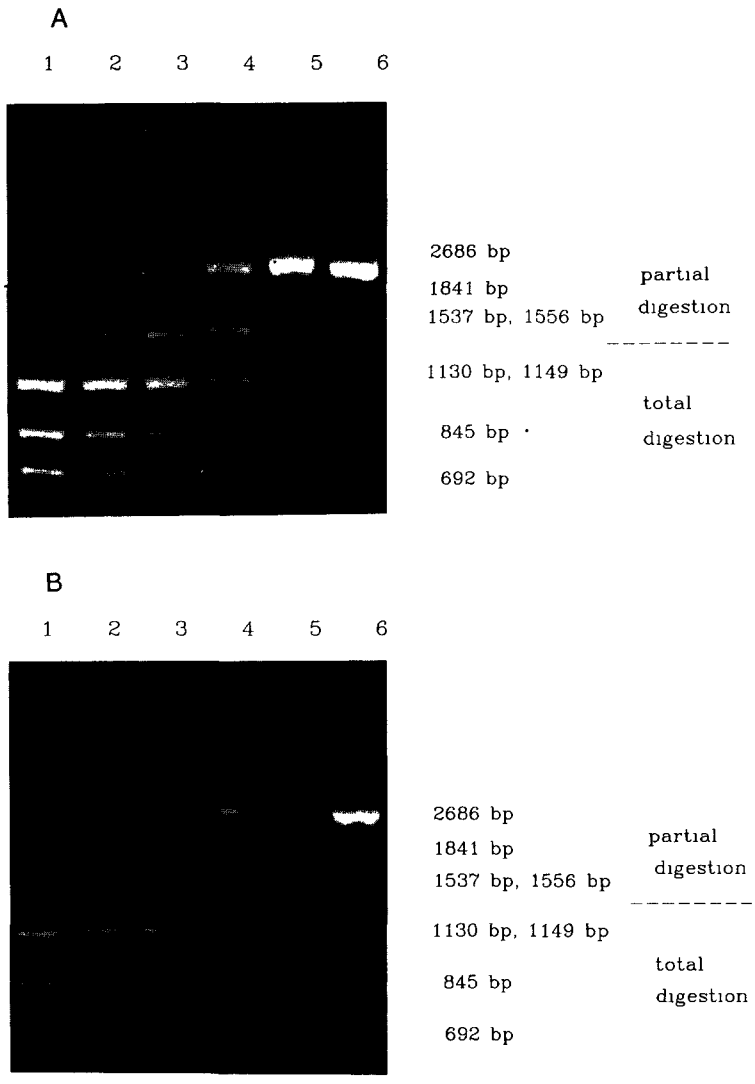

Figure 4. Plasmid pUC19 DNA linearised with *Pst*I, irradiated with 500 Jm^{-2}, 1000 Jm^{-2}, 5000 Jm^{-2} and 20,000 Jm^{-2} (lanes 2–5, respectively) in the presence (*A*) and absence (*B*) of 20 mmol/l Tris-HCl (pH 8.0), and digested with 3 units of *Dra*I. Lane 1, unirradiated DNA digested with *Dra*I; lane 6, undigested DNA.

reacts with hydroxyl radicals (Hicks and Gebicki 1986) so that the present observation of the strong protective effect could easily be explained by a strong capacity of Tris to scavenge water radicals. However, the protective effect sharply vanished in

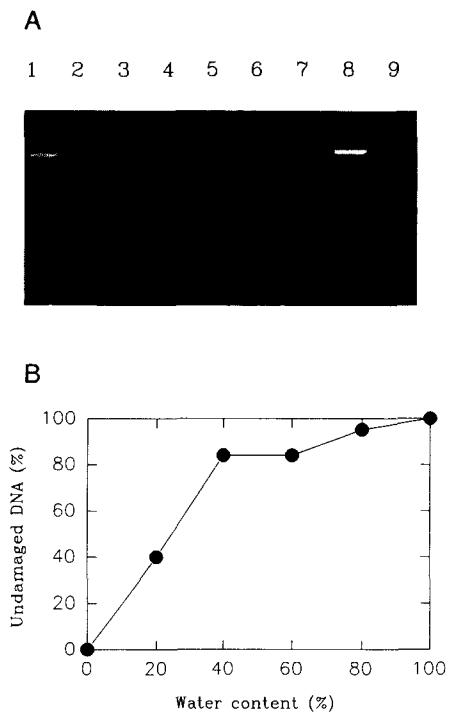

Figure 5. Role of water in the protective effect of Tris-HCl. (*A*) Lane 1, unirradiated lambda phage DNA; lanes 2 and 9, DNA dissolved, respectively, in water and in formamide, and irradiated in the absence of Tris-HCl by 20,000 Jm^{-2}. DNA dissolved in 100%, 80%, 60%, 40% and 20% aqueous formamide containing 20 mmol/l Tris-HCl irradiated with 20,000 Jm^{-2} (lanes 3–7, respectively). (*B*) Dependence of the proportion of lambda phage DNA undamaged by double-stranded breaks on water content in the DNA samples.

aqueous formamide solutions containing Tris if the water content decreased below 40% (Fig. 5). This observation cannot be explained by the above mechanism. Yet it is possible that UV light also generates reactive species from formamide which nick DNA but the reactive species are not captured by Tris. This notion is consistent with the results of all of our present experiments. However, we have not done this study to search for unusual possibilities of DNA nicking or its protection against nicking by UV light. The reported result has rather arisen in the course of development of a new simple photochemical method to detect DNA-protein interactions. Therefore we point out aspects below related to the photofootprinting which have practical rather than theoretical importance.

Photofootprinting, a very powerful approach to study DNA conformation and its interactions with proteins both *in vitro* and *in vivo*, uses sequencing gels to detect specific breaks induced by a chemical reaction in the positions of photodamaged bases. Therefore breaks existing in DNA before the sequencing reaction would lead to artefacts or at least cause a non-specific background in the gels. Here we show that ultraviolet light indeed induces the undesirable breaks while Tris can be used to minimize their number. Fortunately, as far as we know all published photofootprinting studies have been done in buffers containing sufficient concentrations of Tris molecules though their authors probably did not use this buffer

because of the effect described in this paper (Becker and Wang 1984; 1989a; Gale and Smerdon 1988; Wang and Becker 1988; Becker et al. 1989).

Nevertheless, the protective effect of Tris might be of interest to radiobiologists who have this point in the center of their research. Tris buffer is widely used in biological experiments so that it might be of interest to look whether some experiments might not have been affected by using or not using the buffers containing Tris molecules (Cullis et al. 1985; Boullard and Giacomoni 1988).

DNA strand breaking is a biologically interesting phenomenon, taking place due to environmental factors including ultraviolet light but also in the course of many physiological reactions, e.g. during DNA recombination, repair and topoisomerization. However, the DNA strand breaking is also very important from the scientific point of view because the basic idea standing behind the chemical sequencing method of DNA and the many related methods includes detection of sites in DNA, where the bases were damaged, through the DNA strand breakage. However, the breakage is a secondary process, induced by hot piperidine after specific chemical reactions removing bases from DNA. The present and other (Swarts et al. 1992; Hüttermann et al. 1992) results indicate that the DNA backbone breaking by ultraviolet light is sensitive to DNA hydration and therefore conformation which inspires an idea to use this approach in DNA conformation studies. Experimental tests of this idea are in progress in our laboratory.

References

Becker M. M., Wang J. C. (1984): Use of light for footprinting DNA *in vivo* Nature **309,** 682—687

Becker M. M., Wang Z. (1989a): B - A transitions within a 5S ribosomal RNA gene are highly sequence-specific. J. Biol. Chem. **264,** 4163—4167

Becker M. M., Wang Z. (1989b): Origin of ultraviolet damage in DNA. J. Mol. Biol. **210,** 429—438

Becker M. M., Wang Z., Grossmann G., Becherer K. A. (1989): Genomic footprinting in mammalian cells with ultraviolet light. Proc. Nat. Acad. Sci. USA **86,** 5315—5319

Birnboim H. C., Doly J. (1979): A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucl. Acid. Res. **7,** 1513—1523

Boullard A., Giacomoni P. U. (1988): Effect of UV irradiation at defined wavelengths on the tertiary structure of double-stranded covalently closed circular DNA. J. Photochem. Photobiol., B-Biol. **2,** 491—501

Cleaver J. E. (1983): Restriction enzyme cleavage ultraviolet-damaged simian virus 40 and pBR322 DNA. J. Mol. Biol. **170,** 305—317

Cullis P. M., Symons M. C. R., Wren B. W. (1985): Effect of ionizing radiation on deoxyribonucleic acid. Part 3. The effect of iodoacetamide. J. Chem. Soc. Perkin Trans. II, 1819—1825

Gale J. M., Smerdon M. J. (1988): Photofootprinting of nucleosome core DNA in intact chromatin having different structural states. J. Mol. Biol. **204,** 949—958

Hicks M., Gebicki J. M. (1986): Rate constants for reaction of hydroxyl radicals with Tris, Tricine and HEPES buffers. FEBS Lett. **199,** 92—94

Hüttermann J., Röhrig M., Köhnlein W. (1992): Free radicals from irradiated lyophilized DNA: influence of water of hydration. Int. J. Radiat. Biol. **61,** 299—313

Lyamichev V. I., Frank-Kamenetskii M. D., Soyfer U. N. (1990): Protection against UV-induced pyrimidine dimerization in DNA by triplex formation. Nature **344,** 568—570

Swarts S. G., Sevilla M. D., Becker D., Tokar Ch. J., Wheeler K. T. (1992): Radiation-induced DNA damage as a function of hydration. I. Release of unaltered bases. Radiat. Res. **129,** 333—344

von Sonntag C. (1987): The Chemical Basis of Radiation Biology (Taylor and Francis, London, New York, Philadelphia)

Wang Z., Becker M. M. (1988): Selective visualization of gene structure with ultraviolet light. Proc. Nat. Acad. Sci. USA **85,** 654—658

Final version accepted June 17, 1993