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Effect of denaturation on the photochemistry of pyrimidine bases in isolated DNA

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Abstract

The influence of denaturation on DNA photochemistry was studied by quantifying the yield of formation of all possible bipyrimidine photolesions within isolated genomic DNA samples exposed to UVC radiation. Effects of DNA melting was studied either by carrying out irradiation over a wide range of temperature (0–90 °C) or by decreasing the ionic strength of the solution at 30 °C. A first observation was a much larger decrease in the photoreactivity upon increasing the temperature in single-stranded than in double-stranded DNA. Secondly, formation of *trans*,*syn* cyclobutane dimers and, to a lesser extent, modification in the ratio between the yields of cyclobutane dimers and (6-4) photoproducts, were found to be other main features associated with denaturation. These results emphasize the modulating role of structure in the yield and nature of UV-induced DNA damage.

Keywords: DNA damage; UV radiation; Pyrimidine dimers; DNA photochemistry; Ionic strength; DNA structure

1. Introduction

The chemical integrity of DNA is greatly threatened by UVB and UVC radiations because it is one of the main cellular chromophores for photons in these wavelength ranges. DNA damage arising from the resulting photochemical reactions is at the origin of deleterious cellular effects including mutagenesis, carcinogenesis and aging. The photochemistry of DNA has thus been extensively studied both in isolated and cellular DNA and was found to mostly involve dimerization reactions between adjacent pyrimidine bases (thymine (T) and cytosine (C)) [1]. Two main classes of photoproducts (Fig. 1), including cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidone photoproducts (64PPs), are produced as the result of UV-induced excitation of DNA bases. In addition, (6-4) photoproducts can undergo conversion into their Dewar valence isomers by absorption of a second UV photon. Distribution of bipyrimidine photoproducts within UV-irradiated DNA is complicated by the existence of several diastereoisomers for the cyclobutane dimers that can exhibit either *cis*,*syn* or *trans*,*syn* configuration (*c*,*s* and *t*,*s* CPDs, respectively), although the former is the exclusive form in B-DNA. In addition, formation of cyclobutane dimer, (6-4) photoproduct and Dewar valence isomer may occur for each of the four bipyrimidine doublets, leading to the potential formation of a large number of photolesions.

Yet, bipyrimidine photoproducts are not all produced in the same yield within double-stranded DNA. Identification of the main parameters influencing their distribution is a requisite for a better understanding of DNA photochemistry. In that respect, variation in the secondary structure and geometry of DNA has been identified as an important factor. For instance, changes in DNA conformation upon binding of small acid soluble proteins were found to be, at least partly, responsible for the novel photochemistry of DNA in bacterial spores [2–6]. Local DNA deformations

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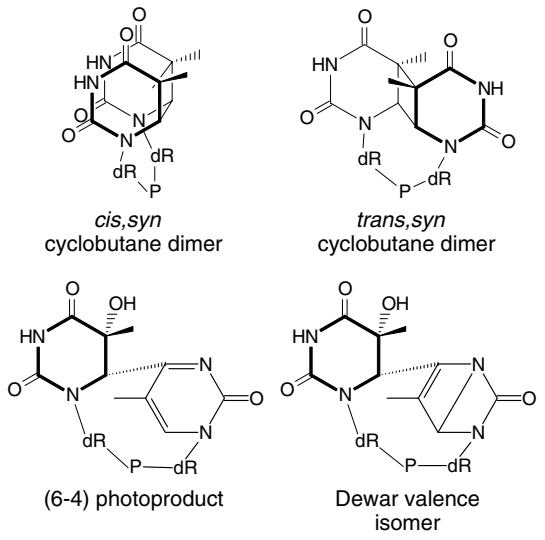

Fig. 1. Structure of UVC-induced dimeric photoproducts at TT sites. Similar lesions can be produced at TC, CT and CC doublets. The bold thymine moiety represents the 5′-end base.

due to either specific sequences [7,8] or formation of loops [9] were also found to affect the formation of bipyrimidine photolesions. In addition, early studies on isolated DNA have shown that freezing the samples [10] and inducing transition from B to A-form [11,12] exhibited strong effects on the yield and the nature of the UV-induced dimeric photoproducts. This result was confirmed by a recent study involving DNA and mixed DNA/RNA hairpins [13]. Inter-strand photoproducts can also be produced in high yield under specific irradiation conditions [14]. Interestingly, structural effects are even observed with DNA monomers as illustrated by the effect of the stacking mode of thymine residues in frozen solutions [15–17] and in dry films [14] on the distribution of UVC-induced dimers.

The most drastic structural modification that DNA may undergo is the denaturation of the duplex into two single-stranded nucleic acids. Experimentally, this process is usually triggered by heating the DNA sample [18]. Heat-induced denaturation experiments are often carried out in order to determine the so-called melting temperature, which corresponds to the temperature at which duplex and single stranded DNA are in equal amount. This parameter is a characteristic feature of a given DNA fragment that may be related to the length and base pair content [18], and to the presence of destabilizing defaults such as mismatches [19–22] and DNA lesions [23–27]. Interestingly, decrease in the melting temperature [28–30] and variation of various thermodynamic parameters of the denaturation process [31–33] have been found to depend on the salt content of the solution, illustrating the fact that the DNA duplexes are less stable when the ionic strength is lowered.

A first work has been reported on the variation in the yield of thymine cyclobutane dimer (TT-CPD) as a function of the denaturation state of DNA [34]. However, like in most of the early studies using photoproduct quantification by acidic hydrolysis of [^{3}H]-thymine labeled DNA, information is lacking on the formation of (6-4) photoproducts, Dewar valence isomers and cytosine-containing cyclobutane dimers. We thus present a more extensive description of the effect of melting on DNA photochemistry based on the use of the versatile and specific HPLC/tandem mass spectrometry technique [35]. This assay makes possible the individual quantification of all dimeric photoproducts and was recently applied to isolated and cellular DNA [36–41]. In order to study the effects of melting on DNA photochemistry, the yields of formation of dimeric pyrimidine photoproducts within UVC-irradiated DNA were correlated over a large range of temperature with the extent of denaturation determined by UV absorption measurements. Additional experiments, involving denaturation of DNA induced by decrease in the ionic strength at room temperature, were designed in order to identify effects observed in the melting study that would be of thermal rather than structural origin. The obtained results showed that the irradiation conditions exhibit effects in terms of overall reactivity of DNA and ratio between the yield of cyclobutane dimers and that of (6-4) photoproducts. However, the major consequence of denaturation was found to be the formation of *trans*,*syn* cyclobutane dimers.

2. Methods and materials

2.1. Chemicals

Calf thymus DNA (used as received), sodium chloride, phosphodiesterase I, phosphodiesterase II and nuclease P1 were purchased from Sigma (St. Louis, MO). Alkaline phosphatase was obtained from Roche Diagnostics (Manheim, Germany). Calibrated solutions of the *c*,*s* and *t*,*s* CPDs, 64PP and Dew of thymidylyl-(3′–5′)-thymidine (TpT), thymidylyl-(3′–5′)-2′-deoxycytidine (TpdC), 2′-deoxycytidylyl-(3′–5′)-thymidine (dCpT) and 2′-deoxycytidylyl-(3′–5′)-2′-deoxycytidine (dCpdC) were prepared as previously reported [36,37].

Thermal Denaturation Analyses. UV absorption of the sample placed in a sealed 400 μl quartz cell was monitored at 260 nm on a 8453 Hewlett–Packard spectrophotometer equipped with a 89090A thermostated cell. Experiments were performed with aqueous solutions of DNA (50 μg ml^{-1}) containing either no, 0.02 or 0.1 M NaCl. The thermal denaturation was followed between 5 and 91 °C. Steps of 5 and 1.5 °C were used within the 5–40 and 40–91 °C ranges, respectively, for samples containing NaCl. Ranges of 5 to 49 at 2 °C min^{-1} and 49 to 91 °C at 3 °C min^{-1} were used for the DNA sample in pure water. Equilibration time at each temperature was 2 min. UV absorption was also measured at 30 °C in 50 μg ml^{-1} DNA samples in the presence of NaCl at a concentration ranging between 0 and 0.2 M.