Induction of DNA Damage by Porphyrin Photosensitizers

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ABSTRACT

The photosensitizing effects of hematoporphyrin derivative, meso-tetra(p-carboxypheny1)porphine, meso-tetra(p-carboxyphenyl)porphine, and meso-tetra(4-N-methylpyridyl)porphine on CoIE1 supercoiled DNA were studied using agarose gel electrophoresis. Photoinduced single- and double-strand breaks were observed to form under neutral conditions. Singlet oxygen was shown to predominate in the mechanism of the induction of these lesions in the case of hematoporphyrin derivative, meso-tetra(p-carboxyphenyl)porphine, and meso-tetra(p-carboxyphenyl)porphine and to play a significant role in the case of meso-tetra(4-N-methylpyridyl)porphine.

The results suggest the possibility that the risk of photodynamic carcinogenesis may accompany photochemotherapy and fluorescence endoscopic procedures involving porphyrin photosensitizers.

INTRODUCTION

The ability of certain compounds to act as photosensitizers in the presence of oxygen (photodynamic effect) is known to be the basis for several diseases (16), including cancer (28). Büngeler (2), for example, showed that HP3 plus exposure to sunlight induced tumors in mice. This work followed the suggestion by Körb1er (20) that the accumulation of porphyrins in the skin with aging leads to photodynamic carcinogenesis.

These observations have gained new significance as a result of recent experimentation and clinical application of HPD and other photosensitizers in photodynamic chemotherapy (8-10, 14, 19) and endoscopic techniques for detection of in situ carcinoma.6-5 Furthermore, there is considerable evidence in support of a correlation between carcinogenic potential and photodynamic activity (6, 11, 24, 25, 27, 29). In view of this and the general assumption that photodynamic carcinogenesis results from biologically significant damage to DNA, it is clear that insight into the basic interactions of porphyrin and nucleic acids can add to our understanding of the molecular basis of photodynamic carcinogenesis.

At the present time, there is very little information available regarding the photodynamic action of porphyrin photosensitizers on DNA. Gutter et al. (15) studied the photodynamic mod-

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2 To whom requests for reprints should be addressed, at Department of Biophysics Research, Roswell Park Memorial Institute, 666 Elm Street, Buffalo, N.Y. 14263.
3 The abbreviations used are: HP, hematoporphyrin; HPD, hematoporphyrin derivative; TSPP, meso-tetra(p-sulfonatophenyl)porphine; TCPP, meso-tetra(p-carboxyphenyl)porphine; T4MPyP, meso-tetra(4-N-methylpyridyl)porphine.
5 D. A. Cortese and D. R. Sanderson. Hematoporphyrin derivative detection studies at Mayo Clinic. In: Proceedings of UICC Workshop (1979), Roswell Park Memorial Institute, Buffalo, N.Y.

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ifications of DNA by HP. These authors found that illumination of DNA with white light (300 to 750 nm) in the presence of hematoporphyrin (5 × 10^-4 M) resulted in the selected degradation of guanine, as determined by measurements of the DNA melting temperature. They also found evidence of single-strand breaks, which were presumed to be secondary to the photodynamic degradation of guanine.

Boye and Moan (1) have shown that breakage of DNA in vitro and inside Escherichia coli cells occurs on exposure to light (365 nm) in the presence of 1 × 10^-5 M HP. Strand breaks were detected under alkaline conditions using sucrose density gradient techniques but were not observed under neutral conditions. More recently, Moan et al. (23) have shown that alkali-labile breaks in DNA and sister chromatid exchange occur in human cells (NHIP 3025) in vitro, following exposure to sublethal doses of light in the presence of HP.

In the present investigation, we have used electrophoresis to detect photosensitized single- and double-strand breaks in CoIE1 DNA under neutral conditions. A comparison was made between HPD and 3 synthetic porphyrins. Two of the synthetic porphyrins, TSPP and TCPP, are well-known porphyrin derivatives which have been studied for both their tissue localization properties (26) and their photodynamic activity (5). No evidence has been found to suggest that these porphyrins bind to DNA. The third synthetic porphyrin, T4MPyP, a known photosensitizer (5), has been identified as a DNA intercalator (12, 13). Because of its ability to bind to DNA, T4MPyP serves as a unique tool to compare the effect of a DNA-bound photosensitizer with that of unbound photosensitizers.

MATERIALS AND METHODS

HPD was provided by Dr. T. Dougherty, Department of Radiation Medicine, Roswell Park Memorial Institute. TSPP and TCPP were synthesized by Dr. N. Datta-Gupta (4). T4MPyP was purchased as the tetraiodide salt from Strem Chemicals, Inc.

CoIE1 DNA was isolated from JC411 cells using the procedure of Clewell and Helinski (3) and purified on a hydroxyapatite column. DNA solutions were illuminated in a light box to eliminate ambient light. The light source was a G.E. H-85A3 mercury vapor lamp fitted with an Oriel long-pass filter (G-772-3900). This filter transmits 50% of the light at 390 nm and 0% of the light at λ < 360 nm. Illuminations were carried out for either 4 or 24 hr. The output (irradiance) was measured with a radiometer (Coherent Radiation, Model 210) to be approximately 100 milliwatts/cm2. This provides an incident energy of 1.4 × 10^8 and 8.6 × 10^7 J/cm2 in the case of 4 and 24 hr, respectively.

Tube gel electrophoresis of DNA was carried out in a Tris-acetate buffer (pH 8.2) (40 mm Tris-5 mm sodium acetate-1 mm EDTA) using 0.7% agarose gels and approximately 70 min running time. The gels were stained with ethidium bromide, and photographs were taken under UV using Polaroid type 665 black and white negative film. The percentage of DNA I remaining after illumination was determined from densitometer tracings of the negatives by normalization to control samples containing porphyrin and maintained in the dark. Control samples (illuminated and dark) always contained less than 20%.
of DNA II. No effect of illumination was noted in control samples not containing porphyrin. The electrophoresis was carried out with duplicate samples, and each experiment was repeated at least once. Reproducibility was estimated to be within ±5%, based on densitometer tracings of duplicate samples, and to within ±10%, based upon repeat experiments.

RESULTS

Damage to DNA is expressed in Tables 1 and 2 as percentage of Form I remaining after illumination, as described in the previous section. In some cases DNA I is converted to Form II and Form III DNA. For example, DNA III is found as a product of photosensitization by TCPP and TSPP at 1 × 10⁻⁴ M and to a lesser extent by T4MPyP at 1 × 10⁻⁶ M. Chart 1 illustrates this effect for TSPP at 4 and 24 hr illumination. The DNA remaining after 24 hr is approximately equally divided between Forms II and III reflecting double- as well as single-strand breaks. Correction for the difference in the fluorescence of ethidium bromide bound to different forms of DNA (22) was not made.

As shown in Table 1, all four porphyrins tested were found to photosensitize the conversion of DNA I to DNA II in the presence of visible light composed of wavelengths longer than 360 nm. This conversion results from a single break in one strand of the double-stranded backbone. The level of the photosensitizing activity of TCPP was found to be similar to that of TSPP. HPD is the least effective and T4MPyP is the most effective. An increase in concentration of HPD of 3 orders of magnitude is required to obtain an effect approximately equivalent to that obtained with T4MPyP.

It has been proposed that the photosensitizing activity of HPD results to a large extent from its ability to generate singlet oxygen, as illustrated in Equations A and B (7, 30).

Table 1

<table>
<thead>
<tr>
<th>Concentration of porphyrin (M)</th>
<th>HPD 24 hr</th>
<th>T4MPyP 24 hr</th>
<th>TCPP 24 hr</th>
<th>TSPP 24 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 × 10⁻⁸</td>
<td>64</td>
<td>58</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>1 × 10⁻⁸</td>
<td>65</td>
<td>55</td>
<td>84</td>
<td>84</td>
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<tr>
<td>1 × 10⁻⁴</td>
<td>24</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>1 × 10⁻³</td>
<td>16</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Concentration of NaN₃ (M)</th>
<th>HPD 24 hr</th>
<th>T4MPyP 24 hr</th>
<th>TCPP 24 hr</th>
<th>TSPP 24 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0 × 10⁻²</td>
<td>64</td>
<td>93</td>
<td>92</td>
<td>94</td>
</tr>
<tr>
<td>2.5 × 10⁻²</td>
<td>49</td>
<td>93</td>
<td>92</td>
<td>94</td>
</tr>
<tr>
<td>5.8 × 10⁻²</td>
<td>32</td>
<td>98</td>
<td>94</td>
<td>94</td>
</tr>
</tbody>
</table>

HPD in its ground state interacts with visible light and is excited to a singlet state. The singlet state decays to a triplet state and reacts with ground-state (triplet) oxygen to produce oxygen in its singlet state. This reactive species can interact with various biological substrates to produce chemical lesions via a photodynamic effect.

No information regarding the mechanism of photosensitization for the other 3 porphyrins is known. Table 2 lists the results of a series of experiments in which the singlet oxygen inhibitor, sodium azide, was used to inhibit the photosensitizing effect. In the case of HPD, TSPP, and TCPP, it was found that the DNA could be completely protected against photosensitization using sodium azide at a concentration of 5.8 × 10⁻² M. The photosensitizing effect of T4MPyP was found to be only partially blocked at this concentration.

DISCUSSION

These experiments clearly demonstrate that HPD, T4MPyP, TCPP, and TSPP, in the presence of visible light, induce strand breaks in DNA under neutral conditions. This is somewhat in contrast to the recent work of Boye and Moan (1). These authors also detected photoinduced breaks in ColEI DNA, but only under alkaline conditions. It should be noted, however, that their measurements were made with HP as the free base, whereas the clinical photosensitizer HPD (9, 21) was used in the present work.

Another interesting aspect of these results is the demonstration that a photosensitizer need not be bound to DNA in order to produce photodynamic damage. This confirms the conclusion of Boye and Moan (1), who reported similar results with the free base of HP. These authors indicated that this result contrasted with the suggestion of Ito and Kobayashi (18) and Ito (17) that binding of the photosensitizer to DNA was a necessary element of subsequent photodynamic damage. It is
important to note, however, that Ito and Kobayashi measured
the photodynamic effect by mutation frequency rather than
strand breaks. This observation underlines the importance of
T4MPyP as a probe to compare with HPD, TCPP, or TSPP for
their ability to induce mutations by photosensitization.

The ability to completely inhibit the photodynamic effect
of HPD, TCPP, and TSPP with sodium azide supports the concept
that singlet oxygen is ultimately responsible for the strand
breaks. The inability to completely block the effect of T4MPyP
suggests either that there is an additional mechanism involved
in this instance or that the tightly DNA-bound porphyrin is able
to generate singlet oxygen so close to the substrate that the
sodium azide is only partially effective as an inhibitor.

The detailed mechanism of the strand breaks produced in
DNA by the porphyrin photosensitizers studied in this investi-
gation is not known, nor do we have information about other
damage such as specific base degradation. This and the im-
portant question of the relationship of photosensitized damage
in DNA and photodynamic carcinogenesis remain to be an-
swered.

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