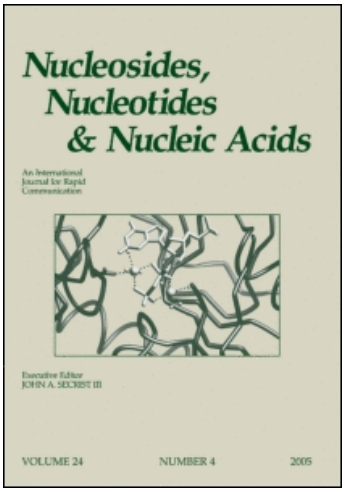


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Photochemical Cleavage of DNA With Phosphorus(V)porphyrin Derivatives

Takao Saito[a]; Masanori Kitamura[a]; Motomasa Tanaka[a]; Minoru Morimoto[a]; Hiroshi Segawa[a]; Takeo Shimidzu[a]
[a] Division of Molecular Engineering, Graduate School of Engineering, Kyoto University, Kyoto, Japan

To cite this Article Saito, Takao , Kitamura, Masanori , Tanaka, Motomasa , Morimoto, Minoru , Segawa, Hiroshi and Shimidzu, Takeo(1994) 'Photochemical Cleavage of DNA With Phosphorus(V)porphyrin Derivatives', Nucleosides, Nucleotides and Nucleic Acids, 13: 6, 1607 — 1615
To link to this Article: DOI: 10.1080/15257779408012175
URL: http://dx.doi.org/10.1080/15257779408012175

NUCLEOSIDES & NUCLEOTIDES, 13(6&7), 1607-1615 (1994)

PHOTOCHEMICAL CLEAVAGE OF DNA WITH PHOSPHORUS(V)PORPHYRIN DERIVATIVES§

Takao Saito, Masanori Kitamura, Motomasa Tanaka, Minoru Morimoto, Hiroshi Segawa and Takeo Shimidzu*

Division of Molecular Engineering, Graduate School of Engineering, Kyoto University, Kyoto 606-01, Japan.

ABSTRACT : Photocleavage of double-stranded circular DNA was examined using a phosphorus(V)porphyrin derivative. The P(V)porphyrin derivative cleaved the DNA upon visible light irradiation both in air and in argon. Photoreactions through an indirect process with singlet oxygen and a direct electron transfer process were suggested.

INTRODUCTION

Synthetic photonuclease is attracting attention as a useful tool in molecular biology and promising agent for phototherapy. The typical synthetic photonucleases reported are made of photosensitizer with a functional moiety interacting with nucleic acid. [1-3] As the photosensitizer, water-soluble porphyrin derivatives have been frequently used, because the triplet excited state of some porphyrin derivatives can efficiently generate singlet oxygen. [4-6] By the use of the porphyrin attached to intercalater [7] or oligonucleotide, [8,9] targetted photochemical reactions on DNA have been reported. These photoreactions ultimately give rise to strand breakdown and denaturation of the DNA.

For the breakdown and denaturation of the DNA, the following two mechanisms are considered as main processes in the photoreaction. (1) An indirect process with reactive oxygen species such as singlet oxygen. (2) A direct electron transfer process between the

§This paper is dedicated to Professor Morio Ikehara.

excited photoactive molecule and the nucleic acid base. The mechanisms depend on the type of photoactive moiety. Although both mechanisms result in the breakdown of DNA, their prospects for the sequence selective cleavage of DNA are quite different. The direct electron transfer is theoretically sensitive to the distance[10] and preferable to the sequence selective cleavage, but the indirect process by the reactive oxygen species should cause sequence dispersive cleavage in principle because of the diffusing tendency of them. In the case of the porphyrin derivatives reported so far, the main process is considered to be the process (1) with reactive oxygen species in the air.

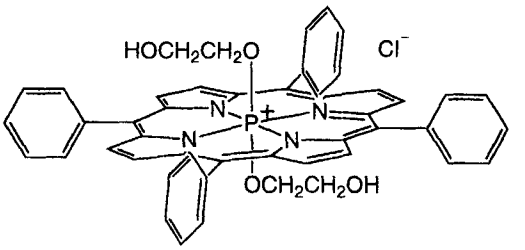


FIG.1. Structure of EG2P(V)TPP.

Recently, we have reported the syntheses and properties of a series of phosphorus(V)porphyrin derivatives.[11-14] The P(V)porphyrin derivatives have a strong oxidizing power in the singlet and the triplet excited states, which may lead to photocleavage through a direct electron transfer process (2), and the lifetimes are comparable to free-base porphyrin because of the absence of the heavy atom. Moreover, they can form a stable axial bond on both sides of the porphyrin ring. Therefore, functionalization with an oligonucleotide is possible in the axial direction.[15, 16] We have already found P(V)porphyrins useful as molecular probes for sensing the DNA sequence by use of the electron transfer mechanism.[15, 16] We also reported the synthesis of oligonucleotide derivatives whose phosphodiester linkage was replaced with P(V)porphyrin, which may lead to a tight interaction between the photoactive moiety and DNA.

In this study, we examined the photoactivity of water-soluble bis(2-hydroxyethoxy)-phosphorus(V)tetraphenylporphyrin ($EG_2P(V)TPP$) as a photoactive unit of the artificial photonuclease (FIG.1).

EXPERIMENTAL

Materials

Bis(2-hydroxyethoxy)phosphorus(V)tetraphenylporphyrin chloride ($EG_2P(V)TPP$) was synthesized according to the published procedure.[11] φx174RFI DNA(originated from bacteriophage φx174am3 containing over 70% covalently closed circular form I) was obtained from Takara Biochemicals. Superoxide dismutase (Sigma Chemicals), double-stranded poly(dA)poly(dT) and poly(dG)poly(dC) (Pharmacia Biotech) were used as

received. Other chemicals were obtained from general laboratory suppliers. The concentrations of porphyrin and DNA were determined by optical density using the following molar extinction coefficients: $EG_2P(V)TPP$, ($\varepsilon = 3.1 \times 10^5 M^{-1}cm^{-1}$, λ = 427nm); ϕx174RFI DNA, ($\varepsilon = 6600M^{-1}cm^{-1}$, λ = 260nm); poly(dA)poly(dT), (ε = $6600M^{-1}cm^{-1}$, λ = 262nm); poly(dG)poly(dC), ($\varepsilon = 8400M^{-1}cm^{-1}$, λ = 254nm).

Photocleavage

The reaction mixtures (total volume 20µl) were prepared in a standard buffer (10mM NaCl, 20mM Tris· HCl buffer, pH6.8) containing ϕx174RFI DNA (4.0×10^{-5}M nucleotide unit) and ($EG_2P(V)TPP$) (2.0×10^{-5}M), where the molar ratio of DNA base pairs to the porphyrin was 2.0. Samples were transferred to small glass tubes for irradiation. Irradiation was carried out at room temperature with a Xe lamp(Ushio DSB-501A) through interference filters (TOSHIBA L-38, IRA-25S) to cut off ultraviolet (UV) light (<380nm) and infrared light (>800nm). For the experiment under oxygen-free conditions, degassing was performed by a series of freeze-pump-thaw operations.

The photoirradiation products were analyzed by typical electrophoresis on a 0.8% agarose gel (gel size : 6.5×10×1.0cm) in Tris· HCl buffer containing 10mM NaCl (adjusted to pH6.8) at 50 V for 90min. The gel was incubated in a solution of ethidium bromide ($3\mu gml^{-1}$) for 20min and DNA bands were detected by fluorescence under an UV TF-20M lamp. Photographs of the gel were taken with a polaroid ACMEL CRT camera (film PID).

Measurement

Steady state absorption and emission spectra were recorded on a UV-2200 spectrophotometer and RF-503A spectrophotometer (Shimadzu), respectively. Fluorescence lifetimes were determined in 10^{-6}M solution by a Horiba NAES-550 time-correlated single-photon counting instrument. The excitation light was passed through a Toshiba band pass glass filter V-42 (maximum transmittance at 420nm) and the emission was detected through the monochromator. Time-resolved transient absorption spectra were measured with a microcomputer-controlled nanosecond laser photolysis system using a pulsed dye laser (FL3002, Lambda Physik, QUI, 390nm, fwhm=20ns) pumped by a XeCl excimer laser (EMG101MSC, Lambda Physik, 308nm, fwhm=20ns).

RESULTS AND DISCUSSION

The photograph in FIG.2 shows typical examples of the photocleavage of DNA by visible light irradiation of ϕx174RFI DNA (original ϕx174RFI DNA contained a small amount of form II DNA as a reference) in the presence of the $EG_2P(V)TPP$. Irradiation of supercoiled covalently closed circular DNA (form I, 4.0×10^{-5}M nucleotide) in the presence of the $EG_2P(V)TPP$ (2.0×10^{-5}M) in aerated solution (10mM NaCl, 20mM Tris· HCl buffer, pH6.8) resulted in the appearance of open circular DNA (form II) and a small

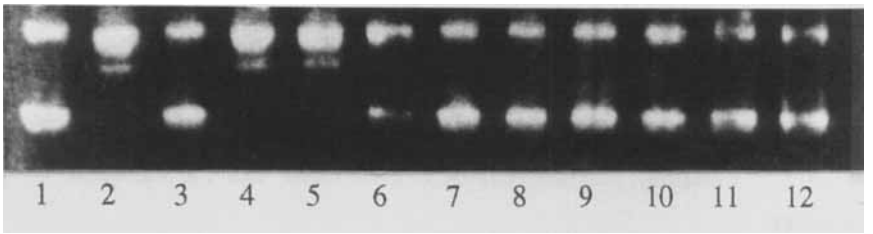


FIG.2.Agarose gel electrophoresis showing relaxation of φx174RFI DNA (40 mM) due to photocleavage by bis(2-hydroxyethoxy)P(V)TPP (20 mM) in 20 mM Tris·HCl buffer, pH 6.8 containing 10 mM NaCl. Lane1, DNA only: Lane2, DNA+P(V)TPP in air: Lane3, Lane2+NaN_3 (100 mM), Lane4, Lane2+SOD (10 ng/ml): Lane5, Lane2+D-mannitol (100 mM): Lane6, DNA+P(V)TPP in argon saturated aqueous solution. All irradiation were performed for 1 hr. Control experiments, lane7~12 were performed without irradiation.

amount of linear DNA (form III). The photocleavage was enhanced by the increase of the concentration of the $EG_2P(V)TPP$ (1.0×10^{-5} ~ 1.0×10^{-4}M) and irradiation time (0 ~ 60min, data not shown). No strand scission was observed in the absence of $EG_2P(V)TPP$ (FIG.2, lane1) and in the dark (FIG.2, lane7). To detect the reactive oxygen species in the photoreaction, the effects of scavengers[17] were examined, using sodium azide (NaN_3), superoxide dismutase (SOD), and D-mannitol. As shown in lanes 3-5, NaN_3 effectively inhibited the DNA cleavage. However, SOD and D-mannitol did not inhibit the DNA cleavage. These findings suggest that the main reactive species in the photocleavage in air is singlet oxygen ($^1O_2$), but not superoxide anion radical or hydroxy radical.[18] Energy transfer from the triplet excited state of the EG_2 P(V)TPP to O_2 is considered to have resulted in the formation of $^1O_2$, which causes oxidative cleavage of the DNA.[19] In fact, the time-resolved absorption spectroscopy indicated that the lifetime of the triplet excited state of the $EG_2P(V)TPP$ was shortened with O_2. Interestingly, the single-strand scission of DNA was also observed even in the absence of O_2 (FIG.2, lane 6). This result suggests the possibility of the photocleavage of DNA by the direct electron transfer between the $EG_2P(V)TPP$ and DNA.

In general, decomposition of DNA by direct electron transfer is initiated from the oxidation of nucleic acid bases. For example, direct UV irradiation and methylene blue photosensitization induced single or double-strand breaks of DNA from the initial formation of oxidized bases, mainly guanine in the absence of O_2.[20, 21] For the electron transfer reaction between the excited state of the photosensitizer and the nucleic acid bases, the photosensitizer should have strong oxidizing power in its excited state. In the case of

the $EG_2P(V)TPP$, the reduction potentials in the singlet and the triplet excited states are estimated +1.83V and +1.44V (vs NHE), respectively. These values are considered to be high enough to oxidize nucleic acid bases.

Whether the predominant process is oxygen mediated or direct electron transfer pathways is dependent on the interaction between the $EG_2P(V)TPP$ and DNA. In the presence of either poly(dA)poly(dT) or poly(dG)poly(dC), the absorption of the Q band decreased and red-shifted in an aqueous solution at a low ionic strength (TABLE 1). Similar absorption changes were also reported for other porphyrins.[22] Since the $EG_2P(V)TPP$ is a mono cation and DNA is an anionic polymer, the absorption changes are considered to be due to the electrostatic interaction between them. In fact, these absorption changes were reduced at a high ionic strength which decreases the electrostatic interaction. It is noteworthy that the interaction is strong enough to cause electron transfer from DNA to short-lived singlet excited $EG_2P(V)TPP$.

To confirm the electron transfer from DNA to the singlet excited $EG_2P(V)TPP$, the fluorescence quenching was measured under the condition that the ground state interaction occurred at a low ionic strength. The fluorescence of the $EG_2P(V)TPP$ was quenched more efficiently with poly(dG)poly(dC) (58%) than with poly(dA)poly(dT) (45%) (TABLE 1) and their decay became multi-components in both cases. In the case of two components analysis, long life components (poly(dA)poly(dT), 3.9ns ; poly(dG)poly(dC), 3.9ns) were assigned to free $EG_2P(V)TPP$ (3.8ns) which has not interacted with double-stranded DNA. The residual short life components (poly(dA)poly(dT), <1.0ns ; poly(dG)poly(dC), < 0.8ns) are considered to have originated from $EG_2P(V)TPP$ interacting to DNA. The strong quenching suggests that the existence of the direct electron transfer from DNA to the singlet excited $EG_2P(V)TPP$. The free energy change for oxidation of nucleic acid bases by the singlet excited $EG_2P(V)TPP$ was sufficient for the electron transfer, that is -0.99V for guanine and -0.59V for adenine.[23]

The electron transfer of the triplet excited $EG_2P(V)TPP$ was directly confirmed by the transient absorption measurements under the same conditions as the fluorescence quenching measurements, where ground state interaction took place. At 150ns after laser pulse excitation, the triplet absorptions of the $EG_2P(V)TPP$ around 480nm (FIG.3, C) were observed in the presence of either poly(dG)poly(dC) or poly(dA)poly(dT) (FIG.3, (a), (b)), but the decay processes are quite different in each case. In the case of poly(dG)poly(dC), the spectra decayed gradually, leaving a long life residual transient absorption peak around 460nm. This absorption peak was assigned to one electron reduced $EG_2P(V)TPP$ based on the absorption spectral change by electrochemical reduction in FIG. 3 (d). It is notable that absorption spectra of the one-electron reduced $EG_2P(V)TPP$ were observed even in the presence of

TABLE 1. Absorption and emission data of P(V)TPP in the presence of polynucleotide.

DNA	Absorption of Q band (nm)	Relative fluorescence intensity	Fluorescence lifetime (ns)
none	558.0	1.00	3.8 (100%)
poly(dA)poly(dT)	564.0	0.55	3.9 (73.9%) < 1.0 (26.1%)
poly(dG)poly(dC)	562.6	0.42	3.9 (85.9%) < 0.8 (14.1%)

Measurements were carried out in aqueous solution (pH 7.0) at room temperature. [$EG_2P(V)TPP$] = 3.0 μM; [poly(dA)poly(dT)] = 9.0 μM (nucleotide); [poly(dG)poly(dC)] = 9.0 μM (nucleotide). Values of the red shift of absorption were measured by the 558 nm absorbance maximum of $EG_2P(V)TPP$. Fluorescence was recorded with an excitation wavelength of 437 nm. Numbers in parenthesis are relative quantum yields.

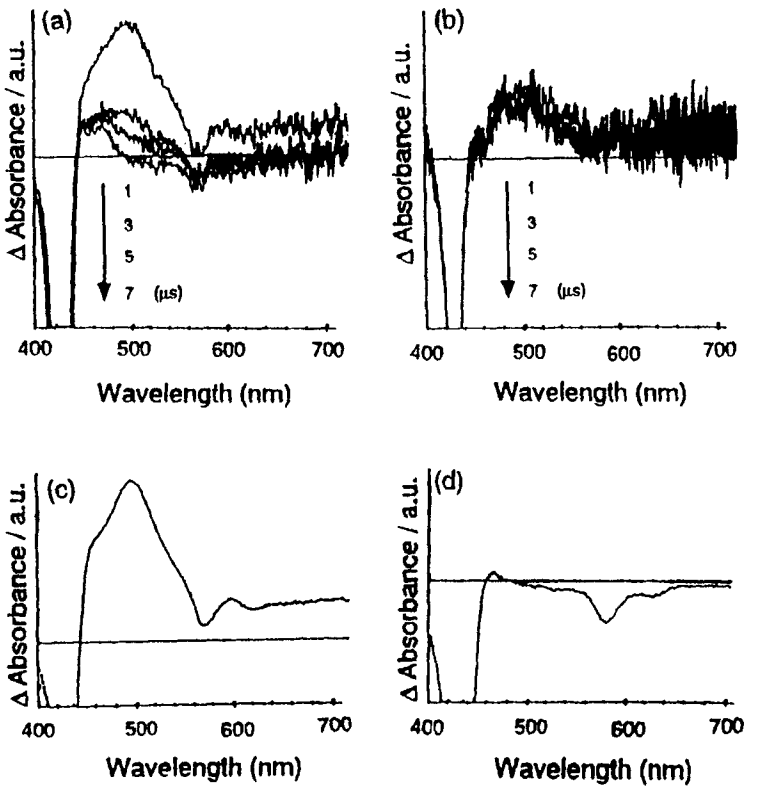
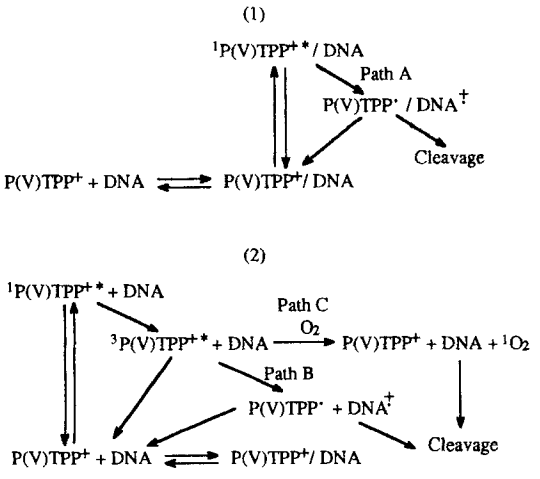


FIG.3. Transient absorption spectral changes of $EG_2P(V)TPP$ after the laser pulse excitation in the presence of poly(dG)poly(dC) (a) and poly(dA)-poly(dT) (b). The reference spectra of triplet-triplet absorption (c) and reduced radical absorption (d) were obtained by laser flash photolysis and electrochemical reduction, respectively.

oxygen. These findings suggest that the direct electron transfer from poly(dG)-poly(dC) to the triplet excited the $EG_2P(V)TPP$ can compete with the formation of $^1O_2$. In the case of poly(dA)poly(dT), the reduced $EG_2P(V)TPP$ cannot be observed. These results can be explained by the free energy change for oxidation of nucleic acid bases by the triplet excited $EG_2P(V)TPP$, where it is -0.60V for guanine but -0.20V for adenine, which is too small for electron transfer.



SCHEME 1. Pathways for photoreaction between P(V)TPP and DNA : (1) Photoreaction from the singlet excited P(V)TPP. (2) Photoreaction from the triplet excited P(V)TPP.

SCHEME 1 summarizes the mechanisms of the photocleavage of DNA. There are three possible pathways in the photoreaction between P(V)TPP and DNA. Path A involves the direct electron transfer from nucleic acid bases to the singlet excited P(V)TPP. As the lifetime of the singlet excited $EG_2P(V)TPP$ is 3.8ns, ground state interaction of DNA and the $EG_2P(V)TPP$ is required for the direct electron transfer effectively. Path B involves the direct electron transfer from nucleic acid bases to the triplet excited $EG_2P(V)TPP$. Since the lifetime of the triplet excited $EG_2P(V)TPP$ is about 186μs, the electron transfer can occur through the encounter of free $EG_2P(V)TPP$ and DNA. Path C involves the indirect process mediated by $^1O_2$ which is produced by energy transfer from the triplet excited P(V)TPP to O_2. This pathway involving a freely diffusive $^1O_2$ which has a relatively long life in aqueous solution, would be less sensitive to the distance between the photosensitizer and the targetted site of DNA.

For a direct electron transfer to the targetted sequence, a singlet excited $EG_2P(V)TPP$ is better because of its short lifetime which minimizes mistargetting. In this case, a tight interaction between DNA (or RNA) and $EG_2P(V)TPP$ at ground state is preferred. The existence of the direct electron transfer path with the singlet state of P(V)TPP suggests the possibility of a strongly sequence targetting photocleavage system, satisfying the requirements for a tight and sequence recognizing interactive moiety to DNA on a photoactive $EG_2P(V)TPP$.[15,16]

ACKNOWLEDGMENT

This work was partially supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.

REFERENCES

(1) Praseuth, D., Chassignol, M., Takusugi, M., Le Doan, T., Thuong, N. T., and Hélène, C. (1987) *J. Mol. Biol., 196,* 939.

(2) Praseuth, D., Le Doan, T., Chassignol, M., Decout, J. L., Habbout, N., Lhomme, J.,Thuong, N. T., and Hélène, C. (1988) *Biochemistry., 27,* 3031.

(3) Praseuth, D., Perrouault, L., Le Doan, T., Chassignol, M., and Thuong, N. T. (1986) *Biochemistry., 25,* 6736.

(4) Praseuth, D., Gaudemer, A., Verlhac, J. B., Kraljic, I., Sissoeff, I., and Guille, E. (1986) *Photochem. Photobiol., 44,* 717.

(5) Sari, M. A., Batttioni, J. P., Dupre, D., Mansuy, D., and Le Pecq, J. B. (1990) *Biochemistry., 29,* 4205.

(6) Croke, D. T., Perrouault, L., Sari, M. A., Battioni, J. P., Mansuy, D., Hélène,C., and Le Doan, T. (1993) *J. Photochem. Photobiol. B: Biol., 18,* 41.

(7) Sentagne, C., Meunier, B., and Paillous, N. (1992) *J. Photochem. Photobiol. B: Biol., 16,* 47.

(8) Le Doan, T., Praseuth, D., Perrouault, L., Chassignol, M ., Thuong, N. T. and., Hélène, C. (1990) *Bioconjugate Chem., 1,* 108.

(9) Vlassov, V. V., Deeva, E.A., Ivanova, E.M., Knorre, D.G., Maltseva, T. V., Frolova, E. I. (1991) *Nucleosides & Nucleotides, 10,* 641.

(10) Cross, G. L., and Miller, J.R. (1988) *SCIENCE, 240,* 449.

(11) Segawa, H., Kunimoto, K., Nakamoto, A., and Shimidzu, T. (1992) *J. Chem. Soc., Perkin Trans. 1, 8,* 939.(14) Kitamura, M., Nimura, A., Kunimoto, K., Segawa, H., and Shimidzu, T. (1991) *Nucleic Acid Res., Symp., 25,* 13.

(12) Segawa, H., Nakayama, N., and Shimidzu, T. (1992) *J. Chem. Soc., Chem. Commun.,* 784.

(13) Segawa, H., Nakamoto, A., and Shimidzu, T. (1992) *ibid.,* 1066.

(14) Kunimoto, K., Segawa, H., and Shimidzu. (1992) *Tetrahedron Lett., 33,* 6327.

(15) Kitamura, M., Nimura, A., Kunimoto, K., Segawa, H., Shimidzu, T. (1991) *Nucleic Acid Res.,Symp., 25,* 13.

(16) Shimidzu, T., Segawa, H., Kitamura, M., and Nimura, A. (1992) *Nucleic Acid Res.,Symp., 27,* 948.

(17) Halliwell, B and Gutteridge, J. M. C. " Free Radicals in Biology and Medicine," Oxford University Press (1985).

(18) Hasty, N., Merkel, P.B., Radlick, P., and Keane, D.R. (1972) *Tetrahedron Lett. 1*, 49.

(19) Piette, J. (1990) *J. Photochem. Photobiol. B: Biol., 4,* 335.

(20) Gorner, H., Wala, M., and Schulte-frohlinde, D. (1992) *Photochem. Photobiol. 55*, 173.

(21) Blau, W., Croke, D.T., Kelly, J.M.,McConnell, D.J., OhUigin, C., and Van derPutten, W. J. M. (1987) *J. Chem. Soc., Chem. Commun.*, 751.

(22) Pasternack, R. F., Gibbs, E. J., and Villafranca, J. J. (1983) *Biochemistry, 22*, 2406.

(23) Dunn, D.A., Lin, V. H., and Kochevar, I. E. (1991) *Photochem. Photobiol. 53*, 47.

Received 12/27/93
Accepted 2/7/94