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Photochemical Covalent Binding of *p*-Methoxycinnamic Acid to Calf Thymus DNA

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Irradiation of E-*p*-[α-^{14}C]methoxycinnamic acid and calf thymus DNA with uv light leads to incorporation of the radiolabel into the DNA. Sephadex chromatography confirms that the label incorporation is due to covalent bond formation. Photobinding is more efficient with denatured vs native DNA and with higher frequency light, e.g., overall quantum efficiencies at 308 nm for denatured and native DNA are 1.1 and 0.6 $\times$ 10^{-5}, respectively, vs 22.0 and 8.0 $\times$ 10^{-5} for 266 nm, respectively (values for 0.7 m*M* pMCA and ca. 0.7 mg/ml DNA). A comparative study with several polyribonucleotides shows the relative selectivity for binding to be poly(C) > poly(A) $\gg$ poly(G). Photolysis of labeled DNA with 254-nm light leads to the release of both isomers of pMCA, evidence that at least a portion of the covalent binding is due to the formation of cyclobutane adducts. Equilibrium dialysis studies give no evidence for preassociation of pMCA and DNA.

INTRODUCTION

E-3-(4-Methoxyphenyl)-2-propenoic acid (E-*p*-methoxycinnamic acid, E-pMCA, Fig. 1) is a naturally occurring compound found in the roots of *Veronica virginica* (*1*) and also as a component of onjisaponin, a triterpenoidal saponin isolated from the root of *Polygala tenuifolia* (*2*). Its esters (primarily 2-ethylhexyl-*p*-methoxycinnamate, 2-EHMC) (*3*), amides (*4*), and metal salts (*5*) are used as the active ingredients of sunscreens used in photoprotective cosmetics. 2-EHMC is typically present in concentrations of 5–10% (w/v) in sunscreen formulations (*6*).

There has been some controversy regarding the potential phototoxicity of 2-EHMC, with an early report of tumor induction (*7*) contradicted by more recent investigations (*8, 9*). We became interested in the photobiological properties of the free acid because it is structurally related to other naturally occurring acrylic acid derivatives which we have shown to photochemically react with DNA. These include E-urocanic acid (E-UA), formed in the skin by the enzymatic deamination of histidine, and E-indoleacrylic acid (E-IA), found primarily in plants as a consequence of the deamination of tryptophan (cf. Fig. 1). Both of these substrates covalently bind to DNA and its component bases under the influence of uv light, there being good evidence for cyloaddition to the pyrimidine bases as one (of perhaps several) mechanisms for such binding (*10–13*).

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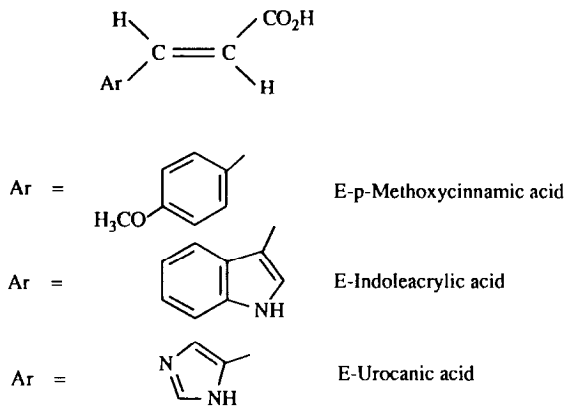

FIG. 1. Chemical structures of the acrylic acid derivatives.

EXPERIMENTAL

Chemicals. E-*p*-Methoxycinnamic acid, *p*-anisaldehyde, and diphenylamine were purchased from Aldrich Chemical Co. (Milwaukee, WI). Calf thymus DNA (Type I: sodium salt, "highly polymerized"), polyribonucleotides, Sephadex G-100-120 (bead size 40–120 μm excludes molecules with MW 100,000), dialysis tubing (12000 MWCO: 250-9U) (cleaned according to the literature procedure) (*14*) and [2-^{14}C]malonic acid (specific activity 2.5 mCi/mmol) were from Sigma Chemical Co. (St. Louis, MO). ACS scintillation fluid was from Amersham (Arlington Heights, IL). HPLC grade methanol was purchased from Burdick and Jackson Laboratories and the deionized water was distilled from a Corning MP-1 water still. All other chemicals were of reagent grade and were used as received.

Instrumentation. Ultraviolet analyses were performed on a Gilford modified Beckman DU, a Hewlett–Packard HP8451A photodiode array, and/or a Perkin–Elmer Lambda 3B UV/VIS spectrophotometer. Centrifugations were carried out with a DuPont Instruments Sorvall RC5B refrigerated superspeed centrifuge and/or a Damon/IEC centrifuge (Model HN-SII). Reversed phase high performance liquid chromatography was performed on a Varian 5000 programmable ternary solvent delivery liquid chromatograph, equipped with a Model 7125 Rheodyne (Cotati, CA) injection valve with either a 0.1- or a 0.2-ml injection loop, and a Varian 2050 variable wavelength detector set at 280 nm (unless otherwise specified). The chromatograms were recorded and processed with a Perkin–Elmer LCI-100 computing integrator. The assays generally used a 5-μm C_8 reversed phase stainless steel column (4.6 × 250 mm; Alltech Associates, Inc., Deerfield, IL), except for analysis of pMCA in the presence of DNA, wherein a 5-μm GFF-S5-80 internal surface reversed phase (ISRP) column (*15*) (4.6 × 250 mm; Regis Chemical Co., Morton Grove, IL) was employed. The following isocratic solvent programs were utilized: 75% 0.05 M sodium phosphate buffer, pH 7.0 : 25% methanol at a flow rate of 1.0 ml min^{-1} (method 1) and 100% 0.1 M sodium phosphate

buffer, pH 7.0, at a flow rate of 0.5 ml min^{-1} (method 2). Radioactive fractions from the column were collected with an Instrumentation Specialties Co. (ISCO) fraction collector and counted on a Packard 300C liquid scintillation counter.

Photolyses. Standard photolysis experiments were done by irradiating solutions in aqueous buffer (0.1 M, pH 7.0) in parafilm-sealed, cylindrical quartz photolysis tubes (13 mm × 12 cm, 8.0 ml vol), in a turntable surrounding a Pyrex ($\lambda > 294$ nm) filtered Canrad-Hanovia (Newark, NJ) 450-W medium pressure Hg lamp (Model 679A-36). The photolysis at $\lambda > 270$ nm utilized a vycor filtered lamp with the quartz photolysis tubes encased within larger corex tubes. The temperature of the cooling bath was maintained at 15–18°C with a Neslab Endocal refrigerated circulating bath. The photolysis tubes were 8.0 cm from the light source; at this distance the intensity was 2.0×10^{16} photons s^{-1} cm^{-2} through air as monitored with a UVX Radiometer and an (uncorrected) UVX-31 sensor (Ultraviolet Products Inc., San Gabriel, CA). Degassed solutions were flushed with argon for a minimum of 25 min prior to photolysis. The dark controls, covered with Al foil, were inserted into the turntable under identical conditions as the photolyzed samples. Experiments at 254 nm, at ambient temperature, utilized cylindrical quartz photolysis tubes and a Canrad–Hanovia (Model 688A-45) low pressure Hg resonance lamp (typical flux into the tubes, $2–3 \times 10^{16}$ photons s^{-1} cm^{-2}). The quantum efficiency measurement for the binding of E-[α-^{14}C]pMCA to DNA at 266 nm was determined using a frequency-quadrupled Quanta-Ray DCR-1 Nd:YAG laser fired at 10 Hz at 8.2 mJ/pulse. The degassed solutions were photolyzed in a 1-cm^2 quartz cuvette. The dose-rate, monitored with a Scientech 362 power energy meter, was 1.26 and 1.27×10^{17} photons s^{-1} into the cell, in the native and denatured DNA experiments, respectively; the dose-rate independently measured with uranyl oxalate actinometry (*16, 17*) was ca. 5% higher. The quantum efficiency measurements at 308 nm employed a XeCl charged Lambda Physik Lasertechnik LPX 100 excimer laser. The degassed solutions were irradiated with the laser operated at 22 kV with a repetition rate of 2 Hz at 43.2 mJ/pulse; the dose-rate was 1.34×10^{17} photons s^{-1} as measured with uranyl oxalate actinometry. The laser beam was diverted with a quartz turning prism placed 10 cm from the beam port. It was focused through a focusing lens (3.8 cm diameter × 10.2 cm focal length) 11 cm from the face of the cuvette and oriented with the cylindrical axis vertical in order to provide horizontal focusing.

Methods. Denatured DNA was prepared by dissolving 30 mg DNA in 10 ml of distilled water overnight. The solution was heated at reflux for 40 min, quickly plunged into an ice bath, and brought to room temperature slowly. The hyperchromicity observed after denaturation was ca. 25%.

Binding assays. The amount of covalent binding between E-[α-^{14}C]pMCA and DNA was determined by exhaustive dialysis of the photolysate against 5 mM Tris buffer (pH 7.2) at 4°C for 3–4 days. The dialysate was changed every day and counted for the released radioactivity. After the dialysate reached background levels, the solution was removed from the dialysis bag and the DNA assayed using absorbance at 260 nm. Standard conversion factors of 21 and 26 AU/mg DNA were used for native and heat-denatured DNA, respectively. In the quantum efficiency experiment at 266 nm, DNA was also quantitated by the Burton assay

(*18*), wherein the DNA was hydrolysed with perchloric acid and the liberated deoxyribose moiety was assayed spectrophotometrically using a diphenylamine reagent. Polyribonucleotides were assayed spectrophotometrically using the following molar extinction coefficients (*19*): λ_{nm} (ε, M^{-1} cm^{-1}), poly(A), 257 (10,700); poly(C), 269 (6300); poly(G), 255 (9500); poly(U), 261 (9600). The radioactivity (dpm) was counted to determine the amount of label which had been bound. This was divided by the specific activity (dpm/mmol) of the irradiated sample and multiplied by 10^6 to get the nanomoles of bound label. The level of covalent binding is reported as the number of nanomoles of pMCA/milligram DNA (more rigorously, nmol of radioactivity/mg nucleic acid).

The DNA and/or polynucleotide was then precipitated with 0.1 vol of 2 M NaCl and 2 vol of 95% EtOH, stored overnight in a freezer, and spun at 2000 rpm for 20 min. The pellet was dissolved in 50 mM Tris buffer, pH 7.2, and the procedure was generally repeated at least three times. At each precipitation–dissolution step, the amounts of nucleic acid and radioactivity were measured and the levels of covalent binding determined.

Quantum efficiencies were derived from the binding levels in the final precipitation, multiplied by the amount of DNA (mg) in the dialysis bag, and are reported as the total moles of bound pMCA per einstein of absorbed light.

The covalent nature of bound radioactivity was further confirmed with size exclusion chromatography. The final precipitate of the labeled nucleic acid was loaded on 1.33 × 45- to 50-cm columns of Sephadex and eluted with 0.1 M sodium phosphate buffer, pH 7.0 (flow rate 1.0 ± 0.05 ml min^{-1}). Fractions (3.0 ml) were collected and each fraction was assayed for its uv absorbance and radioactivity.

Equilibrium dialysis. For equilibrium dialysis studies, 2.0-ml solutions of native and heat-denatured DNA (0.85 mg/ml; both irradiated and unirradiated) were dialyzed against 2.0 ml of E-[α-^{14}C]pMCA (0.86 mM, 0.077 mCi/mmol) in 0.1 M sodium phosphate buffer, pH 7.0. A control was run simultaneously with the dialysis bag containing only the buffer. The solutions were shaken at 4°C at a speed of 150 rpm for 4 days, and each day 2 50-μl aliquots of the outside solutions were counted. On the final day, 2 50-μl aliquots of the solutions inside the bag were also counted.

Synthesis of E-p-[α-^{14}C]Methoxycinnamic acid. This substrate was prepared by the Doebner modification of the Knoevenagel reaction using an adaptation of the literature procedure (*20*) described for "cold" pMCA. A stirred solution of distilled *p*-anisaldehyde (48.4 mg, 0.36 mmol), cold malonic acid (39.5 mg, 0.38 mmol), and [2-^{14}C]malonic acid (0.12 mCi) in pyridine (0.19 ml) containing piperidine (4 μl) was heated at ca. 100°C for 18 h. After cooling, the reaction mixture was diluted with distilled water (1.0 ml) and acidified with 5 N HCl. The mixture so obtained was refrigerated for 6 h, filtered, washed with cold water, and dried with a stream of nitrogen to afford 53.2 mg (0.094 mCi, sp act 0.310 mCi/mmol) of E-[α-^{14}C]pMCA (84 and 78% chemical and radiochemical yield, respectively). The identity of the product was established by comparison with an authentic cold sample of pMCA using TLC and HPLC analysis. TLC showed a single spot with both shortwave uv light and iodine vapor development, using several eluents: *n*-butanol : 95% EtOH : water (4 : 1 : 5, R_f 0.75), diethyl ether (R_f 0.51), and chloro-

form : 95% EtOH (4 : 1, R_f 0.76). The chemical and radiochemical purity, as determined from the combined HPLC–liquid scintillation counting (C_8 analytical column with method 1, t_R 10.5 min) was > 98%.

RESULTS

Photoinduced covalent binding of pMCA to DNA. E-[α-^{14}C]pMCA (0.75–0.88 mM) and calf thymus DNA (0.75 mg/ml) were dissolved in 6.0 ml of phosphate buffer (pH 7.0) and photolyzed with a medium pressure mercury lamp through Pyrex ($\lambda > 294$ nm) for 20 h, or through corex ($\lambda > 270$ nm) for 30 h, in each case at 15°C under argon. The data are presented in Table I.

The time course for label incorporation into native DNA (0.75 mg/ml) was determined using labeled pMCA (0.95 mM) by photolysis under argon at 15°C for 20, 26, 48, 72, and 96 h. The data are plotted in Fig. 2.

The presence of air had no effect on binding; when the pMCA (0.63 mM) and native DNA (0.75 mg/ml) were photolyzed for 30 h at 18°C through corex, binding levels of 190 and 187 nmol pMCA/mg DNA were obtained in argon and air, respectively.

Quantum efficiencies for label incorporation were determined for native and denatured DNA using 266- and 308-nm laser excitation by photolysis of a 4.0-ml solution of E-[α-^{14}C]pMCA (0.70–0.74 mM) and DNA (0.70–0.80 mg/ml) in phosphate buffer at room temperature under argon for 60 min. The data are presented in Table II.

Photoinduced covalent binding of pMCA to polyribonucleotides. E-[α-^{14}C] pMCA (0.70 mM) and each of the polyribonucleotides (1.25 mg/ml) were dissolved in 6.0 ml of phosphate buffer (pH 7.0) and photolyzed with a medium pressure

TABLE I

Photochemical Binding of E-[α-^{14}C]pMCA to Calf Thymus DNA

Sample	DNA[a] (mg)	pMCA (nmol)	Covalent binding (nmol pMCA/mg DNA)[b]	Photobinding yield[c]
		At $\lambda > 294$ nm[d]		
Native DNA	2.49	27	10.8	0.51
Denatured DNA	2.16	58.6	27	1.11
Dark control	2.35	0.8	0.3	0.015
		At $\lambda > 270$ nm[e]		
Native DNA	2.42	313	129	5.50
Denatured DNA	2.07	317	153	5.60

[a] Amount recovered after final purification.
[b] A value of 100 nmol label/mg DNA corresponds to 0.033 label/base.
[c] Percentage of original radiolabel incorporated into DNA recovered after purification.
[d] Pyrex filter.
[e] Corex filter.

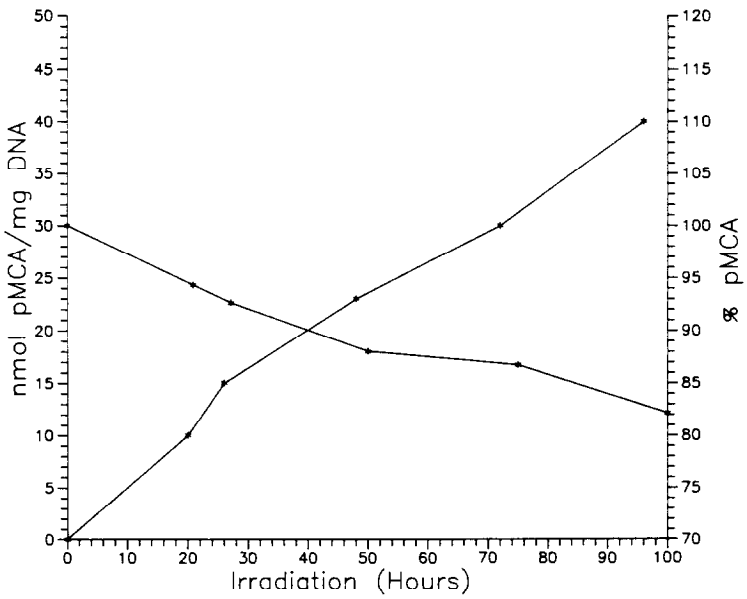

FIG. 2. Time course plots for the photolytic binding of E-[α-^{14}C]pMCA to native DNA and the concomitant disappearance of pMCA at $\lambda > 294$ nm; [DNA]/[pMCA] = 2.3.

mercury lamp through corex ($\lambda > 270$ nm) for 32 h at 17°C under argon. The data are presented in Table III. No value for poly(U) could be obtained because of degradation of the polynucleotide.

Sephadex chromatography. To test the covalent nature of the label incorporation, a sample of native DNA labeled with 30 nmol pMCA/mg DNA (cf. Fig. 2) was subjected to Sephadex chromatography. A 1.0-ml solution of the labeled

TABLE II

Quantum Efficiencies for Radiolabel Incorporation into DNA

Sample	Covalent binding (nmol pMCA/mg DNA)	ϕ^a ($\times 10^5$)
	At λ = 266 nm[b]	
Native DNA	38	8.0
Denatured DNA	52	22.0
	At λ = 308 nm[c]	
Native DNA	2.3	0.6
Denatured DNA	4.5	1.1

[a] Moles of bound pMCA per einstein of light absorbed by the solution.

[b] Frequency quadrupled Quant-Ray DCR-1 Nd : YAG laser.

[c] Lambda Physik XeCl charged excimer laser.

TABLE III

Photochemical Binding of E-[α-^{14}C]pMCA to Polyribonucleotides at λ > 270 nm[a]

Sample	poly(X)[b] (mg)	pMCA (nmol)	Covalent binding (nmol pMCA/mg poly(X))	Photobinding yield[c]
poly(A)	3.31	639	193	15.4
poly(C)	2.14	521	243	12.6
poly(G)	3.54	117	33	2.8
poly(U)			*d*	

[a] Corex filter.
[b] Amount recovered after final purification.
[c] Percentage of original radiolabel incorporated into the polynucleotide recovered after purification.
[d] No precipitation could be induced.

DNA was loaded on a Sephadex column (1.33 × 46 cm) and the eluted fractions (3.0 ml each) were assayed for DNA (A_{260}) and counted (2 0.5-ml aliquots) for pMCA. The elution profile is presented in Fig. 3, where it may be noted that the DNA and the radioactivity coeluted in fractions 6–9, with binding levels of 27, 29, 28, and 35 nmol pMCA/mg DNA, respectively, in good agreement with level of binding measured in the prechromatographed material. These four fractions constitute 92% of the radioactivity loaded onto the column; the total recovery of label

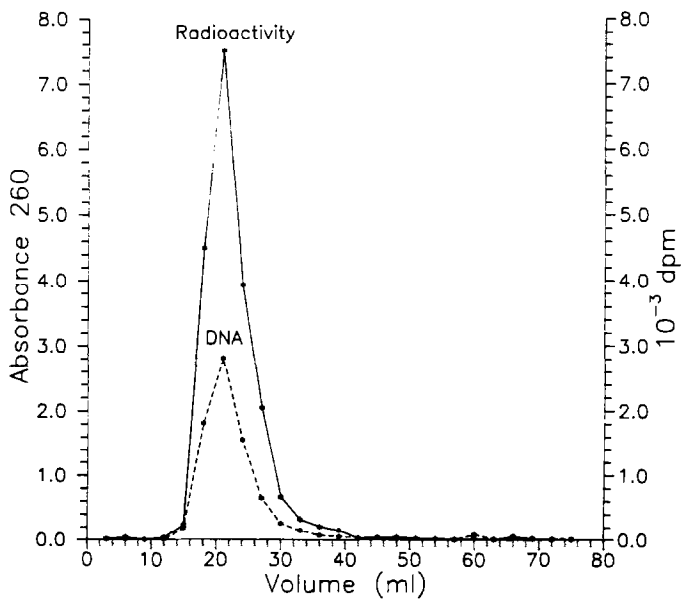

FIG. 3. Sephadex chromatogram of pMCA–native DNA photoadduct isolated after 72 h photolysis (λ > 294 nm) at 15°C of E-[α-^{14}C]pMCA and DNA; [DNA]/[pMCA] = 2.3. Photomodified DNA loaded onto the column, 1.10 mg.

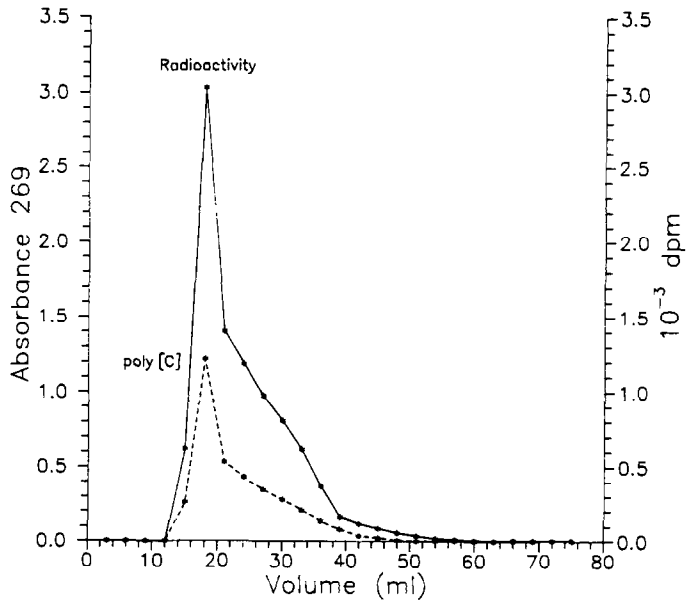

FIG. 4. Sephadex elution profile of pMCA–poly(C) photoadduct isolated after 32 h photolysis ($\lambda > 270$ nm) at 17°C of E-[α-^{14}C]pMCA and poly(C); [poly(C)]/[pMCA] = 3.0. pMCA–poly(C) photoadduct loaded onto the column, 0.54 mg.

for the complete chromatogram was quantitative. Sephadex chromatography of radiolabeled denatured DNA gave rise to analogous results (data not shown).

Radiolabeled poly(C) (cf. Table III) was also subjected to Sephadex chromatography. A 0.5-ml solution of the labeled poly(C) was loaded on the Sephadex column and the eluted fractions (3.0 ml each) were assayed for poly(C) (A_{269}) and radioactivity. The data are plotted in Fig. 4. Fractions 5–7 exhibited binding levels of 235, 243, and 256 nmol pMCA/mg poly(C), respectively, again in good agreement with the level of label incorpoation (243 nmol pMCA/mg poly(C)) measured for the prechromatographed material. These three fractions accounted for 65% of the radioactivity placed on the column; the total recovery in the complete chromatogram was quantitative.

Photolysis of E-[α-^{14}C]pMCA in the absence of DNA ($\lambda > 294$ nm, 15°C, 25 h) resulted in the formation of a nondialyzable material (i.e., a photopolymer of pMCA), which accounted for 8.4 (0.4%) and 5.9 (0.3%) nmol of radiolabel, upon photolysis in argon and air, respectively. The photopolymers produced in argon and air were combined and evaporated under nitrogen. The residue was dissolved in phosphate buffer (1.0 ml) and an 0.8-ml aliquot was loaded onto a Sephadex column (1.33 × 46 cm). The collected fractions (3.0 ml each) were assayed for radioactivity with the results plotted in Fig. 5 (recovery of radioactivity in this experiment was quantitative). The slightly higher pMCA/DNA ratios in fractions 9 and 7 of Figs. 3 and 4, respectively, can be attributed to contamination of these fractions by small amounts of the photopolymer.

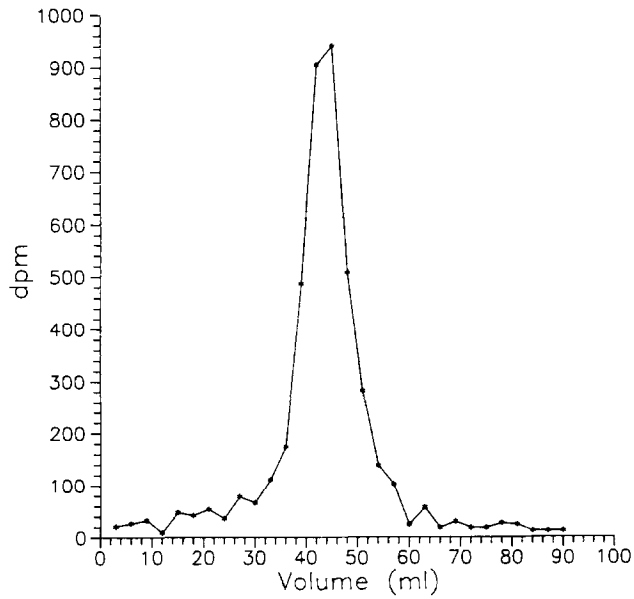

FIG. 5. Sephadex chromatogram of E-[α-^{14}C]pMCA photopolymer.

Photolysis of radiolabeled DNA With 254-nm light. Native DNA, isolated from a 72-h Pyrex-filtered photolysis with radiolabeled pMCA, and purified by Sephadex chromatography, was subjected to further photolysis for a total of 300 min with the 254-nm line of a low-pressure mercury lamp. Two products were detected by HPLC (C_8 analytical column and method 1) with retention times of 6.5 and 10.5 min, corresponding to Z- and E-pMCA, respectively. An analogous result was obtained with the radiolabeled denatured DNA.

Labeled native DNA, isolated from the 266-nm laser quantum efficiency experiment (see above) and containing 38 nmol label/mg DNA (a level comparable to the sample used in the experiment described above), was irradiated with 254-nm light and monitored after 300 min. HPLC analysis showed no evidence of either isomer of pMCA.

Equilibrium dialysis studies. Solutions of radiolabeled pMCA and native DNA were studied by equilibrium dialysis to investigate the possibility of their noncovalent association. The data are summarized in Table IV.

DISCUSSION

As noted in the Introduction, this study was motivated, in part, to determine whether pMCA would photolytically bind to DNA as had been observed with other acrylic acid derivatives. In fact, photolysis of radiolabeled pMCA and DNA indeed leads to extensive incorporation of the radiolabel into the nucleic acid

TABLE IV

Equilibrium Dialysis Studies With E-[α-^{14}C]pMCA and DNA

Sample	Inside bag[a]	Outside bag[a]
Control[b]	6895	6872
Native DNA	6969	6994
Denatured DNA	6668	6931
Preirradiated DNA	6716	6812

[a] All values in units of dpm/0.05-ml aliquot of solution.
[b] Without DNA in the dialysis bag.

(Table I). Levels of incorporation as high as 1 label per 16 bases (190 nmol label/mg DNA) have been observed, a level appreciably higher than that observed with UA (1 label per 38 bases (*10*)) but lower than that seen with IA (1 label per 10 bases (*11*)). However, even the lowest photobinding yield reported in Table I (0.51%) corresponds to a nucleotide/pMCA ratio (280) which is significantly greater than the nucleotide/8-methoxypsoralen ratio (1500) known to be capable of inhibiting DNA polymerase activity (*21*). The necessity for light to bring about label incorporation is evident from the control experiment in Table I, wherein binding in the dark was ca. 3% of that observed for irradiated samples (Pyrex filter). Oxygen does not impede binding, by contrast with UA (*22*) and IA (*11*) where air was found to diminish label incorporation. A time course study for label incorporation shows a virtually linear dependence on dose with an almost parallel disappearance of pMCA (Fig. 2). (Note that the total amount of pMCA bound to DNA in each experiment does not exactly equal the amount of lost pMCA because of a modest amount of competitive photodegradation (<10%).) The levels of incorporation are greater for single-stranded (i.e., denatured) vs native DNA (as has been observed for UA and IA). The greater incorporation into denatured DNA, the extensive binding observed with (single-stranded) poly(C), and the equilibrium dialysis studies (Table IV) all suggest a minor role, if any, for ground state complexation in the photochemistry. (One cannot, of course, exclude the possibility that trace quantities of preassociated complexes do exist and react with high efficiency to give an apparently low quantum efficiency reaction.)

The photolytic incorporation of radiolabel is wavelength dependent (as has been observed for UA (*10, 22*) and IA (*11*)). This is qualitatively evident from the Pyrex vs corex data (Table I), and is quantitatively confirmed by the quantum efficiencies (more accurately "quantum utilizations" since multiple absorbers are involved) determined at 266 and 308 nm (Table II), i.e., the ϕ's are 13–20 times higher at the shorter wavelength. The relative absorption at 308 nm is such that pMCA absorbs 88% of the incident light whereas at 266 nm the situation is almost reversed, i.e., DNA is the major absorber (62%) (cf. Fig. 6). The apparent correlation of ϕ and DNA absorption suggests that photoexcitation of the DNA initiates the requisite chemical reaction(s), i.e., pMCA + DNA* → product(s). Analogous

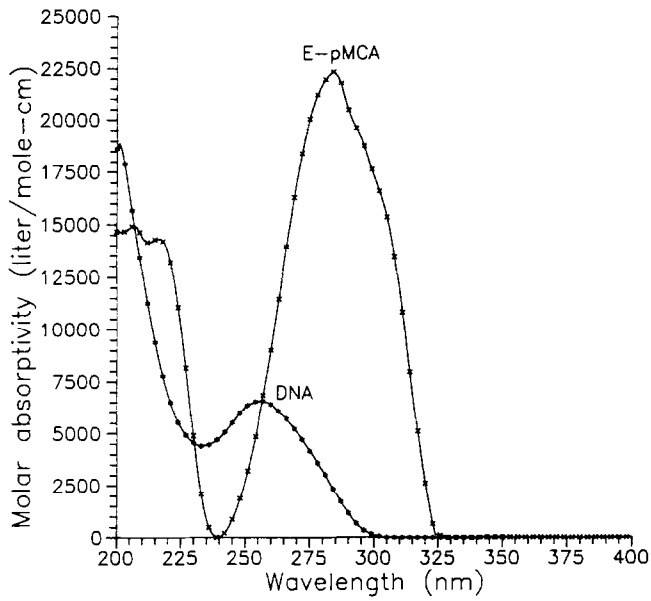

FIG. 6. Ultraviolet absorption spectra of E-pMCA and native calf thymus DNA in 0.1 M sodium phosphate buffer, pH 7.0.

conclusions have been reached for the photolytic binding of UA (*22*) and IA (*11*) to DNA. It is noteworthy that ϕ for photodestruction of pMCA in the absence of DNA is also some 10-fold higher at 266 vs 308 nm, i.e., 3.20×10^{-3} vs 3.34×10^{-4}, respectively.

All the binding levels reported herein were obtained after exhaustive purification of DNA by multiple dialyses and repeated precipitations, at which point the label/DNA ratios had become relatively constant. This would indicate that the label is covalently bound to DNA, a conclusion supported by the coelution of the label with DNA and poly(C) during Sephadex chromatography (Figs. 3 and 4). In addition, there is evidence that the label incorporation involves, at least in part, photocycloaddition of the ethylenic bond of pMCA and the 5,6 double bond of pyrimidine bases in the DNA. Thus, a comparative study of the photolytic binding of pMCA with the polyribonucleotides showed the greatest level of label incorporation with poly(C) (though substantial binding was also noted with poly(A)) (Table III). Furthermore, it is characteristic of photogenerated cyclobutanes that they are cleaved by high frequency light (for example, see (*12*) and (*22*), (*11*), and (*23*) for adducts involving UA, IA, and angelicin, respectively). Indeed, photolysis of the pMCA/DNA products (both native and denatured) with 254-nm light did indeed lead to regeneration of the pMCA. These DNA samples had been generated using Pyrex-filtered light. Interestingly, when the labeled DNA had been prepared using 266-nm light, no pMCA release by 254-nm photolysis was observed. Analogous results have been observed with the IA/DNA products pre-

pared with long wavelength (Pyrex filtered) vs 266 nm light (*24*). These observations are consistent with the existence of multiple mechanisms for reaction of the acrylic acids with DNA, with the 2 + 2 cycloaddition mechanism favored at long wavelengths and one (or more) additional reactions becoming dominant with higher frequency light (*25*) (possibly because the cycloadducts are cleaved more efficiently than they are formed with such light (*24*)). We cannot yet speak to the details of the binding mechanism(s) at these higher frequencies, though there is preliminary evidence from the photolysis of UA with 2′-deoxyadenosine that electron transfer chemistry may be operative (*25*).

Further *in vitro* and *in vivo* studies to determine the photoreactivity of pMCA and other acrylic acids with cellular nucleic acid and protein are in progress.

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