The Photochemistry of Uridylic Acid

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INTRODUCTION

Studies of the action spectra for a variety of biological effects of ultraviolet irradiation have indicated in many instances that the nucleic acids are at least the immediate receptors of the effective radiations (*1*, *2*). Relatively few studies have been made, however, of the effects of ultraviolet irradiation on the nucleic acids or their components. Studies of the effects of irradiation on the free purine or pyrimidine bases of the nucleic acids (*3*) are of limited value because, as will be shown, the attachment of the pentose sugar to the heterocylic ring can profoundly modify the photochemical behavior. Studies of the effects of ultraviolet irradiation on the intact nucleic acids (*4*, *5*) have been difficult to interpret because of the limited criteria of photochemical change which can be applied to these large molecules and the consequent necessity of using doses of radiation considerably in excess of those adequate to produce the biological effects.

In this and a subsequent paper, the results are presented of the effects of ultraviolet irradiation on mononucleotide components of nucleic acids, in the hope that these results may provide a basis for extrapolation to the effects of ultraviolet irradiation on the polymeric nucleic acids. As many of the biological effects of ultraviolet irradiation have been shown to be, at least in part, reversible by light or heat (*6–8*), particular attention has been paid to certain effects of ultraviolet irradiation on nucleotides which have been found to be of such a nature that they are readily reversed.

MATERIALS AND METHODS

Uridylic acid was obtained from Nutritional Biochemicals Corp. This material was found by chromatographic analysis (*9*) to be at least 98% uridylic acid *b* (uridine-3′-phosphate, Fig. 1) (*10*, *11*). This material was used as the uridylic acid *b* of the experiments to be described.

To obtain uridylic acid *a* (uridine-2′-phosphate), 10 mg of the *b* isomer was dissolved in 10 ml of 0.1 *N* HCl, the solution boiled for 1 hour, neutralized, and then the uridylic acids *a* and *b* separated by ion exchange chromatography (*9*). This treatment gave 3.7% uracil plus uridine, 39.4% uridylic acid *a*, and 57.2% uridylic acid *b*. The fractions containing *a* isomer were pooled, readsorbed onto ion exchange resin, and eluted in small volume with 0.2 *M* NaCl.

Uridine was obtained from Nutritional Biochemical Corp.

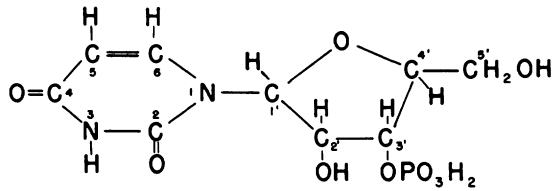

FIG. 1. Uridine-3′-phosphate (uridylic acid *b*).

Thymidylic acid was obtained by ion exchange chromatographic fractionation of an enzymic digest of deoxyribonucleic acid (*12*). This material is believed to be thymidine-5′-phosphate, as it is dephosphorylated by preparations of 5′-nucleotidase (*13*).

Irradiations were carried out with mechanical stirring in quartz cuvettes of the type used in the Beckman ultraviolet spectrophotometer.[1] A low-pressure, quartz, mercury arc, wound in a spiral, made by the Hanovia Mfg. Co., was used as the radiation source. Sources of this type emit over 90% of their radiant energy in the mercury resonance line at 2537 Å (*14*). To remove radiation below 2100 Å (*15*) a filter of a 1-cm path of fresh absolute alcohol was employed in all irradiations. The cuvettes were located at a sufficient distance (ca. 20 cm) from the lamp that the irradiating beam was substantially parallel.

For the determination of quantum efficiency, the uranyl oxalate actinometer of Leighton and Forbes (*16*), as modified by Forbes and Heidt (*17*), was used to measure the beam intensity, following the procedure outlined by Bowen (*18*). For the quantum efficiency measurements, the radiation was filtered through a $NiSO_4$-$CoSO_4$ filter (*19*) which effectively transmitted only radiation in the spectral region 2350 to 3400 Å.

All absorption measurements were made in the Beckman model DU spectrophotometer.

RESULTS

Irradiation. The irradiation of uridylic acid *a* or *b* in neutral or acid solution results in the disappearance of the characteristic absorption band peaked at 260 mμ (Fig. 2). Accompanying the disappearance of this band, there is a slight decrease in the shorter wave absorption below 230 mμ, and a slight increase in absorption at wavelengths longer than 295 mμ. This latter effect, which is best observed in concentrated solution (Fig. 3) may represent the emergence of a weak absorption band centered about 300 to 310 mμ.

The kinetics of degradation, when corrected for the decrease in quanta absorbed by the solution (see the Appendix), are first order (Fig. 4), indicating a "one-hit"

[1] The solutions undoubtedly contained dissolved air; however, irradiation of uridylic acid solutions, which had been de-aerated by prolonged flushing with compressed N_2, gave identical results.

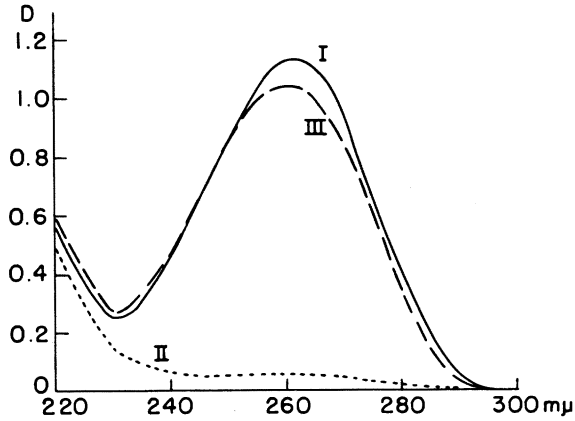

FIG. 2. Effect of irradiation and subsequent addition of acid on uridylic acid *b* spectrum. I, Absorption spectrum before irradiation. II, Absorption spectrum after 40-minute irradiation. III, Absorption spectrum after reversal at pH 0.8 (42 hours at pH 0.8 at 20°C).

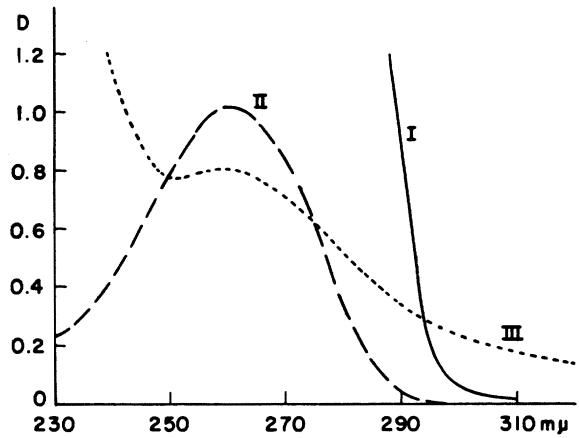

FIG. 3. Increase in absorption of uridylic acid *b* above 290 mμ, produced by irradiation. I, Absorption spectrum before irradiation, 1 mg/ml. II, Absorption spectrum before irradiation, 0.03 mg/ml. III, Absorption spectrum after irradiation, 1 mg/ml.

process. Under similar conditions the rates of degradation are the same for uridylic acid *a* or *b* and the quantum yield is 0.0216 mole/einstein.

Recovery. The product of this ultraviolet irradiation is of an unstable nature and may be caused by various procedures either to revert to the original uridylic acid or to transform to a new substance with an ultraviolet absorption distinct from that of uridylic acid.

If the pH of a neutral solution of irradiated uridylic acid is adjusted to pH 0.8 by addition of HCl, the absorption peak at 260 mμ reappears, returning to 90 to

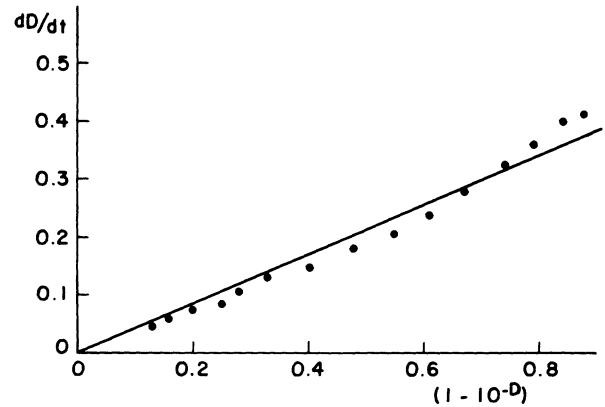

FIG. 4. Plot of the rate of decrease of absorption at 254 mμ of a uridylic acid *b* solution vs. the fraction of incident energy absorbed at 254 mμ ($1 - 10^{-D}$). The straight line indicates a "one-hit" process.

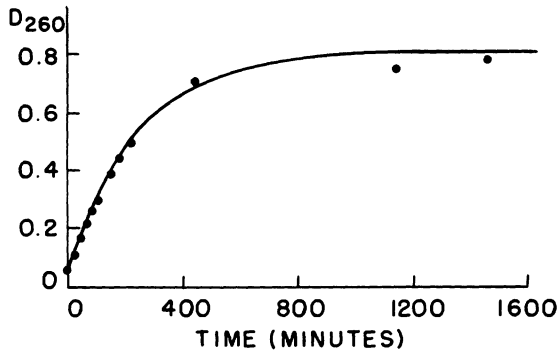

FIG. 5. Recovery of absorption at 260 mμ at room temperature by irradiated uridylic acid *b* when the pH is adjusted to 0.8. Solid line represents the equation

$$D = 0.057 + 0.763(1 - e^{-t/255})$$

100% of its original value (Fig. 2). The kinetics of recovery of the absorption are first order (Fig. 5). The reverted material has an absorption spectrum identical with the initial uridylic acid (*a* or *b* in each case) and migrates with uridylic acid in paper chromatography, using the 5% Na_2HPO_4-amyl alcohol solvent of Carter (*20*).

If a neutral solution of the irradiation product of uridylic acid *a* or *b* is heated at 85° C for 4.5 hours, the absorption peak at 260 mμ gradually reappears, reaching a value 90 to 100% of its original value. Concomitantly the ultraviolet-induced increase in absorption above 295 mμ disappears. The rate of recovery of uridylic acid *b* at 85° C is distinctly faster than that of uridylic acid *a*.

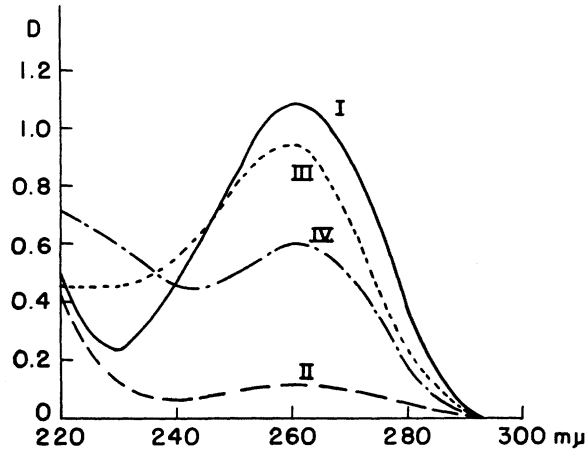

FIG. 6. Recovery of irradiated uridylic acid *b* in alkaline solution. I, Spectrum of uridylic acid *b* at pH 7, before irradiation. II, Spectrum of uridylic acid *b* at pH 7, immediately after irradiation. III, Spectrum of irradiated uridylic acid *b*, 60 minutes after adding alkali to make the solution to *N*/4 NaOH. IV, Spectrum of unirradiated uridylic acid *b* in *N*/4 NaOH, adjusted to same molar concentration as in III.

If a neutral solution of irradiated uridylic acid is made alkaline to pH 13, the ultraviolet absorption at 260 mμ rises abruptly. However, the absorption spectrum of the substance obtained is not that of the original uridylic acid in alkaline solution (Fig. 6).

Stability of the irradiation product. In neutral solution, at room temperature, the irradiation product is not stable but will very slowly revert to the original uridylic acid. The rate is such that approximately 9% of the absorption of an irradiated solution of uridylic acid *b* is recovered in 5 days at room temperature. Studies in solutions of various pH indicate a maximum stability for the product about pH 5. At pH 5.2 the product is stable for at least a week at room temperature. This fact has permitted the separation of the irradiation product of uridylic acid *b* from undegraded uridylic acid *b* by an ion exchange chromatography at pH 5.2 (Fig. 7). The irradiation product may be identified by its absorption at 220 mμ, its lack of absorption at 260 mμ, and the increase in absorption at 260 mμ when its solution is heated at 85° C; it migrates down the column slightly ahead of the undegraded uridylic acid *b*, in almost the same relative position as that of uridylic acid *a*.

Irradiation of related compounds. Irradiation of the pyrimidine base uracil results in a loss of the absorption peak at 260 mμ, with the formation of several products, only one of which is reversible to uracil on acidification or heating (*15*). The quantum yield for the degradation of uracil is 0.0052 mole/einstein, of which the reversible product accounts for approximately half.

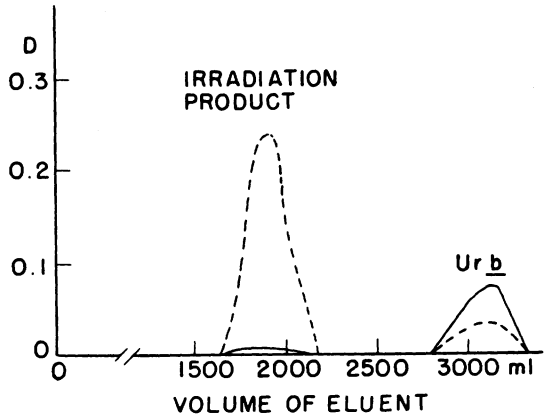

FIG. 7. Chromatographic separation of irradiation product from undegraded uridylic acid *b*. Column: Dowex-1-8x, 10 cm × 1 cm diameter. 9.3 mg irradiated uridylic acid *b* added. Eluent: 0.04 *M* formate buffer, pH 5.2. —— D_{260}. - - - - D_{220}.

Irradiation of the nucleoside uridine yields the same results obtained with uridylic acid. The irradiation product quantitatively recovers its absorption at 260 mμ on acidification or heating. The rate of degradation of uridine is the same as that for uridylic acid *a* or *b*; the rate of recovery is somewhat more rapid than that of the uridylic acids.

Irradiation of thymidylic acid in neutral solution produces a very slow decrease of the ultraviolet absorption peak at 260 mμ without formation of any product reversible to thymidylic acid by the procedures indicated above. It may be estimated that thymidylic acid is about 1/200 as sensitive to irradiation as are the uridylic acids.

DISCUSSION

The nature of the unstable irradiation product of uridylic acid is not known. The most probable reactions which would lead to a loss of the 260-mμ absorption peak would appear to be either a hydrolytic splitting of the pyrimidine ring, or an addition of water across the 5,6 double bond. Chain-like, non-ultraviolet-absorbing molecules are known which will condense readily upon heating (*21*) or in acid (*22*) to form a pyrimidine ring. However, the failure of thymine or of thymidylic acid to form such unstable products, and the corresponding situation with cytidylic and 5-methylcytidylic acids (*23*) suggest that the presence of an unsubstituted 5,6 double bond is essential for this reaction. Saturation of the double bond would be expected to destroy the 260-mμ peak (*24*), and the isolated 2,4 keto groups might then be expected to give rise to a weak band about 300 mμ (*25*). Hydrolytic splitting of the pyrimidine ring with the formation of carboxyl and amine groups

would also be expected to modify the chromatographic behavior of the molecule far more than is observed for the irradiation product.

Uridylic acid is known to occur within cells as a component of certain coenzymes (*26*, *27*) and of ribonucleic acids. The latter are believed to play a role in protein synthesis (*28*); also at least one fraction of ribonucleic acid has been shown to serve as an inhibitor of a cellular deoxyribonuclease, conceivably a very important function (*29*). The formation by ultraviolet irradiation of an altered uridylic acid within one of these substances might significantly affect its function and thereby disturb the cellular behavior. The instability of the irradiation product might account for the reversals of ultraviolet irradiation effects achieved by the various external agents described.

The initial action of the absorbed quantum is undoubtedly an excitation of the uridylic acid molecule to a higher and more reactive energy level. In dilute solution, as employed in this work, the excited molecule will apparently, about one time in forty-six, react with a neighboring solvent molecule. In a ribonucleic acid structure, however, it may well be more likely that the excited molecule will react with a neighboring nucleotide than with the solvent. Preliminary evidence for such a reaction has been obtained on irradiation of uridylic acid in very concentrated solution.

SUMMARY

Upon 2537Å irradiation of uridylic acid *a* or *b*, an essentially quantitative conversion is obtained to an unstable product lacking the characteristic 260-mμ absorption band. The quantum yield for this conversion is 0.0216. The irradiation product can be isolated by ion exchange chromatography. In acid solution, or on heating of a neutral solution, the irradiation product reverts to the original uridylic acid. In alkaline solution the irradiation product is transformed to an unidentified substance.

APPENDIX

If a solution of optical density D at some wavelength (254 mμ) is irradiated with parallel light of that wavelength, the energy absorbed per unit time, I_a, will be

$$I_a = I_0 - I_t = I_0 - I_0(10^{-D}) = I_0(1 - 10^{-D})$$

where I_0 is the incident light intensity and I_t is the transmitted light intensity. Assuming a first-order reaction, constant intensity of illumination, and no absorption by the product of irradiation at the wavelength chosen, the rate of degradation of the absorbing substance, dC/dt, will be proportional to the radiation absorbed per unit time,

$$dC/dt = -kI_a = -kI_0(1 - 10^{-D})$$

and

$$D = k_2 C$$

so

$$\frac{1}{k_2}\frac{dD}{dt} = -kI_0(1 - 10^{-D})$$

or

$$dD/dt = -k_3 I_0(1 - 10^{-D})$$

A graph of dD/dt versus $(1 - 10^{-D})$ should then be a straight line (Fig. 4).

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