Uridine 5'-Phosphate Photohydrate Formation in Irradiated *Escherichia coli* 30S Ribosomes: Photochemistry and Relation to Ribonucleic Acid-Protein Cross-Linking†

Lester Gorelic* and Sydney A. Shain

ABSTRACT: Irradiation of aqueous buffered solutions of *Escherichia coli* 30S ribosomes with doses of 254-nm radiation greater than 10^{19} quanta causes formation of uridine 5'-phosphate (UMP) photohydrates in ribosomal 16S RNA (rRNA). The number of molecules of UMP photohydrate formed at doses less than 2×10^{20} quanta is linearly dependent on dose of absorbed 254-nm radiation. Maximum UMP photohydrate formation is dependent on initial ribosome concentration. When solutions containing 1 A_{260} unit of 30S ribosomes/mL were irradiated with greater than 2×10^{20} quanta of 254-nm radiation, maximum photohydrate formation was equal to 47 residues/ribosome. Irradiation of solutions containing 2 A_{260} units/mL with greater than 7×10^{20} quanta caused formation of 102 UMP photohydrates/ribosome. These values correspond to conversion of either 15 or 33%, respectively, of the total UMP content of 30S ribosome 16S rRNA to photohydrates. Target theory analysis of UMP photohydration in 30S ribosomes showed that UMP photohydrates are formed by single-hit kinetics from two photochemically distinct precursors. Of the total 16S rRNA UMP residues, 10% was included in the most rapidly (low dose) reacting fraction. The respective photohydration cross sections are 0.014 (low dose) and 0.0095 cm^2/μEinstein (high dose) for ribosome solutions containing 2 A_{260} units/mL. UMP photohydrate content of irradiated 30S ribosomes was compared with that of previous data for the extent of RNA-protein cross-linking at equivalent doses of absorbed 254-nm radiation. This comparison showed that at least two UMP photohydrates form per RNA-protein cross-linking event in 30S ribosomes irradiated with a dose of 254-nm radiation (1.5×10^{19} quanta), which causes cross-linking of only three ribosomal proteins to 16S rRNA.

Irradiation of ribosomes with 254-nm radiation causes covalent cross-linking of rRNA[1] and ribosomal proteins (Gorelic, 1975a,b; Moller & Brimacombe, 1975; Turchinsky et al., 1978; Reboud et al., 1978, 1980; Buisson et al., 1979; Terao et al., 1980). This photoreaction has been used to identify RNA-binding ribosomal proteins in prokaryote and eukaryote ribosomes (Gorelic, 1976a,b; Reboud et al., 1978, 1980; Buisson et al., 1979) and to chemically characterize RNA-protein binding sites in ribosomes and derived ribonucleoprotein complexes (Rinke et al., 1976; Moller et al., 1978; Zweib & Brimacombe, 1979; Ehresmann et al., 1980; Maly et al., 1980).

Assignment of RNA-protein interactions by photochemical cross-linking could be compromised (1) by photoreactions that compete with or inhibit formation of cross-links at "native" RNA-protein interaction sites or (2) by photoinduced alterations in ribosome organization abolishing native rRNA-protein binding sites or generating artifactual "new" rRNA-protein binding sites. Potentially compromising photoreactions would include pyrimidine photohydration and photodimerization, deamination of cytosine photohydrates to uracil photohydrates, and rRNA chain breakage. Chain breaks have been identified in rRNA of *Escherichia coli* ribosomes following irradiation at 254 nm (Gorelic & Parker, 1978). Pyrimidine photohydrates and photodimers have been identified in ultraviolet-irradiated synthetic ribopolymers, viral RNA, and intact RNA viruses. These photoproducts have been implicated as causative factors in ultraviolet-mediated alterations in RNA secondary structure (Pearson & Johns, 1966; Gordon et al., 1976). Moreover, uracil photohydrates or photodimers could not participate in the photoaddition reactions required for the production of RNA-protein cross-links. Consequently their formation could eliminate potential sites of cross-linking of ribosomal proteins to complementary rRNA binding sites. Spontaneous deamination of cytosine photohydrate to uracil photohydrate occurs to the extent of 10% in irradiated cytosine solutions (Becker et al., 1967). If a similar C to U transition occurs in base-paired rRNA during irradiation, localized denaturation could occur and either eliminate native rRNA-protein binding sites that might otherwise have undergone cross-linking or generate new cross-linking sites.

In this report we examine the contribution of UMP photohydration to 30S ribosome photochemistry. Additionally, a preliminary assessment has been made of the extent to which UMP photohydration may compromise formation of covalent rRNA-protein cross-links in irradiated *E. coli* 30S ribosomes.

Experimental Procedures

Materials. Sodium [^{3}H]borohydride (sp act. 450-500 Ci/mol), [2-^{14}C]uracil (sp act. 50 Ci/mol), and [5,6-^{3}H]-uridine (sp act. 40 Ci/mmol) were obtained from Amersham/Searle. Proteinase K was from Boehringer-Mannheim and sodium borohydride was from Alpha Inorganics. Potassium ferrioxalate was prepared according to the procedure of Parker (1953). 1,3-Propanediol (Eastman, white label) was from Fisher Scientific, benzophenone (mp 48-49 °C) was from Matheson Coleman and Bell, and silica gel H (TLC grade) was from E. M. Merck. Dowex 50 W-X8 (H^+) and Dowex 1-X10 (OH^-), both 100-200 mesh, and Bio-Gel HT hydroxylapatite were obtained from Bio-Rad Laboratories.

[1,2-^{3}H]Propane-1,3-diol was prepared from [5,6-^{3}H]uridine photohydrate by reaction with sodium borohydride (Cerutti et al., 1969). [2-^{14}C]Uracil-containing 30S ribosomes (16S

† From the Department of Cellular and Molecular Biology, Southwest Foundation for Research and Education, San Antonio, Texas 78284. *Received June 5, 1981.* This study was supported by National Science Foundation Grant PCM 7923950.

[1] Abbreviations: U, uracil; C, cytosine; UMP, uridine 5'-phosphate; CMP, cytidine 5'-phosphate; UMP·H_2O, uridine 5'-phosphate photohydrate; poly(U), poly(uridylic acid); poly(A), poly(adenylic acid); RNA, ribonucleic acid; rRNA, ribosomal ribonucleic acid; $NaDodSO_4$, sodium dodecyl sulfate; TLC, thin-layer chromatography; UV, ultraviolet; SD, standard deviation.

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rRNA labeled) were isolated from *E. coli* D10 grown to midlog phase at 30 °C in complete medium supplemented with 5 μg/mL uracil and 0.35 μCi/mL [2-^{14}C]uracil and purified as previously described (Gorelic & Parker, 1978). Purified 30S ribosomes contained 2700 cpm of $^{14}C/A_{260}$ unit.

Irradiation Conditions. ^{14}C-Labeled 30S ribosomes (110–120 mL, 1 or 2 A_{260} units/mL) were prepared in irradiation buffer (5 mM H_3PO_4, neutralized to pH 7.2 with KOH, 10 mM $MgCl_2$, and 50 mM KCl) and irradiated with 254-nm radiation at 22 °C as previously described (Gorelic & Parker, 1978). The average intensity of 254-nm radiation (1.51×10^{18} quanta/s) to which the initial volume of ribosome solution was exposed was determined by ferrioxalate actinometry (Parker, 1953). The fraction of incident radiation absorbed by the ribosome solutions, determined by uridine actinometry, was 0.220 and 0.403 for 1 A_{260} unit/mL and 2 A_{260} units/mL ribosome solutions, respectively. Uridine is converted to uridine photohydrate by 254-nm radiation with a quantum efficiency of 0.017 (Swenson & Setlow, 1963).

Analysis of UMP Photohydrate Content of Irradiated Samples. Aliquots (2–10 mL) were removed and deproteinized by incubation for 20 min at 30 °C with proteinase K (final concentration 200 μg/mL, preincubated to destroy residual nucleases). The initial 10 min of incubation was in the absence of $NaDodSO_4$, whereas the final 10 min of incubation was in the presence of $NaDodSO_4$ (2 mg/mL). After incubation, samples were cooled to 0 °C and clarified by low-speed centrifugation, and the clarified supernatant was concentrated by ultrafiltration (Amicon type CF-25 Centriflo ultrafiltration cones). Retentates were removed, cones were washed with a small volume of 5% NaCl, the wash and retentate were combined, and the volume of each sample was adjusted to 0.5 mL by addition of 5% NaCl. An aliquot of this solution was used to quantitate [^{14}C]RNA recovery.

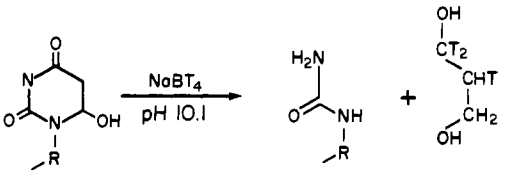
Aliquots (0.2 mL) of the deproteinized, concentrated solutions were adjusted to pH 10.1 by addition of 0.1 M Na_2CO_3, and UMP photohydrates were converted to 1,3-propanediol by reaction with alkaline $NaBT_4$ (Cerutti et al., 1969). We used a modification of the procedure of Cerutti et al. (1969) to isolate and quantitate radiolabeled 1,3-propanediol. At the conclusion of the $NaBT_4$ reaction (16 h at 10 °C) unreacted $NaBT_4$ was decomposed by adjusting the solution pH to 2.0 with 0.1 M HCl, followed by titration to pH 6.0–7.0 with 0.1 M Na_2CO_3. 1,3-Propanediol (20 μL, 50% aqueous solution) was added as carrier and radiolabeled 1,3-propanediol isolated by chromatography of the neutralized solution on a mixed-bed ion-exchange column. Aqueous columns were prepared in 5.0-mL disposable plastic syringes and contained (bottom to top) layers composed of 0.8 mL of hydroxylapatite, 1.0 mL of Dowex 50 W-X8 (H^+), and 1.0 mL of Dowex 1-X10 (OH^-). A 0.4-mL layer of sand was used to separate each column component. Samples were eluted with water, 0.8-mL fractions were collected, and 10-μL aliquots were removed for quantitation of radioactivity.

Fractions were pooled to contain greater than 90% of the eluted radioactivity, and the 1,3-propanediol content was assayed by thin-layer chromatography (TLC) on 1-mm 20 × 20 cm silica gel H plates, which had been activated by heating at 130 °C for 60 min. Aliquots containing equal amounts of radioactivity were applied to each plate. Control samples prepared from nonirradiated ribosomes, which otherwise had been identically treated, were applied to each TLC plate for determination of background radioactivity. Authentic tritiated 1,3-propanediol was applied to each plate as a tracking marker. After sample application, plates were stored overnight in a desiccator over $CaCl_2$. Control studies showed that volatile background radioactivity was reproducibly reduced to a constant level without detectable loss of 1,3-propanediol. The plates were subsequently developed with $CHCl_3$–CH_3OH (18:5 v/v), and the lanes were divided into 1-cm increments and scraped into vials containing 0.5 mL of methanol. These suspensions were incubated at room temperature for 60 min, 5 mL of Amersham/Searle ACS liquid scintillation fluid was added, and sample radioactivity was determined in a Beckman liquid scintillation counter. Active tritium and $NaBH_4$ contents of the alkaline $NaBT_4$ solutions used for reduction were determined by reaction with ethanolic benzophenone and quantitation of the tritium content of the resultant benzhydrol product after purification by TLC (Eastman silica gel G TLC sheets) with $CHCl_3$ (see paragraph at end of paper regarding supplementary material).

Efficiency of the $NaBT_4$ Reduction of UMP Photohydrates. UMP photohydrates were prepared by 254-nm irradiation of poly(uridylic acid) (10 mg, 24.7 μmol of UMP) in 100 mL of irradiation buffer by using the procedures described for irradiation of *E. coli* 30S ribosome 16S rRNA. A 10-min irradiation caused a 67% decrease in the 260-nm absorbance. UMP photohydrate content was quantitated by determining the fraction of products labile to dehydration at 50 °C or by quantitation of 1,3-propanediol formed upon $NaBT_4$ reduction. The efficiency of the $NaBT_4$-reduction reaction was then calculated as the ratio of UMP photohydrates quantitated by the $NaBT_4$ reduction procedure/UMP photohydrates quantitated by the thermal reversal procedure. This analysis was performed for two separate irradiations for which the thermal reversal was done in duplicate and the $NaBT_4$ reduction was done in triplicate.

Results

UMP photohydrate content of polyribonucleotides is quantitated by conversion to radiolabeled 1,3-propanediol by reaction with $NaBT_4$. This reaction causes reductive cleavage of UMP photohydrates of I in ribopolymers to tritiated pro-



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panediol (II) with incorporation of three atoms of tritium per molecule of original photohydrate.

We determined the efficiency of $NaBT_4$ reduction of UMP photohydrate to 1,3-propanediol to be 60 ± 8% (mean ± SD) for our experimental conditions. We also determined that the reduction of benzophenone with $NaBH_4$ (0.5 molar excess of $NaBH_4$) was quantitative, whereas for our experimental conditions (equivalent $NaBH_4$ and benzophenone concentration), the reaction only proceeded to 78 ± 8% (mean ± SD) completion. All UMP photohydration data presented in this report consequently have been corrected to reflect the efficiency of $NaBT_4$ reduction under our conditions. The correction was achieved by multiplying observed data by the factor 2.317 [1/[(0.60)(0.78)]].

Representative chromatograms of samples derived from irradiated ribosomal solutions are presented in Figure 1. The data show that most of the $NaBT_4$-derived radioactivity in the assayed samples chromatographs as a single peak with an R_f identical with that of authentic 1,3-propanediol (diol). The amount of diol obtained increases with increasing time of

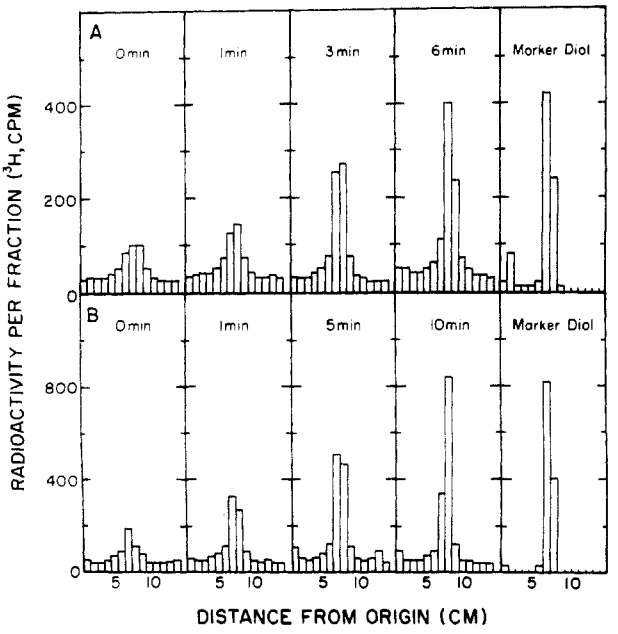

FIGURE 1: Relationship between irradiation interval or 30S ribosome concentration and the yield of tritiated propanediol. Solutions of 30S ribosomes, 1 A_{260} unit/mL (A) or 2 A_{260} units/mL (B), were irradiated at 254 nm, and aliquots containing 0.45 A_{260} unit (A) or 0.90 A_{260} unit (B) were removed from the vessel after the indicated irradiation interval. Samples were reduced with 50 μmol of $NaBT_4$ containing (1.6–1.67) $\times$ 10^6 cpm/μmol. Prepared samples containing 3 $\times$ 10^5 cpm of ^{3}H were applied as 4-cm streaks to silica gel H plates and analyzed for tritium content by the procedures described under Experimental Procedures. Authentic radiolabeled 1,3-propanediol (marker diol) was applied to each plate as a reference. Actual tritium content of diol marker samples was 10 times the value shown in the figure.

irradiation (Figure 1). The ratio of diol:background radioactivity is 0.19:1, 1.09:1, and 1.61:1 for samples from solutions of 30S ribosomes at 1 A_{260} unit/mL irradiated for 1, 3, and 6 min, respectively (Figure 1A). When solutions of 30S ribosomes at 2 A_{260} units/mL were irradiated (254 nm) and processed as described, the ratio of diol:background radioactivity was 0.82:1, 1.82:1, and 2.26:1 for samples from solutions irradiated for 1, 5, and 10 min, respectively (Figure 1B).

The relationship between UMP photohydrate content of irradiated 30S ribosomes and the dose of absorbed 254-nm radiation or initial ribosome concentration is shown in Figure 2. The formation of UMP photohydrates is a linear function of dose of absorbed 254-nm radiation over the range of (0–2) $\times$ 10^{20} quanta (1 A_{260} unit/mL) or (0–5.5) $\times$ 10^{20} quanta (2 A_{260} units/mL). The correlation coefficients for regression analysis of the linear portions of the plots in Figure 2 were 0.98–0.99. The slopes of the high- and low-dose regions, respectively, are 2.74 $\times$ 10^{-21} and 8.16 $\times$ 10^{-22} μmol/quanta (1 A_{260} unit/mL) and 2.24 $\times$ 10^{-21} and 7.4 $\times$ 10^{-22} μmol/quanta (2 A_{260} units/mL). These data (Figure 2) also show that UMP photohydrate formation is independent of initial 30S ribosome concentration over a dose range of (0–1.2) $\times$ 10^{20} quanta of 254-nm radiation.

The data of Figure 2 are replotted in Figure 3 as UMP photohydrates formed per 30S ribosome. The slopes of the regressed lines and the y intercepts of the low-dose regions of the plots are 1.94 $\times$ 10^{-19} photohydrates quanta^{-1} ribosome^{-1} and 4.69 (1 A_{260} unit/mL) and 8.82 $\times$ 10^{-20} photohydrates quanta^{-1} ribosome^{-1} and 2.64 (2 A_{260} units/mL). The ratio of the slopes of these lines (Figure 3) is 2.20 and, within error, is the expected value if UMP photohydrates are forming at the same rate per quanta in solutions differing in initial ribosome concentration by a factor of 2. These data show that 9 and 19 UMP photohydrates form per 30S ribosome per 10^{20} quanta of absorbed 254-nm radiation in ribosome solutions of 2 and 1 A_{260} units/mL, respectively. The data (Figure 3) also show that 47 $\pm$ 8 (mean $\pm$ range) and 102 $\pm$ 14 UMP photohydrates are formed in heavily irradiated ribosome solutions of 1 and 2 A_{260} units/mL, respectively. On the basis of a value of 308 for UMP content of *E. coli* 30S ribosome bound 16S rRNA calculated from the data of Spahr & Tissieres (1959) and Carbon et al. (1979), our data demonstrate an apparent maximum conversion of 15 $\pm$ 3% (mean $\pm$ range), 1 A_{260} unit/mL, and 33 $\pm$ 5%, 2 A_{260} units/mL, of the total 16S rRNA UMP residues to UMP photohydrates.

Target theory analysis shows there are two linear components to the photohydration reaction occurring in ribosome solutions containing either 1 or 2 A_{260} units/mL. The low-dose components, extending over a dose range of (0–1.2) $\times$ 10^{20} quanta (Figure 4A) or (0–5.5) $\times$ 10^{20} quanta (Figure 4B),

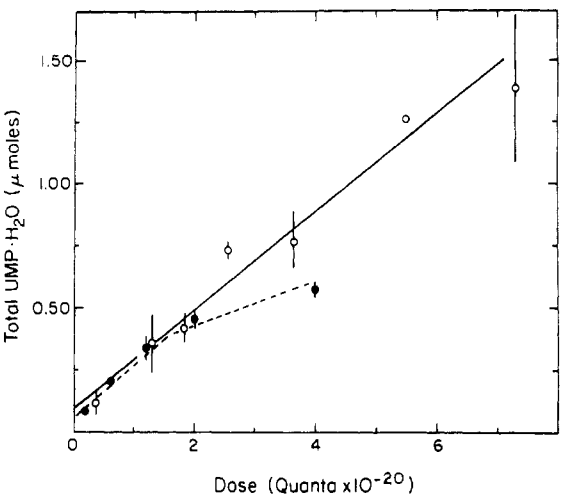

FIGURE 2: Relationship between dose of 254-nm radiation or 30S ribosome concentration and uridine photohydrate (UMP·H_2O) formation. 30S ribosome solutions of 1 A_{260} unit/mL (●) and 2 A_{260} units/mL (○) were irradiated and analyzed for UMP·H_2O content. Observed data for UMP·H_2O formation were corrected for the effects of sample removal upon the amount of radiation absorbed during the irradiation interval. The data for (●) are the result of a single irradiation performed in duplicate, whereas the data for (○) are the result of two independent irradiations. Data are plotted as mean $\pm$ range.

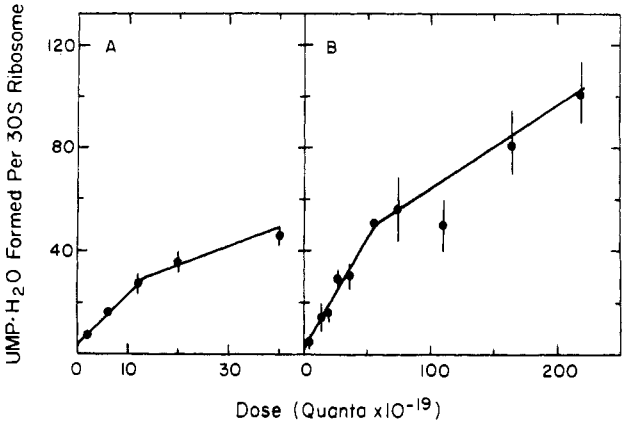

FIGURE 3: Relationship between dose of 254-nm radiation or ribosome concentration and uridine photohydrate (UMP·H_2O) formation per ribosome. The data of Figure 2 were recalculated by using the relationship 1 A_{260} unit/mL = 60 μg of RNA and a molecular weight of 5.5 $\times$ 10^5 for 16S rRNA. The absence of a range bar indicates that the data are from a single determination. (A) 1 A_{260} unit/mL of 30S ribosomes. (B) 2 A_{260} units/mL of 30S ribosomes.

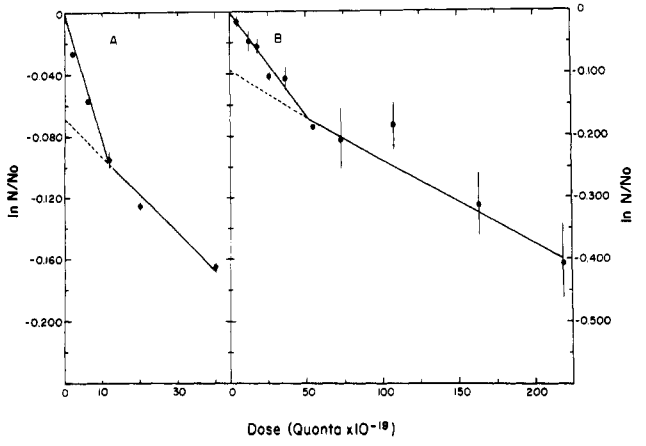

FIGURE 4: Semilogarithmic plot of the fraction (N/N_0) of total 30S ribosome 16S rRNA uracil residues that have not undergone photohydration vs. dose of absorbed 254-nm radiation. Data are the mean ● range for two determinations. Dashed lines are the extrapolated least-squares fit for the linear portions of the plots. The absence of a range bar indicates that the data are from a single determination. (A) 1 A_{260} unit/mL of 30S ribosomes. (B) 2 A_{260} units/mL of 30S ribosomes.

have regression coefficients of −0.9973 and −0.98404, respectively. The high-dose components, beginning at doses greater than 1.2×10^{20} quanta (Figure 4A) and greater than 5.5×10^{20} quanta (Figure 4B), have regression coefficients of −0.9850 and −0.9957, respectively.

Two-component plots, such as those of Figure 4, for which the low-dose components have y intercepts of zero are characteristic of photoproduct formation from two photochemically distinct precursors. The fraction of total precursor population corresponding to the high-dose reactive component is graphically determined as the zero-dose intercept of the linear portion of the plot for this component. The fraction corresponding to the low-dose component is calculated as 1 minus the value for the high-dose component. Photohydration cross section for the high-dose component is determined from the slope of the plot for this component. The corresponding parameter for the low-dose component is evaluated as the slope of the plot for the low-dose region after correction for the contribution from the high-dose component (Rupert & Harm, 1966).

When solutions containing 1 A_{260} unit/mL are irradiated with 254-nm radiation, the photohydration cross section and the reactive fraction for the high-dose reactive component, respectively, are 2.46×10^{-22} quanta^{-1} (0.018 cm^2/μEinstein) and 94% (Figure 4A), whereas the respective values for the low-dose reactive component are 5.05×10^{-22} quanta^{-1} (0.036 cm^2/μEinstein) and 6% (Figure 4A). When solutions containing 2 A_{260} units/mL are irradiated with 254-nm radiation, the photohydration cross section and reactive fraction for the high-dose reactive component, respectively, are 1.33×10^{-22} quanta^{-1} (0.0095 cm^2/μEinstein) and 90% (Figure 4B), whereas the respective values for the low-dose reactive component are 1.89×10^{-22} quanta^{-1} (0.014 cm^2/μEinstein) and 10% (Figure 4B).

Discussion

Ribosome Photochemistry. We used an established assay procedure (Cerutti et al., 1969) to demonstrate the formation of UMP photohydrates in the 16S rRNA component of *E. coli* 30S ribosomes irradiated with 254-nm radiation. We showed that UMP photohydrates form from two photochemically distinct precursors by a process involving single-hit kinetics (Figure 4). The initial rate of formation of UMP photohydrates was linearly related to the dose of absorbed 254-nm radiation and was independent of 30S ribosome concentration (Figure 2).

The photohydration cross sections for UMP photohydrate formation from the major (high-dose) and minor (low-dose) precursors in *E. coli* 30S ribosomes, respectively, were 0.0095 and 0.014 cm^2/μEinstein (2 A_{260} units/mL) or 0.018 and 0.036 cm^2/μEinstein (1 A_{260} unit/mL). These data imply that photohydration cross sections for the minor and major precursors are dependent on initial 30S ribosome concentration. This observation is not the result of a dependence of the quantum efficiency of UMP photohydration on 30S ribosome concentration. The quantum efficiencies for UMP photohydration, as calculated from the slopes of the regressed lines in the high-dose regions in Figure 2, are 4.92×10^{-4} molecules/quanta (1 A_{260} unit/mL) and 4.47×10^{-4} molecules/quanta (2 A_{260} units/mL). After correction for the contribution from the high-dose component, the quantum efficiencies for UMP photohydration, as calculated from the slopes of the regressed lines for the low-dose regions in Figure 2, are 1.16×10^{-3} molecules/quanta (1 A_{260} unit/mL) and 8.99×10^{-4} molecules/quanta (2 A_{260} units/mL). Because photohydration cross section is the product of the quantum efficiency and the absorption cross section of the reacting molecule, it is possible that the apparent concentration dependent change in photohydration cross section is a consequence of changes in the effective absorption cross section of the UMP photohydrate precursors.

The value of 0.0095 cm^2/μEinstein, which we determined to be the photohydration cross section for the major precursor in *E. coli* 30S ribosomes, is comparable to the reported value of 0.008 cm^2/μEinstein for the photohydration cross section of UMP residues in base-paired poly(A)·poly(U); however, it is only 11% of the reported photohydration cross section of 0.085 cm^2/μEinstein for UMP residues in single-stranded poly(U) (Pearson & Johns, 1966). These comparisons suggest that UMP photohydrates principally form from precursors in base-paired regions of *E. coli* 30S ribosome 16S rRNA. However, other explanations are possible. For example, interaction of 16S rRNA with 30S ribosome components could effect a reduction in UMP sensitivity toward photohydration independent of 16S rRNA higher order structure. Additionally, molecular species other than UMP residues could be precursors of UMP photohydrates, and these precursors could be converted to UMP photohydrate with an overall lower efficiency than UMP. One such precursor could be CMP photohydrate (Becker et al., 1967).

E. coli 30S ribosome 16S rRNA showed limited reactivity for UMP photohydrate formation. Maximally, 15–33% of the total 16S rRNA UMP residues were converted to UMP photohydrates after extensive irradiation (2.3×10^{21} quanta). It is unlikely that the indicated resistance of UMP toward photohydration is a result of exclusion of water from the 30S ribosome interior. The hydrodynamic properties of *E. coli* 30S ribosomes have been interpreted to be representative of a fully hydrated nucleoprotein complex (Van Holde & Hill, 1974). It is also unlikely that underestimation of photohydrate formation has occurred because of inaccessibility of UMP photohydrates for reaction with $NaBT_4$ since the RNA was extensively deproteinized prior to reduction with $NaBT_4$. It appears probable that the indicated limited reactivity of 30S ribosome 16S rRNA for photohydration of UMP is a result of effects of ribosome structure on UMP photochemistry. Consistent with this conclusion is the observed enhanced yield of UMP photohydrates, which characterized prolonged irra-

diation. Prolonged irradiation would increase secondary photochemical reactions (Gorelic & Parker, 1978) that may enhance susceptibility of UMP residues to photohydration as a consequence of alterations in ribosome structure.

Relationship of UMP Photohydration to RNA–Protein Cross-Linking. Irradiation of *E. coli* 30S ribosomes with an absorbed dose of 1.5×10^{19} quanta of 254-nm radiation causes covalent cross-linking of ribosomal proteins S7, S17, and S20 to 16S rRNA. The extent of cross-linking is 100% for protein S7, 30% for protein S17, and 50% for protein S20 (Gorelic, 1976a). Our current data demonstrate the formation of 2.65–2.90 UMP photohydrates/ribosome irradiated with 1.5×10^{19} quanta of 254-nm radiation. If it is assumed that each of the three 30S ribosomal proteins is cross-linked to 16S rRNA by a single covalent bond, it can be calculated that there will be 27–29 UMP photohydrates and 18 cross-links formed per 10 30S ribosomes. Our data therefore suggest that UMP photohydrate foramtion occurs almost twice as frequently as RNA–protein cross-link formation during 254-nm irradiation.

The extent to which UMP photohydrate formation, in particular, and pyrimidine photohydration, in general, contribute to 30S ribosome photochemistry may be significantly greater than that estimated by the current determinations. UMP photohydrates undergo slow spontaneous dehydration to the parent nucleotide (Fisher & Johns, 1976), and such losses may have occurred during quantitation of 30S ribosome UMP photohydrates. Additionally, CMP photohydrates may be precursors of UMP photohydrates quantitated in 30S ribosomes. Because model studies with cytidine photohydrate indicate only a 10% maximum conversion to uracil photohydrate (Becker et al., 1967), the total pyrimidine photohydrate content of irradiated 30S ribosomes may be significantly underestimated. These considerations imply that the rate of photohydration of 254 nm irradiated ribosomes may be significantly underestimated. If ribosomal proteins are cross-linked to 16S rRNA by a single photochemical event, our analysis indicates that the actual rate of photohydration may substantially exceed the rate of photochemical cross-linking.

Photohydration may alter RNA secondary and/or tertiary structure as a result of disruption of A–U or G–C hydrogen-bonded base pairs. We conclude that significant potential for the introduction of bias in the identification of ribosomal RNA binding proteins exists, particularly if cross-linking of ribosomal proteins to ribosomal 16S rRNA is the consequence of a single photochemical event. The extent to which photohydration may potentially compromise the utility of the cross-linking reaction as a probe of ribosome structure may be assessed only by rigorous quantitation of the rates of ribosomal 16S rRNA photohydration and cross-linking. This requires quantitation of the number of covalent bonds formed per protein cross-linked to ribosomal 16S rRNA, assessment of the efficiency of detection of UMP photohydrates, and evaluation of the possible contribution of CMP photohydrates to measured photohydration products.

Acknowledgments

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Supplementary Material Available

Derivation and application of relationships used to correct the experimentally determined yield of UMP photohydrates for the effects of sample removal and changes in irradiated solution volume (7 pages). Ordering information is given on any current masthead page.

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