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Studies of the Effects of Ultraviolet Radiation on the Structural Integrities of Ribosomal RNA Components of the *Escherichia coli* 50S Ribosomal Subunit†

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ABSTRACT: The effects of 254-nm radiation on the structural integrities of free and 50S ribosome-bound 5S and 23S ribosomal ribonucleic acids (rRNA) have been elucidated. Irradiation of aqueous solutions of *Escherichia coli* 50S ribosomes with 253.7-nm radiation results in the formation of single-strand breaks in double-stranded regions of the 23S rRNA component, but not in rRNA chain scission, and destabilization of the secondary structure of the 23S rRNA toward denaturation. The minimum doses of 253.7-nm radiation required for the first detection of the two effects are 7×10^{19} quanta for the production of single-strand breaks in double-stranded regions of the 23S rRNA, and $\leq 2.3 \times 10^{19}$ quanta for destabilization of the 23S rRNA secondary structure. Free 23S rRNA is resistant toward photoinduced chain breakage at doses of 253.7-nm radiation up to at least 2.3×10^{20} and is much less sensitive toward destabilization of secondary structure than ribosome-bound 23S rRNA. In contrast to the photosensitivity of 50S ribosome-bound 23S rRNA toward chain breakage, 50S ribosome-bound 5S rRNA is resistant toward chain breakage at doses of 253.7-nm radiation up to at least 2.3×10^{20} quanta. Ribosome-bound 5S and 23S rRNA are also not photosensitive toward intermolecular 5S/23S rRNA cross-linkage.

Recent photochemical studies of *Escherichia coli* 30S ribosomes have indicated that exposure of aqueous buffered solutions of 30S ribosomes to 254-nm radiation results in the introduction of chain breaks into the 16S rRNA[1] component (Gorelic, 1976a,b). Two types of chain breaks were identified in these studies. One type of chain break, designated chain nicks, corresponds to single-strand breaks in base-paired regions of the 16S rRNA and is first introduced into the 16S rRNA at a dose of 254-nm radiation of 6×10^{19} quanta. The second type of chain break is designated chain scission and corresponds to single-strand breaks in single-stranded regions or double-strand breaks in double-stranded regions of the 16S rRNA. This second type of chain break is first introduced into the 16S rRNA at doses of 254-nm radiation of ca. 3×10^{20} quanta. In contrast to the results obtained with 30S ribosome-bound 16S rRNA, RNA chain breaks were not detected in 16S rRNA irradiated in the free state with doses of 254-nm radiation up to and including 3×10^{20} quanta.

The *E. coli* 50S ribosome, like the 30S ribosome, is a macromolecular complex constituted of RNA and protein molecules. It would therefore be reasonable to expect that irradiation of aqueous buffered solutions of 50S ribosomes with 254-nm radiation might result in changes in the structural integrities of the rRNA components of the 50S ribosome analogous to the changes that have been reported for the 16S rRNA component of UV-irradiated 30S ribosomes. There are reasons to believe, however, that the sensitivities of the rRNA components of *E. coli* 30S and 50S ribosomes toward UV-mediated changes in their structural integrities could differ in at least two respects. The recent results of neutron scattering studies of rRNA and protein distributions in the *E. coli* ribosomes suggest that there are substantially more RNA–RNA type interactions in the *E. coli* 50S ribisome than in the 30S ribosome (Moore et al., 1974; Stührmann et al., 1976). Although the molecular natures of the photophysical processes responsible for UV-mediated RNA chain breakage have not been identified, it is likely that either RNA–RNA or RNA–protein interactions are involved in these photoprocesses. Consequently, a *first* difference might be that the rRNA components of the *E. coli* 50S ribosome could be substantially more sensitive than the 16S rRNA of 30S ribosomes toward UV-mediated chain breakage if RNA–RNA type interactions are primarily responsible for chain breakage. The *E. coli* 50S ribosomes contain, in addition to their protein complement, two rRNA species, whereas there is only a single 16S rRNA species in the *E. coli* 30S ribosome. The results of studies of pyrimidine photochemistry in aqueous solutions have indicated that UV irradiation of appropriate mixtures of pyrimidines can

† From the Department of Chemistry, Wayne State University, Detroit, Michigan 48202. *Received September 15, 1977; revised manuscript received April 3, 1978.* This work was supported by National Cancer Institute Grant No. CA 18046 from the National Institutes of Health and United States Public Health Service and Career Development Award No. CA 70999.

[1] Abbreviations used: Me_2SO, dimethyl sulfoxide; tRNA and rRNA, transfer and ribosomal ribonucleic acids, respectively; poly (U), poly(uridylic acid); Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; UV, ultraviolet; CD, circular dichroism; ORD, optical rotatory dispersion; T_m, melting temperature.

0006-2960/78/0417-3152$01.00/0

result in the formation of mixed pyrimidine-pyrimidine photoadducts (Varghese, 1971a,b). It would be reasonable to expect that interstrand photoadducts of these types might also form in mixtures of UV-irradiated polynucleotides and nucleic acid molecules, if the reactive bases in these macromolecules were accessible to each other for covalent bond formation. Consequently, a *second difference* might be the ability of only the *E. coli* 50S ribosomes to participate in the UV-mediated formation of intermolecular RNA–RNA cross-links.

Based upon the conclusions of the above discussions, it seems reasonable to expect that an analysis of the effects of ultraviolet radiation on the structural integrities of the 5S and 23S rRNA components of the *E. coli* 50S ribosomes could provide an insight into the basic nature of the interactions responsible for the previously noted sensitivity of ribosome-bound rRNA toward UV-mediated chain breakage. In addition, since UV-mediated interstrand cross-linkage of the 5S and 23S rRNA components of the *E. coli* 50S ribosome would require the interaction of these two rRNA species in the native topographical state of the ribosome, the indicated analyses could also provide an indication of the relative spatial orientations of the 5S and 23S rRNA components of the *E. coli* 50S ribosomes.

The effects of 254-nm radiation on the structural integrities of the rRNA components of intact *E. coli* 50S ribosomes have been determined in this report. The resultant data have been discussed in terms of the aspects of *E. coli* 50S ribosome topography described above. These data have also been discussed in terms of the contributions the indicated modifications in rRNA structural integrity might make to photoinactivation of ribosome function.

Materials and Methods

Pronase B was obtained from Calbiochem. Sodium lauryl sulfate was obtained from Schwarz/Mann and was recrystallized from ethanol prior to use. Bentonite and urea (Reagent grade) were obtained from Fisher Scientific. Radioactively labeled pyrimidine and purine bases were obtained from Schwarz/Mann. The specific activities of the purine and pyrimidine bases were as follows: [5,6-^{3}H]uracil, 40 Ci/mmol; [5-^{3}H]cytosine, 26 Ci/mmol; [2-^{3}H]adenine hydrochloride, 21 Ci/mmol; [8-^{3}H]guanine, 6 Ci/mmol. Potassium ferrioxalate was prepared according to the procedure of Hatchard & Parker (1956). Bio-Gel A-5.0m (200–400 mesh) was obtained from Bio-Rad Laboratories. Sephacryl S-200 superfine and Sepharose CL-4B were obtained from Pharmacia. [Sephacryl S-200 superfine is a cross-linked dextran with an exclusion limit of 200 000 daltons. Sepharose CL-4B is a cross-linked Sepharose with an exclusion limit of 20×10^6 daltons (proteins) and 5×10^6 daltons (polysaccharides). Both types of gel filtration media are stable in the presence of organic solvents and strongly denaturing media].

Bentonite was prepared for use according to the procedure of Frankel-Conrat et al. (1961). Urea solutions were treated with bentonite prior to use in order to remove contaminating ribonuclease(s). Sepharose CL-4B and Sephacryl S-200 were pretreated with 0.2 and 2 M potassium hydroxide, respectively, for the same reason.

Ribosomal subunits, radioactively labeled in their rRNA components with tritium, were prepared from *E. coli* D10 cells grown in complete media supplemented with radioactively labeled purines and pyrimidines and harvested in midlog phase, according to previously published methods (Gorelic, 1975). The growth media contained, per liter: Difco nutrient broth, 8 g; HyCas SF (Humko Sheffield), 8 g; [5,6-^{3}H]uracil, 350 μCi; [5-^{3}H]cytosine, 350 μCi; [2-^{3}H]adenine, 350 μCi; [8-^{3}H]guanine, 700 μCi. Radioactively labeled cells were mixed with an equal weight of unlabeled *E. coli* D10 cells prior to isolation and purification of the individual ribosomal subunits. Mixtures of ^{3}H-labeled 5 and 23S rRNA were prepared from ^{3}H-labeled 50S ribosomes according to a previously published procedure (Hochkeppel et al., 1976) and further purified by treatment with Proteinase K followed by gel filtration on a Sephacryl S-200 column. Control studies indicated that rRNA samples prepared in this manner did not contain protein-derived material. ^{3}H-labeled 5S rRNA was separated, when needed, from the 23S rRNA component in the resultant mixtures by gel filtration on Sephacryl S-200. A comparison of a PEI-cellulose chromatogram of a total T_1 RNase digest of the low molecular weight material eluted from the gel filtration column, with the published chromatogram of an authentic 5S rRNA sample (Mirzabekov & Griffin, 1972) permitted an unequivocal assignment of the eluted material as 5S rRNA.

Irradiation Conditions. Solutions of *E. coli* 50S ribosomes, or of a mixture of 5S and 23S rRNA, in 100 mL of irradiation buffer (5 mM H_3PO_4, neutralized to pH 7.2 with KOH; 10 mM $MgCl_2$; 30 mM KCl) were prepared and irradiated at 254-nm radiation according to a previously described method (Gorelic, 1975). The final concentrations of 50S ribosomes or rRNA were 1.00 A_{260} unit per mL. The temperatures of the 50S ribosome or rRNA solutions were maintained at 22.0 ± 0.1 °C during the course of the irradiations by circulation of thermostated water through the dipper well. Samples (1.0 mL) of ribosomes or rRNA were removed at regular intervals during the course of the irradiations. The samples were immediately treated with sodium lauryl sulfate and Pronase B as described below in order to minimize the effects of nucleases on the structural integrities of the rRNA molecules and stored at −70 °C until used.

The output of the light source at 254 nm was determined by ferrioxalate actinometry (Parker, 1953) to be $1.28 \pm 0.05 \times 10^{18}$ quanta s^{-1}. The fraction of incident 254-nm radiation actually absorbed by the ribosome or rRNA solutions was estimated by uridine actinometry (Fikus & Shugar, 1953) to be 0.30. All doses reported in this study have been corrected by this factor.

Preparation of Samples for Gel Filtration Analysis. Ribosome samples were incubated for 5 min at 37 °C in the presence of sodium lauryl sulfate (final concentration, 20 mg/mL). The resultant solutions were cooled to room temperature, centrifuged at low-speed to remove precipitated material, and then incubated for 1 h at 37 °C in the presence of Pronase B (final concentration, 1 mg/mL). The Pronase B was added as a 10 mg/mL solution in 10 mM Tris-HCl (pH 7.6), that had been preincubated at 37 °C for 10 min in order to destroy contaminating nucleases. A control gel filtration study, using *E. coli* 50S ribosomes radioactively labeled in their protein components with carbon-14, indicated that treatment of the ribosomes with sodium lauryl sulfate and Pronase B, followed by dialysis, resulted in extensive degradation of the ribosomal proteins and the almost complete disappearance of protein-derived material from the rRNA regions of the elution profile. The resultant samples were used without further treatment in gel filtration studies not denaturing for rRNA secondary structure. Alternatively, the treated ribosome samples were dialyzed overnight at 4 °C against a 10 mM Tris-HCl buffer (pH 7.6) that was 0.1 mM in magnesium concentration, and the resultant samples were used in gel filtration studies under conditions partially denaturing for rRNA secondary structure; or the samples were dialyzed overnight at 4 °C against a urea solution [containing, at final concentrations, urea (8 M), EDTA (2 mM, added as a 0.1 M solution neutralized to pH 7.6 with KOH), and Tris-HCl (20 mM,

neutralized to pH 7.6 with KOH)], incubated at 60 °C for 15 min and used in gel filtration studies under conditions completely denaturing for rRNA secondary structure. Control studies indicated that the recovery of RNA-derived radioactivity from the dialyses was greater than 90%. *E. coli* B tRNA (final concentration, $4A_{260}$ units/sample) was added to the samples applied to the gel filtration columns to serve as an internal marker and standard for variations in column resolution. The tRNA marker was added prior to denaturation of the samples used in gel filtration studies under conditions completely denaturing for rRNA secondary structure, and subsequent to denaturation in gel filtration studies under conditions partially denaturating for rRNA secondary structure. The basis for the selection of the conditions used to partially denature the rRNA secondary structure is a previous report that dialysis of 16S rRNA and 30S ribosomes against a low magnesium buffer completely unfolds the rRNA tertiary structure and results in a partial loss of base-pairing interactions (Spitnik-Elson et al., 1974). The basis for the selection of the conditions used to "completely" denature rRNA secondary structure is a previous report that treatment of *E. coli* rRNA and other RNA molecules with extensive secondary structure under the described condition permits detection of "hidden-breaks" in the rRNA molecules (Reijnders et al., 1973).

Results

Irradiation of aqueous buffered solutions of *E. coli* 30S ribosomes with 254-nm radiation could result in the introduction of two types of chain breaks into the rRNA components. One type of chain break, designated earlier as chain scission (Gorelic, 1976b), would result from the formation of single-strand breaks in single-stranded regions or of double-strand breaks in base-paired regions of the rRNA. The second type of chain break, designated "nicks", would result from the formation of single-strand breaks in base-paired regions of the rRNA. Both types of chain breaks would be expected to affect the physical properties of the rRNA components of the 50S ribosomes related to their molecular weight. The particular parameter of this type that will be discussed in this report will be the elution profiles of the rRNA components on gel filtration columns. Changes in gel filtration properties of the rRNA components due to chain scission should be readily detected under conditions not denaturing for rRNA secondary structure. The only possible exception to this conclusion would be the case where such chain breaks are introduced exclusively into the nonpaired regions of the hair-pin loop structures present in the rRNA molecules (Cox & Kanagalingan, 1967; Cox et al., 1968; Cox, 1970). Changes in the gel filtration characteristics of the 5S rRNA component due to intermolecular 5S/23S rRNA cross-linkage should also be detectable under conditions not denaturing for rRNA secondary structure. Since the molecular weight of the 23S rRNA molecule is ca. 25 times greater than that of the 5S rRNA, the expected change would be a shift in the 5S rRNA peak to the 23S rRNA region of the elution profile. The introduction of "nicks" into base-paired regions of the rRNA should be manifested by changes in the normal gel filtration characteristics of the rRNA molecules only under conditions denaturing for rRNA secondary structure. The severity of the required denaturing conditions would depend upon the extent of base-pairing at the site of the nicks: more severe denaturing conditions would be required for the release of the resultant RNA fragments from extensively base-paired regions than from regions with only limited secondary structure.

Based upon the conclusions of the above analyses, three experimental conditions were selected for studying the effects of 254-nm radiation on the structural integrities of the rRNA components of *E. coli* 50S ribosomes. The *first* experimental *condition* involves the use of a Tris–NaCl buffer to elute the rRNA samples from the gel filtration columns. This buffer system was used in our earlier studies of 30S ribosome photochemistry to test for RNA chain scission (Gorelic, 1976b). The *second* experimental *condition* involves dialysis of the rRNA samples, prior to gel filtration analysis, in order to diminish the magnesium concentration, and elution of the rRNA samples from gel filtration media using a Tris-based buffer lacking magnesium. Previous studies in other laboratories have indicated that reduction of magnesium concentration in rRNA solutions to values less than 1 mM results in an unfolding of rRNA tertiary structure and partial denaturation of rRNA secondary structure, and that these effects can be reversed by incubation of the rRNA samples in the presence of magnesium at concentrations of >10 mM (Brown et al., 1971; Richards et al., 1973). Consequently, these particular conditions will permit detection of chain scission and of nicks occurring in regions of the rRNA with only limited secondary structure. The *third* experimental *condition* involves denaturation of the rRNA samples by incubation at 60 °C in a Tris-based buffer system containing urea (final concentration, 8 M) and EDTA (final concentration, 2 mM), and elution of the rRNA samples from the gel filtration columns utilizing the same buffer system and temperature. Incubation of RNA under these conditions has been used in other laboratories to detect "hidden" breaks in RNA molecules of extensive secondary structures. Consequently, these experimental conditions will be used for the detection of nicks in extensively base-paired regions of the rRNA molecules. [It should be noted that a 1:1 Me_2SO/Tris buffer system was used in our earlier studies of 30S ribosome photochemistry (Gorelic, 1976b) for a similar purpose. A control study run in the present report indicated that, in contrast to 16S rRNA, 23S rRNA is completely insoluble in such a system.]

Gel Filtration Studies of the Effects of 254-nm Radiation on the Structural Integrity of 50S Ribosome-Bound 23S rRNA. The gel filtration elution profiles of the rRNA components of irradiated *E. coli* 50S ribosomes, obtained under conditions either *nondenaturing* or *partially denaturing* for rRNA secondary structure, are presented in Figures 1A–C and 1D–F, respectively. The RNA elution profiles in Figures 1A–C indicate that exposure of *E. coli* 50S ribosomes to doses of 254-nm radiation $<2.3 \times 10^{20}$ quanta does not result in statistically significant changes in the gel filtration characteristics, under nondenaturing conditions, of the 23S rRNA molecule. This conclusion is based upon the absence of statistically significant changes in the initial elution volumes of the peak of 23S rRNA-derived radioactivity (21.6 mL in the control elution profile in Figure 1A and an average value of 21.5 ± 0.5 mL in Figures 1B and 1C) and the absence of a dose-dependent shift in the distribution of 23S rRNA-derived radioactivity toward larger elution volumes. On the other hand, the RNA elution profiles in Figures 1D–F indicate that UV irradiation of the *E. coli* 50S ribosomes does result in a change in the gel filtration characteristics of the 23S rRNA detectable under experimental conditions partially denaturing for rRNA secondary structure. The indicated change is a dose-dependent shift in the fraction of 23S rRNA-derived radioactivity initially detected at larger elution volumes in the control elution profile: the fraction of total RNA-derived radioactivity coeluting with the *E. coli* B tRNA marker (determined by standard techniques of integration) increases from an initial value of 12% for the unirradiated sample (Figure 1D) to values of 16% for

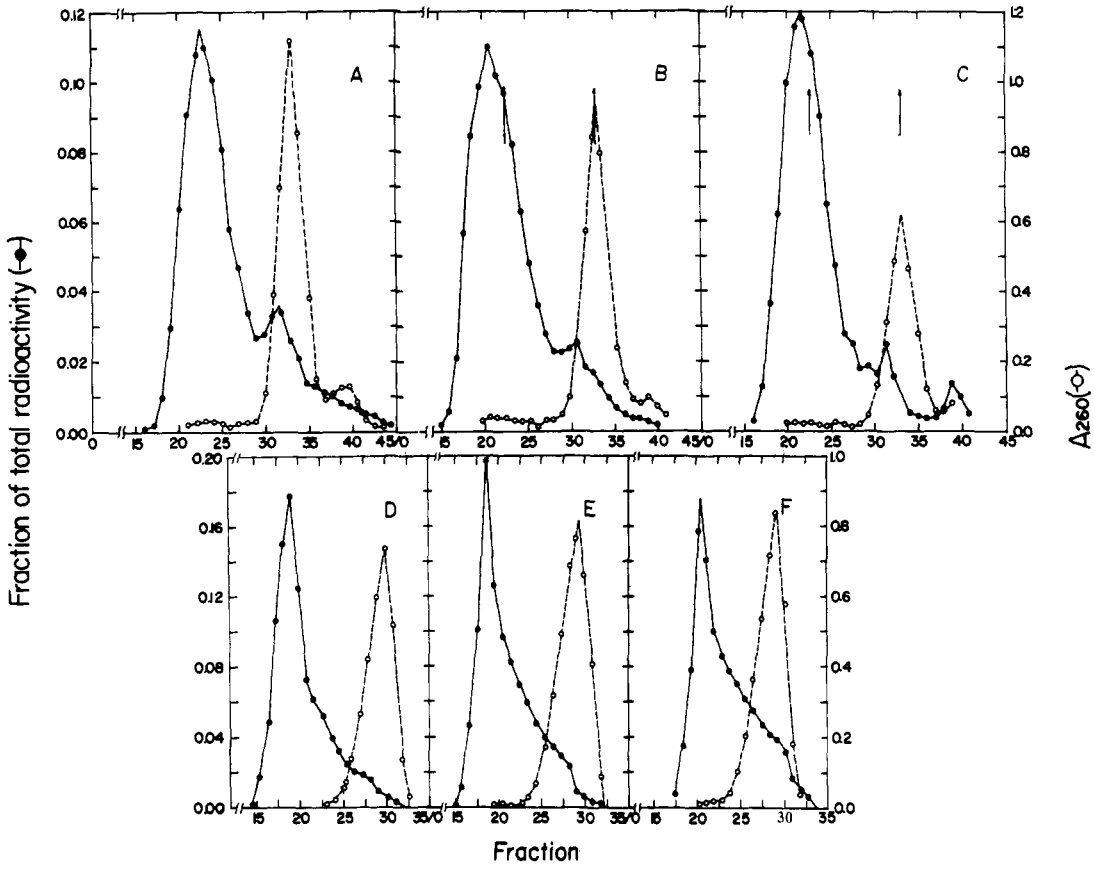

FIGURE 1: Gel filtration characteristics, under conditions not denaturing and partially denaturing for rRNA secondary structure, of the 23S rRNA components of unirradiated and irradiated *E. coli* 50S ribosomes. rRNA samples were prepared for analysis from unirradiated and UV-irradiated *E. coli* 50S ribosomes according to the procedures described in Materials and Method. Aliquots of the samples (0.6 mL for analysis under nondenaturing conditions, and 0.3 mL for analysis under partially denaturing conditions) were applied to the tops of either of two Sepharose CL-4B columns and eluted with the same solutions used in the packing of the columns. One column, used for studies under nondenaturing conditions, had dimensions of 1.1 × 38 cm and was packed at 20 °C in a 10 mM Tris-HCl (pH 7.6)-10 mM $MgCl_2$-0.15 M NaCl buffer. Precalibration of this column with bromophenol blue gave an elution volume for this marker of 38.15 mL. Fractions of 0.95-1.05 mL were collected at 20 °C from this column at a flow rate of 0.33 mL/min. A second column, used for studies under partially denaturing conditions, had dimensions of 1.1 × 50 cm and was packed at 20 °C in a 10 mM Tris-HCl (pH 7.6)-0.15 M NaCl buffer. Precalibration of this column with bromophenol blue gave an elution volume for this marker of 50.26 mL. Fractions of 0.9-1.0 mL were collected at 20 °C from this column at a flow rate of 0.08 mL/min. The fractions containing the *E. coli* B tRNA (○) marker were identified by determining the absorbance at 260 nm (A_{260}) of each fraction. Portions of the collected fractions were added to 5.0 mL of ACS liquid scintillation fluid (Amersham/Searle), and the amount of radioactivity (●) in the resultant samples was determined on a Nuclear Chicago Isocap 300 scintillation counter. The amount of radioactivity in each fraction was expressed as the fraction of total cpm eluted from the column. (A-C) rRNA samples prepared from *E. coli* 50S ribosomes irradiated with 0, 7.0×10^{19}, and 2.3×10^{20} quanta of 254-nm radiation, respectively, and analyzed under nondenaturing conditions. The arrows in B and C indicate the elution volumes of the rRNA and marker tRNA peaks in the control elution profile (A). The amount of radioactivity recovered from each of the columns was 9 of the total input and corresponded to values of 16 704 (A), 20 895 (B), and 22 206 (C) cpm. (D-F) rRNA samples prepared from *E. coli* 50S ribosomes irradiated with 0, 7 ×s 10^{19}, and 2.3×10^{20} quanta of 254-nm radiation, respectively, and analyzed under partially denaturing conditions. The amount of radioactivity eluted from each column was 9 of the input and corresponded to 40 478 (D), 24 792 (E), and 16 591 (F) cpm.

RNA samples prepared from 50S ribosomes irradiated with doses of 254-nm radiation of 7×10^{19} quanta (Figure 1E), and to 32% for an RNA sample prepared from 50S ribosomes irradiated with 2.3×10^{20} quanta of 254-nm radiation (Figure 1F). The observed dose-dependent shift in distribution of RNA-derived radioactivity in the elution profiles in Figures 1D-F is not, however, accompanied by a statistically significant shift in the initial elution volume of the major peak of 23S rRNA derived radioactivity (average value, 19.1 ± 0.9 mL).

The gel filtration elution profiles of the rRNA components of UV-irradiated *E. coli* 50S ribosomes, obtained under conditions *completely denaturing* for rRNA secondary structure, are presented in Figures 2A-G. Irradiation of the *E. coli* 50S ribosomes with 254-nm radiation is seen to result in *substantial* changes in the gel filtration characteristics of the rRNA components that are detected under conditions completely denaturing for rRNA secondary structure. These changes include a shift in elution volumes of the major peaks of rRNA-derived radioactivity from a value of ca. 20 mL for an RNA sample prepared from unirradiated 50S ribosomes (Figure 2A) to an average value of 15.6 ± 0.1 mL for RNA samples prepared from 50S ribosomes irradiated with doses of 254-nm radiation $>4.6 \times 10^{19}$ quanta (Figures 2C-G) and an accompanying marked sharpening of the rRNA elution profile peak in elution profiles of RNA samples prepared from *E. coli* 50S ribosomes irradiated with doses of 254-nm radiation $>4.6 \times 10^{19}$ quanta but $<6.9 \times 10^{19}$ quanta (Figures 2C and 2D). The detection of a new peak of RNA-derived radioactivity at smaller elution volumes in the elution profile in Figure 2B suggests that doses of $\leq 2.3 \times 10^{19}$ quanta of 254-nm radiation are sufficient to initiate the changes in RNA elution profiles observed in Figures 2A through 2D. Irradiation of *E. coli* 50S ribosomes with doses of 254-nm radiation $>1.2 \times 10^{20}$ quanta results in additional changes in the gel filtration characteristics of rRNA samples prepared from unirradiated

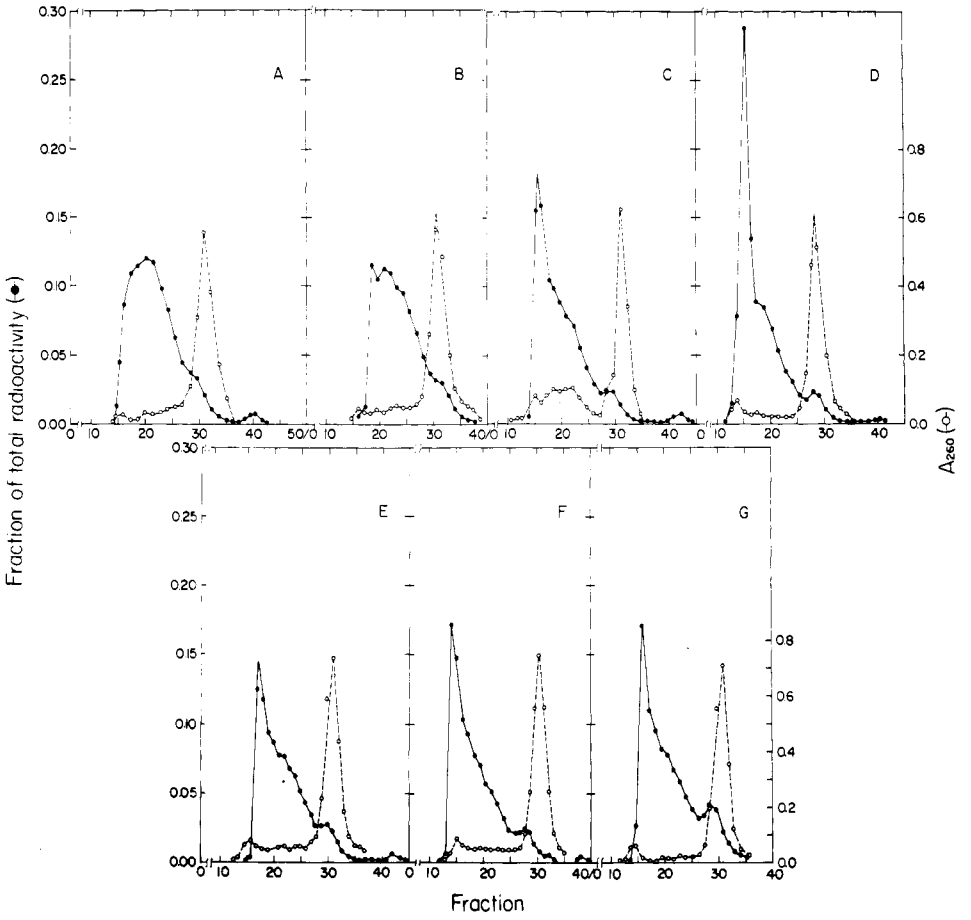

FIGURE 2: Gel filtration characteristics, under conditions "completely" denaturing for rRNA secondary structure, of the 23S rRNA component of unirradiated and irradiated *E. coli* 50S ribosomes. A solution of 50S ribosomes was prepared and irradiated with various doses of 254-nm radiation as described in Materials and Methods. Aliquots (1.0 mL) were removed at specific doses of 254-nm radiation and were prepared for gel filtration under conditions completely denaturing for rRNA secondary structure according to the procedure described in Materials and Methods. Portions (0.5 mL) of the resultant treated samples were applied to the top of a 1.1 × 40 cm Sepharose CL-4B column packed at 60 °C in an 8 M urea–2 mM EDTA–20 mM Tris-HCl (pH 7.6) buffer that had been precalibrated with a bromophenol blue marker. The elution volume of the marker was 47.0 mL. The pretreated samples were eluted at 60 °C with an 8 M urea–2 mM EDTA–20 mM Tris-HCl (pH 7.6) buffer. Fractions of 1.1–1.2 mL were collected at a flow rate of 0.3 mL/min. Fractions containing the *E. coli* B tRNA marker were identified by monitoring the A_{260} of the collected fractions (○). The amount of radioactivity in the collected fractions was determined by adding 0.5 mL of each fraction to 8.0 mL of ACS scintillation fluid and counting the resultant solutions. The radioactivity in each fraction was expressed as the fraction of total cpm eluted from the column (●). (A) Sample prepared from unirradiated ribosome; (B–G) samples prepared from ribosomes irradiated with 2.3×10^{19}, 4.6×10^{19}, 6.9×10^{19}, 1.2×10^{20}, 1.6×10^{20}, and 2.3×10^{20} quanta of 254-nm radiation, respectively. The radioactivity recovered from each of the columns was 90% of the total input and corresponded to 62 040, 56 769, 59 488, 46 219, 36 892, 35 401, and 25 447 cpm for the elution profiles in Figures 2A–G, respectively.

50S ribosomes. These changes include a reduction in the contribution of RNA-derived radioactivity corresponding to the sharp peaks in Figures 2C and 2D to the overall RNA elution profiles, an accompanying appearance of a substantial amount of RNA-derived radioactivity as a "tail" extending to elution volumes comparable to that of the *E. coli* B tRNA marker (Figures 2E–G), and the appearance of a small peak of RNA-derived radioactivity in the tRNA marker regions of elution profiles of RNA samples prepared from 50S ribosomes irradiated with doses of 254-nm radiation $>1.6 \times 10^{20}$ quanta (Figures 2F and 2G). It should finally be noted that the RNA elution profiles, obtained under gel filtration conditions either partially or completely denaturing for rRNA secondary structure, of RNA samples prepared from 50S ribosomes irradiated with doses of 254-nm radiation $>1.2 \times 10^{20}$ quanta (Figures 1E and 2E–G, respectively) are quite similar.

The characteristics of the RNA elution profiles in Figure 2 could be explained in a number of ways. The broad elution profile of the control rRNA sample (Figure 2A) could be a result of the presence of hidden breaks in the RNA sample treated and applied to the gel filtration column or could be due to incomplete denaturation of the rRNA, resulting in the formation of a mixture of rRNA conformers with different gel filtration characteristics. The sharp peaks of RNA-derived radioactivity in the elution profiles in Figures 2C and 2D could be the result of photoinduced modifications in the rRNA structure that either decreased or enhanced the stability of the secondary structure of the 23S rRNA component toward denaturation. Finally, the observed effects of higher doses of 254-nm radiation on the elution profiles of the rRNA components of the *E. coli* 50S ribosomes (Figures 2E–G) could be a result of UV-mediated fragmentation of the rRNA components, or could be due to photoinduced modifications of the rRNA components that affected their gel filtration characteristics but not their structural integrities.

Two additional gel filtration studies were carried out in order to distinguish between the alternative explanations offered above for the RNA elution profiles in Figure 2. In *one study,* samples were removed from different regions of the RNA elution profiles in Figures 2A, 2D, and 2E, treated under conditions reported in other laboratories to permit complete renaturation of rRNA secondary structure (Brown et al., 1971; Richards et al., 1973), and analyzed by gel filtration on Bio-Gel A-5.0m (200–400 mesh) under conditions not denaturing for

rRNA secondary structure. It was reasoned here that the elution profiles would depend upon the region in the elution profiles in Figures 2A, 2D, and 2E from which the RNA samples were originally taken if RNA chain breaks were originally present in the denatured samples, but that there would be considerable overlap in the resultant elution profiles of the different "renatured" RNA samples if the original denatured samples were actually comprised of a mixture of partially denatured rRNA species. The results of such a gel filtration analysis are presented in Figures 3A-G. The RNA elution profiles in this figure indicate that the basis for the RNA elution profile in Figure 2A and for the poorly defined "tail" of radioactivity in Figure 2D is incomplete denaturation of the rRNA samples rather than the presence of the "hidden breaks"; and that the elution profiles in Figures 2E-G reflect the presence of chain breaks in the rRNA samples used to obtain these elution profiles. These conclusions are based upon the findings that there is substantial overlap of the elution profiles of RNA samples obtained from various regions of the elution profiles in Figures 2A and 2D (Figures 3A-C and 3D and 3E, respectively) and that the elution volumes of samples removed from the elution profile in Figure 2E are dependent upon the region in the elution profile from which they were originally taken (Figures 3F and 3G).

In a *second* study, rRNA samples were prepared from unirradiated and UV-irradiated *E. coli* 50S ribosomes, subjected to conditions "completely" denaturing for rRNA secondary structure, and then "renatured." The resultant samples were then analyzed by gel filtration on Bio-Gel A-5.0m (200-400 mesh) under conditions not denaturing for rRNA secondary structure. It was reasoned here that, if the secondary structure of the rRNA is stabilized toward denaturation (as a result of, viz., intrastrand RNA-RNA cross-links), there should be a closer correspondence between the elution profile of a control untreated rRNA sample derived from unirradiated ribosomes and the elution profile of a denatured and subsequently renatured rRNA sample derived from a UV-irradiated ribosome sample than between the elution profile of a control rRNA sample and an rRNA sample in which the secondary structure had been destabilized as a result of photoinduced base modifications.[2] The corresponding RNA elution profiles are presented in Figure 4. The RNA elution profiles in this figure quite clearly indicate that UV irradiation of the *E. coli* 50S ribosomes does not result in an enhancement of the stability of at least the secondary structure of 23S rRNA toward denaturation. The bases for this conclusion are the findings that "renaturation" of a denatured rRNA sample prepared from unirradiated *E. coli* 50S ribosomes does not result in an RNA elution profile that overlaps the elution profile of an untreated rRNA sample (Figures 4B and 4A, respectively); and that UV irradiation of the 50S ribosomes does not change this situation (Figures 4C-F). The transition from the RNA elution profile in Figure 2A to that observed in Figure 2D is therefore a result of UV-mediated destabilization of 23S rRNA secondary structure. The sharpness of the peak of 23S rRNA derived radioactivity in

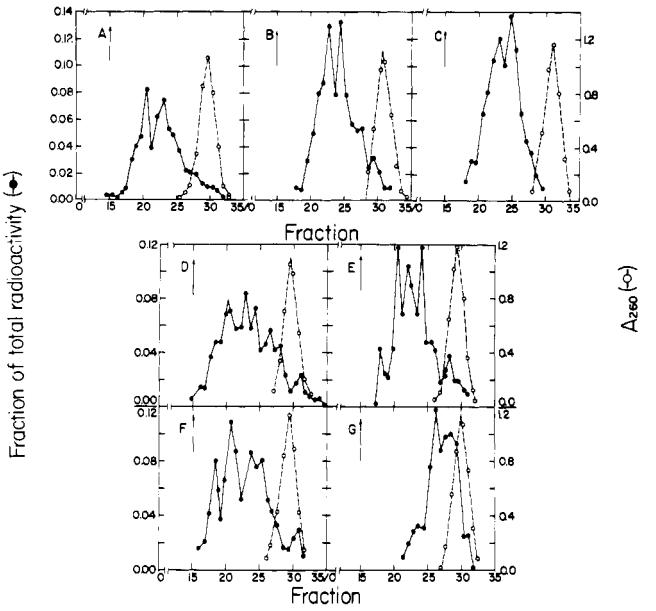

FIGURE 3: Reanalysis, by gel filtration and under conditions not denaturing for rRNA secondary structure, of fractions taken from various regions of the elution profiles in Figure 2. The RNA elution profiles in Figures 2A, 2D, and 2E were arbitrarily divided into different regions. The collected fractions in each of these regions were pooled and "renatured" by dialysis for 8 h at 20 °C and then overnight at 4 °C against a 10 mM Tris-HCl (pH 7.6)-10 mM $MgCl_2$-0.10 M NaCl buffer. Aliquots (0.4 mL) of the dialyzed samples were applied to the top of a 1.1 × 38.5 cm column of Bio-Gel A-5.0m (200-400 mesh) packed at 20 °C in a 10 mM Tris-HCl (pH 7.6)-10 mM $MgCl_2$-0.10 M NaCl buffer. The column was precalibrated using bromophenol blue; the elution volume of this marker was 44.0 mL. The applied samples were eluted at 20 °C with the Tris-HCl-$MgCl_2$-NaCl buffer used in the packing of the column. Fractions of 0.75-0.85 mL were collected at a flow rate of 0.07 mL/min. The location of the *E. coli* B tRNA marker (○) and the amount of radioactivity (●) in each of the collected fractions were determined by the procedures described in the legend to Figure 1. The amount of input radioactivity recovered from each of the columns was determined to be 90%. (A-C) RNA elution profiles of samples obtained from regions I-III (corresponding to elution volumes of 15.0 to 20.25 mL, 20.25-24.25 mL, and 24.25-28.25 mL), respectively, in the elution profile in Figure 2A. The ratio of the amounts of radioactivity in each of the analyzed regions is 2.33:1.82:1. The elution volumes of each of the major peaks of radioactivity were 20.5 and 23.0 mL (A) and 22.8 and 24.6 mL (B and C). The elution volumes of the *E. coli* B tRNA markers were 29.0 mL (A) and 31.5 mL (B and C). (D-E) RNA elution profiles of samples obtained from regions I and II (corresponding to elution volumes of 11.5-20.0 mL and 20.0-25.0 mL, respectively) in the elution profile in Figure 2D. The ratio of the amounts of radioactivity in each of the analyzed regions is 3.2:1. (F-G) RNA elution profiles of samples obtained from regions I and II (corresponding to elution volumes of 16.0-21.0 mL and 21.0-26.0 mL, respectively) in the elution profile in Figure 2E. The ratio of the amounts of radioactivity in each of the analyzed regions is 1.9:1. The arrows in Figures 3A-G denote the elution volumes of a marker undenatured 23S rRNA sample.

Figure 2D and its smaller elution volume relative to the peak of 23S rRNA derived radioactivity in the control RNA elution profile in Figure 2A would be consistent with extensive, if not complete, denaturation of the 23S rRNA secondary structure. The elution profiles in Figure 4 also offer support to the earlier conclusion that exposure of *E. coli* 50S ribosomes to 254-nm radiation does result in chain breakage of at least the 23S rRNA component: there is an increase in the fraction of total RNA-derived radioactivity coeluting with the *E. coli* B tRNA marker in elution profiles of rRNA samples prepared from ribosomes irradiated with doses of 254-nm radiation > 1.2×10^{20} quanta (Figures 4E and 4F), and the elution profile of the rRNA sample prepared from 50S ribosomes irradiated with 2.3×10^{20} quanta of 254-nm radiation is seen to be extremely

[2] An alternative approach that could be used to identify effects of UV radiation on rRNA secondary structure would be to determine the T_m of the rRNA component before and after irradiation by, viz., CD or ORD. Irradiation of an RNA sample can result in the formation of uracil and cytosine photohydrates (Wang, 1976). Both types of photohydrates revert to the unsubstituted pyrimidines at elevated temperatures. Consequently the use of CD or ORD to monitor dose-dependent changes in rRNA secondary structure would not be capable of distinguishing between thermal denaturation of rRNA secondary structure and thermal reversal of pyrimidine photoproducts to UV-absorbing materials.

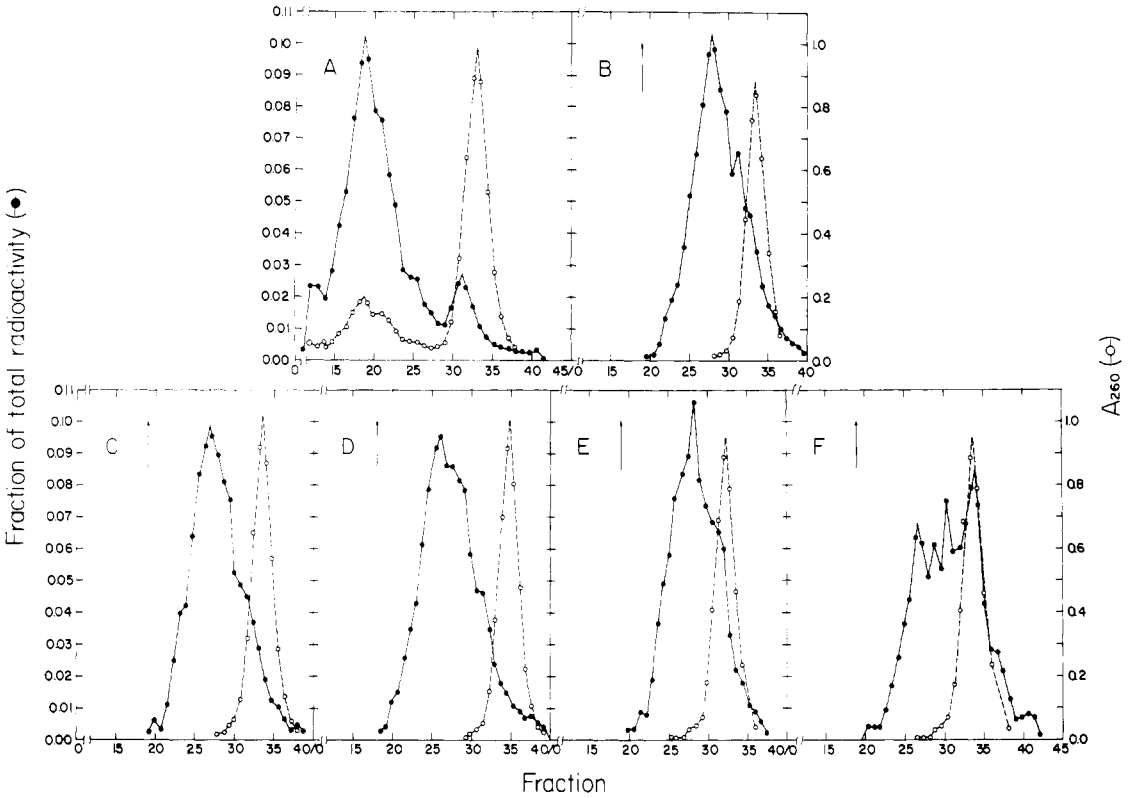

FIGURE 4: Gel filtration characteristics, under conditions not denaturing for rRNA secondary structure, of renatured rRNA from unirradiated and irradiated *E. coli* 50S ribosomes. A solution of *E. coli* 50S ribosomes was prepared and irradiated with various doses of 254-nm radiation as described in Materials and Methods. Aliquots (1.0 mL) were removed at regular intervals and treated with sodium lauryl sulfate and Pronase B according to the procedures described in Materials and Methods. The resultant samples were dialyzed overnight against an 8 M urea–2 mM EDTA–20 mM Tris-HCl (pH 7.6) buffer and then incubated at 60 °C for 30 min. The incubated samples were then dialyzed overnight at 4 °C against a 10 mM Tris-HCl–10 mM $MgCl_2$ buffer, and aliquots (0.2 mL) of the resultant solution applied to the top of a 1.1 × 40 cm Bio-Gel A-5.0m (200–400 mesh) column packed at 20 °C in 10 mM Tris-HCl–10 mM $MgCl_2$ and precalibrated with bromophenol blue. The elution volume of the bromophenol blue marker was 46.5 mL. The applied samples were eluted at 20 °C with a 10 mM Tris-HCl (pH 7.6)–10 mM $MgCl_2$ buffer. Fractions of 0.80–0.90 mL were collected at a rate of 0.07 mL/min. The location of the *E. coli* B tRNA marker (○) and the amount of radioactivity in each of the collected fractions were determined by the procedures described in the legend to Figure 1. The radioactivity in each fraction was expressed as the fraction of total cpm eluted from the column (●). (A) RNA sample prepared from untreated and unirradiated 50S ribosomes; (B–F) RNA samples prepared from treated 50S ribosomes irradiated with 0, 2.3×10^{19}, 6.9×10^{19}, 1.2×10^{20}, and 2.3×10^{20} quanta of 254-nm radiation, respectively. The amount of radioactivity recovered from each of the columns was 90% of the total input and corresponded to 18 401, 21 510, 9324, 9252, 9538, and 6379 cpm, respectively. The arrows in B–F indicate the elution volume of the peak of the rRNA-derived radioactivity in A.

complex (Figure 4G).

Gel Filtration Studies of the Effects of 254-nm Radiation on the Structural Integrity of 50S Ribosome-Bound 5S rRNA. The effects of 254-nm radiation on the gel filtration characteristics of 50S ribosome bound 5S rRNA, monitored under experimental conditions either nondenaturing or "completely" denaturing for rRNA secondary structure, are represented by the RNA elution profiles in Figures 5 and 6, respectively. Irradiation of *E. coli* 50S ribosomes with doses of 254-nm radiation $< 2.3 \times 10^{20}$ quanta does not seem to result in changes in the gel filtration characteristics of the 5S rRNA detectable under nondenaturing conditions. The specific characteristics monitored were the elution volumes of the peaks of 5S rRNA derived radioactivity and the fraction of total rRNA-derived radioactivity detected in the 5S rRNA regions of the elution profiles (see legends to Figure 5 for actual data). The only possible exception to these conclusions is the 5S rRNA sample prepared from *E. coli* 50S ribosomes irradiated with 2.3×10^{20} quanta of 254-nm radiation: the fraction of total rRNA-derived radioactivity detected in the 5S rRNA regions of the corresponding elution profile is ca. 35% larger than the values of the corresponding parameter in the elution profiles in Figures 5A–D. The elution profiles in Figures 6A–E indicate that irradiation of *E. coli* 50S ribosomes with doses of 254-nm radiation up to and including 2.3×10^{20} quanta does not result in statistically significant changes in the structural integrity of the 5S rRNA component detectable under experimental conditions completely denaturing for rRNA secondary structure.

A Gel Filtration Study of the Effects of 254-nm Radiation on the Structural Integrity of Free 23S rRNA. A gel filtration elution profile, obtained under experimental conditions completely denaturing for rRNA secondary structure, of 23S rRNA samples prepared from unirradiated and UV-irradiated *E. coli* 50S ribosomes is presented in Figure 7. The dose of 254-nm radiation used in this particular study (2.3×10^{20} quanta) was found in earlier studies in this report to produce the largest changes in the initial structural integrities of 50S ribosome-bound rRNA (Figure 2F).

Irradiation of free 23S rRNA with 2.3×10^{20} quanta of 254-nm radiation is seen to result in the appearance of a new peak of rRNA-derived radioactivity elution at a volume smaller than the elution volume of the peak of 23S rRNA derived radioactivity in the control elution profile (Figures 7B and 7A, respectively). On the other hand, a statistically significant shift in the fraction of total rRNA-derived radioactivity in the 23S rRNA region of the elution profile to larger elution volumes is not observed.

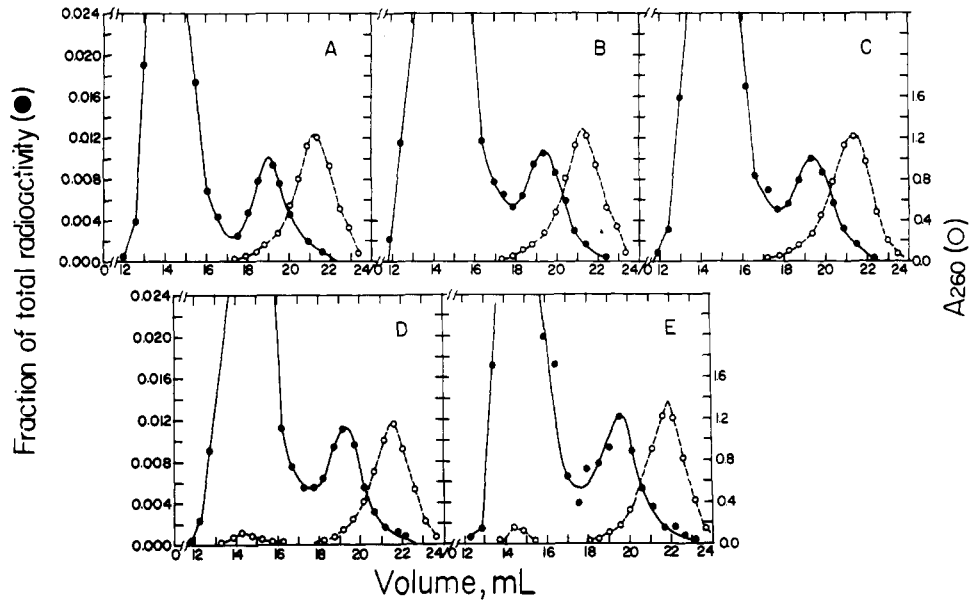

FIGURE 5: Gel filtration characteristics, under conditions not denaturing for rRNA secondary structure, of 5S rRNA prepared from unirradiated and UV-irradiated *E. coli* 50S ribosomes. A solution of *E. coli* 50S ribosomes was irradiated with various doses of 254-nm radiation and prepared for gel filtration analysis under nondenaturing conditions according to the procedures described in Materials and Methods. The samples were dialyzed against the elution buffer (10 mM Tris-HCl (pH 7.6)-10 mM $MgCl_2$-0.15 M NaCl) and aliquots of each sample (0.40 mL) applied to the top of a 1.1 × 37.5 cm Sephacryl S-200 Superfine column packed in the elution buffer. The column was precalibrated using bromophenol blue and authentic samples of 5S and 23S rRNA as markers. The elution volume of the bromophenol blue marker was 103.6 mL; the elution volumes of the 5S and 23S rRNA markers were 19.3 and 14.8 mL, respectively. Fractions of 0.50-0.60 mL were collected at a flow rate of 0.22 mL min^{-1}. The location of the *E. coli* B tRNA marker (○) and the amount of radioactivity (●) in each fraction were determined according to the procedure in the legend to Figure 1. The fraction of total RNA-derived radioactivity under each of the rRNA peaks was determined by integration, using a K & E Model No. 620022 planimeter. (A-E) RNA elution profiles of samples obtained from *E. coli* 50S ribosomes irradiated with 0, 2.3 × 10^{19}, 6.9 × 10^{19}, 1.2 × 10^{20}, and 2.3 × 10^{20} quanta of 254-nm radiation, respectively. The total amount of radioactivity recovered from each of the columns was greater than 90% of the input and corresponded to values of 186 488, 111 758, 108 289, 104 703, and 95 442 cpm for A-E, respectively. The integrated areas under the peaks of radioactivity corresponding to 23S rRNA are (in arbitrary units) 94 ± 7 (A), 96 ± 4 (B), 86 ± 5 (C), 89 ± 6 (D), and 86 ± 6 (E). The integrated areas under the peaks of radioactivity corresponding to 5S rRNA, and corrected for overlap of the 23S rRNA peak, are (in arbitrary units) 100 ± 7 (A), 118 ± 8(B), 94 ± 7 (C), 115 ± 3 (D), and 136 ± 6 (E). The elution volumes of the peaks of 5S rRNA derived radioactivity were 19.0 mL (A) and 19.3 ± 0.3 mL (B-E). The average elution volume of the *E. coli* B tRNA marker was 21.5 ± 0.3 mL.

Discussion

The effects of 254-nm radiation on the gel filtration characteristics of free 23S rRNA and 50S ribosome-bound 5S and 23S rRNA have been determined in this report. The results of this determination indicate that exposure of aqueous solutions of *E. coli* 50S ribosomes to doses of 254-nm radiation < 2.3 × 10^{20} quanta does not result in changes in the gel filtration characteristics of the 5S and 23S rRNA components of unirradiated 50S ribosomes detectable under experimental conditions not denaturing for rRNA secondary structure, or in the gel filtration characteristics of the 5S rRNA detectable under experimental conditions completely denaturing for 5S rRNA secondary structure. On the other hand, UV-mediated changes in the initial gel filtration characteristics of the 23S rRNA component of the *E. coli* 50S ribosomes are detectable under conditions completely denaturing for 23S rRNA secondary structure. These changes include a marked sharpening and a shift to smaller elution volumes of the initial peak of 23S rRNA derived radioactivity in elution profiles of rRNA samples prepared from *E. coli* 50S ribosomes irradiated with < 6.9 × 10^{19} quanta of 254-nm radiation, and a shift to larger elution volumes of a considerable fraction of total rRNA-derived radioactivity initially detected in the 23S rRNA region of a control elution profile in rRNA samples prepared from *E. coli* 50S ribosomes irradiated with > 6.9 × 10^{19} quanta of 254-nm radiation. The second effect is also detected in rRNA samples prepared from *E. coli* 50S ribosomes irradiated with >6.9 × 10^{19} quanta of 254-nm radiation, under gel filtration conditions only partially denaturing for 23S rRNA secondary structure. Finally, irradiation of an aqueous solution of free 23S rRNA with 2.3 × 10^{20} quanta of 254-nm radiation results in changes in the initial gel filtration characteristics of the 23S rRNA, detectable under conditions completely denaturing for 23S rRNA secondary structure. The basic natures of the changes in the gel filtration characteristics of free 23S rRNA are similar to the changes observed for ribosome-bound 23S rRNA; however, the magnitudes of the changes are comparable to the changes observed for ribosome-bound 23S rRNA samples prepared from 50S ribosomes irradiated with only 2.3 × 10^{19} quanta of 254-nm radiation. Based upon arguments developed earlier in this report, these findings indicate that: (1) irradiation of aqueous solutions of *E. coli* 50S ribosomes with >7 × 10^{19} quanta of 254-nm radiation results in the introduction of nicks into regions of the 23S rRNA component with limited secondary structure, by a photoprocess that is apparently ribosome dependent; and (2) irradiation of aqueous solutions of *E. coli* 50S ribosomes with <2.3 × 10^{20} quanta of 254-nm radiation does not result in chain scission of either the 5S rRNA or the 23S rRNA components, or in intermolecular 5S rRNA/23S rRNA cross-linkage. The findings regarding the effect of 7 × 10^{19} quanta of 254-nm radiation on the gel filtration characteristics of the 23S rRNA component, monitored under conditions "completely" denaturing for rRNA secondary structure, indicate that 254-nm radiation exerts an additional effect on the rRNA components of the 50S ribosomes: destabilization of 23S rRNA secondary structure toward denaturation.

It had been shown in an earlier study that irradiation of *E. coli* 30S ribosomes with >6.3 × 10^{19} quanta of 254-nm ra-

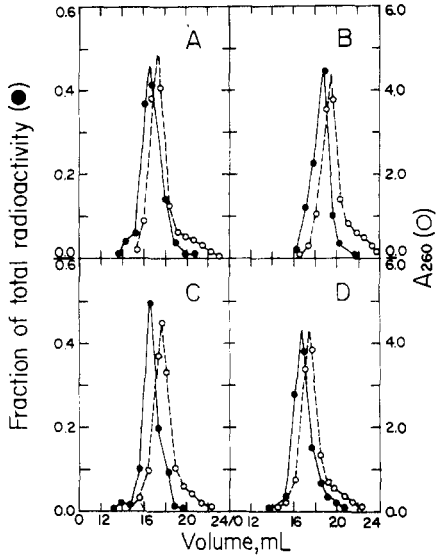

FIGURE 6: Gel filtration characteristics, under conditions "completely" denaturing for rRNA secondary structure, of 5S rRNA prepared from unirradiated and UV-irradiated *E. coli* 50S ribosomes. A solution of *E. coli* 50S ribosomes was irradiated with various doses of 254-nm radiation. Samples (2.0 mL) were removed and prepared for gel filtration analysis under nondenaturing conditions, according to the procedures described in Materials and Methods. Aliquots (1.2 mL) of each of the samples were applied to the top of a 1.5 × 47 cm Sephacryl S-200 superfine column and the samples eluted with Tris-HCl (10 mM, pH 7.6)-$MgCl_2$ (10 mM)-NaCl (0.15 M) buffer. Fractions of ca. 1.2 mL were collected at a flow rate of 0.30 mL min^{-1}. Aliquots (0.2 mL) of the collected fraction were removed and their radioactivity contents determined by the procedure described in the legend to Figure 1. The fractions containing the 5S rRNA component were pooled in such a manner as to minimize contamination with 23S rRNA, placed in a dialysis bag, and concentrated by covering with Carbowax 6000 (Union Carbide Corp.). The concentrated samples were prepared for gel filtration under denaturing conditions according to the procedure described in the legend to Figure 2. Aliquots (0.40 mL) of the treated samples were applied to the top of a 1.1 × 42 cm Sephacryl S-200 Superfine column packed at 60 °C with a Tris-urea-EDTA buffer, the composition of which was the same as described in the legend to Figure 2. The column was precalibrated with bromophenol blue; the elution volume of this marker was 42.5 mL. The samples were eluted at 60 °C with the buffer used in the packing of the column, and fractions of 0.72–0.80 mL were collected at a flow rate of 0.45 mL min^{-1}. The elution volume of the *E. coli* B tRNA marker (○) and the amount of radioactivity in each of the collected fractions (●) were determined by the procedure described in the legend to Figure 1. The amount of radioactivity recovered from the columns was ca. 90% of the input. Controls indicated that less than 10% of the radioactivity in the original 5S rRNA samples were lost during the concentration step with Carbowax and the preparation by dialysis of the rRNA samples for gel filtration analysis. (A–D) Elution profiles of 5S rRNA samples prepared from *E. coli* 50S ribosomes irradiated with 0, 6.9×10^{19}, 1.2×10^{20}, and 2.3×10^{20} quanta of 254-nm radiation, respectively. The amount of radioactivity recovered in each elution profile was 1365 (A), 1561 (B), 1242 (C), and 1888 cpm (D). The average elution volumes of the *E. coli* B tRNA marker and the peak of 5S rRNA derived radioactivity in each of the elution profiles were 17.4 ± 0.20 and 16.6 ± 0.2 mL, respectively.

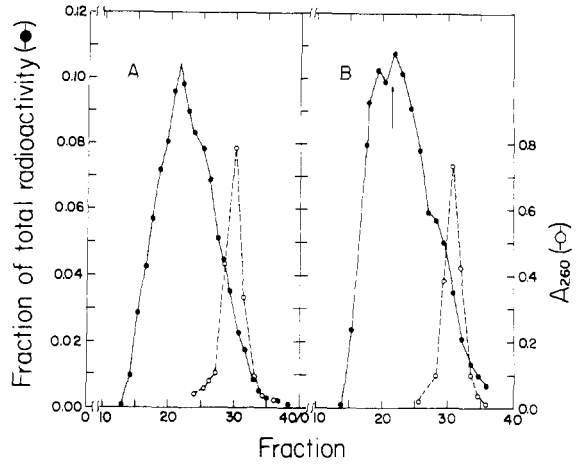

FIGURE 7: Gel filtration characteristics, under conditions completely denaturing for rRNA secondary structure, of unirradiated and irradiated free rRNA. A solution of a mixture of ^3H-labeled 5S and ^3H-labeled 23S rRNA was prepared and irradiated with 2.3×10^{20} quanta of 254-nm radiation, as described in Materials and Methods. Aliquots (1.0 mL) were taken prior to and subsequent to irradiation and were treated with sodium lauryl sulfate and Pronase B according to the procedure described in Materials and Methods. The samples were then denatured and prepared for gel filtration analysis under conditions completely denaturing for rRNA secondary structure, according to the procedures described in the legend to Figure 2. Aliquots (0.3 mL) of the resultant samples were applied to the top of a 1.1 × 38 cm Sepharose CL-4B column packed at 60°C with an 8 M urea-20 mM Tris-HCl (pH 7.6)-2 mM EDTA-0.1 M NaCl buffer. The samples were eluted at 60 °C with the urea-Tris-HCl-EDTA-NaCl buffer described above. Fractions of 1.2–1.3 mL were collected at a flow rate of 0.3 mL/min. The locations of the *E. coli* B tRNA marker (○) and of the radioactivity in each of the collected fractions were determined as described in the legend to Figure 1. The amount of radioactivity in each fraction was expressed as the fraction of total cpm eluted from the column (●). (A) Unirradiated rRNA sample; (B) irradiated rRNA sample. The amount of radioactivity eluted from each of the columns was 90% of the total input and corresponded to 27 385 cpm for the unirradiated RNA sample (A), and 28 053 cpm for the irradiated RNA sample (B). The arrow in B indicates the elution volume of the rRNA peak in the control elution profile (A).

diation results in the introduction of "nicks" into the 16S rRNA component, and that 16S rRNA chain scission occurs after exposure of the 30S ribosomes to doses of 254-nm radiation greater than 1.7×10^{20} quanta (Gorelic, 1976b). The results of the studies presented in this report therefore suggest that the rRNA components of the *E. coli* 50S ribosomes are inherently less sensitive toward UV-mediated chain breakage than the 16S rRNA component of the *E. coli* 30S ribosomes. It is also possible, however, that the sensitivities of the rRNA components toward UV-mediated chain breakage are similar, but the extent of masking of RNA chain breaks in UV-irradiated 50S ribosomes might be greater than in UV-irradiated 30S ribosomes. It is also possible that chain breaks might occur by a combination of photochemistry and the action of ribosome-associated endoribonuclease activities, and the contribution of the latter to the formation of chain breaks might be greater in the 30S ribosomes than in the 50S ribosomes. Control studies in this report indicate that the 23S rRNA secondary structure is fully destabilized toward denaturation prior to the introduction of nicks into the rRNA molecule, and in other reports (Aubert et al., 1968) that 5S rRNA secondary structure should be fully denatured under the completely denaturing conditions used in this study. An additional set of gel filtration control studies (Gorelic, unpublished observation) indicated that pretreatment of unirradiated or UV irradiated 30S and 50S ribosomes with sodium lauryl sulfate and Pronase B removes either most or all of the protein-derived material from the rRNA regions of the resultant elution profiles, and in those cases where protein-derived material remains in the rRNA regions, the amounts of material retained in samples prepared from either 30S or 50S ribosomes are comparable. It is therefore unlikely that masking of rRNA chain breaks as a result of incomplete denaturation of the rRNA components of the *E. coli* 50S ribosome, or as a result of more extensive "covalent bridging" of the RNA fragments constituting the nicks by covalent RNA/protein cross-links, made major contributions to the apparently lower UV sensitivity of 50S ribosome-rRNA toward chain breakage. It is also unlikely that a ribosome-associated nuclease is responsible for the differences in sensitivity toward chain breakage under consideration here: the 16S rRNA component of native or unfolded 30S ri-

bosomes isolated from RNase I⁻ *E. coli* strains is not sensitive to autodegradation (Ceri & Maeba, 1973); and, although evidence for a 50S ribosome-associated nuclease activity has been presented (Staehlin et al., 1969; Ceri & Maeba, 1973), the level of this activity is such that the extent of UV-mediated chain breakage of 50S ribosome-bound 23S rRNA observed in this report cannot be accounted for by the action of such a nuclease on 23S rRNA. It can therefore be concluded that the rRNA components of the *E. coli* 50S ribosomes are, in reality, less sensitive toward UV-mediated chain breakage than 30S ribosome-bound 16S rRNA.

Arguments presented earlier in this report suggested that the rRNA components of *E. coli* 50S ribosomes should be more sensitive than 30S ribosome-bound 16S rRNA toward UV-mediated chain breakage if chain breaks resulted primarily from RNA–RNA interactions. The conclusions of the above analyses therefore indicate that RNA/protein-type interactions make the major contribution to UV-mediated ribosome-dependent RNA chain breakage. The data indicate, however, that the RNA/protein type interactions required for RNA chain breakage are probably not present in the native topographical state of the 50S ribosome. The basis for this conclusion is that chain breakage of 50S ribosome-bound 23S rRNA does not occur at the lowest doses of 254-nm radiation used in the present study but is first detected in the 23S rRNA *only after complete UV-mediated destabilization of 23S rRNA secondary structure* toward denaturation. The photochemical basis for destabilization of 23S rRNA secondary structure has not been defined in this study but could very well be the production of photohydrates and/or photodimers in base-paired regions of the 23S rRNA, as has already been demonstrated to be the case in UV-irradiated polynucleotides (DeBoer et al., 1967). Studies are currently in progress in our laboratory to test the validity of this latter proposal. Interestingly, the data obtained in this report indicate that, like rRNA chain breakage, incorporation of the 23S rRNA into a 50S ribosome particle markedly enhances the photosensitivity of 23S rRNA toward destabilization of its secondary structure.

The lack of reactivity of 5S and 23S rRNA in UV-mediated intermolecular cross-linkage could indicate that the two rRNA components of the *E. coli* 50S ribosomes are not sufficiently close in the native 50S ribosome to permit the intermolecular interactions required for the formation of such cross-links. The required interactions could be of the base–base type, as has already been discussed in this report. Additionally, since UV irradiation of purines and pyrimidines in the presence of primary and secondary alcohols results in the formation of base–alcohol photoadducts (Elad, 1976; Shetlar, 1976), the required interactions could also be of the base/phosphodiester backbone type. Alternatively, photoreactions other than interstrand cross-link formation, viz., photohydration or photodimerization, might have effectively competed for the rRNA bases, or the doses of 254-nm radiation used in the present study might not have been of a sufficient magnitude to permit the formation of detectable amounts of interstrand cross-links. It can, in fact, be shown that this latter point might be a valid concern. Based upon the number of 50S ribosomes used in the irradiations and the molecular weight of the 23S rRNA component, it can be calculated that on the average each rRNA base will have received 20 hits after exposure of the *E. coli* 50S ribosomes to 2.3×10^{20} quanta of 254-nm radiation. The values for the quantum efficiencies for intersystem crossing in the rRNA bases have been reported to vary from 4×10^{-4} to 7×10^{-3} in aqueous solution at 300 K when measured by the europium method (Lamola & Eisenger, 1971). Consequently, from 0.8% to 14% of the 50S ribosome population had the potential of forming one interstrand RNA/RNA cross-link after exposure to a total dose of 254-nm radiation, if cross-link formation occurred through the triplet photo excited states of the rRNA bases. The data presented in this report do not, therefore, permit an unambiguous decision to be made regarding the question of intermolecular 5S/23S rRNA interactions in the native *E. coli* 50S ribosomes.

It is important to note that the 5S and 23S rRNA components of the *E coli* 50S ribosomes could become indirectly associated with each other as a result of UV-mediated cross-linkage to (a) ribosomal protein(s) shared in common. The effect of such a situation on the initial gel filtration characteristics of the 5S rRNA molecule would be the same as predicted for intermolecular 5S rRNA/23S rRNA cross-linkage, if the extant of the intervening non-cross-linked regions of the cross-linked protein were sufficiently small so as to be sterically inaccessible to the action of Pronase B. The results of an earlier study of RNA/protein cross-linking in UV-irradiated 50S ribosomes (Gorelic, 1976c) indicated that most of the 50S ribosomal proteins would be covalently cross-linked to rRNA at the higher doses of 254-nm radiation used in the present study, i.e., doses greater than 9.6×10^{19} quanta. The only possible exception to the latter conclusion is protein L6, a 50S ribosomal protein that has been assigned a 5S rRNA binding protein (Gray et al., 1972). Consequently, the apparent lack of reactivity of 5S and 23S rRNA in UV-mediated intermolecular cross-linkage does indicate that either the 5S and 23S rRNA molecules interact with and become covalently cross-linked to (a) common ribosomal protein(s), but that the distances between the interaction sites of the rRNA molecules on the protein are sufficiently large that the intervening peptide sequences are susceptible to proteolysis by Pronase B, or that the two rRNA molecules only share in common protein L6. An alternative explanation, that the 5S and 23S rRNA molecules do not share (a) protein(s) in common is unlikely, since stable specific complexes of the isolated rRNA molecules and a small number of ribosomal proteins have been isolated from ribonuclease-treated 50S ribosomes and have also been constructed from the rRNA components and individual ribosomal proteins (Gray et al., 1972, 1973; Horne & Erdmann, 1972).

A final question that will be considered in this report is whether the UV-mediated changes in the structural integrities of the rRNA components of the ribosomes observed in this report and in earlier studies of ribosome photochemistry might make significant contributions to photoinactivation of ribosome functions. The changes of concern are rRNA/protein cross-linkage, rRNA chain breakage, and destabilization of rRNA secondary structure toward denaturation. Kagawa et al. (1967) have reported that in vitro irradiation of *E. coli* 30S and 50S ribosomes with 4.6×10^4 erg mm^{-2} of 254-nm radiation results in a 90% loss of the activities of the ribosomes in in vitro poly(U) programmed polyphenylalanine synthesis. The minimum doses of 254-nm radiation required for the introduction of chain breaks into the rRNA components of the *E. coli* ribosomes (expressed in biologically relevant units[3]) are 4×10^4

[3] The calculated surface area of the irradiation apparatus is 1.25×10^4 mm^2. Consequently, the conversion factor between quanta of 254-nm radiation and erg mm^{-2} in our system is 6.2×10^{-16} erg mm^{-2} quanta^{-1}. It should be noted that this value is different from the conversion factor utilized in previous studies to convert quanta to erg mm^{-2} (Gorelic, 1975, 1976b,c). The basis for the difference is a recalculation of the surface area of the irradiation apparatus used in these studies. Consequently, the value for the conversion factor derived here should be accepted as the correct value in all the previous studies carried out in our laboratory on ribosome photochemistry.

and 4.2×10^4 erg mm^{-2} for nicks in ribosome-bound 16S rRNA and 23S rRNA, respectively, and 8.4×10^4 and $>1.4 \times 10^5$ erg mm^{-2} for chain scission of ribosome-bound 16S rRNA and 5S and 23S rRNA, respectively. Destabilization of 23S rRNA secondary structure is initiated after exposure of *E. coli* 50S ribosomes to $<1.4 \times 10^4$ erg mm^{-2} and is completed after a dose of 4.2×10^4 erg mm^{-2} of 254-nm radiation. Finally, the covalent cross-linkage of most of the *E. coli* 30S and 50S ribosomal proteins to the rRNA components of the corresponding ribosomes requires exposure of the 30S and 50S ribosomes to doses of 254-nm radiation up to and including 9.3×10^3 and 3.8×10^4 erg mm^{-2}, respectively (Gorelic, 1976c). It can therefore be concluded that it is unlikely that UV-mediated rRNA chain-breakage could make an important contribution to photoinactivation of in vitro ribosome function. On the other hand, the UV-induced modification responsible for destabilization of 23S rRNA secondary structure toward denaturation, or rRNA/protein cross-linking, could make important contributions to the effect of UV radiation on *E. coli* ribosome function.

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