Demonstration of Ribosome-Dependent Photoinduced Chain Breakage of the 16S Ribosomal Ribonucleic Acid Component of the *Escherichia coli* 30S Ribosomal Subunit†

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ABSTRACT: The effects of 253.7-nm radiation on the structural integrities of free and ribosome-bound 16S ribosomal ribonucleic acid (rRNA) have been elucidated. Exposure of aqueous solutions of *Escherichia coli* 30S ribosomal subunits to 253.7-nm radiation results in RNA-chain scission and the formation of single-stranded breaks in double-stranded regions of the ribosome-bound 16S rRNA. The minimum doses of incident 253.7-nm radiation required for the first detection of the two types of RNA chain breaks are 2×10^{20} quanta for single-strand breaks in double-stranded regions of the ribosome-bound 16S rRNA, and at least 5×10^{20} quanta for RNA-chain scission. In contrast to the photosensitivity of ribosome-bound 16S rRNA toward chain breakage, free 16S rRNA seems to be resistant toward photoinduced chain breakage at doses of incident 253.7-nm radiation up to at least 10^{21} quanta.

Recent photochemical studies of the *Escherichia coli* 30S ribosomes have indicated that exposure of aqueous buffered solutions of the ribosomes to 253.7-nm radiation results in the covalent cross-linkage of the protein components of the 30S ribosomes to the 16S rRNA component (Gorelic, 1975a,b). Since covalent cross-linkage in situ of the individual 30S ribosomal proteins to the 16S rRNA[1] appeared to occur in a sequential manner, and a kinetic analysis of the cross-linkage reaction indicated that there is a good correlation between the photoreactivity of a ribosomal protein in the cross-linkage reaction and the proximity of the protein to the 16S rRNA in the native topographical state of the 30S ribosome, it was concluded that photochemical methods could be used to study RNA-protein interactions in intact ribosomes (Gorelic, 1976).

The conclusions cited above were inferred from studies indicating that exposure of solutions of *E. coli* 30S ribosomes to 253.7-nm radiation results in dose-dependent changes in a number of physical properties of the ribosome components consistent with the covalent cross-linkage of the ribosomal proteins to the high molecular weight and polyanionic 16S rRNA component. The physical properties monitored were separability of the proteins and 16S rRNA in 4 M urea—2 M LiCl, and the gel filtration and electrophoretic characteristics of the proteins. The molecular basis for the observed dose-dependent changes in these properties was that the covalent cross-linkage of the ribosomal proteins and the 16S rRNA should be accompanied by changes in the initial physical properties of the 30S ribosomal proteins related to their overall size and electrical charge, and in the insolubility of the 16S rRNA component and partitioning of the *E. coli* 30S ribosomal proteins into the soluble phase in 4 M urea–2 M LiCl.

The validity of conclusions regarding ribosome structure inferred from the results of studies of the types cited above is contingent upon the assumption that a number of secondary photoinduced modifications of the ribosomal proteins and the 16S rRNA do not take place. The secondary photoinduced modifications of concern here are peptide-bond breakage, protein–protein cross-linkage, loss of charged side-chain groups from the proteins, and the production of chain breaks in the ribosome-bound 16S rRNA molecule. The results of control experiments in the cited photochemical studies indicated that the possible secondary photoinduced modifications of the 30S ribosomal proteins of concern did not occur at doses of 253.7-nm radiation sufficient to covalently cross-link most of the 30S ribosomal proteins to the 16S rRNA. The data obtained in these studies did, however, seem to suggest that photoinduced RNA-chain breakage might have taken place.

The introduction by photochemical means of chain breaks into the 16S rRNA component of UV-irradiated 30S ribosomes could distort a photochemical analysis of RNA—protein interactions in the 30S subunit in two ways. First, the ribosomal proteins initially cross-linked to the 16S rRNA might be released from the 16S rRNA as a result of the chain breaks in such a manner as to covalently retain rRNA fragments too small in size to affect detectable changes in the initial physical properties of the proteins related to size and charge. Consequently, such proteins might not exhibit the changes in gel filtration and electrophoresis properties required for classification by the methods alluded to above as RNA-binding proteins, even though these proteins were in fact reactive in the cross-linkage reaction. Second, chain breakage of the 16S rRNA component might be so extensive as to alter its native tertiary structure. In such an event, it would be difficult, if not impossible, to relate the interactions inferred from the results of studies of the photoreactivities of the individual 30S ribosomal proteins in the cross-linkage reaction to RNA–protein interactions in the native 30S ribosomal subunit.

Based upon the above conclusions regarding the possible effects of photoinduced chain breaks in the 16S rRNA com-

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[1] Abbreviations used: Me_2SO, dimethyl sulfoxide; tRNA and rRNA, transfer and ribosomal ribonucleic acids, respectively; UV, ultraviolet; Tris, tris(hydroxymethyl)aminomethane; CD, circular dichroism; ORD, optical rotary dispersion.

ponent of UV-irradiated 30S ribosomes on a photochemical analysis of the topography of this subunit and the tentative nature of the conclusions regarding RNA chain breaks obtained in an earlier photochemical study of the 30S ribosome, a detailed analysis of the effects of 253.7-nm radiation on the structural integrity of ribosome-bound 16S rRNA has been carried out in this report. The results of this analysis indicate that chain breaks are present in the 16S rRNA component of UV-irradiated 30S ribosomes, but that the dose requirements for the production of these chain breaks are such that it is unlikely that the production of rRNA chain breaks in UV-irradiated *E. coli* 30S ribosomes distorts a photochemical analysis of RNA–protein interactions in this subunit.

Experimental Section

Materials. Pronase type B was obtained from Calbiochem. Dimethyl sulfoxide (analytical reagent grade) was obtained from Mallinkrodt and was distilled prior to use. The fraction boiling at 74 °C (10 mmHg) was collected and used to prepare the buffers used in the gel filtration studies. Sodium lauryl sulfate was obtained from Schwarz/Mann and was recrystallized from ethanol prior to use. Radioactively labeled uracil ([5,6-^{3}H]uracil (specific activity 40 Ci/mmol)) was obtained from Schwarz/Mann. Calf thymus DNA was obtained from Sigma Chemical. Potassium ferrioxalate was prepared according to the method of Hatchard and Parker (1956). Sepharose CL-4B was obtained from Pharmacia. All other chemicals were analytical reagent grade.

Unlabeled ribosomal subunits were prepared from *E. coli* D10 cells grown in complete media and harvested in mid-log phase according to previously described methods (Gorelic, 1975a). Ribosomal subunits labeled in their rRNA components with [5,6-^{3}H]uracil were prepared from *E. coli* D10 cells grown in complete media containing 2 μg/ml of labeled uracil and harvested in mid-log phase. Radioactively labeled 16S rRNA was prepared from the ^{3}H-labeled 30S ribosomes by dialysis of the ribosomes against 8 M urea–4 M LiCl according to a previously published procedure (Gorelic, 1975a).

Irradiation Conditions. Solutions of *E. coli* 30S ribosomal subunits or 16S rRNA (1 A_{260} unit/ml) in 100 ml of irradiation buffer (5 mM H_3PO_4, neutralized to pH 7.4 with KOH; 10 mM $MgCl_2$; 30 mM KCl) were irradiated at 253.7 nm according to methods that have been previously described (Gorelic, 1975a). The temperatures of the ribosome and 16S 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. The output of the light source at 253.7 nm was determined by ferrioxalate actinometry (Parker, 1953) to be $1.53 \pm 0.04 \times 10^{18}$ quanta s^{-1}. The fraction of incident 254-nm radiation actually absorbed by the ribosome and rRNA solutions was determined by uridine actinometry (Fikus and Shugar, 1966) to be 0.30.

Two types of irradiation experiments were performed. In one type, individual samples of 30S ribosomal subunits were each exposed to specific doses of 253.7-nm radiation. The ribosomes in the samples were then concentrated by ultrafiltration using an Amicon no. XM100A ultrafilter followed by ultracentrifugation in a Beckmann Type 65 angle-head rotor on a Beckmann L2-65B ultracentrifuge. Each of the resultant pelleted ribosome samples was resuspended in an appropriate volume of irradiation buffer and stored at −70 °C. In a second type, a single sample of either radioactively labeled 30S ribosomal subunits or radioactively labeled 16S rRNA was irradiated with a variety of doses of 253.7-nm radiation. Samples (1.0 ml) were removed at regular intervals during the course of the irradiations and were stored at −70 °C until used.

Preparation of Samples for Gel Filtration Analysis. Ribosome or 16S rRNA samples were prepared for gel filtration analysis in one of three ways. First, the samples were incubated with sodium lauryl sulfate (final concentration, 20 mg/ml) for 5 min at 37 °C and cooled to room temperature, and the precipitated material was removed by low-speed centrifugation. Second, the sodium lauryl sulfate treated ribosome or 16S rRNA samples were incubated with Pronase B (final concentration 1 mg/ml) for 1 h at 37 °C, cooled to room temperature, and subjected to low-speed centrifugation in order to remove precipitated material. The Pronase B was added to the sodium lauryl sulfate treated samples 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 any contaminating ribonuclease activity. Third, sodium lauryl sulfate and Pronase treated ribosome or 16S rRNA samples were diluted with an equal volume of dimethyl sulfoxide, incubated at 37 °C for 10 min, cooled to room temperature, and centrifuged at low speed in order to remove any precipitated material.

Control studies using *E. coli* DNA indicated that treatment of an extensively helical nucleic acid molecule such as 16S rRNA in Me_2SO according to the third method would completely disrupt its native secondary structure.

Results

Exposure of aqueous solutions of *E. coli* 30S ribosomes to 253.7-nm radiation could conceivably result in the introduction of two types of breaks into the ribosome-bound 16S rRNA. The first type is chain scission and would result from the introduction of either single-strand breaks into single-stranded regions or double-strand breaks into double-stranded regions of the 16S rRNA. The second type is "nicking" and would result from the introduction of single-strand breaks into double-stranded regions of the 16S rRNA. The protocols selected for detection of these two different types of RNA-chain breaks are as follows. First, in order to test for chain scission, ribosome samples were pretreated with concentrations of sodium lauryl sulfate sufficient to dissociate most of the noncovalently bound ribosomal proteins from the 16S rRNA but not sufficient to result in the denaturation of the native secondary structure of the 16S rRNA molecule, and with Pronase in order to remove as much of the covalently cross-linked proteins as possible. The sodium lauryl sulfate and Pronase pretreated samples were then subjected to gel filtration under conditions not denaturing for the native secondary structure of the 16S rRNA. Second, in order to test for "nicks" the 16S samples pretreated with sodium lauryl sulfate and Pronase were diluted with an equal volume of Me_2SO in order to denature the native secondary structure of the 16S rRNA; the resultant samples were subjected to gel filtration using an elution buffer 50% v/v in Me_2SO.

The basis for the selection of a Me_2SO-based buffer for denaturation of the native secondary structure of the 16S rRNA resides in findings in other laboratories that Me_2SO is an effective denaturant for the double-stranded Sindbis virus RNA molecule and the helical MS2 RNA molecule (Strauss et al., 1968; Dobos et al., 1971).

The results of a gel filtration study, under conditions not denaturing for the native secondary structure of the 16S rRNA molecule, of the effects of 254-nm radiation on the structural integrity of the 16S rRNA molecule in intact *E. coli* 30S ribosomes are presented in Figures 1A–D. Irradiation of aqueous buffered solutions of *E. coli* 30S ribosomes with a dose of in-

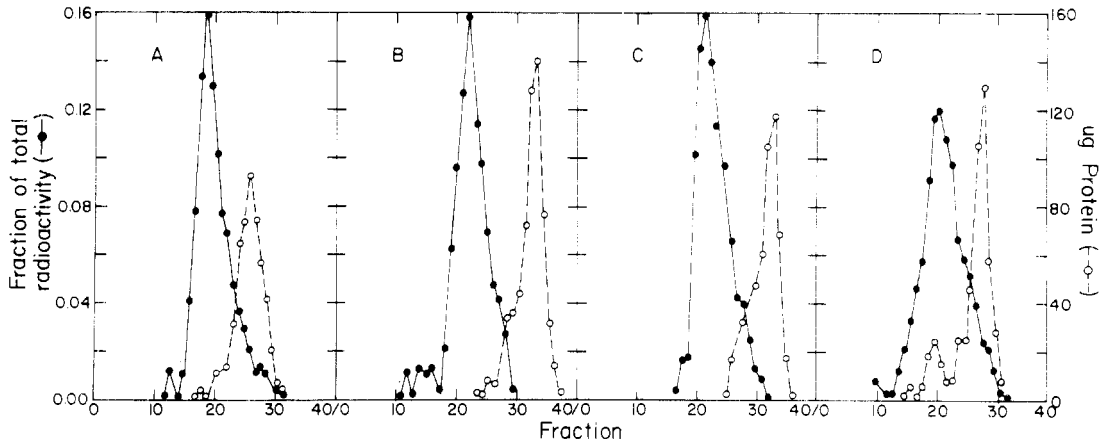

FIGURE 1: Gel filtration characteristics, under nondenaturing conditions, of the protein and 16S rRNA components of unirradiated and irradiated *E. coli* 30S ribosomes. Three solutions of 30S ribosomes labeled with [^{3}H]uracil in their 16S rRNA components were prepared. One solution was not irradiated, a second solution was exposed to an incident dose of 253.7-nm radiation of 5×10^{20} quanta, and a third solution to an incident dose of 10^{21} quanta. The ribosomes were reisolated and prepared for analysis by gel filtration according to the procedure in Methods. Aliquots (0.6 ml) of the prepared samples were applied to the top of a 1.1 × 38 cm Sepharose CL-4B column packed in a 10 mM Tris-HCl (pH 7.6) 0.15 M NaCl buffer. The column was precalibrated using a bromphenol blue marker. The elution volume of this marker was 37.36 ml. The pretreated ribosome samples were eluted with a 10 mM Tris-HCl (pH 7.6)–0.15 M NaCl buffer. Fractions of 1.0 ml were collected at a rate of 0.33 ml/min. The collected fractions were split into two portions. One portion was assayed for protein content by the Lowry method (Lowry et al., 1951), and the resultant data were expressed as μg of protein per fraction (--o--). The second portion of the collected fractions was assayed for radioactivity in a standard toluene-based scintillation fluid using NCS solubilizer (Amersham/Searle) in order to prepare a homogeneous cocktail. The radioactivity in each fraction was expressed as the fraction of total cpm eluted from the column (—●—). The radioactivity recovered from each of the columns was 90% of the total input and corresponded to 11 159 and 10 546 cpm for the unirradiated ribosome samples, and 9519 cpm and 10 702 cpm for the ribosome samples irradiated with incident doses of 5×10^{20} and 10^{21} quanta, respectively. (A) Unirradiated ribosome sample treated with sodium lauryl sulfate; (B) unirradiated ribosome sample treated with sodium lauryl sulfate and Pronase; (C) ribosome sample irradiated with an incident dose of 5×10^{20} quanta and treated with sodium lauryl sulfate and Pronase; (D) ribosome sample irradiated with an incident dose of 10^{21} quanta and treated with sodium lauryl sulfate and Pronase.

cident 254-nm radiation of 5×10^{20} quanta (1.5×10^{20} quanta, corrected; or 1.2×10^4 ergs mm^{-2}) is seen not to result in detectable changes in the initial elution profile of the 16S rRNA component of the *E. coli* 30S ribosome (Figures 1B and 1C). Irradiation of aqueous buffered solutions of *E. coli* 30S ribosomes with a dose of incident 254-nm radiation of 10^{21} quanta (3×10^{20} quanta, corrected; or 2.4×10^4 ergs mm^{-2}) results, on the other hand, in *substantial changes* in the initial elution profile from gel filtration media of the 16S rRNA component of the 30S subunit. The peak height of the 16S rRNA elution profile of the irradiated sample is reduced ca. 25% relative to its initial value, and the breadth of the 16S rRNA peak in the elution profile of the irradiated sample is substantially larger relative to the breadth of the 16S rRNA elution profile of the unirradiated sample (Figures 1B and 1D). In addition to the changes in the initial 16S rRNA elution profiles observed in the more heavily irradiated sample, there is also a substantial overlap between the 16S rRNA elution profile and the Lowry reactive material in this sample (Figure 1D). [In view of the absence of suitable controls (Gorelic, 1975a,b), it is not possible to ascertain what fraction of the Lowry reactive material coeluting with the 16S rRNA-derived material in the gel filtration studies represented by the data in Figure 1D corresponds to peptides covalently and noncovalently associated with the 16S rRNA-derived material.] Finally, the differences observed between the elution volumes of the Lowry reactive materials and extents of overlap between the Lowry reactive and 16S rRNA derived materials in Figures 1A and 1B indicate that the Pronase B used in the studies represented by the data in Figure 1 was active under the experimental conditions.

The results of a gel filtration study, under conditions denaturing for the native secondary structure of the 16S rRNA component of the *E. coli* 30s ribosomes, of the effects of 254-nm radiation on the structural integrity of the 16S rRNA molecule in intact *E. coli* ribosomes are presented in Figure 2. Exposure of the *E. coli* 30S ribosomes to a dose of incident 254-nm radiation of 5×10^{20} quanta (1.5×10^{20} quanta corrected; or 1.2×10^4 ergs mm^{-2}) is seen to result in changes in the structural integrity of the 16S rRNA component of the 30S ribosome that are detectable under denaturing conditions by gel filtration techniques: marked diminution in the peak height and substantial increase in the breadth of the 16S rRNA elution profile relative to their initial values (Figures 2B and 2C). The effects of 254-nm radiation observed by gel filtration techniques on the initial structural integrity of the 16S rRNA component of *E. coli* 30S ribosomes are substantially greater in 30S ribosomes irradiated with a dose of 10^{21} quanta (3×10^{20} quanta corrected; or 2.4×10^4 ergs mm^{-2}). The peak height of the 16S rRNA elution profile is reduced ca. 75% relative to its initial value, and almost one-half of the 16S rRNA derived material is detected at elution volumes substantially larger than the 16S rRNA material in the elution profile of an unirradiated 30S ribosome sample (Figures 2B and 2D). Finally, the differences in the elution volumes of the Lowry reactive materials in Figures 2A and 2B quite clearly indicate that the Pronase B added to the irradiated 30S ribosome samples was active under the experimental conditions used in the studies represented by the data in Figure 2.

The gel filtration studies represented by the data in Figure 2 indicate that exposure of *E. coli* 30S ribosomes to a dose of incident 254-nm radiation of 5×10^{20} quanta does result in changes in the gel filtration properties of the 16S rRNA component of the 30S ribosomes that are detectable under conditions denaturing for the native secondary structure of the 16S rRNA molecule. However, these data do not define a minimum dose of 254-nm radiation below which photoinduced changes in the initial gel filtration properties of the 16S rRNA component of the *E. coli* 30S ribosomes cannot be detected. In order to obtain this information, 30S ribosomes were exposed to a larger number of doses of 254-nm radiation than

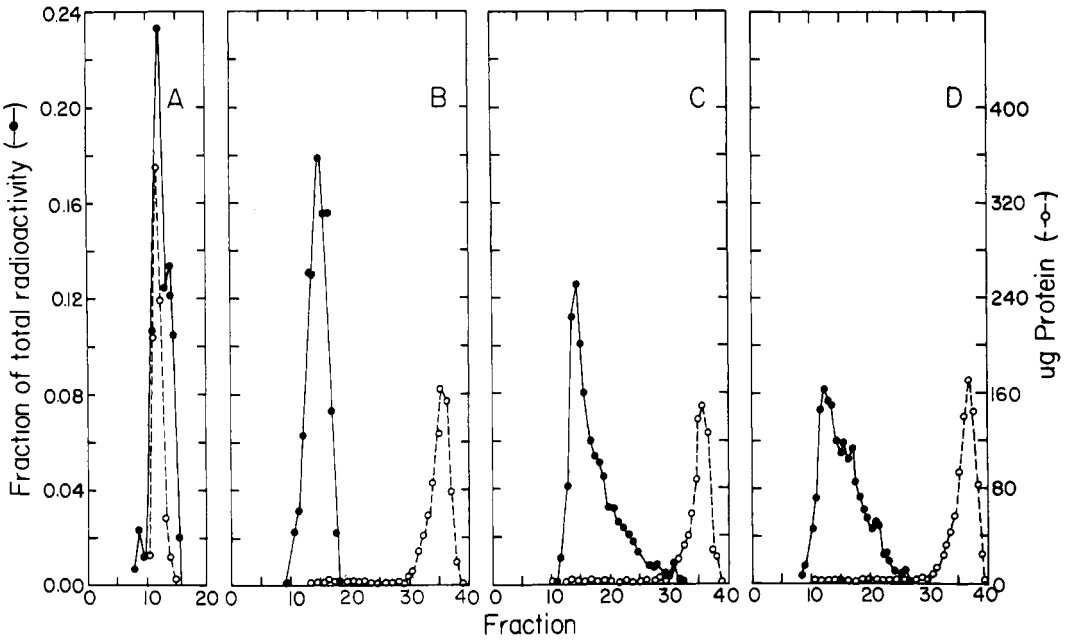

FIGURE 2: Gel filtration characteristics under denaturing conditions of the protein and 16S rRNA components of unirradiated and irradiated *E. coli* 30S ribosomes. Solutions of *E. coli* 30S ribosomes were prepared, irradiated, and pretreated with sodium lauryl sulfate and/or Pronase according to the conditions described in the legend to Figure 1. Portions of the treated samples were diluted with an equal volume of dimethyl sulfoxide and incubated according to the procedure described in Methods, and aliquots (0.6 ml) were applied to the top of a 1.1 × 38 cm Sepharose CL-4B column packed in a 1:1 solution of 10 mM Tris-HCl (pH 7.6)–dimethyl sulfoxide. The column was precalibrated using bromphenol blue as a marker. The elution volume of this marker was 36.95 ml. The applied pretreated ribosome samples were eluted with a 1:1 10 mM Tris-HCl (pH 7.6)–dimethyl sulfoxide buffer at a flow rate of 0.088 ml/min. Fractions of 0.78 ml were collected and analyzed for radioactivity and protein content according to the methods described in the legend to Figure 1. The recoveries of input radioactivity from the columns were 90% and corresponded to 5502 and 4934 cpm for the unirradiated samples, 5561 cpm for the sample irradiated with a dose of 5×10^{20} quanta, and 3962 cpm for the sample irradiated with a dose of 10^{21} quanta. The amount of radioactivity in each of the collected fractions was expressed in the same manner as in Figure 1. (A) Unirradiated ribosome sample treated with sodium lauryl sulfate but not with Pronase; (B) unirradiated ribosome sample treated with sodium lauryl sulfate and Pronase; (C) ribosome sample irradiated with an incident dose of 5×10^{20} quanta of 253.7-nm radiation and treated with sodium lauryl sulfate and Pronase; (D) ribosome sample irradiated with an incident dose of 10^{21} quanta of 253.7-nm radiation and treated with sodium lauryl sulfate and Pronase.

used in the studies represented by the data in Figure 2. The resultant samples were treated with sodium lauryl sulfate and Pronase and analyzed by gel filtration under conditions denaturing for the native secondary structure of the 16S rRNA molecule. *E. coli* tRNA was added to each of the samples applied to the gel filtration columns as an internal standard for calibration of the columns with respect to elution volumes of applied components and resolution in successive passes through the columns. The resultant elution profiles, presented in Figure 3, are in qualitative agreement with the elution profiles in Figure 2. both sets of elution profiles indicate that irradiation of the *E. coli* 30S ribosomes with 254-nm radiation does result in changes in the initial gel filtration properties of the 16S rRNA component of the 30S ribosomes that are readily detectable under conditions denaturing for the native secondary structure of the 16S rRNA molecule. The net results of the changes in gel filtration properties are a dose-dependent decrease in the initial peak height and a dose-dependent increase in the breadth of the initial elution profile of the 16S rRNA component of the *E. coli* 30S ribosomes.

Finally, in order to determine whether free 16S rRNA is sensitive toward photoinduced chain breakage, an aqueous buffered solution of free 16S rRNA was irradiated under the same conditions as used in the studies represented by the data in Figure 3, and the gel filtration characteristics of the UV-irradiated samples were determined under conditions denaturing for the native secondary structure of the 16S rRNA. The resultant elution profiles are presented in Figure 4. These elution profiles quite clearly indicate that exposure of free 16S rRNA to doses of 253.7-nm radiation of up to 10^{21} quanta (3×10^{20} quanta corrected; 2.4×10^4 ergs mm^{-2}) does not result in the introduction of "nicks" into double-stranded regions of the 16S rRNA or RNA chain scission.

Discussion

Gel filtration techniques have been used in this report to study the effects of 253.7-nm radiation on the structural integrities of the 16S rRNA molecule in intact *E. coli* 30S ribosomes and free 16S rRNA. The results of these studies indicate that the 16S rRNA in intact *E. coli* 30S ribosomes is sensitive toward photoinduced chain scission and the introduction of single-strand breaks into double-stranded regions, whereas free 16S rRNA is resistant toward chain breakage at doses of incident 253.7-nm radiation up to 10^{21} quanta (3×10^{20} quanta corrected, or 2.4×10^4 ergs mm^{-2}). These conclusions are based upon the following observations: (1) elution, under nondenaturing conditions, of the 16S rRNA samples prepared from *E. coli* 30S ribosomes irradiated with doses of incident radiation greater than 5×10^{20} (1.5×10^{20} quanta corrected, or 1.2×10^4 ergs mm^{-2}) quanta gives elution profiles with RNA peak heights significantly reduced and peak widths considerably broader than an elution profile of a 16S rRNA sample prepared from an unirradiated 30S ribosome sample; (2) elution, under denaturing conditions, of 16S rRNA samples prepared from 30S ribosomes irradiated with doses of incident 253.7-nm radiation of greater than or equal to 2×10^{20} quanta (6×10^{19} quanta corrected, or 2.4×10^3 ergs mm^{-2}) gives elution profiles with RNA peak heights significantly reduced and peak widths significantly broader than in an elution profile of a 16S rRNA sample prepared from an

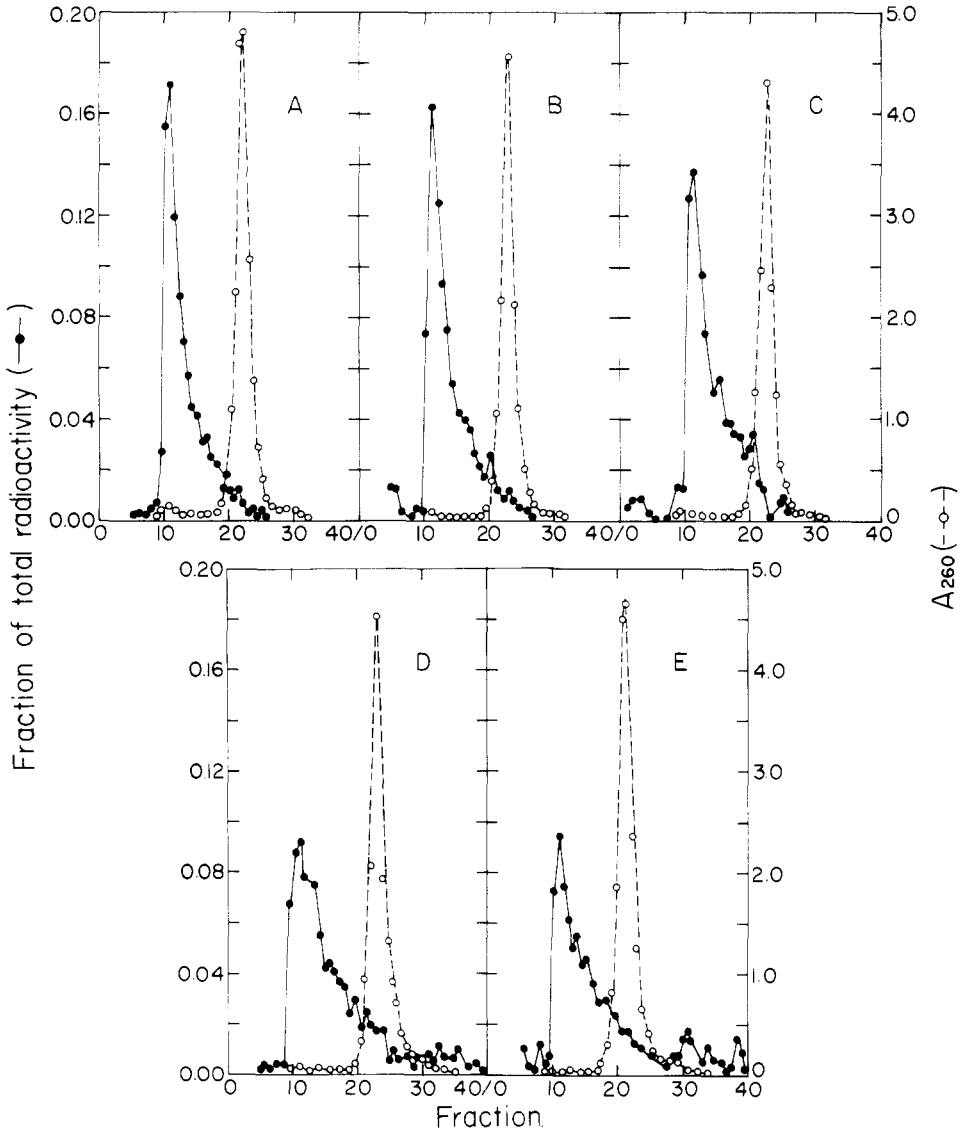

FIGURE 3: Gel filtration characteristics, under denaturing conditions, of the 16S rRNA component of unirradiated and irradiated *E. coli* 30S ribosomes. A solution of *E. coli* 30S ribosomes was exposed to various incident doses of 253.7-nm radiation. Aliquots (1.0 ml) were removed at specific doses of 253.7-nm radiation and were prepared for gel filtration analysis by treatment with sodium lauryl sulfate and Pronase according to the procedures described in Methods and in the legend to Figure 2. *E. coli* B tRNA was added to each of the treated samples in an amount of 4 A_{260} units per sample prior to gel filtration. The resultant solutions were then applied to the top of a 1.1 × 38 cm Sepharose CL-4B column packed in a 1:1 10 mM Tris-HCl (pH 7.6)–dimethyl sulfoxide buffer and that had been precalibrated using bromphenol blue as a marker. The elution volume of the marker was 36.95 ml. The pretreated ribosome samples were then eluted with a 1:1 10 mM Tris-HCl (pH 7.6)–dimethyl sulfoxide buffer. Fractions of 0.75 ml were collected at a flow rate of 0.083 ml/min and were analyzed for radioactivity according to the method described in the legend to Figure 1. The amount of radioactivity in each fraction was expressed in the same manner as in Figure 1. The recoveries of input radioactivity from the columns were 90% and corresponded to 10 543, 8346, 7124, 9595, and 6585 cpm for the samples irradiated with doses of 0, 2×10^{20}, 5×10^{20} 7×10^{20}, and 10^{21} quanta, respectively. Radioactivity (—●—); *E. coli* B tRNA (--○--). (A) Unirradiated ribosome sample; (B) ribosome sample irradiated with an incident dose of 2×10^{20} quanta; (C) ribosome sample irradiated with an incident dose of 5×10^{20} quanta; (D) ribosome sample irradiated with an incident dose of 7×10^{20} quanta; (E) ribosome sample irradiated with an incident dose of 10^{21} quanta.

unirradiated 30S ribosome sample; (3) changes similar in nature to those observed for the 16S rRNA molecule in intact *E. coli* 30S ribosomes are not detected in gel filtration studies of free 16S rRNA samples exposed to doses of incident 253.7-nm radiation as large as 10^{21} quanta (3×10^{20} quanta corrected; 2.4×10^4 ergs mm^{-2}). The data presented in this report also indicate that the 16S rRNA molecule in intact *E. coli* 30S ribosomes is more sensitive toward the photoinduced formation of nicks than toward photoinduced RNA-chain scission. This conclusion is based upon the observation that lower doses of incident 253.7-nm radiation are required for detectable changes in the 16S rRNA elution profile obtained under gel filtration conditions denaturing for the native secondary structure of the 16S rRNA than under nondenaturing gel filtration conditions and receives additional support from the finding in this report that substantially larger changes in the elution profile of the 16S rRNA prepared from 30S ribosomes irradiated with an incident dose of 10^{21} (3×10^{20} quanta corrected, or 2.4×10^4 ergs mm^{-2}) quanta are detected under denaturing conditions than under conditions not denaturing for the native secondary structure of the 16S rRNA.

The sensitivity of the 16S rRNA molecule in intact *E. coli* 30S ribosomes toward chain cleavage could be a property conferred upon the 16S rRNA molecule by its secondary and/or tertiary structure or could result from physical interactions of the 16S rRNA with the protein components of the

30S ribosome. Alternatively, the photosensitivity of the ribosome-bound 16S rRNA toward chain cleavage could result from the interaction of the 16S rRNA molecule with a ribosome-bound endonuclease. Since 16S rRNA can be isolated intact from *E. coli* 30S ribosomes, this latter interaction would probably require prior photoinduced modifications in ribosome topography to take place in order for the 16S rRNA to assume an orientation sensitive toward nucleolytic attack by such a ribosome-bound activity.

It is unlikely that the secondary structure of the 16S rRNA component of the *E. coli* 30S ribosome is a primary determinant of the photosensitivity toward chain cleavage of the 16S rRNA molecule in intact *E. coli* 30S ribosomes. Sarkar et al. (1967) and Yang (1967) have shown by CD and ORD studies that the helical contents of free 16S rRNA and 16S rRNA incorporated into the intact 30S ribosome are quite similar; yet the free state of the 16S rRNA molecule has been demonstrated in this report to be substantially less sensitive toward photoinduced chain breakage than the incorporated state.

It is not possible to develop arguments that completely rule out the involvement of a ribosome-bound endonuclease at least in the initial stages of 16S rRNA chain breakage. The photoinduced changes in the 16S rRNA elution profiles observed in this report would be consistent with chain cleavage occurring in a random fashion. However, the obvious low resolving power of the Sepharose CL-4B column does not permit a decision to be made as to whether an initial step in the chain-cleavage process involved an endonucleolytic attack on the 16S rRNA molecule to give two RNA fragments of comparable molecular weights that subsequently underwent photoinduced chain cleavage to a random distribution of lower molecular weight RNA fragments.

Based upon the conclusions of the above discussion, it would seem reasonable to conclude that it is probably the tertiary structure of the ribosome-bound 16S rRNA or interactions of the 16S rRNA with the ribosomal proteins that primarily confer upon the ribosome-bound 16S rRNA photosensitivity toward chain cleavage, but that the action of ribosome-bound endonucleases—viz., ribonuclease III (Robertson et al., 1968)—might also contribute to this photosensitivity. It should be noted that the first two explanations are not necessarily mutually exclusive since the ribosomal proteins could participate in chain breakage by affecting a condensation of the native tertiary structure of the 16S rRNA to a conformation inherently more reactive toward photoinduced rRNA-chain cleavage than free 16S rRNA as well as by participating chemically in the chain-breakage process. These suggestions are conjectural in nature and require further experimental verification. Studies of a chemical nature are currently in progress to test the validity of the mechanisms for chain breakage proposed here. These studies are being coupled with comparative studies of *E. coli* strains wild type and mutant with respect to RNase III activities in order to assess the extent to which a ribosome-bound endonuclease activity might contribute to chain breakage of the 16S rRNA in UV-irradiated *E. coli* 30S ribosomes.

The initial objective of this study was to determine whether photoinduced chain breakage and/or chain scission of the 16S rRNA molecule in intact *E. coli* 30S ribosomes would distort an analysis of 30S ribosome structure in terms of the in situ photoreactivities of individual 30S ribosomal proteins in covalent cross-linkage with the 16S rRNA molecule. Earlier reports (Gorelic, 1975a,b, 1976) indicated that most of the proteins in irradiated intact *E. coli* 30S ribosomes are completely cross-linked to the 16S rRNA molecule at incident

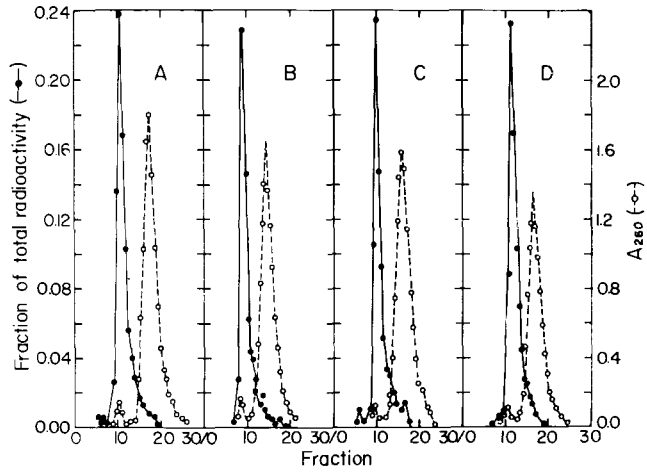

FIGURE 4: Gel filtration characteristics, under denaturing conditions, of unirradiated and irradiated 16S rRNA. A solution of 16S rRNA was irradiated with incident doses of 253.7-nm radiation up to 10^{21} quanta. Aliquots (1.0 ml) were removed at doses of 5×10^{20} and 10^{21} quanta and were prepared for gel filtration analysis by sodium lauryl sulfate and Pronase pretreatments and dilution with dimethyl sulfoxide according to the procedures described in the legend to Figure 3 and in Methods. Aliquots of the prepared samples were then applied to the top of a 1.1×38 cm Sepharose CL-4B column packed in a 1:1 Tris-HCl (pH 7.6)–dimethyl sulfoxide buffer precalibrated with a bromphenol blue marker. The elution volume for this marker was 36.95 ml. The applied 16S rRNA samples were then eluted with the 1:1 10 mM Tris-HCl–dimethyl sulfoxide buffer. Fractions of 0.58 ml were collected at a flow rate of 0.096 ml/min and were analyzed for radioactivity. The amount of radioactivity in each fraction was expressed in the same manner as in Figure 1. The recoveries of input radioactivity from the columns were 90% and corresponded to 2778, 2152, and 2061 cpm for the unirradiated 16S rRNA sample and the 16S rRNA samples exposed to incident doses of 5×10^{20} and 10^{21}, quanta, respectively. 16S rRNA (—●—); *E. coli* B tRNA (--○--). (A,B) Unirradiated 16S rRNA sample; (C) 16S rRNA sample irradiated with an incident dose of 5×10^{20} quanta; (D) 16S rRNA sample irradiated with an incident dose of 10^{21} quanta.

doses of 254-nm radiation of 2×10^{20} quanta (6×10^{19} quanta, corrected; or 2.4×10^3 ergs mm^{-2}). On the other hand, it was observed in this study that photoinduced chain scission of the 16S rRNA component in intact *E. coli* 30S ribosomes appears to require a dose of incident 254-nm radiation of ca. 10^{21} quanta (3×10^{20} quanta, corrected). In addition, although photoinduced 16S rRNA chain "nicking" was detected in this study at doses of 254-nm radiation of ca. 2×10^{20} quanta (6×10^{19} quanta, corrected), the elution volume of the released 16S rRNA-derived material was consistent with the production of fragments substantially larger in molecular weight than the added *E. coli* tRNA marker. It would therefore seem reasonable to conclude that neither photoinduced 16S rRNA chain scission nor chain "nicking" would distort an analysis of 30S ribosome structure based upon the photoinduced cross-linkage in situ of the protein components of the 30S ribosome to the 16S rRNA component.

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Carbon-13 Nuclear Magnetic Resonance Spectroscopy of Suspensions of Chinese Hamster Ovary Cells Specifically Enriched with [methyl-^{13}C]Choline†

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ABSTRACT: The mammalian Chinese hamster ovary cell line (CHO) was labeled with [methyl-^{13}C]choline. Under the conditions used, ~42% of the cellular choline was found to be associated with cellular lipids and the remaining 58% was present in the water-soluble fraction of the cell, primarily as phosphorylcholine. The spin–lattice relaxation time (T_1) of this pool of phosphorylcholine indicates an intracellular viscosity ~1.2 times that of H_2O. Cells fixed with formaldehyde and washed several times did not exhibit ^{13}C resonances corresponding to free choline or phosphorylcholine and were sufficiently stable to be studied up to 43 °C. The T_1 and line width behavior of the methyl resonance was studied as function of temperature in the fixed cells. The T_1 data exhibited an Arrhenius dependence with an activation energy of 4.3 kcal/mol, similar to that observed for free choline. The line width does not conform to an Arrhenius law and the values obtained for the CHO cells are similar to those previously reported for sonicated lecithin vesicles and to the value obtained in sonicated lecithin vesicles prepared from the extracted CHO cell lipids. The values are significantly smaller than the values reported for unsonicated lecithin dispersions. The data are discussed in terms of a theoretical model involving multiple internal rotations. A measurement of the ^{13}C–^{1}H nuclear Overhauser enhancement for the choline methyl carbons in vesicles prepared from the extracted CHO cell lipids gave a value of 3.0 ± 0.3.

Carbon-13 nuclear magnetic resonance spectroscopy has been used extensively to study the architecture and dynamics of the lipid bilayers in aqueous suspensions of liposomes and synthetic single-walled vesicles (Oldfield and Chapman, 1971; Birdsall et al., 1972; Levine et al., 1972a,b; Williams et al., 1973; Assmann et al., 1974; Gent and Prestegard, 1974; Godici and Landsberger, 1974, 1975; Sears et al., 1974; Stoffel et al., 1974). In contrast to the situation generally encountered in proton magnetic resonance spectroscopy (for a review, see Horwitz, 1972), the ^{13}C NMR[1] spectra of these lipid bilayers exhibit a remarkably high degree of resolution, frequently allowing the extraction of line width, spin–lattice relaxation (T_1), and chemical-shift data for individual lipid carbon atoms. These systems have been offered as models for the state of the lipids in natural membranes, but whether they are appropriate ones is questionable, given the high protein content of natural membranes and the important contributions that lipoprotein interactions probably make to membrane structure. In addition, Chan et al. (1973) have pointed out that the small liposomes and vesicles (250–500 Å) have a much shorter radius of curvature than cells (20 000 Å or more) and this could lead to packing disorders in the former which would strongly affect lipid mobilities.

Recently, ^{13}C NMR studies of natural membrane systems have been reported (Metcalfe et al., 1971; Metcalf, 1972; Robinson et al., 1972; Lee et al., 1973; Keough et al., 1973; Williams et al., 1973; Brown et al., 1975; Nicolau et al., 1975), but, due to many overlapping resonances and the low natural abundance and low magnetogyric ratio of ^{13}C, the generally poor quality of the spectra recorded has prevented a proper appreciation of the great potential of ^{13}C NMR spectroscopy for investigating the structure of the components of suspensions of intact cells, such as those considered here. These problems of low sensitivity and large natural background can be overcome to a large extent by employing systems uniformly or specifically labeled with ^{13}C. Thus, in an early study, Metcalfe

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[1] Abbreviations used are: CHO, Chinese hamster ovary; NMR, nuclear magnetic resonance; EPR, electron paramagnetic resonance; NOE, nuclear Overhauser effect; FT, Fourier transform.