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Composition of the *Escherichia coli* 70S Ribosomal Interface: A Cross-Linking Study†

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ABSTRACT: 70S tight-couple ribosomes from *Escherichia coli* were cross-linked by using the bifunctional reagent phenyldiglyoxal (PDG). The reaction was stopped after 4-h incubation while still in the linear range. In comparison with untreated ribosomes, 30% of those treated with PDG were shown, by sucrose gradient experiments, not to be separable into their subunits, but remained as 70S particles. There was no detectable change in the structure of the reacted particles when their sedimentation behavior was compared with that of native 70S controls. When the cross-linking reaction was performed in the presence of $tRNA^{Phe}$ and poly(U), the reacted ribosomes retained 40–50% of their tRNA binding activity. The reaction leads predominantly to the formation of RNA–protein cross-links but protein–protein as well as RNA–RNA cross-links could also be detected. Cross-linked material was extracted, and the individual RNAs were separated into 23S, 16S, and 5S RNAs. Proteins were identified electrophoretically after reversal of the RNA–protein cross-links. Proteins were found to be cross-linked to RNAs within and across the ribosomal subunits; the latter are considered to be close to or at the 70S subunit interface. The arrangement of RNA and protein at the subunit interface is discussed.

There is growing evidence that the interacting domains of the two ribosomal subunits, the so-called subunit interface, are of central importance in protein synthesis. In a series of affinity labeling and cross-linking studies (Ofengand et al., 1980; Girshovich et al., 1981; Maassen & Möller, 1981; Gimautdinova et al., 1981), the interface has been shown to harbor binding sites for substrates such as tRNA, mRNA, and the initiation, elongation, and termination factors of protein synthesis. Furthermore, the reversible association–dissociation of the ribosomal subunits is an important step during the initiation and termination of the protein synthesis cycle (Grunberg-Manago & Gros, 1977; Weissbach, 1980).

A number of interface components have already been identified by using different approaches. Important information has been obtained from immune electron microscopy studies (Stöffler et al., 1980; Kahan et al., 1981), partial nuclease digestion studies (Santer & Shane, 1977), chemical modification (Herr & Noller, 1979; Herr et al., 1979), and direct chemical cross-linking (Lambert & Traut, 1981; Bäumert et al., 1978).

Although these studies yielded valuable information, there is still a lack of information about which components are involved in subunit association and how these components are arranged topographically. In fact, little is known about how much RNA and/or protein contribute(s) to the formation of the interacting domain of the two ribosomal subunits. Clearly, more data have to be collected if a detailed picture of the topography of the subunit interface is to be constructed.

To gain a better understanding of how RNA and protein are arranged between the interacting sites of the two subunits, we have undertaken a cross-linking study using the bifunctional reagent phenyldiglyoxal (PDG). This reagent seems to be especially suitable for topographical studies of particles consisting of both RNA and protein: (i) the reaction can be performed under conditions optimal for protein synthesis; (ii) the reagent does not noticeably affect the structure of the particles, and reacted ribosomes retain 40–50% of their tRNA binding activity; (iii) the reaction with RNA is specific for guanosine while proteins react through their lysine and arginine residues; consequently, the reaction yields defined cross-linking products between RNA–protein, protein–protein, and RNA–RNA components of the ribosome; (iv) the reaction with RNA (guanosine) is completely reversible under mild alkaline conditions which allows a direct analysis of proteins cross-linked to RNA (Wagner et al., 1980; Wagner & Garrett, 1978).

In this study, we describe the identification of proteins cross-linked to the 23S, 16S, and 5S RNAs of the *Escherichia coli* ribosome. Cross-linking occurred within and across the individual subunits and allowed us to draw conclusions about the arrangement of the proteins and RNA in the free and associated states of the subunits, and which components are involved in the formation of the ribosomal interface.

Materials and Methods

Materials. PDG was synthesized as described (Wagner & Garrett, 1978). Acrylamide, *N,N'*-methylenebis(acrylamide), and sodium dodecyl sulfate ($NaDodSO_4$) were from Bio-Rad; sucrose (ribonuclease free) and $AgNO_3$ were from Merck. Urea (ultra pure) was from BRL. Dithiothreitol was from

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Serva; glutaraldehyde was from BDH Chemicals. Dialysis tubing (11.5 mm, molecular weight cutoff 3500) was from Spectrapor, Los Angeles, CA. [^{14}C]Lysine (sp act. 336 mCi/mmol) was from Amersham Buchler.

Preparation of Ribosomes. Ribosomes were prepared from *E. coli* MRE600 cells. Cells were disrupted by grinding with aluminum oxide in 20 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), pH 7.2, 10 mM $MgCl_2$, 60 mM NH_4Cl, and 10 mM 2-mercaptoethanol. DNase I was added to a final concentration of 5 μg/mL. After the aluminum oxide and cell debris were removed by centrifugation, the supernatant was centrifuged for 90 min at 15 000 rpm (SS34 Sorvall rotor). The supernatant was layered on top of a sucrose cushion (1.1 M sucrose in 50 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 0.5 M NH_4Cl, and 10 mM 2-mercaptoethanol) and centrifuged at 38 000 rpm for 24 h (45 Ti rotor). The pellets with the ribosomes were dissolved in 20 mM Tris-HCl, pH 7.5, 6.3 mM $MgCl_2$, 0.3 mM ethylenediaminetetraacetic acid (EDTA), 100 mM NH_4Cl, and 10 mM 2-mercaptoethanol. 70S tight-couple ribosomes were isolated according to Hapke & Noll (1976) by zonal centrifugation in 6 mM $MgCl_2$. The 70S tight-couple peak was pelleted at 40 000 rpm for 24 h (45 Ti rotor). The pellets were dissolved in 50 mM sodium cacodylate, pH 7.2, 10 mM $MgCl_2$, 150 mM KCl, and 3 mM 2-mercaptoethanol at a concentration of ~480 A_{260} units/mL.

Cross-Linking Reaction. In a typical cross-linking reaction, 70S tight-couple ribosomes at a final concentration of 30 A_{260} units/mL were incubated in 100 mM sodium cacodylate, pH 7.2, and 20 mM $MgCl_2$ together with an equal volume of a saturated PDG solution in the same buffer, the reagent concentration being approximately 2.5×10^{-5} M. Incubation was carried out at 37 °C for 4 h. The reaction was stopped by the addition of 0.67 volume of ethanol (−20 °C), and the ribosomes were precipitated at −20 °C for 1 h.

Test for Structural and Functional Integrity. After the cross-linking reaction, ribosomes were separated on a 5–30% sucrose gradient in 40 mM Tris–acetate, pH 7.2, 20 mM sodium borate, and 1 mM magnesium acetate (SW27 rotor, 17 000 rpm, 16 h at 4 °C). Cross-linked material, which sedimented as a 70S peak, was precipitated with 1 volume of ethanol after the Mg^{2+} concentration was adjusted to 20 mM. Ribosomes were dissolved in 50 mM sodium cacodylate, pH 7.2, 150 mM KCl, 20 mM $MgCl_2$, and 6 mM 2-mercaptoethanol and assayed for poly(U)-dependent Phe-tRNA^{Phe} binding according to Nirenberg & Leder (1964).

The structural homogeneity of the cross-linked material was tested by cosedimenting the cross-linked ribosomes with a native ^{14}C-labeled 70S preparation that had not been treated with PDG.

Extraction of Cross-Linked Material. Ribosomes were ethanol precipitated after the reaction to remove excess reagent. They were then centrifuged through a 5–30% sucrose gradient in 40 mM Tris-HCl, pH 7.5, 20 mM sodium borate, 1 mM $MgCl_2$, and 6 mM 2-mercaptoethanol at 4 °C (SW27 rotor, 17 000 rpm, 14 h. Under these conditions, non-cross-linked ribosomes dissociated completely into subunits. Material that remained as a 70S peak was further analyzed for RNA–protein cross-links. The dissociated 50S and 30S subunits were analyzed separately. Proteins, covalently linked to the ribosomal RNAs, were analyzed after removal of the unbound proteins. Four different methods of protein extraction were compared.

(*a*) *Phenol Extraction.* Cross-linked ribosomes were dissolved in BSCE buffer (50 mM Tris–borate, pH 7.2, 150 mM NaCl, 15 mM sodium citrate, 10 mM EDTA, and 1% NaDodSO$_4$), and a bentonite solution was added to a final concentration of 0.2%. Samples were extracted 3 times with an equal volume of phenol saturated with BSCE buffer. The RNA was precipitated with 2.5 volumes of ethanol (−20 °C, 2 h) and separated into 23S, 16S, and 5S RNAs on 5–30% sucrose gradients in 40 mM Tris–acetate, pH 7.2, and 20 mM sodium borate (SW27 rotor, 25 000 rpm, 16 h at 15 °C). The individual RNA peaks were precipitated twice with 2.5 volumes of ethanol.

(*b*) *NaDodSO$_4$ Extraction.* Proteins could alternatively be stripped off by centrifuging the cross-linked ribosomes twice through a sucrose gradient containing 0.2% NaDodSO$_4$. Ribosomes were dissolved in 10 mM sodium acetate, pH 6, and 2% NaDodSO$_4$ and incubated 10 min at 50 °C. They were layered on top of a 5–30% sucrose gradient and centrifuged for 17 h (SW27 rotor, 25 000 rpm, 15 °C). The individual RNA peaks were pooled and precipitated with ethanol, and centrifugation was repeated a second time under identical conditions. The isolated RNA fractions were completely free of noncovalently bound proteins except for the 5S RNA fraction. The 5S RNA obtained from NaDodSO$_4$ gradients was therefore additionally phenol extracted.

(*c*) *LiCl–Urea Extraction.* For the analysis of RNA–protein and protein–protein cross-links, the ribosomes could also be extracted according to Spitnik-Elson (1965) by using 4 M urea and 2 M LiCl.

(*d*) *Acetic Acid Extraction.* Proteins could also be extracted by using the standard acetic acid extraction procedure of Hardy et al. (1969).

In experiments where proteins were extracted by either LiCl–urea or the acetic acid extraction method, the RNA pellets that contained the RNA–protein cross-links were dialyzed against 40 mM Tris–acetate, pH 7.2, and 20 mM borate and separated into 23S, 16S, and 5S RNAs as described for the phenol-extracted material.

Electrophoretic Elution of RNA from Agarose–Acrylamide Gels. RNA bands were cut from the gel according to their UV shadowing. They were put into dialysis bags together with 200 μL of 20 mM Tris–acetate, pH 7.5, 10 mM sodium borate, and 1 mM EDTA and exposed to electrophoresis in the same buffer for 1 h at 20 mA and 4 °C. The RNA was then recovered from the solution inside the dialysis bag by ethanol precipitation.

Reversal of RNA–Protein Cross-Links. Proteins that had been linked to the ribosomal RNAs could be analyzed directly, using electrophoretic methods. The cross-link had to be reversed before, however. The reaction of PDG with RNA (guanosine) is completely reversible under mild alkaline conditions (Wagner & Garrett, 1978). For cleavage of the RNA–protein cross-links, the individual ribosomal RNAs were incubated in 0.2% ammonia for 2 h at 37 °C. The RNA samples were lyophilized twice to remove traces of ammonia. So that reassociation of some of the proteins that form specific RNA–protein complexes (Zimmermann, 1980) could be avoided, the RNA samples were digested with RNase T_1 (250 units/100 μL of sample) or, alternatively, acetic acid extracted.

Identification of Cross-Linked Proteins by Two-Dimensional Polyacrylamide Gel Electrophoresis. For identification of the cross-linked ribosomal proteins, the two-dimensional separation system of Geyl et al. (1981) was used, because it gave the best resolution, and effects due to protein modification with PDG, as well as aggregation in the first dimension, were minimal. An equivalent of not more than 12 A_{260} units of the extracted RNA fraction to be analyzed was loaded onto the first-dimension gels to avoid smearing.

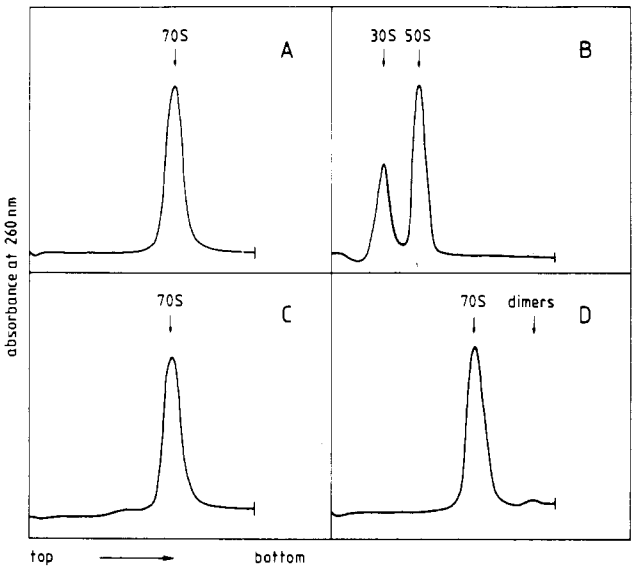

FIGURE 1: Separation of PDG-reacted ribosomes on sucrose gradients. (A) Unreacted control 70S ribosomes; separation at 10 mM Mg^{2+}. (B) Unreacted 70S ribosomes; separation at 1 mM Mg^{2+}. (C) 70S ribosomes reacted at 15×10^{-6} M PDG for 4 h at 37 °C; separation at 10 mM Mg^{2+}. (D) 70S ribosomes reacted at 25×10^{-6} M PDG for 4 h at 37 °C; separation at 10 mM Mg^{2+}.

Staining Procedure. When the sensitive silver staining procedure of Oakley et al. (1980) was used, the proteins cross-linked to the ribosomal RNAs could be identified without using radioactively labeled proteins. In some cases, the yields of cross-linked proteins were high enough to be stained with Coomassie Brilliant Blue.

Results

Determination of the Reaction Conditions. Cross-linking of the subunits could be demonstrated by running the reacted ribosomes on sucrose gradients at 1 mM $MgCl_2$ (see Materials and Methods). Under these conditions, only ribosomes that had been cross-linked via their subunit interface remained as a 70S peak. Unreacted ribosomes dissociated completely into 30S and 50S subunits (Figure 1). The yield of interface cross-linking could thus be determined by measuring the amount of 70S subunits present after gradient centrifugation in the presence of 1 mM $MgCl_2$. Figure 2A shows the time dependence of the interface cross-linking, and Figure 2B shows the yield of interface cross-linking as a function of the PDG concentration. From both graphs, it can be seen that the reaction is linear over the range measured. This can be taken as an indication that the reaction is still at an initial stage and not at a point where distortion of the conformation of the 70S particle enlarges the number of reactive sites. On the basis of these results, the standard reaction conditions, 2.7×10^{-5} M PDG and 4 h at 37 °C, were selected. There is good evidence that the ribosomes retain their structure under these conditions. The yield of cross-linked 70S ribosomes is approximately 30%, while the formation of dimers or aggregates is negligible (Figures 1 and 2).

The conformation of the reacted ribosomes was tested by cosedimenting unreacted ^{14}C-labeled 70S ribosomes with the reacted 70S fraction at 10 mM $MgCl_2$ (Figure 3a). Figure 3b shows unreacted ribosomes from the same ribosome batch, sedimented under the same conditions. There was no detectable shift in the sedimentation pattern of the reacted ribosomes compared to the unreacted ^{14}C-labeled 70S tight-couple ribosomes. The formation of dimers and aggregates could, however, be observed at longer reaction times.

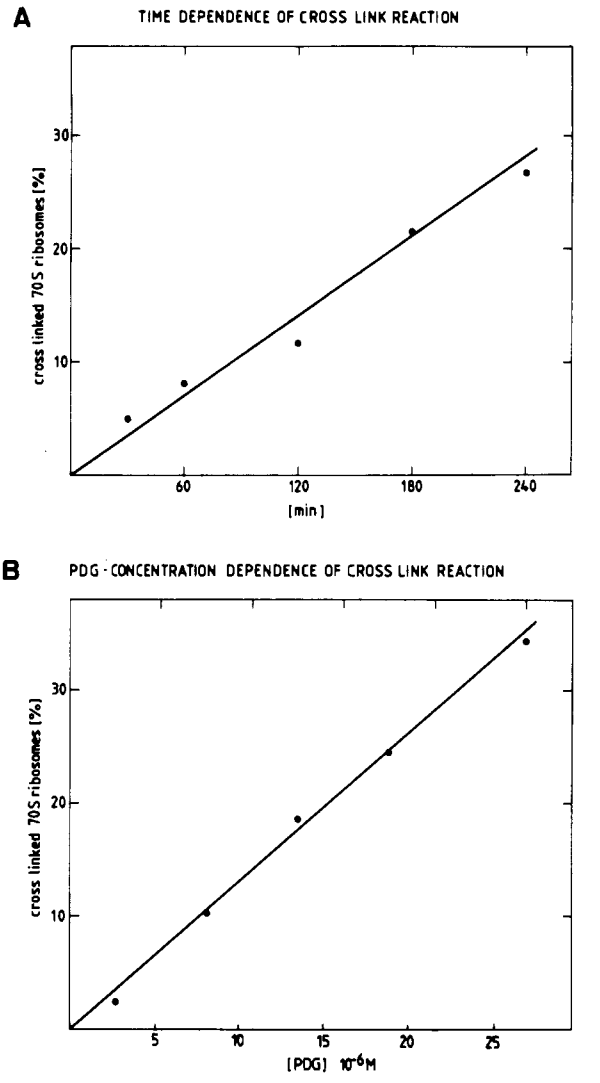

FIGURE 2: Yields of 70S cross-linked ribosomes. (A) Time dependence of the cross-link reaction. (B) PDG concentration dependence of the cross-link reaction. 70S ribosomes were reacted for various times at 2.7×10^{-5} M PDG concentration (A) or for 4 h at various PDG concentrations (B). Ribosomes were then centrifuged through 5–30% sucrose gradients in 40 mM Tris-HCl, pH 7.5, 20 mM sodium borate, 1 mM $MgCl_2$, and 6 mM 2-mercaptoethanol, and the amount of remaining 70S material was quantified by integrating the OD absorbance profiles.

Functional Activity of the Reacted Ribosomes. The tRNA binding activity of PDG-reacted 70S ribosomes was tested at the various PDG concentrations employed. Figure 4 shows that the tRNA binding activity drops rapidly at higher reagent concentrations. It is known, however, that ribosomes modified with α-dicarbonyl reagents rapidly lose their tRNA binding activity, due to modification of a number of the 16S RNA guanosines. The ribosomes retain their activity, however, over a wide range of reagent concentrations when the modification is performed in the presence of bound tRNA (Noller & Chairs, 1972; Wagner et al., 1980). The drop in activity of PDG-reacted ribosomes, therefore, cannot be taken as an indication that the cross-linking reaction has distorted the conformation of the 70S ribosomes. When the cross-linking reaction was performed in the presence of bound tRNA, the activity of the reacted ribosomes was only reduced to 40–50% (Figure 4).

Analysis of RNA–Protein Cross-Links. Proteins cross-linked to the individual ribosomal RNAs were identified by isolating the RNAs under conditions where all the nonbound proteins had been extracted completely and the ribosomal

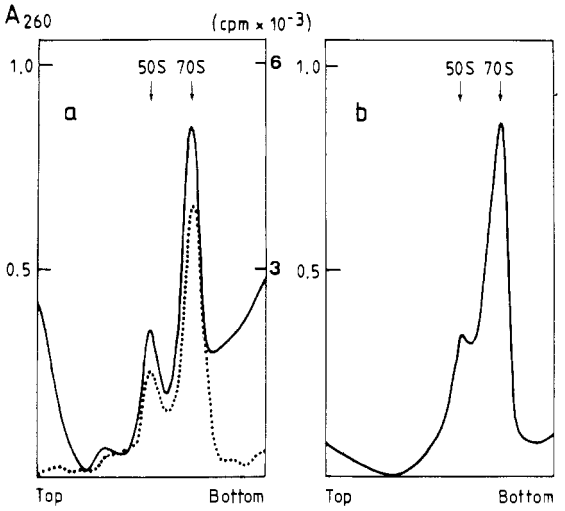

FIGURE 3: Cosedimentation of reacted 70S ribosomes with unreacted ^{14}C-labeled 70S ribosomes. (a) Cross-linked 70S ribosomes were isolated at 1 mM Mg^{2+} adjusted to 10 mM Mg^{2+} and cosedimented with unreacted ^{14}C-labeled 70S tight-couple ribosomes. The dotted line represents ^{14}C counts: the unbroken line is the absorption at 260 nm. (b) Unreacted 70S control ribosomes run on a sucrose gradient at 10 mM Mg^{2+}.

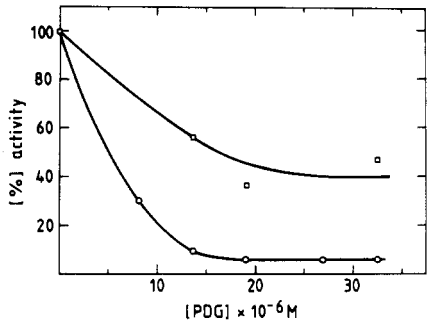

FIGURE 4: Functional activity of PDG-reacted 70S ribosomes. Cross-linked 70S ribosomes were tested for their Phe-$tRNA^{Phe}$ binding activity as described under Materials and Methods. (□) Cross-link reaction in the presence of poly(U) and $tRNA^{Phe}$; (○) cross-link reaction in the absence of poly(U) and $tRNA^{Phe}$.

RNAs were subsequently separated into 23S, 16S, and 5S RNAs. Several methods for the extraction of RNA and protein from the isolated PDG-reacted ribosomes were compared (see Materials and Methods). The RNA yields of the four extraction methods are similar and significantly lower than those with unreacted material. In case of the $NaDodSO_4$ gradient extraction, the RNAs are already separated into 23S, 16S, and 5S RNAs, and no further separation is required (Figure 5). For the other extraction systems, two consecutive gradient centrifugations are necessary after the extraction for an optimal separation of the large ribosomal RNAs. Consequently, the yield of RNA recovered from the phenol extraction, the LiCl–urea extraction, or the acetic acid extraction was only about 15%, while the yield of RNA isolated from $NaDodSO_4$ gradients was usually more than 30% (see Table I).

Although the $NaDodSO_4$ gradient extraction method gives a much better recovery of the individual RNAs, compared to the other methods, considerable problems were encountered during the electrophoretic identification of the proteins. The $NaDodSO_4$ could not always be completely removed, which resulted in loss or smearing of the protein samples in the two-dimensional gel system. The 5S RNA fraction is not free of unbound protein and had to be further extracted with phenol.

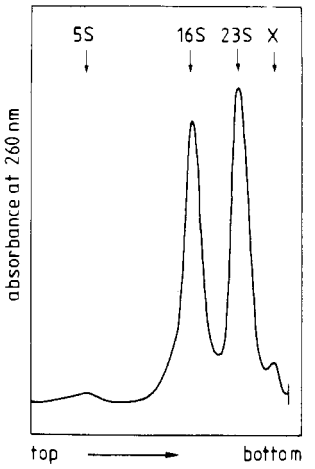

FIGURE 5: Separation of ribosomal RNAs on sucrose gradients. Ribosomal RNAs were separated on sucrose gradients after unbound proteins were extracted. X denotes a high molecular weight RNA fraction formed by RNA–RNA cross-linking.

Table I: Comparison of the Yields of RNA from the Different RNA-Protein Extraction Methods for PDG-Cross-Linked and Unreacted 70S Ribosomes

method of extraction	total RNA from reacted 70 S (%)	total RNA from control 70 S (%)	RNA after gradient separations (%)
phenol	40	83	13
LiCl-urea	60	97	17
acetic acid	65	~80[a]	~15[a]
$NaDodSO_4$ gradient	32	81	32

[a] RNA extracted with acetic acid could sometimes not be dissolved completely.

LiCl–urea and acetic acid extractions were used when the RNA and protein fraction had to be analyzed simultaneously. Sometimes it was difficult, however, to get the RNA pellets back into solution.

The advantage of phenol extraction is that it is fast, and the action of nucleases on the ribosomal RNAs is reduced to a minimum. As a disadvantage, however, the recovery of RNA is low with heavily cross-linked material (see Table I). RNAs with several cross-linked proteins may be selectively lost in the phenol layer (especially true for 5S RNA). The data shown in Figure 8 were obtained by using the LiCl–urea extraction method.

A systematic comparison of the individual proteins recovered by the various extraction methods was, however, not performed due to the difficulty of exact quantification of the cross-linked proteins.

Yields of the Cross-Linked Proteins. Cross-linking at the interface was efficient, and under standard conditions, 30% of the 70S ribosomes were cross-linked (Figure 2). The yield of the ribosomal proteins cross-linked to ribosomal RNA is difficult to assess precisely. Not all the cross-linking can be attributed to RNA–protein linkage. There is a considerable amount of RNA–RNA and protein–protein cross-link. The overall RNA–protein cross-linking yield is 4% if determined directly after extraction. It falls when the RNAs are separated (see Table II). The yield of the individual cross-linked proteins can be estimated roughly from the staining intensities of the protein spots. Care has to be taken with the interpretation of the staining intensities, because some of the ribosomal proteins stain with different intensities with the silver staining method of Oakley et al. (1980). A comparison of the Coo-

Table II: Yields of RNA and Covalently Bound Proteins at Individual Purification Steps

procedure	RNA (%)	protein (%)[a]
starting 70 S	100	100
70 S cross-linked	30	24[b]
extraction (LiCl–urea)	20	4
sucrose gradient separations		
23S RNA[c]	6	0.6
5S RNA[c]	0.6	0.1
sucrose gradients + gel separation		
16S RNA[c]	1.2	0.07

[a] Yields are calculated from [^{14}C]lysine-labeled proteins with the assumption of an even distribution of lysines among the ribosomal proteins. [b] During the first steps of purification, some proteins are lost from the 70S particle. [c] Yields are calculated according to the A_{260} values. No corrections are made for the molecular weights of the individual RNAs.

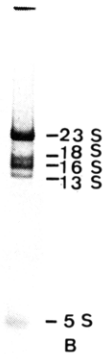

FIGURE 6: Agarose–acrylamide gel electrophoresis of extracted ribosomal RNAs. Separation of extracted ribosomal RNA on a combination 3% acrylamide–1% agarose gel. Separation was performed at 4 °C, 40 mA, for 4 h. The gel was stained with toluidine blue. B denotes the bromophenol blue marker dye. The positions of the individual RNAs and RNA fragments are indicated.

massie Blue vs. the silver staining intensities had to be done before the yields of the proteins could be estimated.

Isolation of the Individual RNAs. The extracted ribosomal RNA which contained the covalently bound proteins had to be fractionated into 23S, 16S, and 5S RNAs. The separation was performed on sucrose gradients (see Materials and Methods) with a maximum of 30 A_{260} units in each SW27 tube (Figure 5). Two consecutive centrifugation cycles were carried out in order to keep cross-contamination of the RNAs to a minimum. In some of the gradients, the 16S RNA peak was not homogeneous, and shoulders mainly on the lower molecular weight side of the 16S RNA peak could be detected. The composition of the individual RNA peaks was therefore tested by using gel electrophoresis on composite agarose–acrylamide gels (Wagner et al., 1980). The shoulders near the 16S RNA peak could be identified as 13S and 18S fragments. Both fragments are broken-down products of 23S RNA (Spierer et al., 1975). We found that these fragments existed in some of the 70S preparations which had no detectable loss of function. They were not introduced during the incubation with PDG or the workup procedure. When there was evidence of broken-down 23S RNA, the 16S RNA fraction was repurified on composite agarose–acrylamide gels and eluted electrophoretically (Figure 6).

Due to the extensive purification of the ribosomal RNAs, only small amounts of cross-linked proteins are recovered at the end of the isolation procedure. Table II gives a comparison

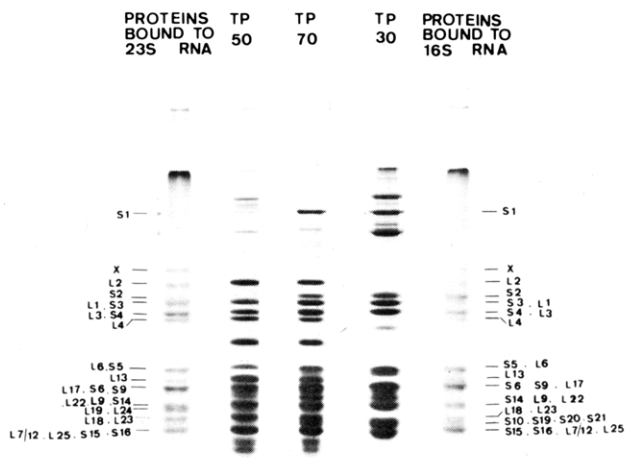

FIGURE 7: Separation of proteins cross-linked to 23S and 16S RNAs on a one-dimensional $NaDodSO_4$-containing slab gel. The tracks TP30, TP50, and TP70 show total proteins extracted from unreacted 30S, 50S, or 70S ribosomes. The band X does not correspond to any of the individual ribosomal proteins and is very likely a protein dimer cross-linked to the ribosomal RNAs. In cases where more than one protein migrates in a single band, all the possible proteins are indicated in the margin.

of the yields of RNA and covalently bound proteins at the individual purification steps. It should be noted that only a fraction of the proteins cross-linked are interface proteins. The data are given for all the proteins cross-linked to the RNA.

Identification of Cross-Linked Proteins. For identification of the proteins that had been linked to the ribosomal RNAs, first the cross-links were broken, and then the freed proteins were characterized according to their known mobility in various electrophoretical systems. Some proteins (e.g., L2, L4, L13, S1, and S2) could be identified unambiguously on one-dimensional $NaDodSO_4$ slab gels according to Dijk & Littlechild (1979) (Figure 7). However, the resolution of the system was not sufficient to enable all the cross-linked proteins to be identified.

Complementary information was obtained, using cellulose acetate gel electrophoresis, where the separation is directed mainly by the proteins' charge (Zubke et al., 1977). Although proteins L1–L3–L25, and S10, and S8 could be identified as cross-linked to the rRNAs, an unambiguous identification of all the cross-linked proteins was impossible. All the proteins could be identified, however, by using two-dimensional gel electrophoretic separation methods. The best resolution was obtained with the system described by Geyl et al. (1981). This system resolved all of the 70S proteins, and smearing of the cross-linked proteins was minimal. Smearing may have been caused by (i) relatively large amounts of RNA present during the electrophoretic separation and (ii) modification of some of the protein side chains by the cross-linking reagent, resulting in a slight change in their mobility in the first dimension of the electrophoretical separation, where the mobility is charge dependent. Figure 8 shows the identification of proteins on two-dimensional gels. Gels were stained by using the silver staining method of Oakley et al. (1980), except for proteins linked to the 5S RNA, where autoradiography was used (Figure 8C,F).

The list of proteins shown in Table III was obtained from Figure 8. Table III also contains a list of proteins that were cross-linked in the isolated subunits. The identification of these proteins is also shown in Figure 8. Protein S1 was not well resolved on the gels but could be identified on the one-di-

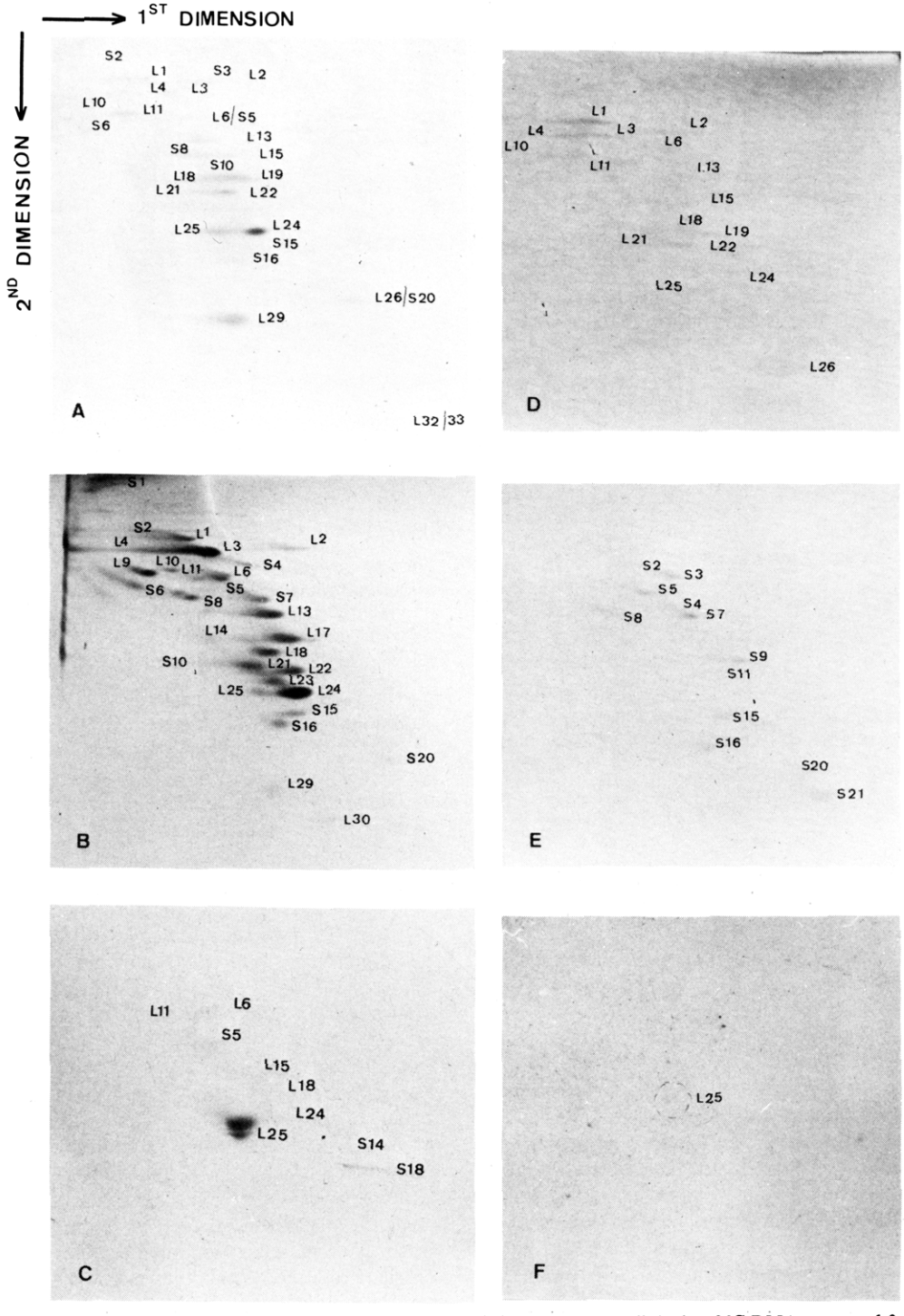

FIGURE 8: Identification of cross-linked proteins on two-dimensional gels. (A) Proteins cross-linked to 23S RNA extracted from 70S ribosomes. (B) Proteins cross-linked to 16S RNA extracted from 70S ribosomes. (C) Proteins cross-linked to 5S RNA extracted from 70S ribosomes. (D) Proteins cross-linked to 23S RNA extracted from 50S subunits. (E) Proteins cross-linked to 16S RNA extracted from 30S subunits. (F) Proteins cross-linked to 5S RNA extracted from 50S subunits. Panels A, B, D, and E were obtained from gels after staining with silver, while in panels C and F autoradiograms are shown. Some of the weaker spots do not show up clearly on the figures but could be seen on the original gels. The proteins from the weaker spots are given in parentheses in Table III.

mensional NaDodSO$_4$ gels (Figure 7).

Discussion

Subunit Interface Proteins. L proteins cross-linked to the RNA of the small subunit, as well as S proteins cross-linked to the RNA of the large subunit, are considered to be at or close to the subunit's interface. Many proteins from the large subunit cross-link to 16S RNA in good yield, while only a limited number of S proteins cross-link to 23S RNA, with low yield. Two explanations are consistent with this result. First, the L proteins may be more accessible to the cross-linking reagent and more reactive than the S proteins. Second, the interfaces of the two subunits may have different compositions. At the small subunit interface, large portions of the RNA are accessible while the 50S subunit interface seems to consist predominantly of proteins. This idea is consistent with the findings of Gualerzi et al. (1981) and Giocanti & Ekert (1981). The latter authors could not detect 30S proteins cross-linked to the large subunit's RNA but identified eight 50S proteins linked to the 16S RNA when they investigated

Table III: Proteins Cross-Linked to Individual RNAs[a]

	70 S			50 S		30 S
protein	23S RNA	16S RNA	5S RNA	23S RNA	5S RNA	16S RNA
S1	(+)	(+)				nd
S2	+	(+)				+
S3	+					+++
S4		+				+
S5	+	+	(+)			++
S6	+	+				
S7		+				+++
S8	(+)	+[b]				+
S9						++
S10	(+)	(+)				
S11						+
S14			(+)			
S15	+	+				+
S16	+	+				+
S18			+			
S20/L26	+	+				+
S21						+
L1	+	(+)		+++		
L2	+	(+)		++		
L3	+	+++		++		
L4	+	(+)		(+)		
L6	+	(+)	(+)	+		
L9		++[b]				
L10	(+)	+		+		
L11	(+)	(+)	(+)	(+)		
L13	+	++		+		
L14		(+)				
L15	(+)		(+)	+		
L17		++				
L18	++	++	(+)	+		
L19	++			+		
L21	(+)	+		+		
L22	+	+		+		
L23		+				
L24	+++	+++	(+)	(+)		
L25	++	+	+++	(+)	(+)	
L26/S20	+	+		+		
L29	++	+				
L30		(+)				
L32/33	+					

[a] The relative yields of the cross-linked proteins are indicated by the following symbols: (+), very weak; +, weak; ++, intermediate; +++, strong; nd, not determined. [b] This protein was found occasionally as a double spot.

interface proteins by radiochemical cross-linking; except for L5, our results confirm theirs.

There is a striking homology between the sets of L proteins cross-linked to 16S and 23S RNAs. This cannot be explained by cross-contamination, because extreme care was taken during the purification of the individual RNAs (compare Figures 5 and 6). If there were small amounts of undetected contaminating 23S RNA in the 16S RNA preparation, they would not be sufficient to account for the strong L-protein spots in the 16S RNA fractions analyzed. Furthermore, a number of L proteins were found to cross-link only to 23S RNA (L19, L32/33) or to 16S RNA (L9, L14, L17, L23, L30), again ruling out cross-contamination. Cross-linking to both RNAs is more likely a specific effect. Giocanti & Ekert (1981), in their radiochemical cross-linking study, obtained a very similar result; all the L proteins which linked to 16S RNA were also found to cross-link to 23S RNA.

Comparison of Cross-Linked Proteins within 70S Ribosomes and Isolated 30S and 50S Subunits. Comparison of the proteins cross-linked within 70S ribosomes and in the isolated subunits (Figure 9, Table III) reveal some interesting differences. Some proteins are exclusively cross-linked within 70S ribosomes (S6 and S10 to 16S RNA, L29 and L32/33

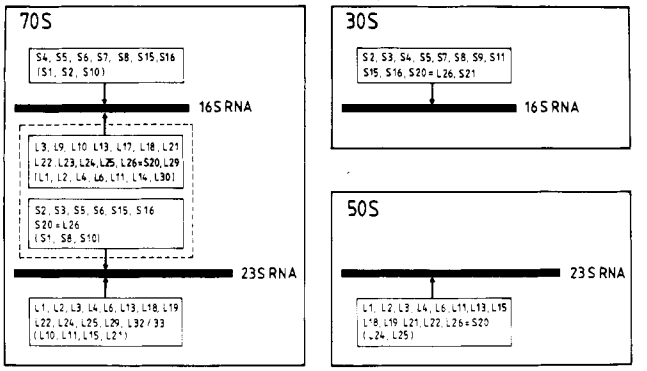

FIGURE 9: Schematic arrangement of RNA and protein at the ribosomal interface and in the isolated subunits. Proteins in the boxes cross-link to the RNAs as indicated by arrows. The dotted line represents the interface regions. Ribosomal RNAs are indicated by a solid bar.

to 23S RNA), while others are only cross-linked in the isolated subunits (S3, S9, S11, and S21 to 16S RNA). In the 50S subunit, no additional proteins were found to link to 23S RNA. Moreover, the yield of the protein which cross-links to 23S RNA at the highest level, in 70S ribosomes, L24, is drastically reduced in the free 50S subunit. This may reflect structural differences between the associated and dissociated states of the ribosome, as well as protection of some of the 30S proteins from the reaction when the two subunits join to form a 70S particle. It seems as if structural changes or exposure or protection effects are smaller in the 50S subunit than in the 30S subunit.

Comparison with Other Cross-Linking Results. A number of cross-linking studies have already been performed to investigate the internal topography of the ribosome. Cross-linking has been found to occur between proteins or between RNA and proteins, depending on the kind of reagent employed. Cross-linking has been found mainly within the ribosomal subunits but has also been detected across the interface [e.g., see Lambert & Traut (1981), Sköld (1981), and Kenny et al. (1979)]. The very similar results of Giocanti & Ekert (1981) have already been discussed.

Moreover, the proteins S2, S6, S8, S10, S15, and L1, L2, L6, L10, L18, and L21, identified in our study as interface proteins by RNA–protein cross-linking, have already been shown by the protein–protein cross-linking studies of Lambert & Traut (1981) to be located at the subunit interface.

In a different study, using diepoxybutane as a cross-linking reagent, a number of proteins have been identified as interface components which we failed to find (Sköld, 1981). Eight proteins from the small subunit were identified as being cross-linked to 23S RNA, but of these, we found only protein S6. From the large subunit, there are only four proteins cross-linked to 16S RNA of which L17 is the only one we found. However, our results and Sköld's results agree well for proteins cross-linked within the subunits. Six of the ten proteins they found cross-linked within the 30S subunit are the same as those we have found (S3, S4, S5, S7, S8, and S9). There is an even greater similarity for the L proteins cross-linked within the 50S subunit (L1, L2, L4, L13, L15, L18, L21, and L19/22/24). The differences between the interface proteins identified in both studies may be explained by the different stereochemical properties of the two reagents employed (the length of PDG is about 10 Å, that of diepoxybutane approximately 4 Å), but it may also reflect different functional states of the two ribosome preparations tested.

Interface components have also been determined by using different approaches, such as chemical modification (Gualerzi

et al., 1981) and immunological methods (Morrison et al., 1973). There is a good correlation between these studies for quite a number of proteins. Our results and other investigations (Herr & Noller, 1979; Herr et al., 1979) show that the ribosomal RNAs have to be considered as important interface components. It would be of value to know the exact locations of the cross-link points between the interface proteins and the ribosomal RNAs. Moreover, PDG as a cross-linking reagent results in discrete RNA-RNA cross-links via the ribosomal interface, and we are currently trying to localize these RNA contact points by RNA sequencing methods.

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