Petermann, M. L., and Pavlovec, A. (1966), *Biochim. Biophys. Acta 114*, 264.
Petermann, M. L., and Pavlovec, A. (1967), *Biochemistry 6*, 2950.
Petermann, M. L., Pavlovec, A., and Weinstein, I. B. (1969), *Fed. Proc., Fed. Amer. Soc. Exp. Biol. 28*, 725.
Pritchard, P. M., Gilbert, J. M., Shafritz, D. A., and Anderson, W. F. (1970), *Nature (London) 226*, 511.
Rebound, A.-M., Hamilton, M. G., and Petermann, M. L. (1969), *Biochemistry 8*, 843.
Tashiro, Y., and Siekevitz, P. (1965), *J. Mol. Biol. 11*, 149.
Tengerdy, R. P., and Faust, C. H., Jr. (1971), *Anal. Biochem. 42*, 248.
Weller, D. L., Shechter, Y., Musgrove, D., Rougvie, M., and Horowitz, J. (1968), *Biochemistry 7*, 3668.

Glutaraldehyde Reactivity of the Proteins of *Escherichia coli* Ribosomes†

Lawrence Kahan* and Eberhard Kaltschmidt

ABSTRACT: The topography of the small and large subunits of *Escherichia coli* ribosomes has been investigated by use of glutaraldehyde as a chemical probe. All of the ribosomal proteins react with glutaraldehyde when the proteins are present as a concentrated mixture of free proteins to give products which do not give spots in a defined two-dimensional electrophoresis pattern. The proteins of the intact 30S subunit are reactive in the order S1, S10 > S2, S3, S6, S13, S18, S19, S21 > S5, S7, S8, S9–S11 (part), S14 > S4, S9–S11 (part), S12, S15, S16, S17, S20. The proteins of the 50S subunit are reactive in the order L19, L27, L33 > L7, L12, L25, L26, L30, L31 > L4, L5, L6, L8, L9, L10, L11, L14, L18, L22, L23, L24, L29, L32 > L1, L2, L3, L13, L15, L16, L17, L20, L21, L28. Since ribosomes treated with low concentrations of glutaraldehyde retain substantial functional activity and normal sedimentation properties, it is concluded that the most reactive proteins in the intact subunits are at least partly external.

The small and large subunits of *Escherichia coli* ribosomes are complex structures containing about 21 and 33 distinct proteins and 1 and 2 molecules of RNA, respectively. Knowledge of the three-dimensional organization of these structures should be helpful in elucidation of the mechanism of protein synthesis and the control of translation. Some information about the topography of the 30S and 50S subunits has been obtained by modification of the external proteins of the intact ribosome (Craven and Gupta, 1970; Chang and Flaks, 1970, 1971; Moore, 1971; Noller *et al.*, 1971; Spitnik-Elson and Breiman, 1971; Crichton and Wittmann, 1971); however the results of these studies are sometimes ambiguous due to the difficulty of identification and quantitation of native and modified proteins in the complex mixture of proteins obtained after modification and due to the uncertain stability of the ribosome structure during tryptic digestion.

We have developed a simple, generally applicable technique for the modification of some external proteins and the subsequent identification of the unmodified proteins by two-dimensional electrophoresis (Kaltschmidt and Wittmann, 1970a), and have applied this technique to the examination of the topography of the 30S and 50S subunits of *E. coli* ribosomes. Our method consists of derivatization of the external polypeptides with glutaraldehyde, extraction of the protein moiety of the ribosomes into 67% acetic acid, and identification of unmodified proteins by two-dimensional acrylamide gel electrophoresis. Glutaraldehyde reacts almost exclusively with free amino groups and can react with proteins and organelles without causing substantial modification of funtional activity or structure (Avrameas and Ternynck, 1969; Avrameas, 1969; Sabatini *et al.*, 1963). Since modified proteins apparently do not give spots in the gel pattern, they do not interfere with identification of the unmodified proteins (see below).

Materials and Methods

Preparation of Ribosomes. *E. coli* Q13 were broken by alumina grinding, and 70S ribosomes were prepared and dissociated into 30S and 50S subunits as described by Traub *et al.* (1971). 30S subunits were purified by differential centrifugation and density gradient centrifugation (Traub *et al.*, 1971). In some cases 30S ribosomes were further purified by a second density gradient centrifugation step. 50S ribosomes were purified from the material remaining after purification of 30S ribosomes by two cycles of density gradient centrifugation (Traub *et al.*, 1971). Total 70S proteins used as reference proteins were prepared as described by Hardy *et al.* (1969).

Reaction of Ribosomes with Glutaraldehyde (Large Volume Method). Ribosomes were dialyzed against 0.32 M KCl, 0.01 M $MgCl_2$, and 0.01 M sodium cacodylate buffer (pH 7.0) for 18 hr at 4°. The ribosome concentration was adjusted to 0.5

† From the Institute for Enzyme Research, and Departments of Biochemistry and Genetics of the University of Wisconsin, Madison, Wisconsin 53706. The paper is No. 1521 of the Laboratory of Genetics. *Received December 9, 1971.* This work was supported in part by the College of Agriculture and Life Sciences, University of Wisconsin, and by grants from the National Institute of General Medical Sciences (GM-15422), National Science Foundation (GB-31086X), and Petroleum Research Fund.

* Postdoctoral Fellow of the American Cancer Society 1970–1972.

TABLE I: Correspondence between Protein Nomenclatures for the 30S Ribosomal Proteins.[a]

Berlin Code	Madison Code	Uppsala Code	Geneva Code
S1	P1	1	13
S2	P2	4a	11
S3	P3	9 (+5)	10b
S4	P4a	10	9
S5	P4	3	8a
S6	P3b,P3c	2	10a
S7	P5	8	7
S8	P4b	2a	8b
S9[b]	P8	12	5
S10	P6	4	6
S11[b]	P7	11	4c
S12	P10	15	
S13	P10a	15b	
S14	P11	12b	
S15	P10b	14	4b
S16	P9a	6	4a
S17	P9b	7	3a
S18	P12	12a	2b
S19	P13	13	2a
S20	P14	16	1
S21	P15	15a	0

[a] As determined by Wittmann *et al.* (1971). [b] In our patterns, S9 and S11 are both found at the position of spot S9.

mg/ml with the above buffer, and 0.1 volume of 1 M sodium cacodylate buffer (pH 7.0) was added. A solution of glutaraldehyde (Fisher, biological grade) (0.04 volume) at the appropriate dilution in 0.32 M KCl, 0.01 M $MgCl_2$, and 0.1 M sodium cacodylate buffer (pH 7.0) was added and the samples were incubated 30 min at 0°. The samples were dialyzed overnight at 4° against two changes of 50 volumes of 0.03 M NH_4Cl, 0.01 M $MgCl_2$, 0.006 M 2-mercaptoethanol, and 0.01 M Tris buffer (pH 7.8) and then pelleted by centrifugation. In some experiments 0.05 volume of 2 M Tris buffer (pH 7.4) was added to stop the fixation reaction prior to dialysis.

Reaction of Ribosomes with Glutaraldehyde (Small Volume Method). Ribosomes were treated as described in the preceding method with the following changes. The ribosome concentration was adjusted such that 0.25 ml of ribosome suspension contained the desired amount of ribosomes (7 mg of 30S subunits or 10 mg of 50S subunits for two-dimensional electrophoresis or 2 mg of 50S subunits for one-dimensional electrophoresis). The samples were dialyzed against two changes of 500 volumes of 0.03 M NH_4Cl, 0.01 M $MgCl_2$, 0.006 M 2-mercaptoethanol, and 0.01 M Tris buffer (pH 7.8) and directly extracted with acetic acid without concentration by centrifugation.

Reaction of Free Proteins with Glutaraldehyde. A mixture of all 70S ribosomal proteins (5 mg) was dissolved in 0.2 ml of 8 M urea and 4 M LiCl and dialyzed against three changes of 500 volumes of 1 M KCl, 0.01 M $MgCl_2$, and 0.1 M cacodylate buffer (pH 7.0). The resulting fine suspension was divided into four samples of 1 mg of protein in 50 μl of buffer and 10 μl of solutions of glutaraldehyde in the same buffer was added such that the final concentration of glutaraldehyde was 0, 0.03, 0.1, or 0.3%. The samples were incubated at 0° for 30 min, transferred to dialysis bags with an additional 0.4 ml of the same concentrations of glutaraldehyde in buffer and dialyzed 12 hr against 1 l. of 0.03 M NH_4Cl, 0.01 M $MgCl_2$, 0.006 M 2-mercaptoethanol, and 0.01 M Tris buffer (pH 7.8) at 4°. The samples were then dialyzed against sample gel for disc electrophoresis in order to solubilize all protein. S13 in 1 M KCl, 0.01 M $MgCl_2$, 0.03 M Tris (pH 7.8), and 0.006 M 2-mercaptoethanol was dialyzed against cacodylate buffer and treated with glutaraldehyde in a similar manner.

Analysis of Reaction Products. Acetic acid extraction of proteins from ribosomes was carried out as described by Hardy *et al.* (1969). One-dimensional disc electrophoresis was performed as described by Leboy *et al.* (1964). Two-dimensional acrylamide gel electrophoresis was performed as described by Kaltschmidt and Wittmann (1970a). Protein nomenclature is that of Kaltschmidt and Wittmann (1970b). The correspondence between this nomenclature for 30S proteins and other nomenclatures as determined by Wittmann *et al.* (1971) is given in Table I.

Determination of Radioactivity in Acrylamide Gels. Gel slices (0.5 cm) were air-dried at 37° in counting vials. One-half milliliter of 30% H_2O_2 was added and the vials were capped and incubated at 70° for 2–4 hr until all gel was dissolved. The vials were incubated overnight at 4°, 10 ml of Bio-Solv (Beckman) scintillation fluid was added, and the radioactivity was measured in a Packard TriCarb scintillation counter.

Measurement of 30S Functional Activity. Poly(U)-directed phenylalanine polymerization and poly(U)-directed phenylalanyl-tRNA binding activities were measured as described by Traub *et al.* (1971).

Results

Reaction of 30S Ribosomes with Glutaraldehyde. 30S ribosomes were treated with 0.03, 0.1, and 0.3% glutaraldehyde (fixed ribosomes) or with buffer alone (control ribosomes) as described in Materials and Methods. The gel patterns obtained after treatment with glutaraldehyde are shown in Figure 1. The spots given by two proteins, S1 and S10, were present in the control preparation pattern but at least fourfold reduced in the patterns given by all of the fixed ribosome preparations. A second set of proteins, S2, S3, S6, S13, S18, S19, and S21, was reduced fourfold only by 0.1 or 3% glutaraldehyde. A third class, S5, S7, S8, and S14, and a part of S9–S11 were reduced fourfold only in the pattern given by ribosomes treated with 0.3% glutaraldehyde. S4, S12, S15, S16, S17, S20, and a part of S9–S11 were not reduced fourfold by any concentration of glutaraldehyde tested. The apparent decrease in intensity of these spots in the 0.3% glutaraldehyde-treated ribosomal protein pattern is due in part to an accidental loss of material from this one sample; however, lesser decreases in intensity were observed in other experiments. At the highest concentration of glutaraldehyde some alteration of the proteins giving these spots is apparent from the smearing of the spots in the direction of the anode in both dimensions. The results of these experiments are summarized in Table II.

Comparison of Figures 1D,E and 2D,E indicates that addition of Tris at a final concentration of 0.067 M immediately after glutaraldehyde fixation alters the final gel pattern. Some spots such as S5 and L22 which were reduced in intensity at this glutaraldehyde concentration were present in normal

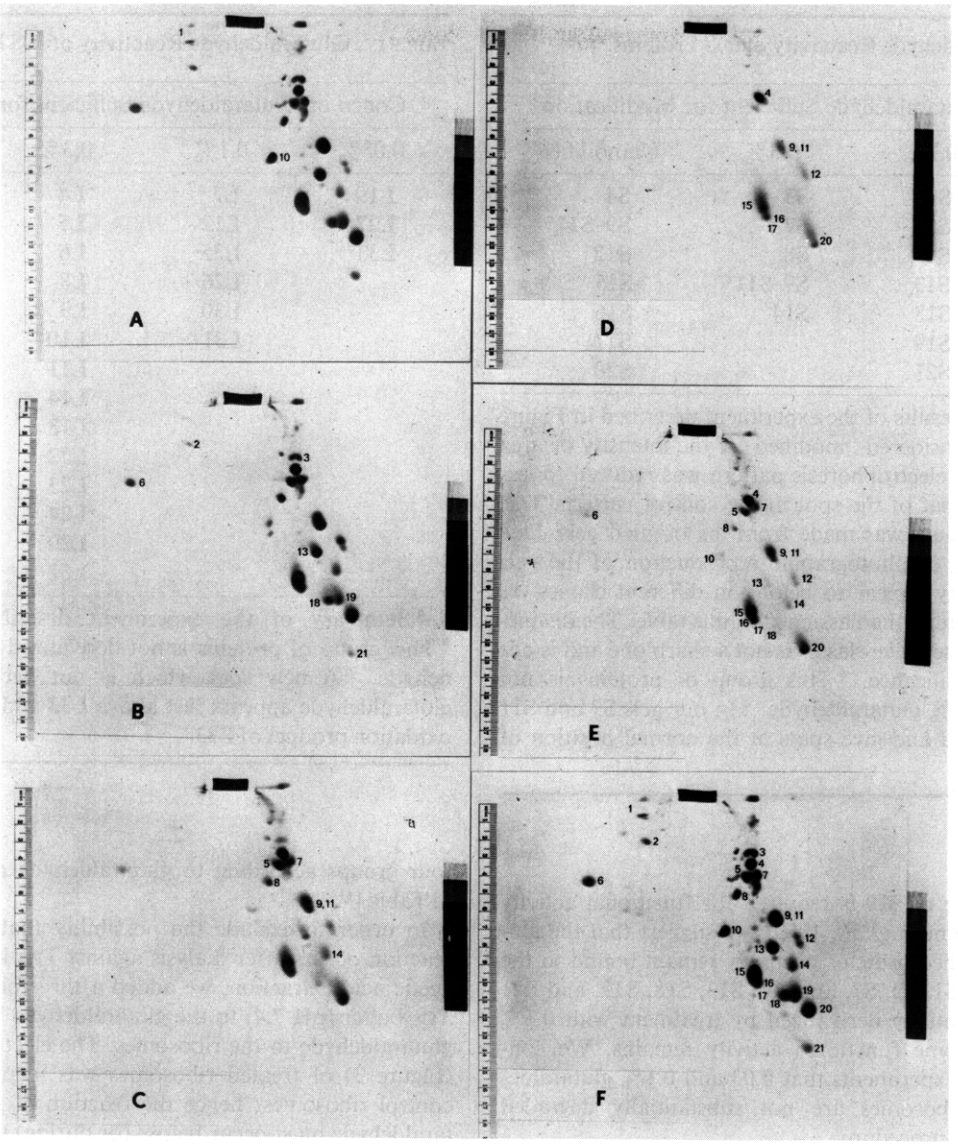

FIGURE 1: Two-dimensional acrylamide gel electrophoresis patterns of control and glutaraldehyde-treated 30S ribosomes obtained as described in Materials and Methods (large volume method). In A–D proteins are numbered in the gel pattern obtained at the highest concentration of glutaraldehyde not giving a fourfold reduction in the intensity of the particular spot. Due to the subjective nature of such a classification system, some proteins did not clearly fall into a single category as indicated in Table II. The object on the right side of each gel is a negative exposed for various lengths of time which served as a standard in the photographic reproduction process. (A) 100 A_{260} of control (buffer treated) 30S ribosomes. (B) 100 A_{260} of 30S ribosomes treated with 0.03% glutaraldehyde. (C) 100 A_{260} of 30S ribosomes treated with 0.1% glutaraldehyde. (D) 100 A_{260} of 30S ribosomes treated with 0.3% glutaraldehyde. (E) 100 A_{260} of 30S ribosomes treated with 0.3% glutaraldehyde. 0.033 volume of 2 M Tris (pH 7.4) was added after 30-min fixation at 0° to stop the fixation reaction. All visible 30S protein spots are numbered. (F) 100 A_{260} of control (buffer treated) 30S ribosomes. All 30S protein spots are numbered. Under our electrophoresis conditions S17 gives two spots, one at the normal position and one partly resolved from S15 and S16 on the anodal side. In this figure the prefix "S" has been omitted from the spot numbers for the sake of clarity.

intensity if the fixation reaction was terminated by the addition of Tris. In addition, some proteins such as S8 and L29 which were completely modified at the given glutaraldehyde concentration were not completely modified when Tris was added. Hence, when the fixation reaction was terminated with Tris, the observed pattern was similar to but not identical with that of a sample treated with a lesser concentration of glutaraldehyde. The effect of addition of Tris to the reaction mixture was greater on spots having partially reduced intensity than on completely missing spots, thus increasing the distinction between modified and unmodified proteins.

In order to determine the extent of degradation of the 30S ribosomes during the glutaraldehyde fixation procedure, the sedimentation properties of the fixed ribosomes in 5–20% sucrose gradients were determined. No slow sedimenting degradation products were observed. Some broadening of the 30S peak in the direction of faster sedimentation was observed suggesting the presence of cross-linked ribosomes. However, the existence of interribosome cross-links should not affect the distinction between unmodified and modified proteins. In addition, the activities of the fixed ribosomes in poly(U)-directed phenylalanine incorporation and phenylalanyl-tRNA binding assays were determined. The observed activities are listed in Table III. When 30S ribosomes are treated with 0.03% glutaraldehyde, S1 and S10 are substantially modified with only a small effect on functional activity.

TABLE II: Glutaraldehyde Reactivity of 30S Proteins.[a]

Concn of Glutaraldehyde Sufficient for Modification			
0.03%	0.1%	0.3%	Unmodified[b]
S1	S2	S5	S4
S10	S3	S7	S9–S11[c]
	S6	S8	S12
	S13	S9–S11[c]	S15
	S18	S14	S16
	S19		S17
	S21		S20

[a] Summary of results of the experiment described in Figure 1. Proteins are considered "modified" if the intensity or area of the spot in the electrophoresis pattern was reduced to less than one-fourth that of the spot in the control pattern. This subjective assignment was made from the original gels. Due to the inaccuracy of photographic reproduction of the gels some proteins may appear to belong in different classes (as judged from Figure 1) than assigned in this table. The distinctions between consecutive classes is not a sharp one and is not of particular significance. [b] This group of proteins is not derivatized by 0.3% glutaraldehyde. [c] In our gels S9 and S11 are partly resolved and give spots at the normal position of S9.

TABLE IV: Glutaraldehyde Reactivity of 50S Proteins.[a]

Concn of Glutaraldehyde Sufficient for Modification			
0.03%	0.1%	0.3%	Unmodified[b]
L19	L7	L4	L1
L27	L12	L5	L2
L33[c]	L25	L6	L3
	L26	L8	L13
	L30	L9	L15
	L31	L10	L16
		L11	L17
		L14	L20
		L18	L21
		L22	L28
		L23	
		L24	
		L29	
		L32	

[a] Summary of the experiment described in Figure 2. [b] This group of proteins is not derivatized by 0.3% glutaraldehyde. [c] A new spot which is not derivatized by 0.3% glutaraldehyde appears just above L33 and may represent an oxidation product of L33.

Since the presence of S10 is required for functional activity of ribosomes (Nomura *et al.*, 1969), we suggest that the glutaraldehyde modified proteins probably remain bound to the ribosome. When S1, S2, S3, S6, S10, S13, S18, S19, and S21 have been substantially derivatized by treatment with 0.1% glutaraldehyde, some functional activity remains. We conclude from these experiments that 0.03 and 0.1% glutaraldehyde modified ribosomes are not substantially degraded during the fixation procedure.

Reaction of 50S Ribosomes with Glutaraldehyde. 50S ribosomes were reacted with several concentrations of glutaraldehyde and the ribsomal proteins extracted and analyzed as previously described. The electrophoresis patterns are shown in Figure 2. The 50S proteins may be divided into four groups according to glutaraldehyde reactivity as listed in Table IV.

In order to exclude the possibility that the modification reaction occurs after dialysis against Tris buffer, *e.g.*, during acetic acid extraction, we added a threefold molar excess of Tris buffer (pH 7.4) to the glutaraldehyde before addition of glutaraldehyde to the ribosomes. The electrophoresis pattern (Figure 3) of treated ribosomes was identical with that of control ribosomes; hence the reaction of proteins with glutaraldehyde must occur before (or during) the dialysis against Tris buffer before the ribosomal proteins are extracted.

We tested the glutaraldehyde reactivity of the ribosomal proteins *in situ* at high concentration (35–50 mg/ml) in small volumes (200 μl) in experiments similar to those described in Figures 1 and 2. Under these conditions a threefold greater concentration of glutaraldehyde was required to obtain the same degrees of modification observed in the preparations shown in Figures 1 and 2. Figure 4 illustrates this point showing that ribosomes at high concentration treated with 0.1% glutaraldehyde in small volumes are modified to the same extent as ribosomes at low concentration in a greater volume modified by 0.03% glutaraldehyde (Figures 1B and 2B). Similarly, ribosomes at high concentration treated with 0.3 and 1% glutaraldehyde give patterns very similar to those shown in Figures 1C and 2C, and 1D and 2D, respectively. We attribute this effect to more rapid neutralization and dilution of glutaraldehyde during the dialysis against Tris buffer after glutaraldehyde treatment due to the use of smaller diameter dialysis tubing.

While the preceding experiments provide sufficient evidence to identify some external proteins of the 30S and 50S ribosomal subunits, they do not preclude the possibility that there are other external proteins which do not react with glutaraldehyde, or which react with glutaraldehyde to yield products which give normal spots in electrophoresis patterns. We have tested the reactivities of the free ribosomal proteins

TABLE III: Functional Activity of Glutaraldehyde-Treated 30S Ribosomes.

	Act.	
Treatment[a]	$(Phe)_m$ Synthesis[b]	Phe-tRNA Binding[b]
Control	(100)[c]	(100)[d]
0.03% glutaraldehyde	66	80
0.10% glutaraldehyde	6	27

[a] 30S ribosomes were treated for 30 min at 0° with buffer or glutaraldehyde in buffer as described in Materials and Methods. [b] Activities are expressed as percent of control activity determined as described in Materials and Methods. [c] 8200 cpm/A_{260} of 30S ribosomes. [d] 4500 cpm/A_{260} of 30S ribosomes.

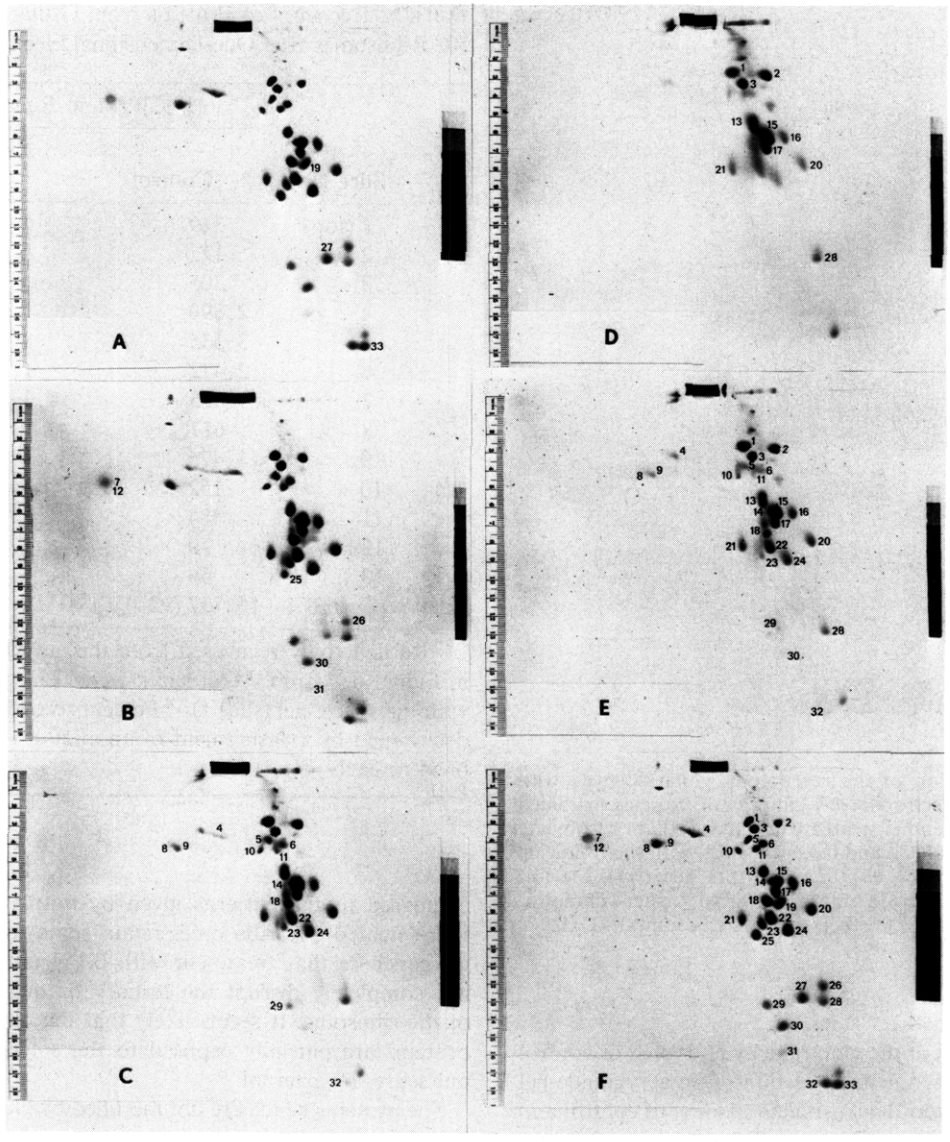

FIGURE 2: Two-dimensional acrylamide gel electrophoresis patterns of control and glutaraldehyde-treated 50S ribosomes obtained as described in Materials and Methods (large volume method). In A–D proteins are numbered in the gel pattern obtained at the highest concentration of glutaraldehyde not giving a fourfold reduction in the intensity of the particular spot. (A) 150 A_{260} of control (buffer treated) 50S ribosomes. (B) 150 A_{260} of 50S ribosomes treated with 0.03% glutaraldehyde. (C) 150 A_{260} of 50S ribosomes treated with 0.1% glutaraldehyde. (D) 150 A_{260} of 50S ribosomes treated with 0.3% glutaraldehyde. (E) 150 A_{260} of 50S ribosomes treated with 0.3% glutaraldehyde. 0.033 volume of 2 M Tris (pH 7.4) was added after 30-min fixation at 0° to stop the fixation reaction. All visible 50S protein spots are numbered. (F) 150 A_{260} of control (buffer treated) 50S ribosomes. All 50S protein spots are numbered. In this figure the prefix "L" has been omitted from the spot numbers for the sake of clarity.

toward glutaraldehyde in order to estimate the extent to which these reactivities are decreased by ribosome structure. Since the concentration of protein at the ribosome surface is extremely high, we tested the effect of glutaraldehyde on a concentrated solution of total 70S ribosomal proteins.

As shown in Figure 5, all of the ribosomal proteins reacted with 0.3% glutaraldehyde to yield products which do not give spots in stained electrophoresis patterns. This result was also obtained with two-dimensional electrophoresis (results not shown). Most of the proteins reacted with 0.1% glutaraldehyde. Since only a small number of ribosomal proteins are modified when 30S or 50S ribosomes are treated with 0.1% glutaraldehyde under reaction conditions similar to those giving essentially complete modification of free ribosomal proteins (Figure 4), we conclude that the inclusion of the ribosomal proteins in the ribosome structure protects these proteins from modification by glutaraldehyde and that the apparent protection of these proteins is not due to alteration in the position of spots given by reaction products of other proteins.

In order to elucidate the properties of the products of the reaction between glutaraldehyde and ribosomal protein which cause the disappearance of the expected stained spots from electrophoresis patterns we have carried out the following experiments. S13 was treated with 0.1% glutaraldehyde at concentrations of 20 μg/ml and 200 μg/ml in an identical manner as described for total 70S proteins. Ten micrograms of each preparation was subjected to one-dimensional acrylamide gel electrophoresis as described in Materials and Methods. No stained bands were observed anywhere in these gels in contrast to the single sharp band given by identical control preparations incubated in buffer alone. However,

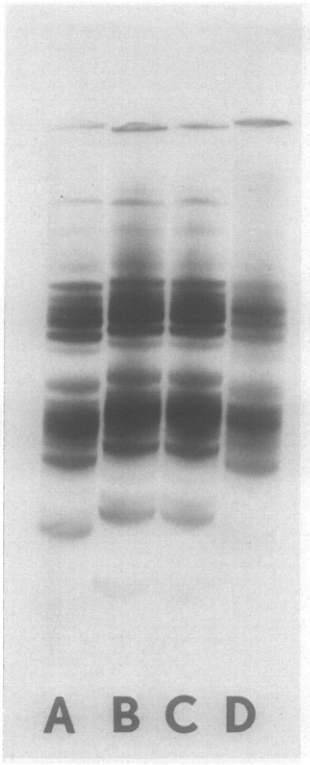

FIGURE 3: Tris inhibition of the reaction of glutaraldehyde with ribosomal proteins. Electrophoresis patterns of proteins extracted from 1 mg of 50S ribosomes treated with 0.1 M sodium cacodylate buffer (pH 7.0), 0.29 M KCl, and 0.009 M $MgCl_2$ with the following additions: (A) no additions; (B) 0.2 M Tris (pH 7.4); (C) 0.2 M Tris (pH 7.4)–0.3% glutaraldehyde (mixed at 10-fold greater concentration and incubated 5 min at 0°, then added to ribosomes); (D) 0.3% glutaraldehyde.

TABLE V: Recovery of Proteins from Glutaraldehyde-Treated 50S Ribosomes after One-Dimensional Electrophoresis.[a]

Slice No.	[^{14}C]Protein Recovered[b]	
	Control	Glutaraldehyde Treated
1 (top)[c]	319	88
2	127	914
3	705	985
4	2,890	985
5	3,535	3,342
6	2,472	1,230
7	3,825	3,133
8	617	1,112
9	1,475	446
10	152	305
11	484	225
12	70	107
13	66	45
Total recovered	16,737 (92.4%)[d]	12,917 (78.8%)[d]

[a] Radioactivity recovered from the acrylamide gels shown in Figure 6. [b] Cpm. [c] Gel slices were 0.5 cm long beginning with the spacer gel (slice 1). [d] Percent recovery of [^{14}C]protein determined by measurement of an aliquot of the sample applied to each gel.

when 10 μg or 100 μg of the glutaraldehyde-treated or control S13 was polymerized in a small section of an acrylamide gel and stained with Amido Black, similar staining of control and treated preparations was observed. We conclude that the staining properties of glutaraldehyde-treated proteins are not responsible for the observed absence of spots from our electrophoresis patterns.

In order to test this conclusion [^{14}C]protein-labeled 50S subunits were mixed with unlabeled 50S subunits in 0.25 ml of buffer and treated with 0.3% glutaraldehyde or buffer as described in Materials and Methods. The ribosomal proteins were extracted and subjected to one-dimensional acrylamide gel electrophoresis giving the results shown in Figure 6. The gels were cut in 0.5-cm sections and the amount of radioactivity in each section measured as described in Materials and Methods. As shown in Table V there is a significant difference in the electrophoretic mobility of radioactive protein from glutaraldehyde-treated and control ribosomes. This mobility difference could be due to interprotein cross-linking or to neutralization of free lysine groups. It is possible that glutaraldehyde-treated proteins migrate anodally in the second dimension of two-dimensional electrophoresis and are lost from the gel.

Discussion

We have shown above that ribosomes which have been treated with glutaraldehyde give two-dimensional acrylamide gel electrophoresis protein patterns which lack certain spots compared to the patterns given by untreated preparations. Since treated 30S ribosomes retain some functional activity, we conclude that treatment with 0.1% glutaraldehyde does not completely disrupt the tertiary or quaternary structure of the ribosome. It seems likely that the modified ribosomal proteins are partially exposed to the solvent and hence, in one sense, are external.

The proteins which are not modified by glutaraldehyde may either be completely internal, or may be external but not contain any primary amino groups which are accessible to and reactive with glutaraldehyde. In addition, if cross-linking of proteins is required for detection of modification by electrophoresis, proteins must have external reactive amino groups favorably oriented for cross-linking with other proteins or RNA in order to be classified as external proteins by this technique.

As we have shown, the glutaraldehyde-reactive ribosomal proteins differ in reactivity towards glutaraldehyde. In part, this difference may be due to differences in the original intensity of the spots, *e.g.*, S21 is a relatively faint spot in the control pattern and derivatization may be more easily observed. However, even among spots of equal intensity such as S10 and S14, there are clear differences in reactivity. It is possible that some proteins have many more exposed free amino groups in favorable positions for cross-linking to other proteins or RNA than do others, and hence, are more likely to be modified during the 30-min reaction period. It is also possible that minor conformational changes in the ribosomes occur during the fixation reaction and that these conformational changes cause an increase in reactivity of certain proteins as the reaction proceeds.

Our observations concerning the glutaraldehyde reactivity of the 30S ribosome are in general agreement with the studies of methoxynitrotropolone reactivity reported by Craven and

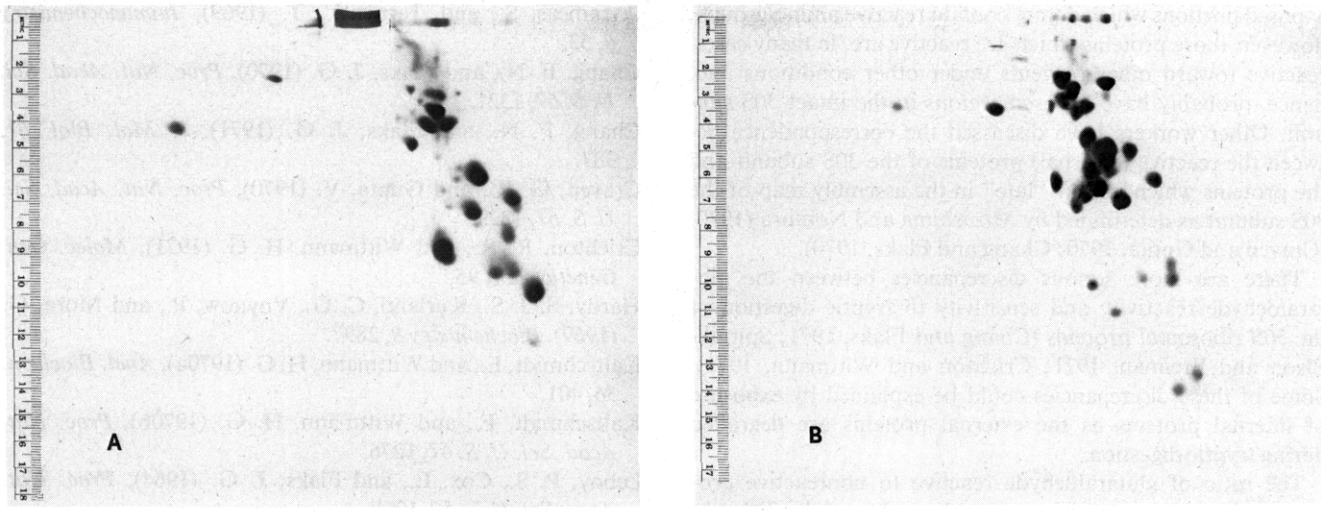

FIGURE 4: Two-dimensional electrophoresis of glutaraldehyde-treated 30S and 50S ribosomes obtained as described in Materials and Methods (small volume method). (A) 100 A_{260} of 30S ribosomes treated with 0.1% glutaraldehyde. (B) 150 A_{260} of 50S ribosomes treated with 0.1% glutaraldehyde.

Gupta (1970) with a few exceptions. S10, S2, and S17 (see Table I) are derivatized by glutaraldehyde and not methoxynitrotropolone. However, both S10 and S2 were reported by these workers to be susceptible to trypsin digestion in in the 30S ribosome. Our results with respect to S13 and S15 are in agreement with those of Craven and Gupta (1970) since it has been shown that S15 corresponds to protein 14 in the nomenclature of Kurland (Wittmann *et al.*, 1971). S13 was not studied by Craven and Gupta, and we have found that this protein does react with glutaraldehyde. S9 and S11 are not resolved in our gel patterns and give a single spot. However, at least one of these proteins reacts with glutaraldehyde which is consistent with the methoxynitrotropolone reactivity of S11 (Craven and Gupta, 1970). Some proteins which are not modified by glutaraldehyde may have

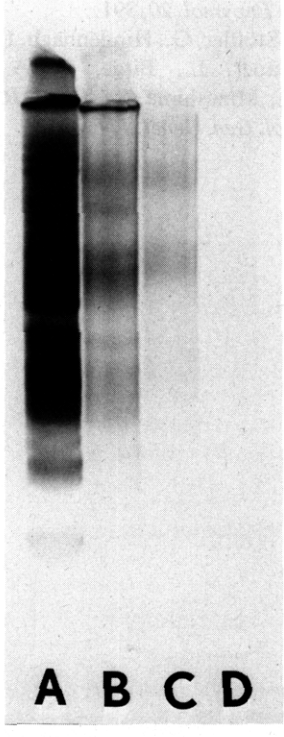

FIGURE 5: Electrophoresis patterns of free total 70S ribosomal proteins treated with glutaraldehyde as described in Materials and Methods. These gels were overloaded with protein so that greatly reduced protein bands would still be visible. (A) 1 mg 70S proteins treated with buffer alone; (B) 1 mg of 70S proteins treated with 0.03% glutaraldehyde; (C) 1 mg of 70S proteins treated with 0.1% glutaraldehyde; (D) 1 mg of 70S proteins treated with 0.3% glutaraldehyde.

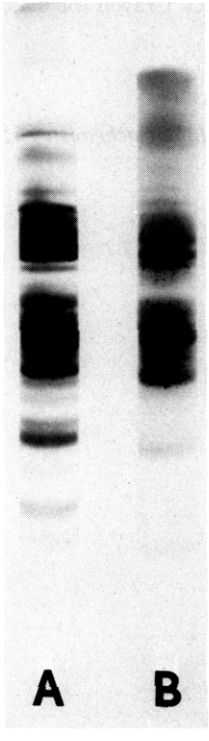

FIGURE 6: Electrophoresis patterns of ^{14}C-labeled proteins extracted from control and glutaraldehyde-treated 50S subunits. ^{14}C-Labeled 50S ribosomes were mixed with 2 mg of unlabeled 50S subunits and treated with buffer (A) or 0.3% glutaraldehyde (B); the proteins extracted from 1 mg of each sample were separated by one-dimensional electrophoresis. The distribution of radioactivity in these gels is described in Table V.

exposed portions which do not contain reactive amino groups; however, those proteins which are reactive are, in many cases, reactive toward other reagents under other conditions and, hence, probably have exposed regions in the intact 30S subunit. Other workers have discussed the correspondence between the reactive (external) proteins of the 30S subunit and the proteins which appear "late" in the assembly map of the 30S subunit as determined by Mizushima and Nomura (1970) (Craven and Gupta, 1970; Chang and Flaks, 1970).

There are more serious discrepancies between the glutaraldehyde reactivity and sensitivity to tryptic digestion of the 50S ribosomal proteins (Chang and Flaks, 1971; Spitnik-Elson and Breiman, 1971; Crichton and Wittmann, 1971). Some of these discrepancies could be explained by exposure of internal proteins as the external proteins are degraded during tryptic digestion.

The ratio of glutaraldehyde reactive to nonreactive proteins in the 50S subunit is comparable to that of the 30S subunit suggesting that there are similarities in the structural organization of these particles. In the case of the 30S subunit, the proteins which bind specifically to 16S RNA in the absence of other proteins, S4, S7, S8, S15, S20, and possibly S13 or S17 (Mizushima and Nomura, 1970; Schaup *et al.*, 1971), are among the proteins which do not substantially react with 0.1% glutaraldehyde. It is tempting to speculate that the group of proteins of the 50S subunit which do not react with 0.1% glutaraldehyde includes the proteins which bind specifically to RNA in the 50S subunit.

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References

Avrameas, S. (1969), *Immunochemistry 6*, 43.

Avrameas, S., and Ternynck, T. (1969), *Immunochemistry 6*, 53.

Chang, F. N., and Flaks, J. G. (1970), *Proc. Nat. Acad. Sci. U. S. 67*, 1321.

Chang, F. N., and Flaks, J. G. (1971), *J. Mol. Biol. 61*, 387.

Craven, G. R., and Gupta, V. (1970), *Proc. Nat. Acad. Sci. U. S. 67*, 1329.

Crichton, R. R., and Wittmann, H. G. (1971), *Molec. Gen. Genetics 114*, 95.

Hardy, S. J. S., Kurland, C. G., Voynow, P., and Mora, G. (1969), *Biochemistry 8*, 2897.

Kaltschmidt, E., and Wittmann, H. G. (1970a), *Anal. Biochem. 36*, 401.

Kaltschmidt, E., and Wittmann, H. G. (1970b), *Proc. Nat. Acad. Sci. U. S. 67*, 1276.

Leboy, P. S., Cox, E., and Flaks, J. G. (1964), *Proc. Nat. Acad. Sci. U. S. 52*, 1367.

Mizushima, S., and Nomura, M. (1970), *Nature (London) 226*, 1214.

Moore, P. B. (1971), *J. Mol. Biol. 60*, 169.

Noller, H. F., Chang, C., Thomas, G., and Aldridge, J. (1971), *J. Mol. Biol. 61*, 669.

Nomura, M., Mizushima, S., Ozaki, M., Traub, P., and Lowry, C. V. (1969), *Cold Spring Harbor Symp. Quant. Biol. 34*, 49.

Sabatini, D. D., Bensch, K., and Barrnett, R. S. (1963), *J. Cell Biol. 17*, 19.

Schaup, H. W., Green, M., and Kurland, C. G. (1971), *Mol. Gen. Genet. 112*, 1.

Spitnik-Elson, P., and Breiman, A. (1971), *Biochim. Biophys. Acta 254*, 457.

Traub, P., Mizushima, S., Lowry, C. V., and Nomura, M. (1971), *Methods Enzymol. 20*, 391.

Wittmann, H. G., Stöffler, G., Hindennach, I., Kurland, C. G., Randall-Hazelbauer, L., Birge, E. A., Nomura, M., Kaltschmidt, E., Mizushima, S., Traut, R. R., and Bickle, T. A. (1971), *Mol. Gen. Genet. 111*, 327.