Spin-Labeled Ribosomes†

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ABSTRACT: *Escherichia coli* ribosomes and subunits have been labeled with the nitroxide containing reagent 4-maleimido-2,2,6,6-tetramethylpiperidinooxyl (MAL-6). Electron spin resonance spectra of the labeled organelle reveal two classes of sulfhydryl environments, one weakly and the other strongly immobilized. The weakly immobilized environment is highly polar and more exposed to the solvent than the strongly immobilized environment. The resonance spectra are sensitive to structural alteration of the ribosomal architecture. For example, the agents urea and EDTA both unfold the ribosome but each in a different manner, the former releasing the structural constraints which cause the strongly immobilized nitroxide environment, the latter favoring this class of labeled ribosomes. Association of the 30S and 50S ribosomes to form an intact 70S structure brings about conformational changes in both subunits. Interaction of the organelle with streptomycin, which binds to the 30S subunit, concurs structural alterations in the 50S subunit. Thus the effect of ligand binding to the ribosome does not have to be localized to the site of attachment but can be relayed through the macromolecular assembly by coupled structural changes. When the individual ribosomal protein L7/L12 is labeled with 4-(2-iodoacetamide)-2,2,6,6-tetramethylpiperidinooxyl (IA-6), the ESR spectra reveal that this functionally important protein has a domain with a high degree of motional flexibility when it is bound in the 70S ribosome.

The spin labeling method has been widely applied in biochemistry, particularly in protein and membrane systems (see Berliner (1976) and Likhtenshtein (1976) for recent comprehensive monographs). Placement of an unpaired electron, usually as a nitroxide, into a macromolecule has several advantages. First, there is generally no interfering electron spin resonance (ESR) signal which is naturally occurring. Second, if the nitroxide can be covalently placed into a known location, information about that specific site can be obtained as opposed to structure averaged information available from the common forms of optical spectroscopy (UV, vis, CD, IR). Most importantly, the ESR line shapes are very sensitive to both the structure of its immediate environment and to local motional properties. Thus this type of spectroscopy provides a type of insight which is not readily attainable by other techniques.

This paper presents some exploratory studies on the use of nitroxide-labeled ribosomes. The radical moeity is introduced into the ribosome by reaction with an *N*-ethylmaleimide derivative attached to a nitroxide functionality (Figure 1). This reagent reacts with reduced sulfhydryl groups of proteins. Since the sulfhydryl reactivity of the *E. coli* ribosome has been well characterized (e.g., Moore, 1971; Ginzburg & Zamir, 1976), this labeling procedure provides a convenient way to introduce the spectral probe in a known way. The labeled ribosomes can then be used to study various ligand binding reactions and conformational dynamics.

Materials and Methods

Preparation and Assay of Ribosomes and Protein L7/L12. The 70S ribosomes were purified from *E. coli* MRE600 as previously described (Tritton & Crothers, 1976). The 30S and 50S subunits were obtained by zonal centrifugation in a Beckman Ti15 rotor essentially as reported by Sypherd & Wireman (1974). Protein L7/L12 was stripped from 50S and 70S ribosomes by the procedure of Hamel et al. (1972). The resulting preparation was at least 90% free of other ribosomal proteins as judged by polyacrylamide gel electrophoresis. Reincorporation of the protein back into the stripped ribosomes was accomplished by incubating core particles and the L7/L12 derived from them in 50 mM Tris, pH 7.5, 10 mM magnesium acetate, and 150 mM KCl (TMK) at 37 °C for 30 min. The activity of the various ribosomal preparations was assayed in a poly(U)-dependent polyphenylalanine synthesizing system (Staehelin & Maglott, 1971).

Buffers. Ribosomes for spectral analysis were contained in TMK (50 mM Tris, 10 mM magnesium acetate, 150 mM KCl, pH 7.5) or TM/10 K (50 mM Tris, 1 mM magnesium acetate, 150 mM KCl, pH 7.5). Sedimentation assays were done in 10–30% sucrose gradients using these buffer systems.

Spin-Labeling Reactions. Ribosomes were spin labeled by covalent reaction with the reagent 4-maleimido-2,2,6,6-tetramethylpiperidinooxyl (MAL-6, Figure 1) obtained from Syva. The reaction was carried out in TMK (4 °C, 16 h) at 100–800 A_{260} units/mL and 1–2 mM MAL-6. The reaction was terminated by exhaustive dialysis vs. several liters of TMK. Protein L7/L12 was labeled with the reagent 4-(2-iodoacetamide)-2,2,6,6-tetramethylpiperidinooxyl (IA-6, Figure 9) by overnight reaction in 50 mM potassium carbonate (pH 9.4) followed by extensive dialysis vs. TMK. This protein preparation was concentrated on Amicon B15 "Minicon" ultrafilters and the protein concentration estimated by the Lowry method.

Electron Spin Resonance Measurements. ESR spectra were run at ambient temperature in a JEOL ME series spectrometer operating at X band (9.28 GHz and 3150 G). Samples were drawn into 25-μL capillary tubes (Kimble), sealed with wax, and placed in standard Quartz ESR tubes. Instrumental conditions which gave optimum sensitivity with least distortion of line shape were 2.0 G modulation amplitude, 10 mW incident microwave power, 10 min sweep of a 100 G window, and 0.3 s response time. Duplicate runs were always superimposable on the original run. In all figures comparing two spectra, the solution conditions and ribosome concentrations (generally $0.5–2 \times 10^{-5}$ M) are identical for each case.

Results

Labeling of 70S Ribosomes. When 70S ribosomes are

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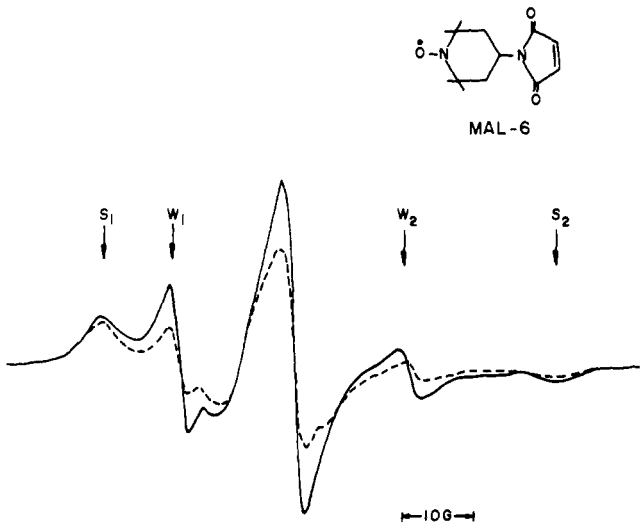

FIGURE 1: ESR spectra of MAL-6 labeled 70S ribosomes in TMK (—) and TMK plus 0.5 M EDTA (- - -). The arrows indicate some of the components of the weakly (W) and strongly (S) immobilized lines.

TABLE I: Relative Activities of Various Ribosomal Preparations in Poly(U)-Directed Polyphenylalanine Synthesis.

sample	rel act. (% of control)
70S ribosomes, control	100
70S ribosomes, labeled with MAL-6	1-5
70S ribosomes (stripped), −L7/L12	9
70S ribosomes (stripped) reconstituted with L7/L12	100
70S ribosomes (stripped) reconstituted with IA-6 labeled L7/L12	147

reacted under standard conditions with the nitroxide spin label MAL-6, the resulting product yields an ESR spectrum as shown in Figure 1. Spectra of this type are a composite of at least two types (Schneider & Smith, 1970): one due to spin labels strongly immobilized (whose components are labeled S) and the other due to spin labels which are weakly immobilized (labeled W). The ratio (*W/S*) between the two amplitudes has been shown to be a reasonable estimate of the relative amounts of the two types of states as long as the value does not exceed 20 (Schneider & Smith, 1970), a criterion met in all of the studies presented here. In previous labeling studies with *N*-ethylmaleimide, which is a congener of the reactive portion of MAL-6, it was shown that the reagent reacts almost exclusively with reduced sulfhydryl groups (Moore, 1971). Furthermore, under the conditions used in the present experiments, Moore's (1971) results predict an uptake of about 20 NEM molecules per 70S ribosome. Since preincubation of the ribosomes with NEM blocks uptake of MAL-6 it is concluded that this spin labeling reagent has a similar pattern of reactivity as NEM.

Moore (1971) has shown that NEM partially inactivates the ability of *E. coli* 70S ribosomes to synthesize polyphenylalanine directed from a poly(U) message. Likewise, MAL-6 in the present study inactivates the ribosome in this assay (Table I). The spin-labeling reagent does not, however, alter the gross structural organization of the ribosomes or subunits as shown by the normal sedimentation properties of the labeled particles (Figure 2). Furthermore, the 70S labeled with MAL-6 is not impaired in its ability to participate in the reversible subunit equilibrium reaction (Figure 2). Thus, although the reagent blocks protein synthesizing function,

FIGURE 2: Sucrose density gradient analysis of MAL-6 labeled 70S ribosomes. The samples were layered on top of 10-30% sucrose gradients in TMK (70S) or TM/10K (30-50S) and run at 21 000 rpm for 17 h in a Beckman SW40Ti rotor. The tubes were fractionated with a Buchler Auto-Densi Flow II system and the absorbance at 260 nm determined for each fraction. Unlabeled ribosomes and subunits were run in parallel tubes to provide the sedimentation constant standards indicated by the arrows.

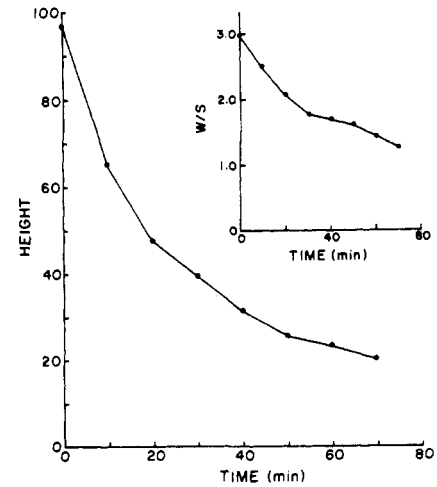

FIGURE 3: The time course of the ascorbate reduction of the nitroxide center in MAL-6 labeled 70S ribosomes in TMK. The concentration of ascorbate is 0.1 mm and the height of the center line in the spectrum is plotted with time. Insert: the effect of ascorbate on the ratio of weakly to strongly immobilized nitroxides as a function of time. The same spectra were used to provide data for both center line height and *W/S* ratio.

MAL-6 leaves the ribosome structurally intact and the spin label should provide a probe for conditions or agents which themselves change the ribosome conformation.

The nitroxide radical is easily reduced with consequent loss of the paramagnetic signal (Gaffney, 1976). In order to ascertain the relative accessibilities of the MAL-6 labeled sulfhydryl positions, radical decay studies via ascorbate reduction were carried out. With 10 mM ascorbate the ESR signal of 70S ribosomes is completely lost in the time required for mixing and placing the sample in the spectrometer (about 2 min). At 0.1 mM ascorbate the reduction in signal proceeds with a half-time of about 30 min when measuring the centerline intensity (Figure 3). If the weakly and strongly immobilized spin labels are reduced at the same rate, then the ratio of their intensities should remain constant as the total intensity decreases. The

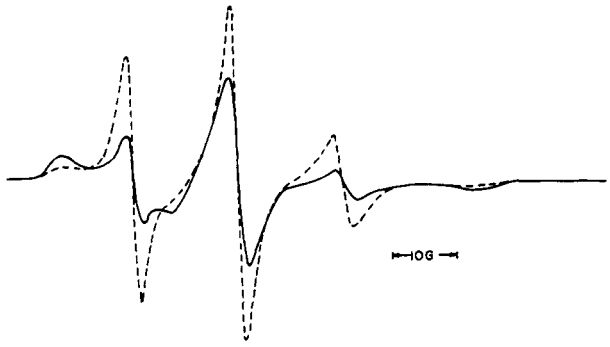

FIGURE 4: The effect of urea on the ESR spectra of MAL-6 labeled 70S ribosomes in TMK. (—) With 0.5 M urea; (- - -) 3 M urea.

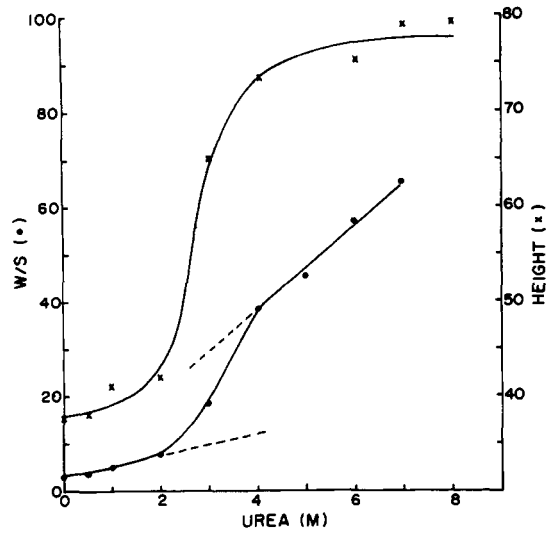

FIGURE 5: Titration of the center line height (×) and ratio of weakly (W) to strongly (S) immobilized environments (●) urea in MAL-6 labeled 70S ribosomes in TMK.

insert to Figure 3 shows, however, that the *W/S* ratio decreases with time of reduction indicating that the weakly immobilized nitroxides are more accessible to reduction by ascorbate than the strongly immobilized centers. Both classes of labels though, eventually are completely reduced and yield no ESR resonance.

Addition of urea to MAL-6 labeled 705 ribosomes leads to large changes in the ESR spectrum (Figure 4). All of the lines narrow and become larger in amplitude with increasing amounts of urea. Also, the *W/S* ratio measured at the lowest field peaks increases with the urea concentration so that at 8 M urea the nitroxide radical reports that all the sulfhydryl environments are only weakly immobilized. A titration curve of these phenomena is shown in Figure 5. Addition of EDTA, which also unfolds the ribosomes (Wong & Dunn, 1974), changes the MAL-6 spectrum, but even at 0.5 M (Figure 1) does not eliminate the distribution between weekly and strongly immobilized sites of labeling.

Labeling of 30S and 50S Ribosomal Subunits. When labeled with MAL-6 under standard conditions, both the small and large ribosomal subunit yield a spectrum qualitatively similar to the labeled 70S ribosome. Thus the distribution of nitroxide labeled sulfhydryl groups between weakly and strongly immobilized environments is approximately the same in the 30S and 50S subunit, although the relative amounts of each may be different (see Discussion). Also, judging from Moore's (1971) studies of *N*-ethylmaleimide uptake, the individual subunits take up about half as many moles of label as

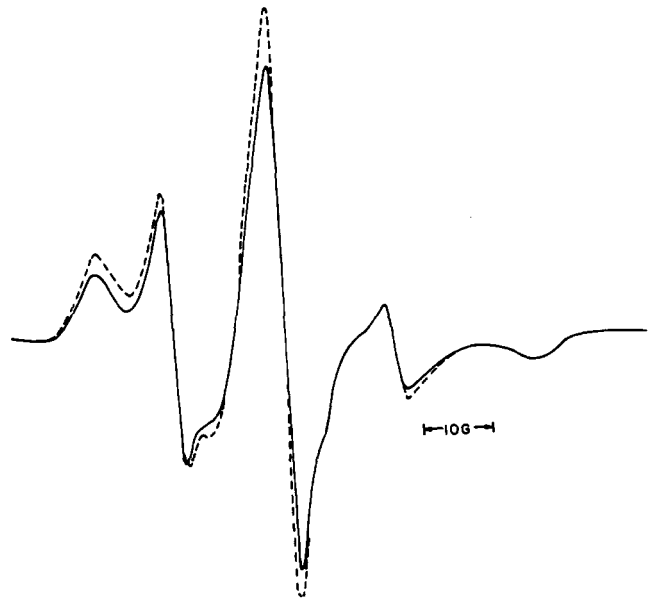

FIGURE 6: The effect of subunit association when the MAL-6 label is contained only in the 30S subunit. (- - -) TMK buffer promoting 70S ribosomes; (—) TM/10K buffer causing dissociation into 30S + 50S subunits.

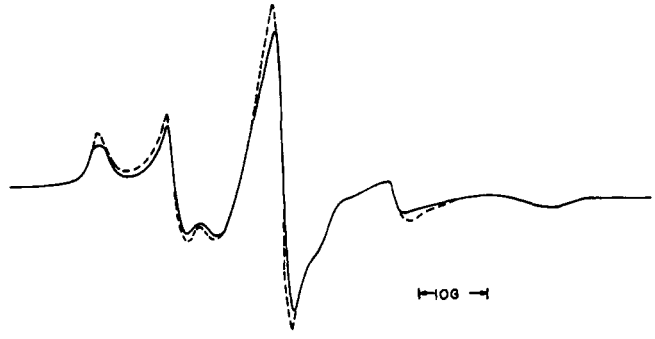

FIGURE 7: The effect of subunit association when the MAL-6 label is contained only in the 50S subunit. (- - -) TMK buffer promoting 70S ribosomes; (—) T M/10 K buffer causing dissociation into 30S + 50S subunits.

the intact 70S ribosome, or about ten MAL-6 molecules per subunit under the conditions of these experiments.

It is known from other work (Zitomer & Flaks, 1972; Ball et al., 1973; Litman et al., 1974) that association of the 30S and 50S subunits to 70S ribosomes produces conformational changes in the particles. The effect of association with an unlabeled subunit on the ESR spectrum of MAL-6 covalently bound to the other subunit is shown in Figures 6 and 7. Association is brought about by raising the magnesium concentration from 1 to 10 mM. The increased Mg^{2+} concentration does not perceptibly alter the spectra when either labeled subunit is present alone. However, when a MAL-6 labeled subunit is associated with an unlabeled partner, the result is to increase the intensity of all the ESR lines and to shift the weakly/strongly immobilized distribution toward a slightly higher fraction of weakly immobilized labels (the *W/S* ratio increases by 20.9 and 7.7% when the label is in the 30S and 50S subunit, respectively). Similar changes, although smaller in magnitude, are seen when the nitroxide is resident in both subunits (data not shown).

The ribosome is the receptor site for many antibiotics. One of the more important of these, both because of its widespread clinical use and because of its special role in the development

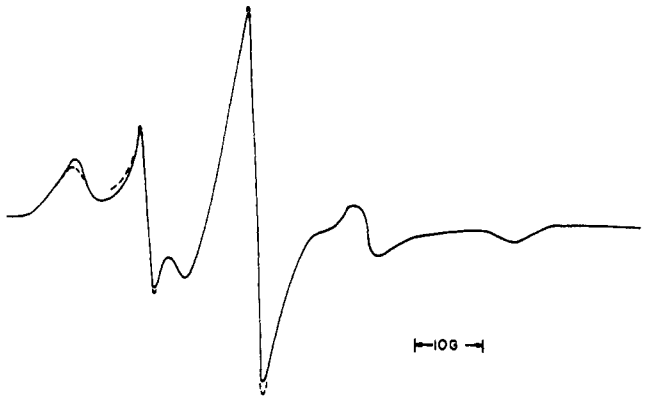

FIGURE 8: The effect of streptomycin on the ESR spectrum when the MAL-6 label is contained only in the 50S subunit. (—) With 70S ribosomes in TMK; (- - -) 70S ribosomes in TMK plus 0.1 mM streptomycin.

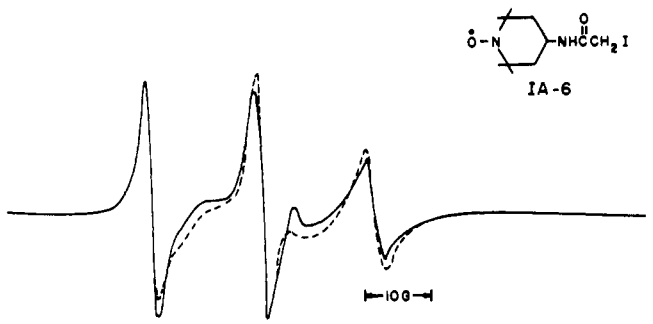

FIGURE 9: ESR spectra of IA-6 labeled L7/L12 in the absence (—) of and reconstituted (- - -) in 70S ribosomes "stripped" of this protein. The labeled protein was reassociated into the ribosome prior to running the spectrum as described in Materials and Methods.

of molecular biology, is streptomycin. This drug has several effects on the bacterial ribosome, most notably a blockade of aminoacyl-tRNA binding and a stimulation of miscoding (reviewed by Schlessinger & Medoff, 1975). At 0.4 mM the drug has no discernible effect on the ESR spectrum of 70S, 30S, or 50S ribosomes. Likewise when labeled 30S are combined with unlabeled 50S the drug does not significantly perturb the spectrum. Surprisingly though, streptomycin does alter the observed 70S spectrum when MAL-6 is present only in the 50S subunit. Figure 8 shows that upon binding to streptomycin these 50S labeled ribosomes show small but reproducible increases in both the center line intensity and the *W/S* ratio (percent increase is 10.7 ± 4.0).

Labeling of Protein L7/L12. The two ribosomal proteins designated L7 and L12 differ from each other only by the presence of a single acetylated amino acid and are necessary for the interaction of the ribosome with initiation, elongation, and termination factors (Möller, 1974). In other words, these proteins are critical to the functioning of the ribosome at every stage of protein synthesis. Moreover it is relatively easy to purify L7/L12 and then reconstitute back the stripped activity (Hamel et al., 1972). For these reasons it was decided to spin label L7/L12 so that studies could be carried out with the label in a specified location within the ribosome. Since this protein contains no free sulfhydryl groups (Moore, 1975), the reagent IA-6 was used to covalently attach the nitroxide. The reactive portion of this molecule is iodoacetamide (Figure 9) which, under the conditions employed, probably reacts either with the unblocked N terminus, a lysine, or the single methionine of L7/L12 (Langlois et al., 1976). The fraction of L7/L12 which is labeled by the reagent is estimated from the signal intensity

TABLE II: Correlation Times Calculated (see Text) for the MAL-6 Nitroxide Groups on the Weakly Immobilized Sulfhydryl Centers and for the IA-6 Labeled Protein L7/L12.

sample	$\tau_c \times 10^9$ (s)
1. 70S ribosome	1.51
2. 50S subunit	1.49
3. 30S subunit	1.50
4. 70S + 8 M urea	1.44
5. L7/L12	4.96
6. L7/L12 reconstituted into stripped 70S ribosomes	3.66

under denaturing conditions to be between 0.5 and 1 spin label per mol of protein monomer. In the ESR reconstitution studies the stripped 70S ribosomes are in a 1:1 molar ratio to promote complete binding of the protein, even though four copies are required to reconstitute a native 70S ribosome.

The resonance spectra obtained for free and ribosome bound L7/L12 are shown in Figure 9. These spectra are typical of a nitroxide which is only weakly immobilized, i.e., the label probably has motion independent of the overall rotation of the protein. Upon reconstitution with 70S ribosomes the spin label spectrum changes indicating that the environment it senses has altered. However, the spectrum still is indicative of a rather mobile nitroxide radical indicating that binding to the bulky ribosome does not immobilize the labeled portion of the L7/L12 protein.

Discussion

The electron spin resonance pattern of *E. coli* ribosomes labeled with the sulfhydryl specific spin label MAL-6 is characteristic of at least two classes of sulfhydryl groups. One class (S) is due to spin labels which have no motion independent of the slowly moving ribosomal assembly; the second (W) is characteristic of spin labels which have some independent, faster motions of their own. It is possibly to estimate the correlation time for the weakly immobilized labels from an equation given by Likhtenshtein (1976) derived from the theory of Freed & Frenkel (1963)

$$\frac{1}{\tau_c} = \frac{1.2 \times 10^{-9}}{\left(\sqrt{\frac{h(+1)}{h(-1)}} - 1\right) \Delta H(+1)} \text{ s}^{-1}$$

where $\Delta H(+1)$ is the width of the most downfield line in Gauss, $h(+1)$ and $h(-1)$ are the intensities of the lower and upper field weakly immobilized lines, and τ_c is the correlation time. The values so calculated are shown in Table II. The τ_c values are essentially the same for both 70S ribosomes and isolated 30S and 50S subunits and are made only slightly faster by denaturation with urea. All of these correlation times are about three orders of magnitude faster than the overall correlation time calculated for the ribosome itself (Tritton & Armitage, 1978); thus these weakly immobilized nitroxides are moving quite rapidly relative to the overall tumbling, most likely because the covalent linkage is not rigid. The second distinct class of nitroxides, the strongly immobilized, takes on the correlation time of the ribosome. In addition there is probably a third class of sulfhydryls which are inaccessible to the MAL-6 reagent (Moore, 1971).

In addition to the correlation times derived from the relative peak *heights*, one can also obtain information from the peak *splittings* in the ESR spectra. For example, the splitting of the outer hyperfine extrema (labeled S_1 and S_2) in the ribosome spectra is 32.8 ± 0.3 G for both ribosomes and subunits. Comparing with the T_{zz} principal values of 32.9 and 31.8 G

for the rigid glass spectra of 2-doxylpropane and di-*tert*-butyl nitroxide in host crystals where motion is negligible (Jost et al., 1971), these values clearly indicate a highly immobilized environment for this class of sulfhydryls in the ribosome. The second, weakly immobilized, environment is characterized by a splitting, one-half of which is a measure of A_N, the nitrogen isotropic hyperfine coupling constant. This value in all MAL-6 labeled ribosome couples is 16.7 ± 0.2 G. Butterfield et al. (1976) have reported the A_N values for MAL-6 in buffer and dodecane as 16.7 and 13.6 G, respectively. Consequently, the weakly immobilized nitroxides in the ribosome must be in a highly polar environment and therefore not likely to be buried in the interior of the organelle. The ascorbate reduction studies support this conclusion and show that, although both classes of MAL-6 labeled sulfhydryls are accessible to reduction, the weakly immobilized nitroxides are reduced more rapidly than the strongly immobilized ones (Figure 3) and thus are probably somewhat more accessible to the surface.

The ratio of the amplitudes of the weakly and strongly immobilized spectral lines (*W/S*) is a measure of the relative population in each class (Butterfield et al., 1976; Schneider & Smith, 1970). The exact value of *W/S* varies somewhat from preparation to preparation (±25%) and also changes slowly (time scale of days to weeks) upon storage of the labeled ribosomes. This may be due to nitroxide reduction by unreacted sulfhydryls. Repeated spectra of the same sample on the same day, however, give highly reproducible values of *W/S*. The numerical value of *W/S* for different preparations of subunits and ribosomes always lies between 2.5 and 3.5. There is a tendency for the value to be somewhat higher for the 30S subunit than either the 50S or 70S particle, but this observation cannot be established with certainty. Nonetheless, the qualitative conclusion to be drawn from this data is that about three times more sulfhydryl groups are in the weakly immobilized than the strongly immobilized location in ribosomes.

The *W/S* ratio and other spectral characteristics are altered by subjecting the ribosomes to various conditions and reagents. Urea, which denatures and unfolds the ribosome, converts all the strongly immobilized nitroxides into the weakly immobilized environment. In 8 M urea the correlation time for the now identical nitroxide population (Table II) is only slightly faster than the weakly immobilized spectrum in intact 70S ribosomes. Hence this population is not constrained in its mobility even by the intact assembly of macromolecules. The midpoint of the urea titration curves is somewhat different when measuring different spectral characteristics. The center line intensity and the *W/S* ratio give midpoints of about 2.8 and 3.1 M, respectively. Both of these are somewhat lower than the 3.5 M midpoint determined by Langer et al. (1975) in thiol accessibility studies. These differences are probably due to the fact that the unfolding of the ribosome by urea is a complex process whose apparent progress depends on the method of observation.

Addition of sufficient EDTA to complex all the essential divalent metal ions also unfolds the ribosome (Wong & Dunn, 1974). The electron spin resonance spectra reveal that these unfolded ribosomes are quite different than those obtained from urea denaturation (Figures 1 and 4); whereas urea converts all of the MAL-6 labels into a weakly immobilized environment (increased *W/S* value), EDTA has the opposite effect leading to a decrease in the *W/S* value. Hence the relative proportion of nitroxides in the strongly immobilized state has increased even though the ribosome is "unfolded" by EDTA. The difference between the two reagents lies in the fact that urea causes proteins to be "split" from the core particle (Langer et al., 1975) and disrupts the secondary structure of the RNA while EDTA causes unfolding with retention of the full set of ribosomal proteins (Spirin, 1974) and with only minimal disruption of RNA secondary structure (Eilam & Elson, 1971). The ESR results suggest that the EDTA unfolded ribosomes retain a good deal of tertiary structure, although the protein environment has rearranged somewhat so that a larger fraction of the labels are in the immobilized environment. Urea, on the other hand, removes the structural constraints which lead to the strongly immobilized nitroxides.

The large structural changes elicited by urea and EDTA affect the overall organization of the ribosome. The association of subunits and binding of drug molecules, on the other hand, produce subtler, more localized conformational effects. Consequently, the labeled 70S ribosomes, with 10–20 nitroxides distributed throughout the structure, do not provide very large spectroscopic changes when only localized effects are wrought in the organelle. For this reason, the effects of subunit association and streptomycin were assessed in ribosomes with MAL-6 in only one subunit so that the extent of labeling per total 70S ribosome is lower than before.

It is apparent from Figures 6 and 7 that association of subunits produces conformational changes in both organelles. With MAL-6 in either the 50S and 30S subunit, Mg^{2+}-induced association with an unlabeled partner produces similar changes including a shift of the nitroxide distribution toward slightly higher *W/S* ratios. Thus there is indicated a general loosening of structure and correspondingly more mobility, especially in the regions of the protein sulfhydryls, when the two subunits join to form a 70S ribosome. This relaxation of structure may be important functionally if the ribosome must flex or switch among different conformational states as it proceeds through the various steps of polypeptide synthesis.

The effect of streptomycin on the ESR spectra is unusual. This drug acts exclusively on the 30S subunit and elicits several responses, the most important of which are to block the binding of aminoacyl-tRNA, to cause misreading of the genetic code, and to prevent dissociation of 30S:50S couples (reviewed by Schlessinger & Medoff, 1975). Binding of the drug elicits no change in the ESR spectrum of MAL-6 labeled 30S subunits either alone or combined with unlabeled 50S subunits. When the nitroxide is resident only in the 50S subunit, however, interaction of the 70S ribosome with streptomycin stimulates a change in the spectrum. These changes (increase in both the *W/S* ratio and the center line intensity) are qualitatively similar, although smaller in magnitude, to the change brought by subunit association. Hence, when the subunits are fully associated, as they are in this experiment, binding of streptomycin induces an additional increment in the distribution favoring the weakly immobilized sulfhydryl environment. Apparently there is a fine balance between the interactions promoting competent association of subunits and the disruption caused by the protein synthesis inhibitor streptomycin. This apparent "loosening" of structure by streptomycin was deduced by Sherman & Simpson (1969) from tritium exchange studies. Likewise, Brakier-Gingras el al. (1974) found that adding streptomycin before labeling ribosomes with MAL-6 lead to a decrease in the rotational correlation time of the labels, also indicative of a "loosened" ribosomal structure.

The present data also indicate that the structural effect of streptomycin is not localized to its site of attachment but can be propagated through the interacting macromolecules of the ribosomal assembly by induced structural changes. Previous studies have shown (Chang & Flaks, 1972; Schreiner & Nierhaus, 1973) that there is only one streptomycin site per 70S or 30S ribosome, even at high input ratios. The drug does not

bind significantly to the 50S subunit. The ESR spectra reveal that, when the drug binds to its receptor site on the small subunit, the effect is "felt" at distant sites on the large subunit. Thus the receptor site may be conformationally coupled to the site of ultimate disruption and the two sites do not have to be close neighbors or even on the same subunit. This is consistent with immune electron microscopic evidence (Stöffler & Tischendorf, 1974) suggesting that binding of a drug molecule can affect the environment of several proteins at once. This observation may pose somewhat of a problem for interpreting certain kinds of experiments aimed at identifying ribosomal active sites. For example, Schreiner & Nierhaus (1973) have identified proteins S3 and S5 as streptomycin binding proteins and Pongs & Lanka (1975) have identified S7, S14, and S16/S17 in the streptomycin receptor site by affinity labeling. Such an identification of the proteins which contact streptomycin does not ensure that these sites are where the ultimate disruption of ribosomal function occurs. Likewise the demonstration that the drug binds to isolated rRNA (Biswas & Gorini, 1972) does not prove a functional role for the nucleic acid but simply suggests that it may form a part of the binding site. Lastly, the fact that ligand binding can simultaneously affect more than a single location within the organelle may help to explain why several kinds of functional effects can be wrought by interaction of ribosomes with a single drug.

The studies with MAL-6 labeled ribosomes have two drawbacks. First, the locations of the nitroxides within the ribosome are not precisely known except insofar as they are at sulfhydryl positions. Secondly, this reagent inactivates ribosome function, although it does not alter the overall integrity of the structure. To overcome these limitations some preliminary work with IA-6 labeled L7/L12 is reported here. IA-6 was chosen because L7/L12 has no sulfhydryl residues and will not react with MAL-6 under standard conditions; also iodoacetamide is not as vigorous at inactivating the ribosome as *N*-ethylmaleimide (Moore, 1971). Indeed, in the present work the IA-6 labeled L7/L12 is capable of reconstituting full activity to 70S ribosomes stripped of the protein by NH_4Cl/ethanol (Table I). The spectrum of the labeled protein reveals that the nitroxide radical is only weakly immobilized. Reconstitution with stripped 70S ribosomes changes the environment around the nitroxide probe; that is, the protein conformation changes upon binding, but the fast correlation time (Table II) is only slightly decreased when the protein is bound to the very bulky 70S ribosome.

Studies of the L7/L12 protein in solution show a very elongated, possibly dumbbell shaped, particle (Gudkov et al., 1977; Osterberg et al., 1976). Immune electron microscopy (Tischendorf et al., 1975; Boublik et al., 1976) confirms that the L7/L12 complex is also elongated when it is in place in the ribosome. The present ESR work demonstrates that at least a portion of the L7/L12 domain maintains a good deal of flexibility when localized in the ribosome and thus this study supports the idea put forth by Boublik et al. (1976) that flexibility in the L7/L12 region may be considered as an explanation of the mechanism of ribosomal movement along messenger RNA.

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