Mandel, M., Schildkraut, C. L., and Marmur, J. (1968), *Methods Enzymol. 12B*, 184.
Marmur, J. (1961), *J. Mol. Biol. 3*, 208.
Martinson, H. G. (1973), *Biochemistry 12*, 145.
Martinson, H. G., and McCarthy, B. J. (1975), *Biochemistry 14*, 1073.
Marushige, K., and Bonner, J. (1966), *J. Mol. Biol. 15*, 160.
McConaughy, B. L., and McCarthy, B. J. (1972), *Biochemistry 11*, 998.
Ohlenbusch, H. H., Olivera, B., Tuan, D., and Davidson, N. (1967), *J. Mol. Biol. 25*, 299.
Olafsson, P. G., and Bryan, A. M. (1974), *Arch. Biochem. Biophys. 165*, 46.
Paul, J., Gilmour, R. S., Fava, M. F., Birnie, G., Harrison, P., Hell, A., Humphries, S., Windass, J., and Young, B. (1974), *Cold Spring Harbor Symp. Quant. Biol. 38*, 885.
Renz, M. (1975), *Proc. Natl. Acad. Sci. U.S.A. 72*, 733.
Riggs, A. D., Suzuki, H., and Bourgeois, S. (1970), *J. Mol. Biol. 48*, 67.
Rutter, W. J., Pictet, R. L., and Morris, P. W. (1973), *Annu. Rev. Biochem. 42*, 601.
Samuels, H. H., and Tomkins, G. M. (1970), *J. Mol. Biol. 52*, 57.
Schlitz, J. R., Mayne, R. M., and Holtzer, H. (1973), *Differentiation 1*, 97.
Schubert, D., and Jacob, F. (1970), *Proc. Natl. Acad. Sci. U.S.A. 67*, 247.
Schwartz, S. A., Horio, D., and Kirsten, W. J. (1974), *Biochem. Biophys. Res. Commun. 61*, 927.
Sheehan, D. M., and Olins, D. E. (1974), *Biochim. Biophys. Acta 353*, 438.
Shih, T. Y., and Bonner, J. (1970), *J. Mol. Biol. 48*, 469.
Simpson, R. T., and Seale, R. L. (1974), *Biochemistry 13*, 4609.
Spelsberg, T. C., Hnilica, L. S., and Ansevin, A. T. (1971), *Biochim. Biophys. Acta 228*, 550.
Stein, G., Park, W., Thrall, C., Mans, R., and Stein, J. (1975), *Nature (London) 257*, 764.
Stellwagon, R. H., and Tomkins, G. M. (1971), *J. Mol. Biol. 56*, 167.
Strom, C. M., and Dorfman, A. (1976), *Proc. Natl. Acad. Sci. U.S.A. 73*, 1019.
Vogel, R., and Singer, M. F. (1975), *Proc. Natl. Acad. Sci. U.S.A. 72*, 2597.
Walther, B. T., Pictet, R. L., David, J. D., and Rutter, W. J. (1974), *J. Biol. Chem. 249*, 1953.
Wataya, Y., Negishi, K., and Hayatsu, H. (1973), *Biochemistry 12*, 3992.
Weintraub, H., Campbell, G., and Holtzer, H. (1972), *J. Mol. Biol. 70*, 337.
Yarus, M., and Berg, P. (1970), *Anal. Biochem. 35*, 450.

Reactivity of Ribosomal Sulfhydryl Groups in 30S Ribosomal Subunits of *Escherichia coli* and 30S–IF-3 Complexes†

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ABSTRACT: The reaction of 30S subunits with the SH group reagent *N*-ethylmaleimide (NEM) causes the loss of approximately 60% of their synthetic activity, but has little or no effect on the ribosomal binding of initiation factor IF-3. The ribosomal binding of this factor, on the other hand, was found to significantly influence the rate and the extent to which several 30S ribosomal proteins react with radioactively labeled NEM when the reaction kinetics of individual ribosomal proteins toward NEM were compared in 30S and 30S–IF-3 complexes. Of the nine 30S ribosomal proteins which react with NEM, proteins S1, S11, S12, and S18 were found to have lower reactivities, while proteins S4 and S21 displayed higher reactivity in the presence of IF-3. The reactivity of proteins S8, S13, and S17, on the other hand, appeared to be influenced little or not at all by the presence of the factor. These results are interpreted as supporting evidence for the premise that the binding of IF-3 results in a conformational change of the 30S subunit.

Studies aimed toward the identification of the nature of the ribosomal binding site for IF-3 have suggested that this factor binds to the 16S rRNA[1] (Gualerzi and Pon, 1973; Pon and Gualerzi, 1976) in a region probably adjacent to the subunit interface (Gualerzi et al., 1973). Cross-linking experiments have indicated that, when bound to the ribosomes, IF-3 neighbors the 3′ end of the 16S rRNA (van Duin et al., 1975) as well as ribosomal proteins S1, S11, S12, S13, S14, S18, S19, and S21 (Hawley et al., 1974; Traut et al., 1974). Although data from another laboratory yield a different pattern of cross-linking, with S7 being the major cross-linked product (van Duin et al., 1975), recent immune electron microscopy data seem to confirm the localization of IF-3 in the proximity of proteins S11, S13, and S19 (Lake and Kahan, 1975).

Several lines of evidence suggest that the binding of IF-3 to the 30S ribosomal subunit causes a conformational change of the ribosomal particle (Pon and Gualerzi, 1974; Gualerzi et al., 1975). This change in conformation may be the molecular basis for the functional activity of the initiation factor in promoting ribosomal binding of mRNA (Iwasaki et al., 1968; Revel et al., 1968; Wahba et al., 1969; Sabol et al., 1973; Noll and Noll, 1974; Jay and Kaempfer, 1975) and in preventing

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[1] Abbreviation used: NEM, *N*-ethylmaleimide; rRNA, ribosomal ribonucleic acid; mRNA, messenger RNA; Tris, tris(hydroxymethyl)-aminomethane; poly(U), poly(uridylic acid).

ribosomal subunit association (Kaempfer, 1972; Noll and Noll, 1972; Sabol et al., 1973; Godefroy-Colburn et al., 1975; Gottlieb et al., 1975). Since circular dichroism studies have suggested that the binding of IF-3 alters the secondary structure of some 30S ribosomal proteins without appreciably affecting the rRNA secondary structure (Gualerzi et al., 1975), it appeared possible that the binding of IF-3 might also influence the reactivity of some sulfhydryl groups toward *N*-ethylmaleimide either by altering their accessibility to the reagent or by causing a modification of the microenvironment in which the SH groups are situated. Such a finding would then prove useful in identifying some of the ribosomal proteins which are affected by IF-3 binding and may, in turn, shed some light on the molecular mechanism by which IF-3 exercises its physiological action at the ribosomal level.

Materials and Methods

Buffers. Buffer A was composed of: 10 mM Tris-HCl (pH 7.7), 10 mM magnesium acetate, 60 mM NH_4Cl, 6 mM 2-mercaptoethanol. Buffer B was: 10 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, 100 mM NH_4Cl. Ribosomal subunits (30S) were prepared from 1 M NH_4Cl washed 70S ribosomes of *Escherichia coli* MRE600 and initiation factor IF-3 was purified to electrophoretic homogeneity from the 1 M NH_4Cl ribosomal wash as previously described (Gualerzi et al., 1971).

The standard poly(U)-dependent polyphenylalanine synthesis was measured as described (Pon and Gualerzi, 1976).

Before the reaction with NEM, the SH groups of the 30S ribosomal subunit were reduced by incubating the ribosomes (heat-reactivated in buffer A at 50 °C for 5 min) at 37 °C for 4 h in the presence of 2-mercaptoethanol (1% v/v). This treatment was necessary in order to minimize the variation between the reactivity of the ribosomal proteins from one reaction to the next and from one preparation of ribosomes to the next. In addition, this pretreatment was found to result in an overall increase (approximately twofold) of the reactivity of the 30S particle. This is accounted for by an enhanced reactivity of all the 30S ribosomal proteins; in particular, proteins S12 and S13, which are otherwise only slightly labeled, show a comparatively greater enhancement after the reductive incubation (cf. Figure 2) enabling a more meaningful comparison of their reactivity in the presence and absence of the factor. Following the reduction with 2-mercaptoethanol, the ribosomes were exhaustively dialyzed at 4 °C against buffer B under a stream of N_2.

The ribosomes were divided into two equal portions and incubated for 10 min at 37 °C in the presence and in the absence of a 1:1 molar input ratio of IF-3. Following this incubation, which resulted in the binding of approximately 0.7 mol of IF-3 per mol of 30S subunits, the ribosomes were precipitated by addition of 0.7 volume of ethanol, and the pellets were dissolved in buffer B to approximately 6 mg/ml and dialyzed 2 h at 4 °C against the same buffer. After this dialysis, the 30S ribosomal subunits and the 30S–IF-3 complexes were incubated with either 1 or 2 mM [^{14}C]-*N*-ethylmaleimide (4 mCi/mmol, New England Nuclear) or, when specified, unlabeled *N*-ethylmaleimide (Sigma). The reaction was allowed to proceed in an ice bath for 1 to 18 h and stopped by addition of dithiothreitol to a final concentration of 5 mM. The reacted ribosomes were then loaded onto a 1.6 × 30 cm Sephadex G-50 column. The 30S or 30S–IF-3 complexes were recovered in the void volume and the ribosomal proteins were extracted by the standard acetic acid–magnesium procedure (Hardy et al., 1969). After lyophilization, the proteins were dissolved in 6 M urea and the amount of proteins recovered in each sample (Lowry et al., 1951) and the specific radioactivity were determined. NEM-labeled proteins (200–400 μg) were applied to each gel after addition of 30S carrier protein (400–200 μg). The 30S ribosomal proteins were separated by two-dimensional electrophoresis (Kaltschmidt and Wittmann, 1970), using second-dimension plates with the size of 10 × 10 × 0.2 cm. In addition, *N,N'*-diallyltartardiamide (2%) was used in place of bisacrylamide as cross-linker and the electrophoreses were run at 4 °C. Following staining and destaining, the slabs were thoroughly rinsed for 4 days in deionized water and the individual spots cut out, incubated for 48 h at 30 °C with 1 ml of H_5IO_6, and counted after addition of 10 ml of toluene–2,5-diphenyloxazole–Triton X-100 (Anker, 1970). Alternatively, in the case of double-labeling experiments, the stained spots were cut out and dried and their radioactivity was determined after combustion in a Tri-Carb sample oxidizer. The radioactivity recovered from the gels ranged from 60 to 70%.

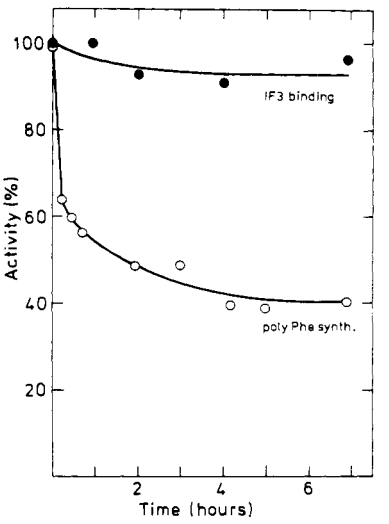

FIGURE 1: Effect of NEM reaction on the activity of the 30S ribosomal subunits in poly(U)-dependent polyphenylalanine synthesis and binding of IF-3. The 30S ribosomal subunits were reduced by incubation with 2-mercaptoethanol, dialyzed, and incubated with 1 mM NEM as described in Materials and Methods for the indicated periods of time. After stopping the reaction by addition of 5 mM dithiothreitol, the ribosomes were dialyzed against buffer A and their activity in poly(U)-dependent polyphenylalanine synthesis and binding of radioactive IF-3 was determined (Pon and Gualerzi, 1976). One hundred percent activity represented 426 666 cpm (406 pmol) of [^{14}C]phenylalanine incorporated and 13 663 cpm (45 pmol) of [^{14}C]labeled IF-3 bound per 1 A_{260} unit of 30S ribosomes.

Results

The experiment of Figure 1 was designed to determine the effect of an NEM treatment analogous to that used in the labeling experiments on the functional activity of the 30S ribosomal subunits in poly(U)-dependent polyphenylalanine synthesis and binding of IF-3. It can be seen from the figure that, similar to that reported by other authors (Traut and Haenni, 1967; Moore, 1973), treatment of 30S subunits with NEM results in a rapid drop in the synthetic activity of the 30S ribosomal subunits which lose approximately 40% of their activity within 30 min of incubation at 2 °C with 1 mM NEM. Following this initial loss, the inactivation proceeds at a slower rate to 60% inactivation after 4 h, after which the activity remains constant at this level for up to 7 h or longer. The basis for this inactivation has been attributed to the early modification of protein S18 (Moore, 1971). It is noteworthy that our

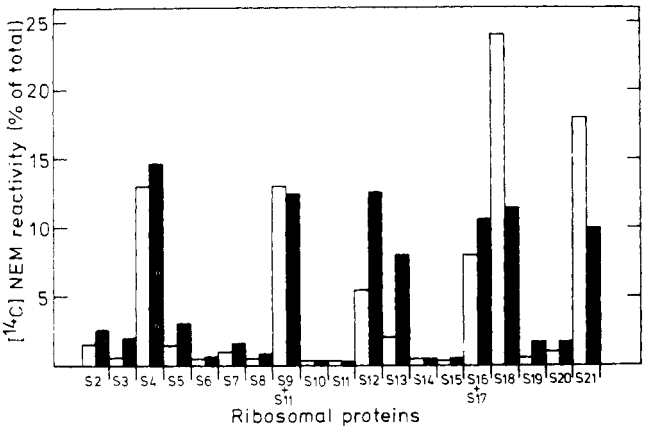

FIGURE 2: Effect of preincubation of the 30S subunits with 2-mercaptoethanol on the extent of reaction of individual ribosomal proteins with [^{14}C]NEM. The 30S ribosomes preincubated with (■) or without (□) 2-mercaptoethanol (cf. Materials and Methods) were incubated for 18 h in an ice bath with 1 mM [^{14}C]NEM. The extent of labeling for the samples reduced and nonreduced with 2-mercaptoethanol was 68 000 and 30 100 cpm, respectively, in 400 μg of protein. Recovery of radioactivity was 60% for both samples. The radioactivity associated with each protein was determined as described in Materials and Methods. It should be noted that the bulk of protein S11 coelectrophoreses with protein S9 which does not contain half-cystine residues, and that modified protein S17 partially coelectrophoreses with protein S16 which also does not contain half-cystine (Moore, 1975). Furthermore, the possibility exists that the NEM radioactivity associated with protein S5 (which does not contain half-cystine) is due to modified protein S8. Finally, protein S12 constantly appeared in our gels as double, triple, or occasionally quadruple spots. The extent of S12 reaction was calculated from the sum of radioactivity in all these spots. Note that in the figure the radioactivity is presented as percent of total label associated with each protein and not as absolute values.

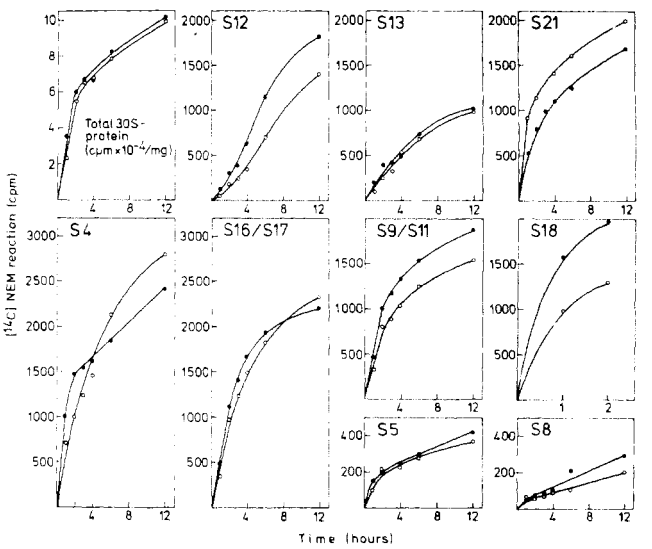

FIGURE 3: Effect of ribosome-bound IF-3 on the reaction kinetics of individual ribosomal proteins with [^{14}C]NEM. The reaction with 1 mM [^{14}C]NEM was carried out as described (see Materials and Methods) and stopped by the addition of dithiothreitol at the indicated times. Each point represents the average of four different electrophoretic slabs: (●) 30S; (○) 30S–IF-3 complexes.

labeling kinetics (cf. Figure 3) also indicate that protein S18 has the fastest rate of reaction. Contrary to the synthetic activity, the IF-3 binding capacity of the 30S ribosomal subunits is only slightly affected by NEM treatment (Figure 1). This indicates that the SH groups of the 30S ribosomal proteins which react with NEM are not involved either directly or indirectly with the binding of the factor. It is known that of the 21 30S ribosomal proteins, only proteins S1, S2, S4, S8, S11, S12, S13, S14, S17, S18, and S21 contain half-cystine groups (Kahan et al., 1974; Moore, 1975) and, of these proteins, only S11, S12, and S17 contain more than one cysteine residue. As seen in Figure 2 and in agreement with other reports (Moore, 1971; Gavrilova and Spirin, 1974), not all the above proteins become labeled upon reaction of the 30S subunits with [^{14}C]NEM and the extent of blocking the SH groups does not reflect the known content of cysteine residues of each protein, indicating that not all the 30S SH groups are equally accessible to the reagent. It is noteworthy, on the other hand, that, under our labeling conditions, none of the proteins lacking SH groups becomes labeled to any significant extent (cf. legend to Figure 2).

The reaction kinetics of ribosomal proteins with [^{14}C]NEM in 30S subunits and 30S–IF-3 complexes are shown in Figure 3. It can be seen from this figure that the amount of [^{14}C]NEM reacting with the 30S ribosomes increases rapidly within the first 2 to 3 h of incubation and then continues to increase but at a slower rate without reaching a plateau even after 12 h of incubation. Figure 3 also shows that the extent of labeling in the presence and absence of IF-3 is fairly similar, although the 30S–IF-3 complex seems to display a slightly reduced reactivity in the early period of incubation. This difference becomes more pronounced if one considers that approximately 7% of the total radioactivity associated with the 30S–IF-3 complex is accounted for by the labeling of the factor. On the other hand, when the reaction kinetics for individual ribosomal proteins are examined (Figure 3), it can be seen that different ribosomal proteins react with NEM at different rates. Thus, proteins S4, S11, S17, S18, and S21 appear to be labeled at a fast rate, while others (S8, S12, and S13) are labeled at a slower rate. From the same figures, it can be seen that, in the presence of IF-3, several proteins (S11, S12, and S18) appear to have a reduced reactivity, while the reactivity of other proteins (S5, S8, S13, and S17) seems to be affected very slightly or not at all by the presence of the factor. The reactivity of protein S4, on the other hand, was lower in the presence of IF-3 for up to 4–5 h of incubation but became much higher after longer periods of incubation (cf. also Table I). Finally, protein S21 definitely displayed a higher reactivity when the factor was bound to the ribosomes.

The validity of the conclusions which can be drawn from the above experiments depends upon the assumption that no major difference exists in the recovery of individual ribosomal proteins from the same electrophoretic slab. In order to ascertain this point, the experiment of Figure 4 was performed. In this experiment the extent of labeling of individual ribosomal proteins with [^{14}C]NEM in the presence and absence of IF-3 was compared by reference to an internal standard (the same ribosomal protein labeled with tritium) rather than by direct comparison of the radioactivity in the various ribosomal proteins derived from 30S and 30S–IF-3 complexes as in the experiment of Figure 3. The results of Figure 4, where the early reaction kinetics (1–4 h) of ribosomal proteins are presented, are clearly in agreement with those obtained in Figure 3, thus confirming that proteins S9–S11, S12, and S18 react at a slower rate and protein S21 at a faster rate in the presence of IF-3, while the reactivity of the other proteins appears to be almost unaffected by the presence of the factor. Concerning protein S4, this experiment confirmed the later increase in reactivity of this protein in the presence of IF-3, but could not confirm the initial IF-3-dependent protection which could thus have been due to an artifact.

Since the unequivocal resolution and identification of protein

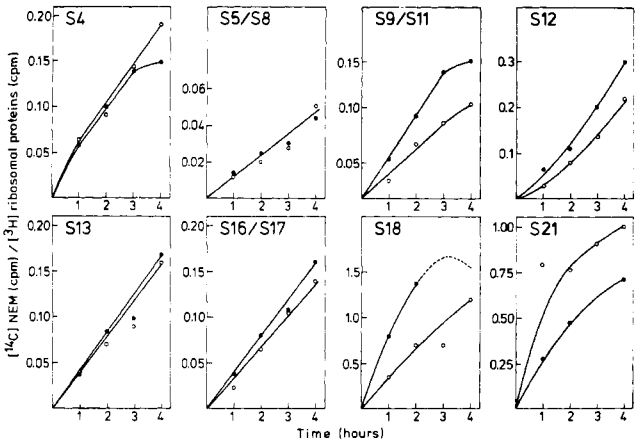

FIGURE 4: Effect of ribosome-bound IF-3 on the initial rate of reaction of ribosomal proteins with [^{14}C]NEM. The experiment was identical with that of Figure 3 with the exception that prior to the electrophoresis, equal amounts (150 000 cpm) of 30S ribosomal proteins labeled by methylation with [^{3}H]formaldehyde (Rice and Means, 1971) were added to each sample containing the [^{14}C]NEM labeled ribosomal proteins. After electrophoresis, ^{3}H and ^{14}C radioactivities associated with each spot were determined following combustion in a Tri-Carb sample oxidizer. The results are plotted as the ratio of the ^{14}C/^{3}H radioactivity vs. time of reaction: (●) 30S; (○) 30S-IF-3 complexes.

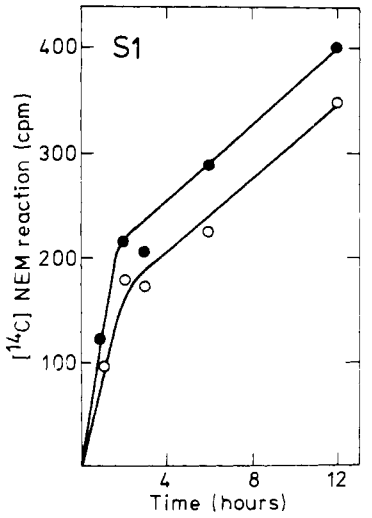

FIGURE 5: Effect of ribosome-bound IF-3 on the [^{14}C]NEM reactivity of protein S1. The experiment was analogous to that of Figure 4 with the exception that S1 was resolved from the other ribosomal proteins by sodium dodecyl sulfate disc gel electrophoresis (Weber and Osborn, 1969). One hundred micrograms of ribosomal protein was applied to each gel. The points represent the average of four electrophoretic runs: (●) 30S; (○) 30S-IF-3 complexes.

S1 in the standard two-dimensional electrophoresis are difficult, the extent of labeling of this protein by NEM was determined in a separate experiment following sodium dodecyl sulfate gel electrophoresis. As seen in the experiment of Figure 5 which shows the kinetics of labeling of this protein, the NEM reactivity of S1, like that of S11, S12, and S18, is also decreased in the presence of IF-3.

In the previous experiments (cf. Figures 3 and 5), the reactivity of the 30S ribosomal proteins toward NEM was followed for a total incubation period of 12 h, but, since it is evident that after 12 h of incubation no plateau is reached for most of the ribosomal proteins, in the experiment presented in Table I, the incubation with [^{14}C]NEM was continued up to 18 h. The figures of the table represent the average of four independent electrophoretic runs (three slabs each) and indicate the percent distribution of total NEM radioactivity among the individual ribosomal proteins which react with NEM in the 30S or 30S-IF-3 complex after this longer period of incubation. It should be noted that, after 18 h incubation with [^{14}C]NEM, the total radioactivity associated with 30S and 30S-IF-3 complexes is virtually the same. Statistical treatment of the data indicates that after 18 h of reaction the differences in the [^{14}C]NEM labeling in the presence and absence of IF-3 are highly significant (99% probability) for proteins S4, S11, and S21 and significant (95% probability) for protein S12. Of the above proteins, S11 and S12 appear to be protected by IF-3, while S4 and S21 appear to have reacted to a greater extent in the presence of IF-3. These data are in complete agreement with those shown in Figures 3 and 4. Protein S18, on the other hand, did not show a statistically significant difference in reactivity after 18 h of incubation in the presence or absence of the factor, although it was strongly protected by the presence of IF-3 during the first 2-3 h of incubation (cf. Figures 3 and 4). This can be explained by the fact that the recovery of S18 in the gels drops as the reaction with NEM proceeds so that the data for this protein become unreliable for periods of incubation longer than 2-3 h and the figures concerning the extent of S18 reaction after 18 h which appear in Table I are certainly underestimated. In spite of this complication, however, the calculation of the distribution of NEM labeling among the other ribosomal proteins is not affected since, after approximately 3-4 h of reaction, similar plateaus of radioactivity are reached for S18 labeled in the presence and absence of IF-3.

TABLE I: Effect of IF-3 on the Distribution of [^{14}C]NEM Radioactivity in Different Ribosomal Proteins (18 Hours of Incubation).

Ribosomal Proteins	Distribution of [^{14}C]NEM Radioactivity (%)[a]		
	30S-IF-3	30 S	Δ
S4	21.59 ± 0.43	19.43 (± 0.61)	(+2.16)[c]
S9-11	10.82 ± 0.63	14.20 (± 0.69)	(−3.38)[c]
S12	11.98 ± 0.74	14.53 (± 0.53)	(−2.55)[b]
S13	6.21 ± 0.44	6.77 (± 0.50)	(−0.56)
S16-17	16.99 ± 1.27	14.80 (± 0.77)	(+2.19)
S18	16.43 ± 1.03	16.73 (± 0.92)	(−0.30)
S21	15.98 ± 0.45	13.54 (± 0.60)	(+2.44)[c]

[a] The total [^{14}C]NEM radioactivity associated with the ribosomal proteins listed in the table represents over 90% of the total radioactivity penetrating the gels (after subtraction of the background) and it is taken as 100% for the calculation of the distribution of [^{14}C]NEM radioactivity among the various ribosomal proteins. The values in the table represent the average percentage (± standard error) derived from four independent electrophoretic runs (total of 12 gel slabs) with samples originating from two independent labeling experiments. [b] Significant difference (P = 0.05). [c] Highly significant difference (P = 0.01).

The experiment of Figure 6 was performed to determine whether any exchange of ribosome-bound IF-3 takes place during the 18-h period of incubation. The results of the figure clearly indicate that nearly complete equilibration of the bound radioactive IF-3 with the unbound unlabeled IF-3 takes place during the course of the incubation both in the presence and absence of 1 mM NEM.

Discussion

The finding that some ribosomal proteins of the 30S subunit display altered reaction kinetics with NEM when IF-3 is

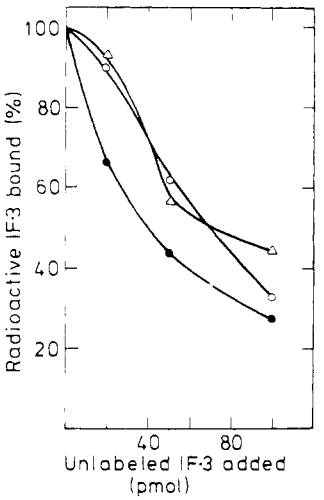

FIGURE 6: Exchange of radioactive ribosome-bound IF-3 with free unlabeled IF-3 during NEM reaction. The experiment was performed by incubating 30S-[^{14}C]IF-3 complexes in the presence of the indicated amounts of nonradioactive IF-3 in the presence (Δ) and in the absence (O) of 1 mM NEM. After 18 h of incubation in an ice bath, the amount of radioactive IF-3 remaining ribosome-bound was determined by sucrose gradient centrifugation as previously described (Pon and Gualerzi, 1976). The curve (●) represents the amount of radioactivity theoretically expected to be associated with the 30S ribosomes if complete equilibration of the radioactive and nonradioactive IF-3 had taken place during the incubation.

ribosome bound poses the problem of analyzing the possible reasons for the modified reactivities. Although the factors which control the reactivity of the ribosomal proteins toward different chemical reagents are still obscure, changes in reactivity of individual ribosomal proteins toward NEM as well as other chemical reagents are normally taken as an indication that a conformational change of the ribosome or a shielding of the reactive site has occurred (Huang and Cantor, 1972; Chang, 1973; Ginzburg et al., 1973; Littman et al., 1974; Ginzburg and Zamir, 1975).

In the present paper it was shown that the binding of initiation factor IF-3 to the 30S ribosomal subunits produces an enhancement of the reactivity toward NEM of protein S21. This is most likely due to an increased exposure of the SH group of this protein which could result from either a local conformational change or a more general structural reorganization involving more than one protein of the 30S ribosome. The latter possibility seems to be favored by the fact that a conformational modification of some 30S ribosomal proteins upon binding of IF-3 has been implicated by circular dichroism studies (Gualerzi et al., 1975). The increase in the reactivity of S21 is paralleled by a substantially decreased reactivity of the SH groups of proteins S18, S11, and S12. Proteins S18 and S21 can be cross-linked with SH bifunctional reagents (Chang and Flaks, 1972; Lutter et al., 1972) and, therefore, their SH groups must be very close to each other on the ribosome. Proteins S11 and S12 are also likely to be adjacent to S18 and S21 since a cross-linked product containing proteins S11, S18, S21, and probably S12 has been found to be induced by tetranitromethane (Shih and Craven, 1973).

If the SH group of a protein were completely shielded by IF-3, one would expect total protection from NEM reaction only provided that (a) all 30S subunits bear an IF-3 molecule bound to identical ribosomal sites and (b) no exchange of IF-3 takes place during the incubation reaction. In light of the fact that the 30S–IF3 complexes obtained for the present studies contained only 0.7 equiv of IF-3 per 30S subunit and that nearly complete exchange of IF-3 takes place during the 18-h period of incubation, one can consider the extent of the decrease in reactivity of proteins S11, S12, and S18 (40–60% for the shortest periods of incubation) as compatible at least from the quantitative point of view, with the possibility of a direct shielding of these proteins by IF-3.

It should be noted, however, that the differential effect of IF-3 on the reactivity of two adjacent SH groups seems to suggest that the IF-3 induced protection of protein S18 (and probably of proteins S11 and S12) results from a conformationally induced shielding rather than from a direct "physical" shielding of these SH groups by the ribosome-bound factor.

In addition to the effect on the reactivity of proteins S11, S12, S18, and S21, IF-3 was shown to affect the reaction of NEM with proteins S1 and S4. Concerning protein S1, the presence of IF-3 on the ribosome induces a moderate decrease in reactivity which could hardly be explained by a direct shielding also in consideration of the fact that the isolated 30S ribosomal subunit contains only 0.2–0.3 equiv of S1 and two to three times more IF-3. The effect of IF-3 on protein S4 is more complex. During the earlier reaction times, IF-3 seems to either protect (cf. Figure 3) or to have no effect at all (cf. Figure 4), while, from 4 to 5 h of incubation on, the amount of NEM labeling becomes higher in the presence than in the absence of IF-3. This seems to be due to the fact that, while the reaction in the absence of IF-3 tends to reach a plateau, in the presence of IF-3, the reaction continues to proceed at a relatively high rate. The simplest explanation for this phenomenon could be that the leveling off of the reaction in the absence of IF-3 is a delayed and secondary consequence of the difference in reaction with or without the factor of some other ribosomal protein. Alternatively, some other chemical group in addition to the SH group may react with NEM as the incubation is prolonged and this nonspecific reaction may be stimulated by IF-3.

Regardless of the molecular mechanism by which all the above changes in reactivity are brought about, the data of this paper clearly indicate that the ribosomal binding of IF-3 somewhat perturbs the secondary structure of proteins S1, S4, S11, S12, S18, and S21. It is interesting to note that, in addition to being found in cross-linked products with IF-3 (cf. introductory section), most of these proteins (S1, S12, S18, and S21) have been implicated in the binding of mRNA to the 30S subunits (Held et al., 1974; van Duin and van Knippenberg, 1974; Fiser et al., 1975) and that proteins S11 and S12 have been indicated as ribosomal subunit interface proteins (Morrison et al., 1973). This suggests that the ultimate mechanism by which IF-3 exercises its physiological function in mRNA binding and in preventing ribosomal subunit association might be to induce a conformational rearrangement of the 30S particle involving these proteins.

References

Anker, H. S. (1970), *FEBS Lett. 7*, 293.
Chang, F. N. (1973), *J. Mol. Biol. 78*, 563–568.
Chang, F. N., and Flaks, J. G. (1972), *J. Mol. Biol. 68*, 177–180.
Fiser, I., Scheit, K. H., Stöffler, G., and Kuechler, E. (1975), *FEBS Lett. 56*, 226–229.
Gavrilova, L. P., and Spirin, A. S. (1974), *FEBS Lett. 39*, 13–16.
Ginzburg, I., Miskin, R., and Zamir, A. (1973), *J. Mol. Biol. 79*, 481–494.
Ginzburg, I., and Zamir, A. (1975), *J. Mol. Biol. 93*, 465–

476.
Godefroy-Colburn, T., Wolfe, A. D., Dondon, J., and Grunberg-Manago, M. (1975), *J. Mol. Biol. 94,* 461-478.
Gottlieb, M., Davis, B. D., and Thompson, R. C. (1975), *Proc. Natl. Acad. Sci. U.S.A. 72,* 4238-4242.
Gualerzi, C., Grandolfo, M., Paradies, H. H., and Pon, C. (1975), *J. Mol. Biol. 95,* 569-573.
Gualerzi, C., and Pon, C. (1973), *Biochem. Biophys. Res. Commun. 52,* 792-799.
Gualerzi, C., Pon, C., and Kaji, A. (1971), *Biochem. Biophys. Res. Commun. 45,* 1312-1319.
Gualerzi, C., Wabl, M. R., and Pon, C. (1973), *FEBS Lett. 35,* 313-316.
Hardy, S. J. S., Kurland, C. G., Voynow, P., and Mora, G. (1969), *Biochemistry 8,* 2897-2905.
Hawley, D. A., Slobin, L. I., and Wahba, A. J. (1974), *Biochem. Biophys. Res. Commun. 61,* 544-550.
Held, W. A., Gette, W. R., and Nomura, M. (1974), *Biochemistry 13,* 2115-2122.
Huang, K.-H., and Cantor, C. R. (1972), *J. Mol. Biol. 67,* 265-275.
Iwasaki, K., Sabol, S., Wahba, A. J., and Ochoa, S. (1968), *Arch. Biochem. Biophys. 125,* 542-547.
Jay, G., and Kaempfer, R. (1975), *J. Biol. Chem. 250,* 5742-5748.
Kaempfer, R. (1972), *J. Mol. Biol. 71,* 583-598.
Kahan, L., Held, W. A., and Nomura, M. (1974), *J. Mol. Biol. 88,* 797-808.
Kaltschmidt, E., and Wittmann, H. G. (1970), *Anal. Biochem. 36,* 401-412.
Lake, J. A., and Kahan, L. (1975), *J. Mol. Biol. 99,* 631-644.
Littman, D. J., Lee, C. C., and Cantor, C. R. (1974), *FEBS Lett. 47,* 268-271.
Lowry, O. H., Rosebrough, N. J., Farr, A. J., Randall, R. J. (1951), *J. Biol. Chem. 193,* 265-275.
Lutter, L. C., Zeichhardt, H., Kurland, C. G., and Stöffler, G. (1972), *Mol. Gen. Genet. 119,* 357-366.
Moore, P. B. (1971), *J. Mol. Biol. 60,* 169-184.
Moore, P. B. (1973), *J. Mol. Biol. 79,* 615-632.
Moore, P. B. (1975), *J. Mol. Biol. 98,* 439-444.
Morrison, C. A., Garrett, R. A., Zeichhardt, H., and Stöffler, G. (1973), *Mol. Gen. Genet. 127,* 359-368.
Noll, M., and Noll, H. (1972), *Nature (London), New Biol. 238,* 225-228.
Noll, M., and Noll, H. (1974), *J. Mol. Biol. 89,* 477-494.
Pon, C. L., and Gualerzi, C. (1974), *Proc. Natl. Acad. Sci. U.S.A. 71,* 4950-4954.
Pon, C. L., and Gualerzi, C. (1976), *Biochemistry 15,* 804-811.
Revel, M., Lelong, J. C., Brawerman, G., and Gros, F. (1968), *Nature (London) 219,* 1016-1021.
Rice, R. H., and Means, G. E. (1971), *J. Biol. Chem. 246,* 831-832.
Sabol, S., Meier, D., and Ochoa, S. (1973), *Eur. J. Biochem. 33,* 332-340.
Sabol, S., Sillero, M. A. G., Iwasaki, K., and Ochoa, S. (1970), *Nature (London) 228,* 1269-1273.
Shih, C. T., and Craven, G. R. (1973), *J. Mol. Biol. 78,* 651-663.
Traut, R. R., and Haenni, A. L. (1967), *Eur. J. Biochem. 2,* 64-73.
Traut, R. R., Heimark, R., Sun, T. T., Hershey, J. W. B., and Bollen, A. (1974), *Ribosomes Meet. 1973,* 271-308.
van Duin, J., Kurland, C. G., Dondon, J., and Grunberg-Manago, M. (1975), *FEBS Lett. 59,* 287-290.
van Duin, J., and van Knippenberg, P. M. (1974), *J. Mol. Biol. 84,* 185-196.
Wahba, A. J., Iwasaki, K., Miller, M. J., Sabol, S., Sillero, M. A. G., and Vasquez, C. (1969), *Cold Spring Harbor Symp. Quant. Biol. 34,* 291-299.
Weber, K., and Osborn, M. (1969), *J. Biol. Chem. 244,* 4406-4412.