Topography of *Escherichia coli* Ribosomal Proteins THE ORDER OF REACTIVITY OF THIOL GROUPS*

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1. 30S and 50S ribosomal subunits of *Escherichia coli* were treated with *N*-[2,3-¹⁴C]-ethylmaleimide and iodo[¹⁴C]acetamide. 2. The proteins in the native subunits which reacted with the reagents were S1, ‡ S2, S12, S13, S18, S21, L2, L5, L6, L10, L11, L15, L17, L20, L26+28 and L27. 3. Several proteins, such as S1, S12, S14, S18, L2, L6, L10, L11 and either L26 or 28, had thiol groups in an oxidized form and reacted to a greater extent after reduction with β -mercaptoethanol or dithiothreitol. 4. The total number of thiol groups in 30S and 50S subunits was determined as 16–17 and 26–27 respectively. The total number of thiol groups in each ribosomal protein was also determined. 5. The reaction of 30S and 50S subunits with iodoacetamide under several different conditions established the order of reactivity of thiol groups.

The effect of thiol inhibitors on the structure and function of ribosomes has been studied for some years, since they play an important role in the function of many proteins and are present in very low amount in ribosomal proteins (Spahr, 1962). The earliest studies on the effects of thiol reagents established the role of thiol groups in the process of dissociation of 100S particles (Wang & Matheson, 1966) and 70S ribosomes (Tamaoki & Miyazawa, 1967; Miyazawa & Tamaoki, 1967). Treatment of ribosomes with low concentrations of *p*-chloromercuribenzoate, *N*-ethylmaleimide or dithiobis-(2-nitrobenzoic acid) (Heintz *et al.*, 1966; Traut & Haenni, 1967; McAllister & Schweet, 1968; Retsema & Conway, 1969) led to a 40–80% loss of ribosomal activity in amino acid incorporation in poly(U)-directed synthesis of poly-phenylalanine.

A chemical study of the distribution and reactivity of thiol groups of *Escherichia coli* ribosomal proteins was first carried out by Moore (1971). The reagent used was *N*-ethylmaleimide and the proteins of the 70S ribosomes which reacted were tentatively identified. The uptake of *N*-ethylmaleimide by protein S18 induced loss of activity of the ribosomes in poly(U)-directed polyphenylalanine synthesis, suggesting that this protein might be the site of inhibition. In contrast,

treatment of ribosomes with iodoacetamide did not result in loss of function. The fact that iodoacetamide-treated ribosomes were insensitive to *N*-ethylmaleimide inactivation once protein S18 had been fully modified by iodoacetamide reinforced the view that protein S18 was the *N*-ethylmaleimide-sensitive protein in the ribosomes.

In a subsequent study dithiobis-(2-nitrobenzoic acid) was used (Acharya & Moore, 1973) to titrate thiol groups, and the total number of thiol groups in each subunit was established under denaturing conditions. In native 70S ribosomes the thiol groups were classified into three groups: fast-reacting, slow-reacting and buried. It was also established that a fast-reacting thiol in protein S1 could be protected from the reagent in the 30S subunit by addition of poly(U). The total number of thiol groups in 30S and 50S ribosomes was also determined by analysis of ribosomal proteins from cells that had been grown in a medium in which half-cystine was labelled with ³⁵S. Iodoacetic acid was used by Slobin (1971) to determine the total number of thiol groups in 30S and 50S proteins, as well as the number of thiol groups that were available for modification in native 30S and 50S subunits. Pongs *et al.* (1973) labelled 70S ribosomes with iodoacetamide and found that proteins S18, S21 and S13 were the primary targets of this reaction. Treatment of subunits with radioactive *p*-chloromercuribenzoate resulted in labelling of 11–12 proteins in the 30S subunit and 8–9 proteins in the 50S subunit (H. Cronenberger & V. A. Erdmann, personal communication).

In the present paper we report on the results of the treatment of *E. coli* ribosomal subunits with iodo[¹⁴C]acetamide and *N*-[¹⁴C]ethylmaleimide. The

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‡ The nomenclature established for the 30S and 50S subunits by Wittmann *et al.* (1971) and by Kaltschmidt & Wittmann (1970a) is used throughout.

treated ribosomes were either in the native or denatured state with or without reduction with β -mercaptoethanol or dithiothreitol. Ribosomal proteins were fractionated by the two-dimensional polyacrylamide-gel-electrophoresis procedure of Kalkschmidt & Wittmann (1970b), and the absolute radioactivity of ^{14}C for each protein was measured after appropriate treatment. The data were used to calculate the number of thiol groups available for the reagents on the surface of the native 30S and 50S subunits as well as the total number of thiol groups of each protein. An attempt was also made to distinguish between the reactivity of thiol groups of different ribosomal proteins when the reaction with iodoacetamide was allowed to proceed at 25° and 37°C.

Materials and Methods

Cells and ribosomes

Ribosomal subunits from *E. coli* A19, derived from strain K12, RNAase I⁻ (Gesteland, 1966), were prepared by centrifugation through linear 10–30% (w/v) sucrose gradients in 1M-magnesium acetate in a Spinco SW27 rotor at 97000g for 15 h at 2°C. 30S and 50S subunits were recovered by ethanol precipitation (Staehelin *et al.*, 1969) after the solution had been adjusted to a concentration of 0.1 M-magnesium acetate and 70% (v/v) ethanol and centrifuged at 10000g for 25 min at 2°C. The pelleted ribosomes were dissolved in 1 ml of TMA buffer (10mM-Tris-HCl–1 mM-MgCl₂–50mM-NH₄Cl), pH 8.0. The same buffer, unless stated otherwise, was used as a medium for the reaction. *E. coli* A19 cells (Gesteland, 1966) labelled with ^3H were grown, harvested and the ribosomes isolated by a method to be described in detail elsewhere (B. B. Ulbrich & K. H. Nierhaus, unpublished work). In summary, the cells were grown in minimal medium, and 0.5 ml of this culture containing 2×10^8 cells/ml was used to inoculate 80 ml of minimal medium containing 1 mCi of ^3H -labelled amino acid mixture (New England Nuclear Corp., Frankfurt, Germany) instead of the 0.05% casamino acids in the original medium. After 5–6 generations the culture was diluted with 80 ml of a second medium containing the same ingredients as the minimal medium and in addition 0.005% casamino acids and 0.5% bactotryptone broth (1.3% bactotryptone, 120mM-NaCl and 0.5% glucose). After three generations 250 ml of standard rich medium was added and the cells were harvested in mid-exponential phase (2.5×10^8 cells/ml). The radioactivity of the ribosomes isolated from such a preparation was between 25000 and 100000 c.p.m./ $E_{260}^{1\text{ml}}$ of 70S ribosomes.

Reagents

Iodo[^{14}C]acetamide (sp. radioactivity 52 mCi/mmol) and *N*-[2,3- ^{14}C]ethylmaleimide (sp. radio-

activity 3 mCi/mmol) were obtained from Amersham-Buchler, Braunschweig, Germany. The Insta-Gel used for counting the radioactivity of the samples was from Packard Instrument Co., Geneva, Switzerland.

Reaction with iodoacetamide

Different reaction times and molar excesses of the reagent were tested; the reaction was found to be complete after 24 h at 4°C with a tenfold molar excess of reagent. To establish the availability and the state of the thiol groups (reduced or oxidized) on the surface of the native particles as well as their total number, three types of samples were prepared, each containing 1 nmol of 30S or 50S subunits (an $E_{260}^{1\text{ml}}$ of 15 was taken to correspond to 1 mg of subunit). The first contained native subunits in which the thiol groups were reduced. The second contained native subunits, reduced with 1% β -mercaptoethanol, which had subsequently been removed by dialysis under N₂ against TMA buffer, pH 8.0. The rRNA of both types of samples was precipitated, after alkylation, with 67% (v/v) acetic acid, in the presence of high Mg²⁺ concentration as described by Hardy *et al.* (1969), and the proteins were subsequently dialysed into 2% (v/v) acetic acid at 4°C. The third type of sample consisted of ribosomal proteins which had been extracted from 30S and 50S subunits with 67% acetic acid before the reaction. After dialysis into 2% acetic acid and freeze-drying, the proteins in the third sample were dissolved in TMA buffer, pH 8.0, containing 6M-guanidine and 1% (v/v) β -mercaptoethanol, incubated at 37°C for 4 h and dialysed into the same buffer without β -mercaptoethanol overnight at 4°C.

Reduction of any native disulphides should yield two new thiol groups. If the amount of β -mercaptoethanol was not adequate, a disulphide might be formed between a protein thiol group and the β -mercaptoethanol. Also during the dialysis to remove the β -mercaptoethanol some re-formation of disulphide might occur.

Accordingly, in another set of experiments the β -mercaptoethanol was replaced by a slightly greater than stoichiometric amount of dithiothreitol (20 mol of dithiothreitol/mol of 30S subunits and 30 mol of dithiothreitol/mol of 50S subunits). The reducing agent was not removed before alkylation, and a tenfold molar excess of iodoacetamide over the total thiol content of the sample was used. The same experiment was carried out with ribosomal proteins obtained from ^3H -labelled ribosomes; unlabelled ribosomal proteins were added before the reaction. The reaction proceeded in all cases under the conditions described above.

Fractionation of the proteins was performed by the two-dimensional polyacrylamide-gel-electrophoresis method of Kalkschmidt & Wittmann (1970b), after

addition of 2mg of unlabelled carrier ribosomal protein to each sample. The 30S and 50S nomenclature follows that of Kaltschmidt & Wittmann (1970a).

Reaction with N-ethylmaleimide

The same three types of ribosomal samples, each containing 5–6nmol of ribosomes prepared as described above in TMA buffer, pH7.0, were treated with a tenfold molar excess of *N*-ethylmaleimide for 24h at 4°C. As in the studies with iodoacetamide, a series of experiments with *N*-ethylmaleimide was carried out with dithiothreitol instead of β -mercaptoethanol as a reducing agent. Ribosomal proteins labelled with ^3H were also modified with *N*-ethylmaleimide after addition of unlabelled protein. The separation of the proteins was done in the same manner as described for iodoacetamide treatment, but without addition of unlabelled carrier ribosomal proteins to each sample.

Time-course of reaction with iodoacetamide

Samples of native ribosomes containing 1nmol of 30S particles were treated with a tenfold molar excess of reagent at 25°C for 2 or 6h, or at 37°C for 30, 60, 120 or 180min. The extraction and separation of the protein mixture was done as described above.

Radioactivity determination

The proteins, fractionated in the two-dimensional gel slabs, were stained with Amido Black, and the spots cut out from the gel and dried at 70°C for 24h. The samples were oxidized with 0.5ml of 30% (v/v) H_2O_2 at 70°C for 24h. After cooling, 5ml of Insta-Gel was added to each sample and the samples were counted in a Packard Tricarb scintillation counter for ^{14}C . The ^{14}C counts in each sample were determined in the scintillation counter with automatic quench control and the d.p.m. were calculated. The error of measurements was $\pm 10\%$ (Weber, 1972). Samples that were doubly labelled with ^3H and ^{14}C were oxidized after drying by pure O_2 in a sample oxidizer (Packard model 305). They were counted for radioactivity in the same way as described above. The counting efficiency was 45% for ^3H and 90–95% for ^{14}C .

Calculation of the 'real' numbers of copies for ribosomal proteins

Ribosomal proteins are not all present in the same amount in isolated 30S and 50S subunits (Voynow & Kurland, 1971). The number of copies varies in different ribosomes and there are some proteins that appear to be present in amounts of more than one

copy per ribosome and others in amounts of less than one copy per ribosome. In addition the apparent stoichiometry of ribosomal proteins changes after two-dimensional gel electrophoresis. This is due to a partial denaturation of the proteins during polymerization of the sample gel, which causes a variable amount of each of the proteins to remain at the origin of the slab. There is a linear relationship between the apparent number of copies and the amount remaining at the origin. Plots of the apparent number of copies versus the amount remaining at the origin for each ribosomal protein show large differences in slope, indicating that the different proteins are retained in different amounts at the origin (Weber, 1972). The graphical method of Weber (1972) was used for calculation of stoichiometry of the proteins on each gel. The ^{14}C d.p.m. for each protein were subsequently normalized by multiplication by an individual factor for each protein, which converts into ^{14}C d.p.m./mol for that particular gel. The experiments with the ribosomal proteins labelled with ^3H before the reaction with iodoacetamide or *N*-ethylmaleimide were done to avoid the problem of lack of stoichiometry. The radioactivity for each protein corresponding to the amount of thiol groups present in it was calculated as a ratio of ^{14}C to ^3H counts multiplied by the molecular weight of each protein.

Results and Discussion

Two-dimensional gel electrophoresis allows fractionation of almost all the *E. coli* ribosomal proteins. Modification of ribosomal proteins with iodoacetamide or *N*-ethylmaleimide does not change the charge of the proteins. Thus after modification the pattern obtained on the gels was the same as in control experiments without reagents. Two pairs of proteins from 50S subunits were not consistently separated, namely proteins L8 and L9 and proteins L26 and L28. The results for these proteins are presented together. In several other cases there is a partial overlapping of the spots, for proteins S15 and S16, L6 and L11, L14, L15 and L17. The data for proteins S9 and S11 are also presented together, since protein S11 gives two spots on electrophoresis, one at the position of protein S11, the other at the position of protein S9 (Wittmann *et al.*, 1971).

Reactivity of ribosomal proteins in native subunits

The results obtained with iodoacetamide and *N*-ethylmaleimide for the 30S and 50S subunits are given in Tables 1 and 2. For each protein the d.p.m./equivalent of protein is given after correction for the amount remaining at the origin and for stoichiometry, as described in the Materials and Methods section. The data for iodoacetamide and for *N*-ethylmaleimide are the means of four or three separate

Table 1. *Reactivity of 30S-subunit proteins to alkylation*

Native 30S subunits with and without prior reduction with β -mercaptoethanol and 30S-subunit proteins in 6M-guanidine hydrochloride after reduction with β -mercaptoethanol were used. The results with the reagents iodoacetamide and *N*-ethylmaleimide are expressed as d.p.m./equivalent of each protein after correction for stoichiometry as described in the Materials and Methods section.

Protein	Iodoacetamide			<i>N</i> -Ethylmaleimide		
	Native subunit	Reduced native subunit	Denatured reduced protein	Native subunit	Reduced native subunit	Denatured reduced protein
S1	330	580	2200	100	570	1350
S2	185	630	2300	119	350	1175
S3	128	200	2060	27	121	405
S4	78	190	2110	65	220	1030
S5	97	100	700	46	80	216
S6	34	83	370	34	61	95
S7	18	46	228	28	34	105
S8	13	19	2250	31	54	1245
S9	47	231	1790	88	340	1182
S10	8	34	40	5	21	48
S11	80	60	300	27	70	260
S12	1870	4600	8217	1360	2100	6500
S13	340	430	1890	132	370	1870
S14	297	244	1688	105	300	1246
S15	5	22	604	15	80	355
S16	9	38	425	44	62	295
S17	6	45	306	30	42	166
S18	1815	2420	1610	1585	1815	1770
S19	74	58	250	30	40	113
S20	32	53	225	10	37	39
S21	992	1870	4280	1450	2785	3600

experiments respectively. Deviation from the mean was in no case greater than 10%. On the basis of these data we can readily classify the reactivity of thiol groups in the two subunits as being exposed, exposed but oxidized, or buried, where these terms are defined in the following way. Exposed thiol groups are those that react completely in the native subunit under the conditions of reaction (24h at 4°C); exposed but oxidized are those that react completely in the native subunit after reduction with β -mercaptoethanol; buried are those that react only in denatured protein. As discussed below we may assume a value of 2100 d.p.m./thiol group for the iodoacetamide results and 1500 d.p.m./thiol group in *N*-ethylmaleimide-treated samples. In the native 30S subunit only three proteins are alkylated to any marked extent (Table 1): proteins S12, S18 and S21. The extent of alkylation corresponds in each of the proteins to one thiol group/protein, except for protein S21 with iodoacetamide (0.45 thiol group/protein). The same three proteins are the only proteins extensively labelled after reduction of 30S subunits. One additional thiol appears in proteins S12 and S21, although in the case of protein S21 we cannot be sure whether the residual part of the first thiol group is reacting after reduction with iodoacetamide, or whether it represents the second thiol group, whose

reaction with *N*-ethylmaleimide is well defined. On denaturation of the 30S subunit the reactivity of a second thiol group in protein S21 towards iodoacetamide increases and a total of ten buried thiol groups, one each in proteins S1, S2, S3, S4, S8, S9 (or S11), S13 and S14 and two in protein S12, is found. The thiol group in protein S3 reacts much less well with *N*-ethylmaleimide than with iodoacetamide.

In the 50S subunit there are no exposed thiol groups in the native particle (Table 2). After reduction with β -mercaptoethanol six thiol groups can be alkylated, one each in the proteins L2, L10, L11, L17, L27 and either L26 or L28. The reactivity of proteins L10, L17 and L27 to iodoacetamide is low compared with the reactivity with *N*-ethylmaleimide. There are a number of other proteins which react to some extent in reduced native 50S subunits, such as L6 and L15. A total of 20–21 buried thiol groups is found in proteins L2 (2 thiol groups), L5 (2), L6 (3), L10 (2), L11 (2), L14 (2), L15 (1), L17 (1–2), L20 (1), L26+L28 (3–4), L27 (0–1). These results are not inconsistent with a somewhat more compact structure for the 50S than for the 30S subunits, although the 50S subunit would be expected to have a smaller surface/volume ratio than the 30S subunit and thus more buried groups. Acharya & Moore (1973) found six reactive thiol groups in native 30S subunits, five reactive

Table 2. *Reactivity of 50S-subunit proteins to alkylation*

Native 50S subunits with and without prior reduction with β -mercaptoethanol and 50S-subunit proteins in 6M-guanidine hydrochloride after reduction with β -mercaptoethanol were used. The results with the reagents iodoacetamide and *N*-ethylmaleimide are expressed as d.p.m./equivalent of each protein after correction for stoichiometry as described in the Materials and Methods section.

Protein	Iodoacetamide			<i>N</i> -Ethylmaleimide		
	Native subunit	Reduced native subunit	Denatured reduced protein	Native subunit	Reduced native subunit	Denatured reduced protein
L1	22	60	680	9	42	105
L2	420	1480	8660	120	1250	5960
L3	30	120	468	0	75	144
L4	28	106	260	0	95	120
L5	80	320	4400	0	345	3000
L6	92	660	5700	30	480	3736
L7	0	0	28	0	16	10
L8+9	20	80	10	0	78	380
L10	60	264	5415	0	1030	3725
L11	126	920	5760	0	1025	3730
L12	0	10	82	0	33	72
L13	20	74	450	0	96	626
L14	0	84	3500	0	115	2900
L15	66	240	1750	0	480	1350
L16	36	90	640	0	88	550
L17	188	285	3537	24	826	2885
L18	0	34	150	0	25	151
L19	0	58	638	0	80	375
L20	220	274	2300	72	282	2040
L21	0	30	176	0	42	70
L22	0	40	352	0	36	120
L23	0	11	100	0	30	84
L24	0	35	315	0	50	554
L25	0	16	152	0	27	88
L26+28	328	1097	9100	30	1100	8540
L27	132	560	1750	34	1890	2966
L29	44	29	130	10	28	720
L30	24	65	238	0	52	346
L32	10	0	1100	0	65	882
L33	14	0	1120	0	30	720

thiol groups in 50S subunits and a total of seven buried thiol groups in the 30S subunits and 18 buried thiol groups in 50S subunits, by using titration with 5,5'-dithiobis-(2-nitrobenzoic acid), which is in quite good agreement with the results presented here.

Effect of reduction with dithiothreitol

In order to exclude the possibility that some oxidation of disulphide bridges might be taking place during the incubation with the reagents some experiments were carried out with dithiothreitol. After reduction with a slight excess of dithiothreitol, alkylation with iodoacetamide or with *N*-ethylmaleimide was carried out in the presence of the reducing agent. The results of these experiments for the native subunits and for the isolated proteins in 6M-guanidine hydrochloride were essentially identical with those found by using β -mercaptoethanol reduction. No additional thiol-containing proteins were found by

this treatment. The enhanced reactivity of protein S18 in the native subunit compared with the isolated denatured protein was also observed after reduction with dithiothreitol, and indeed in these experiments the same effect was observed with *N*-ethylmaleimide.

Total number of thiol groups in ribosomal proteins

From the data of Tables 1 and 2 we have calculated the total number of thiol groups in each ribosomal protein. The ^{14}C d.p.m./equivalent obtained after alkylation of the reduced denatured protein was used for this purpose. In the case of alkylation with iodoacetamide our assumption was that protein S4 contained one thiol group, as is apparent from the determination of the complete sequence (Reinbolt & Schiltz, 1973). Thus a value of 2100 d.p.m. was taken to correspond to one thiol group in the experiments with iodoacetamide and 1500 d.p.m. in those with *N*-ethylmaleimide. In Table 3 the number of mol of

Table 3. *Total number of thiol groups in ribosomal proteins*

The basis for the calculation is described in the Results and Discussion section. The results are given as mol of thiol group/mol of protein.

Protein	No. of thiol groups				
	From reaction with iodoacetamide		From reaction with <i>N</i> -ethylmaleimide		Average
	After reduction with β -mercaptoethanol	After reduction with dithiothreitol	After reduction with β -mercaptoethanol	After reduction with dithiothreitol	
S1	1.1	1.0	0.9	0.9	1.0
S2	1.1	1.1	0.8	1.0	1.0
S3	1.0	0.9	0.3	1.5	0.9
S4	1.0	0.9	0.7	0.8	0.85
S8	1.1	0.9	0.8	1.1	1.0
S9+11	0.9	1.1	0.8	0.9	0.9
S12	3.9	3.9	4.3	4.0	4.0
S13	0.9	1.0	1.2	1.0	1.0
S14	0.8	0.9	0.8	0.9	0.85
S18	0.8	1.2	1.2	1.1	1.1
S21	2.0	2.1	2.4	2.1	2.15
L2	4.1	4.0	4.0	4.0	4.0
L5	2.1	1.9	2.0	2.0	2.0
L6	2.7	2.8	2.5	2.6	2.65
L10	2.6	2.8	2.1	2.5	2.5
L11	2.7	2.5	2.5	2.7	2.6
L14	1.7	2.0	2.0	2.1	1.95
L15	0.8	0.8	0.9	1.0	0.9
L17	1.7	2.0	1.9	1.9	1.9
L20	1.1	0.9	1.3	1.1	1.1
L26+28	4.3	4.9	5.7	5.4	5.1
L27	0.8	1.0	2.0	1.3	1.3

thiol group per mol of protein is given for each of the thiol-containing proteins of the ribosomes. The data from alkylation of denatured proteins reduced with dithiothreitol, as described above, is also included in Table 3. Here a value of 1600 d.p.m./thiol group was used for the results with *N*-ethylmaleimide. The agreement between the four sets of data is rather good, with one or two exceptions. Thus, for example, the value found for protein S3 with *N*-ethylmaleimide differs markedly between the case where reduction was by β -mercaptoethanol and where dithiothreitol was used. Nonetheless the average value for protein S3 suggests that this protein contains only one thiol group. Again for proteins L26+L28 there is a variation between 4.3 and 5.7 thiol groups. However, the average for these two proteins is close to five. In Table 4 the results of the experiments with ^3H -labelled proteins are presented. They represent the average of three determinations. The advantage of using double labelling is that the problems of stoichiometry are avoided. The results obtained by double labelling (Table 4) agree in most cases rather well with those found by the other method. The notable exceptions are the 30S-subunit protein S12, for which the difference is very large (four residues compared with one

or two) and the 50S-subunit proteins L10, L11 and L14. Whether this reflects an error in the stoichiometric factor used, or less homogeneous labelling of these proteins in the ^3H -labelled medium, is not clear. A value of four thiol groups has been found from preliminary sequence data (G. Funatsu, unpublished work) for protein S12. For proteins L10 and L11 the ^3H -labelling data give values for each protein close to unity, which is much less than for the data based on stoichiometry. In contrast the value for protein L14 from the ^3H -labelling data is higher.

We conclude that there are 15 thiol groups in the 30S subunit, distributed between 11 proteins, and that the 50S subunit contains 26–28 thiol groups which are distributed between 11–12 proteins. Previously published estimates of the total number of ribosomal thiol groups range from 10.6 to 15.6 for the 30S subunit and from 19.5 to 30 for the 50S subunit (Spahr, 1962; Slobin, 1971; Acharya & Moore, 1973). The value of 15 found by Spahr (1962) from analysis of cysteine content of total 30S protein and also the values of 15.6 and 16 thiol groups reported by Acharya & Moore (1973) are in good agreement with those reported here. The values of Slobin (1971)

Table 4. Total number of thiol groups in ribosomal proteins obtained from the experiments with ^3H -labelled proteins

For details see the text. Results are expressed as the mean of three determinations $\pm$ S.E.M.

Protein	Mean value of the radioactivity from the reaction with iodoacetic acid and <i>N</i> -ethylmaleimide	Number of thiol groups
S1	3.91 ± 0.97	1
S2	3.48 ± 0.99	1
S3	3.00 ± 1.54	1
S4	3.10 ± 0.15	1
S8	3.69 ± 2.00	1
S9+11	2.76 ± 0.84	1
S12	4.27 ± 2.65	1-2
S13	3.07 ± 0.47	1
S14	2.70 ± 0.40	1
S18	3.28 ± 0.03	1
S21	6.98 ± 0.58	2
L2	21.72 ± 1.43	4
L5	8.50 ± 1.73	1-2
L6	15.63 ± 2.01	3
L10	5.50 ± 0.75	1
L11	5.86 ± 1.24	1
L14	13.75 ± 3.27	2-3
L15	2.52 ± 0.61	0.5
L17	7.38 ± 2.49	1-2
L20	2.80 ± 0.26	0.5
L26+28	23.35 ± 0.37	4-5
L27	8.99 ± 1.49	1-2

are lower for both subunits than those of any other authors, and may reflect the inability of 6M-urea to denature the ribosomal proteins fully. However, these results do not take into account the stoichiometry of different proteins.

Identification of thiol-containing proteins

The only previous study in which the identification of ribosomal proteins containing thiol groups was undertaken of which we are aware is that of Acharya & Moore (1973), and although there is a good agreement for many of the 30S proteins there are some notable differences. Thus we found one thiol group each in proteins S3, S9 and S13, and two to four thiol groups in protein S12, whereas Acharya & Moore (1973) found no thiols in proteins S3 or S9 and a total of 2.5 in proteins S12+S13. One thiol group was found by Acharya & Moore (1973) in protein S15, which we do not find, and in proteins S11 and S17 their study reported two thiol groups in each protein. We find one in either protein S11 or S9 and none in protein S17, and this finding is confirmed in our studies with dithiothreitol. We have no explanation for this discrepancy, except to point out that in our investigations we have used the two-dimensional gel-electrophoresis method to analyse the distribution of alkyl groups, whereas Acharya & Moore (1973) used

Table 5. Reaction of ribosomal proteins in 30S and 50S subunits with iodoacetamide as a function of time at 25°C and 37°C

Results are presented as ^{14}C d.p.m./equivalent of protein after correction for stoichiometry as described in the Materials and Methods section.

Protein	Temperature ...		25°C		37°C			
	Time (min)	...	120	360	30	60	120	180
S1			900	1540	370	1040	1200	1720
S2			288	176	244	468	360	760
S3			60	62	44	84	60	190
S4			110	75	115	172	121	197
S8			0	5	4	11	15	18
S9			80	100	126	233	215	317
S12			735	812	763	1355	1922	2198
S13			920	816	938	1940	2060	1940
S14			198	188	156	411	228	465
S18			1160	1829	1990	1980	1900	1894
S21			2100	3120	1800	3040	3520	4330
L2			97	192	306	170	490	2170
L5			5	14	18	15	47	19
L10			58	54	70	46	100	46
L11			49	98	60	56	1040	1630
L14			46	73	49	46	92	34
L15			26	48	35	30	90	26
L17			80	167	104	148	349	1933
L20			57	37	74	44	1054	1810
L26+28			312	421	867	720	1761	3168
L27			238	402	236	370	940	1614

a combination of column chromatography and one-dimensional gel electrophoresis.

Order of reactivity of thiol groups

By using a number of different conditions for the reaction with iodoacetamide we obtained the results shown in Table 5. It is clear that in the 30S subunit the most reactive groups that are alkylated are in proteins S18 and S21. These are followed in order of reactivity by one thiol group in proteins S13 and S1, one group in protein S12 and the second thiol group in protein S21. These results are in contrast with the results obtained at 4°C, where the thiol groups of proteins S1 and S13 do not react (most probably due to oxidation) and protein S21 reacts only to the extent of one thiol group. In agreement with the results obtained at 4°C (Table 1), proteins S2, S3, S4, S8, S9 and S14 react to a much lesser extent than the rest, and only one of the thiol groups of protein S12 reacts.

In earlier studies (Craven & Gupta, 1970; Moore, 1971) it was found that the thiol groups in the 30S subunit reacting most rapidly towards iodoacetate and *N*-ethylmaleimide were in proteins S1, S11, S18 and S21. Again we have no explanation for the finding of an active thiol group in protein S11. In none of our experiments have we observed any appreciable radioactivity in the area of the gel occupied by protein S11. Similarly, Pongs *et al.* (1973) did not find any reaction of protein S11 with iodoacetamide.

In the 50S subunit the results are less clear, although the most reactive thiol groups are in proteins L26+L28, followed by protein L27 and then by proteins L2, L6, L4, L17 and L20. There is a considerable increase in the extent of reaction of all these 50S-subunit proteins after 2h, which probably reflects a loosening of the conformation of the 50S particle towards a less compact structure.

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