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Surface Topography of the *Escherichia coli* Ribosome Enzymatic Iodination of the 50S Subunit†

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ABSTRACT: The surface topography of the 50S subunit of the *Escherichia coli* ribosome has been investigated by lactoperoxidase iodination. Heavily iodinated ribosomes (40 iodines/70S particle) retain substantial protein synthesis activity. The free 50S subunit incorporates about 15% more ^{125}I than the 50S moiety of the 70S ribosome under comparable conditions of labeling. The pattern of protein reactivity was determined by two-dimensional gel electrophoresis analysis of iodinated ribosomal proteins. The labeling pattern is quite reproducible from sample to sample. This pattern should reflect some measure of surface exposure. The proteins most reactive to enzymatic iodination are L2, L5, L6, L10, L11, and L26. Several proteins, notably L2, L26, L28, and L18, are labeled substantially more in the isolated 50S particle than in the 70S ribosome. The results indicate that these proteins are at least partially protected from iodination by the 30S subunit.

More than 50 different proteins from the 70S *Escherichia coli* ribosome have been identified (Garret and Wittmann, 1973). The location of these proteins is not yet known in any detail. One of the simplest approaches is to explore the surface structure of the ribosome. A technique that allows one to compare exposed proteins of the 70S ribosomes with those of the individual subunits is especially useful.

This paper describes the use of lactoperoxidase to investigate proteins on the surface of the 50S subunit of the 70S ribosome and on the surface of the isolated 50S particle. Lactoperoxidase enzymatically iodinates tyrosines (Morrison *et al.*, 1970). The large size of this enzyme (78,000) allows it to selectively label surface tyrosines. For example, a class of exposed proteins on the intact human erythrocyte was investigated with this enzymatic iodination system (Phillips and Morrison, 1971). While our studies on iodination of ribosomes were in progress, a preliminary report appeared comparing the one-dimensional gel patterns of iodinated native and unfolded 50S particles (Michalski *et al.*, 1973). This work showed that only a few proteins were highly modified. A one-dimensional gel, however, does not permit an unequivocal identification of these proteins.

A number of previous studies of ribosomal protein exposure have been reported (Huang and Cantor, 1972; Craven and Gupta, 1970; Kahan and Kaltschmidt, 1972; Crichton and Wittmann, 1971; Chang and Flaks, 1971; Hsiung and Cantor, 1973). What would be especially useful is a technique which allows greater resolution and quantitation. We shall show results to indicate that lactoperoxidase iodination monitored by two-dimensional gels offers significant advantages.

One problem with any chemical modification approach to structure is the risk of distortion of the particle by covalent reactions. Sedimentation coefficients provide one criterion of native ribosomes but the most stringent criterion available is functional activity. It will be shown that heavily iodinated ribosomes sediment normally and show significant protein synthesis activity. This lends considerable weight to any structural conclusions which can be drawn from the pattern of protein iodination.

Materials and Methods

Ribosomes. 70S ribosomes were prepared from mid-logarithmic phase *Escherichia coli* A19 as previously described (Traub *et al.*, 1971). The following buffers were used: A [10 mM Tris (pH 7.6)–30 mM NH_4Cl–10 mM $Mg(OAc)_2$]; B [A plus 6 mM β-mercaptoethanol]; C [10 mM Tris (pH 7.6)–30 mM NH_4Cl–0.3 mM $Mg(OAc)_2$–6 mM β-mercaptoethanol]. 30S and 50S ribosomal subunits were prepared from 70S ribosomes by dialysis against buffer C for 48 hr at 4° and were separated on 10 → 30% 38 ml sucrose gradients in an SW 27 rotor at 25K for 16 hr. Analytical gradients (5 ml) were centrifuged in an SW 50.1 rotor at 45K for 1–3 hr.

Iodination of Ribosomes. Lactoperoxidase was obtained from Sigma Chemical Co. and purified as described by Morrison and Hultquist (1963); its concentration was determined by absorbance at 412 nm using an extinction coefficient of 114 mM^{-1} (Morrison *et al.*, 1957).

70S or 50S ribosomes were dialyzed against buffer A for 36 hr. A typical 30-ml reaction mixture contained 150 mg of 70S or 50S ribosomes, 300 μg of lactoperoxidase, 1.13 ml of 9.75 mM H_2O_2, and 3 ml of 2×10^{-3} M $Na^{125}I$. The specific activity of the $Na^{125}I$ was usually 10 Ci/M. The reaction mixture was stirred for 30 min. The reaction was stopped by adding 25 μl of β-mercaptoethanol (375 μmol). Ribosomes were pelleted, resuspended, and dialyzed against buffer B.

Extraction of Ribosomal Proteins. 50S ribosomes in buffer C were stripped of proteins either by the acetic acid method (Hardy *et al.*, 1969) or by a modification of LiCl and urea

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method (Kaltschmidt and Wittmann, 1972). LiCl and urea were added to 50S ribosomes to a final concentration of 4 and 8 M, respectively, then allowed to stand at 4° for 48 hr. After centrifuging for 15 min at 20,000*g* the 50S protein containing supernatant was removed from the rRNA pellet. Both methods yielded equivalent results.

Two-Dimensional Gel Electrophoresis and Sample Counting. Two-dimensional polyacrylamide gel electrophoresis was performed as previously described (Kaltschmidt and Wittmann, 1970). The gel slabs were stained in 0.025% Coomassie Blue and 12.5% Cl_3CCOOH until protein spots were visible. The spots were cut out, placed in scintillation vials, dried in an oven (70°) for 24 hr, and dissolved in 1 ml of 30% H_2O_2 by incubation for 24 hr at 50°; 13 ml of Kinards (1152 ml of xylene, 1152 ml of distilled dioxane, 696 ml of ethanol, 240 g of naphthalene, 12 g of 2,5-bis-2-(5-*tert*-butylbenzoxazolyl)-thiophene) solution was added and the vials were counted in a Packard TriCarb scintillation counter at a gain setting of 17%. Efficiency for ^{125}I was typically ~60%.

Approximately 75% of the ^{125}I that runs into the first dimension sample gel of the two-dimensional gel is subsequently found as discrete spots in the second dimension. The stained gels of iodinated proteins are visually identical with stained gels of noniodinated proteins. No new spots are seen. These facts make it appear reasonable, but not certain, that we have located all significant iodinated products. The loss in yield is comparable to what we have observed in the laboratory with ribosomal proteins modified with other radioactive reagents. The loss in yield is probably due to some smearing of the gels.

Protein Synthesis Assay. The activity of iodinated ribosomes was assayed, using a poly(U) dependent phenylalanine incorporation system (Nirenberg, 1963). The assay was performed under conditions of limiting ribosomes (70–90 μg of 70S particle; 80 μg of 50S subunits; 40 μg of 30S subunits). Each 250-μl reaction mixture contained 50 mM ATP, 7.5 mM phosphoenolpyruvate, 5 μg of phosphoenolpyruvate kinase, 6 mM β-mercaptoethanol, 0.05 mM [^{12}C]amino acid mix, 0.05 mM [^{14}C]phenylalanine, 1 mg of soluble enzyme fraction, 250 μg of poly(U), and 250 μg of unfractionated *E. coli* tRNA.

Ribosomes to be assayed for polyphenylalanine synthesis were iodinated with ^{127}I, a stable isotope. Two identical samples of 70S ribosomes were enzymatically iodinated as described above. One sample contained ^{125}I, the other contained ^{127}I. Both were pelleted to remove lactoperoxidase and dialyzed to remove iodine. The ^{125}I labeled sample was counted to determine the iodine/ribosome ratio, and the ^{127}I sample was used for protein syntheis assays.

Results

Effect of Lactoperoxidase and Iodination on Ribosome Activity. We have observed that the commercially available lactoperoxidase (Sigma Chemical Co.) contains a heat stable, high molecular weight contaminant that is capable of rapidly inactivating ribosomes in the absence of H_2O_2 or I^-. When the enzyme is purified by carboxymethylcellulose and Sephadex chromatography (Morrison and Hultquist, 1963), it no longer has any effect on ribosome activity, as determined by poly(U) dependent polyphenylalanine synthesis.

70S A19 *E. coli* ribosomes, derived from a RNase deficient strain, were incubated at 37° for 30 min with H_2O_2 or crude lactoperoxidase, or purified lactoperoxidase or heat-treated (100° for 10 min) lactoperoxidase; 10 μl of β-mercaptoethanol was then added to each reaction mixture to reduce any residual H_2O_2. The samples were next dialyzed against buffer B, pelleted, and assayed for poly(U) dependent polyphenylalanine synthesis. 70S ribosomes iodinated to the extent of 40 iodines per ribosome were also assayed. The results are depicted in Table I. As can be seen, purified lactoperoxidase does not substantially affect protein synthesis, whereas the crude enzyme preparation severely inhibits ribosomes. It is interesting to note that the heat-treated enzyme preparation, which is totally inactive in the iodination system, still contains the active agent responsible for the inactivation of the ribosomes. H_2O_2 and purified enzyme alone or in conjunction do not affect the ribosome activity.

TABLE I: Effect of Chemical and Enzymatic Pretreatment on Ribosome Activity.

Sample	Additions	% Activity[a]
1	None	100
2	5 μg of crude LPE	42
3	5 μg of heat-treated crude LPE	40
4	5 μg of purified LPE	89
5	15 μl of 9.75 mM H_2O_2, 5 μg of crude LPE	44
6	15 μl of 9.75 mM H_2O_2, 5 μg of purified LPE	87
7	15 μl of 9.75 mM H_2O_2	94
8	Iodinated with pure LPE to 40 I/particle	56

[a] Poly(U) directed polyphenylalanine synthesis, assayed as described in Materials and Methods. All samples contain 1 mg of 70S ribosomes. LPE, lactoperoxidase. Iodinated samples were labeled with ^{127}I as described in Materials and Methods. Parallel samples were iodinated with ^{125}I to determine iodine incorporation.

The polyphenylalanine incorporation activity of heavily iodinated 70S particles is less than uniodinated controls. Considering the high level of modification, however, the particles are still significantly active in protein synthesis.

Extent of Iodination. Ribosomes were usually stored in buffer B prior to iodination, then dialyzed extensively against buffer A in order to remove β-mercaptoethanol. The iodination was performed as described in Materials and Methods. No incorporation of radioactive iodine into ribosomal protein was found when either H_2O_2 or lactoperoxidase was omitted from the reaction mixture.

The extent of iodination is controlled by the amount of H_2O_2 added to the reaction mixture. Figure 1 shows the number of iodines incorporated per 70S ribosome as a function of H_2O_2. The reaction mixture contained 5 mg of 70S ribosomes, 10 μg of lactoperoxidase, H_2O_2 as indicated, and a total volume of 1 ml. After an initial lag, the iodine incorporation varies roughly linearly with H_2O_2 concentration.

It is important to have an accurate measurement of the I/R ratio, iodines per subunit, for each sample of iodinated ribosomes. To this end, a carefully determined amount of labeled 50S subunits was counted. A knowledge of the specific activity of the iodine used, the counting efficiency of the scintillation counter, the molecular weight of the 50S subunit, and the cpm/mg of 50S subunits yield the iodines/ribosome ratio for that subunit. The iodines/ribosome ratio varied slightly from sample to sample since it is such a sensitive function of H_2O_2 addition to the reaction mixture. The iodines per 50S particle labeled as subunit, I/50S ratio, was typically between 29 and

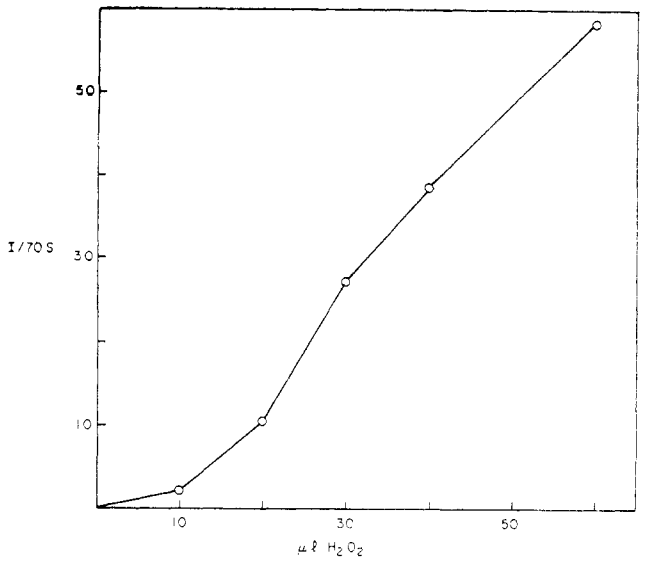

FIGURE 1: Iodine incorporated into 70S ribosomes as a function of H_2O_2; the reaction mixture contained 5 mg of 70S ribosomes and 10 μg of lactoperoxidase in 1 ml of buffer, and H_2O_2 (9.75 mM) was added as indicated.

32. The iodines incorporated into the 50S moiety of the 70S ribosome, I/50S *via* 70S, was typically between 25 and 28. The ratio of the amount of iodines incorporated into the isolated 50S subunit to the amount of iodines incorporated into the 50S moiety of the 70S ribosome, (I/50S)/(I/50S *via* 70S), however was found to be quite reproducible. Four independent determinations gave incorporation ratios of 1.10, 1.13, 1.13, and 1.23. The mean value, 1.15 ± 0.06, was used to standardize the polyacrylamide gel analyses discussed later.

It is important to note that all iodinations were carried out at less than saturation conditions. The numbers of iodines incorporated into 50S subunits in this study were substantially below saturation levels.

Sedimentation of Iodinated Subunits. Sucrose gradients of iodinated 50S particles were compared to gradients of unlabeled 50S subunits in order to determine whether extensive iodination causes detectable conformational distortion. All gradients were run 1.5 hr at 45,000 rpm, in an SW 50.1 rotor, using a 5–20% sucrose gradient. The iodinated 50S particles,

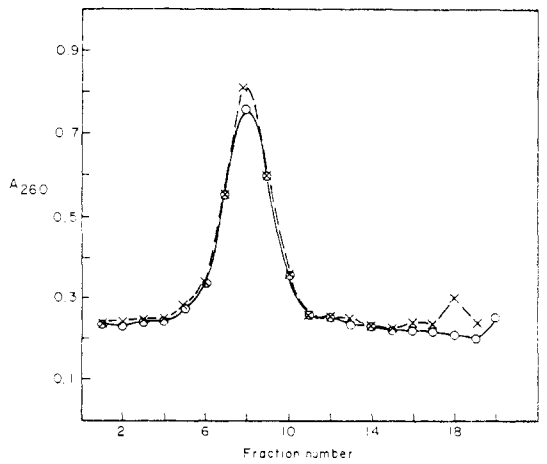

FIGURE 2: Sucrose gradient sedimentation profiles of iodinated and untreated 50S subunits. 5 → 20% sucrose, 45,000 rpm, in SW 50.1 rotor, for 1.5 hr: (×--×) control 50S; (○—○) iondinated 50S.

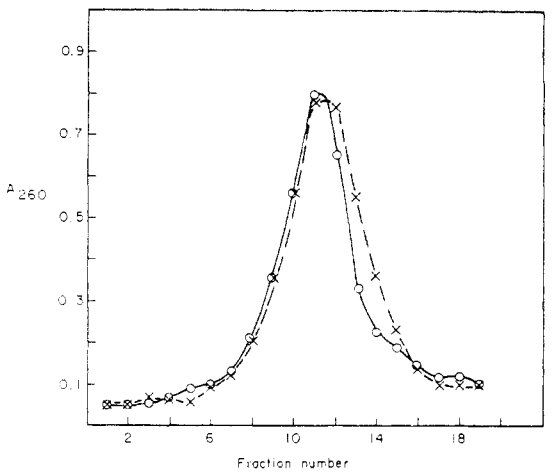

FIGURE 3: Sucrose gradient sedimentation profiles of iodinated and untreated (control) 30S subunits. 5 → 20% sucrose, 45,000 rpm, SW 50.1 rotor, 1.75 hr: (×---×) control 30S; (○—○) iodinated 30S.

labeled to the extent of 25 iodines/subunit, were derived from labeled 70S ribosomes.

As can be seen from Figure 2 there is virtually no difference between iodinated and control 50S ribosomes. Equivalent results were obtained using 50S particles that were labeled as isolated subunits.

Figure 3 shows analogous gradients of 30S subunits. The control 30S particles were derived from uniodinated 70S ribosomes, the iodinated 30S subunits were prepared from iodinated 70S ribosomes. These are labeled to the extent of 22 iodines/30S particle. The figure shows that labeled 30S subunits have almost the same sedimentation velocity as uniodinated 30S. This indicates that no gross conformational distortion has occurred.

Identification of Iodinated 50S Ribosomal Proteins. 70S (or 50S) ribosomes were iodinated as described in Materials and Methods. Routinely 300 mg of 70S and 150 mg of 50S ribosomes were labeled in each reaction mixture. Prior to iodination the ribosomes were dialyzed against buffer A. After iodination ribosomes were dialyzed to remove I^- and pelleted to remove lactoperoxidase. Labeled ribosomes were counted to determine the iodine/ribosome ratio and assayed to determine protein synthesis activity.

Four to six milligrams of ribosomal proteins were used for each two-dimensional gel. Gels were stained for 1.5 hr in 12.5% trichloroacetic acid and Coomassie Blue. The protein spots were carefully cut from the gel, dried, dissolved in 30% H_2O_2, and counted.

Table II compares the data obtained from two experiments. In the first reaction 50S subunits were labeled as isolated particles. For the second experiment, 70S ribosomes were labeled and the 50S subunits were isolated from these ribosomes. The data shown for each experiment are averages of nine gels from three independent reaction mixtures.

Since gels of iodinated subunits can vary as to iodine specific activity and total amount of protein that actually ran onto each gel, a weighted average had to be used. First consider the samples in which the 50S subunit was labeled directly. Let C_{ij} equal the counts observed in the ith protein spot of the jth gel. Then $C_j^T = \Sigma_i C_{ij}$ is the total counts found on the jth gel. The sum is taken over 31 spots on the two-dimensional gel. Proteins L8 and L9 were not resolved from each other. L31 did not appear on our gels. We chose to scale the data to the particular gel with the maximum total counts. This maximum

was $C_{max}{}^{T}$. Therefore the average counts in the ith protein spot can be calculated as

$$\overline{C_i} = \frac{1}{9} C_{max}{}^{T} \sum_{j=1}^{9} C_{ij}/C_j{}^{T} \quad (1)$$

An exactly equivalent procedure was used to find the average counts per protein spot in a sample of 50S particles derived from iodinated 70S complex. These we shall call $\overline{C_i}'$. They are scaled to the gel with the maximum total counts of these samples, $C'_{max}{}^{T}$. Now, to compare 70S and 50S labeled samples we must take cognizance of the fact that not only were the total counts on the gels different but the average number of iodines per 50S particle in the two samples differed as well. As discussed earlier, iodinated free 50S particles pick up ~1.15 times as much iodine per particle as the 50S moiety of the 70S complex. Therefore we adjusted the 70S gel data as shown below to permit an accurate comparison of 50S and 70S data.

$$\overline{S_i} = \frac{\overline{C_i}'}{1.15} \frac{C_{max}{}^{T}}{C'_{max}{}^{T}} \quad (2)$$

In eq 2 $\overline{S_i}$ is the scaled counts per 50S protein of an iodinated 70S particle. It is the quantity one can directly compare with $\overline{C_i}$. Note that the above procedure will compensate for variations in protein sample size as well as changes in specific activity.

As can be seen from Table II the 50S proteins vary greatly with respect to their degree of iodination. Most of the 50S proteins are labeled very similarly in both the 50S and 70S particles. These proteins, presumably, occupy the same environment in 50S and 70S ribosomes and are not protected by the 30S subunit. Several proteins, notably L2, L13, L26, L27, and L28, are labeled significantly more in the free 50S than in the 70S ribosome. We believe that these proteins are protected by the 30S subunit of the 70S ribosome from the enzyme lactoperoxidase.

Figure 4 shows a three-dimensional projection drawing of a typical 50S gel. The blocks represent protein spots on a two-dimensional gel and their height represents cpms of iodine. It is easily seen that smearing of one large spot cannot account for the data obtained. For example, L1 and L3 which are close to heavily labeled L2, L6, and L11 exhibit very small amounts of radioactivity. L33, which is adjacent to L32, contains less than one-tenth as many counts.

TABLE II: ^{125}I Labeling of 50S Proteins.

Protein	$\overline{C_i}$ cpm 50S ($\times 10^{-2}$)	Tyr Equiv	$\overline{S_i}$ cpm 50S *via* 70S ($\times 10^{-2}$)	Tyr Equiv	Tyr[a] (50S)-(50S *via* 70S)
L1	49	0.54	53	0.59	N.D.
2	204	2.30	148	1.65	0.65
3	49	0.54	39	0.43	0.11
4	34	0.44	39	0.43	N.D.
5	480	5.40	486	5.42	N.D.
6	182	2.00	163	1.82	0.18
7	2	0.02	2	0.02	N.D.
89	85	0.95	109	1.21	−0.26
10	199	2.20	217	2.42	−0.22
11	378	4.20	411	4.6	−0.40
12	3	0.04	4	0.04	N.D.
13	85	0.95	59	0.65	0.30
14	44	0.49	35	0.38	0.11
15	60	0.66	51	0.56	0.10
16	39	0.44	36	0.40	N.D.
17	81	0.90	66	0.73	0.17
18	88	0.98	66	0.73	0.25
19	28	0.32	24	0.26	N.D.
20	40	0.44	32	0.36	N.D.
21	38	0.42	36	0.40	N.D.
22	53	0.59	48	0.53	N.D.
23	31	0.35	23	0.26	N.D.
24	32	0.36	28	0.31	N.D.
25	45	0.50	43	0.48	N.D.
26	200	2.24	127	1.42	0.82
27	64	0.71	39	0.43	0.28
28	84	0.93	37	0.41	0.52
29	19	0.21	17	0.19	N.D.
30	10	0.11	10	0.11	N.D.
32	77	0.85	62	0.69	0.16
33	8	0.08	6	0.06	N.D.

[a] Tyrosine values less than 0.1 are denoted by N.D. I/50S = 31.5, I/50S *via* 70 S = 28.

Discussion

These studies indicate that enzymatic iodination can provide useful quantitative data on the reactivity of individual ribosomal proteins analyzed by two-dimensional electrophoresis. Gentle iodination by lactoperoxidase offers some distinct advantage over other currently used modification methods. Techniques that distort or destroy ribosome structure, such as protease or nuclease digestion, yield results that are extremely difficult to quantitate. Reactions with small amine or sulfhydryl reagents can be quantitated but the small size of these reagents casts doubts on their ability to act as surface (external) specific reagents. The specificity of these reagents may, in some cases, be dependent on local reactivity rather than protein accessibility. The wealth of quantitative information obtained from iodination makes the lactoperoxidase technique a good, quick screening method for protein reactivity.

The major question which must be faced is whether or not a pattern of lactoperoxidase iodination can be correlated with the exposure of proteins on the ribosome surface. One way to clarify this question is to compare reactivity of ribosomal protein components with their tyrosine content. If these are strictly parallel it would seem unlikely that iodination could really be measuring surface exposure. In Figure 5 the extent of iodination of selected proteins is compared with their tyrosine content. The ribosomal proteins used in the illustration were chosen for two reasons. First, they are believed to be either unitary or repeated fractional proteins (Garrett and Wittmann, 1973), and second, their mole % of tyrosine and molecular weights are known (Mora *et al.*, 1971). All cpms are taken from the same gel of 50S proteins iodinated as isolated subunits. From Figure 5, it is quite clear that certain ribosomal proteins are preferentially iodinated in the 50S particle and that the degree of iodination correlates little, if at all, with tyrosine content. Similar results were reported by Michalski *et al.* (1973). They found that 50S particles unfolded at low Mg^{2+} concentration showed a much less specific iodination pattern than the native subunits.

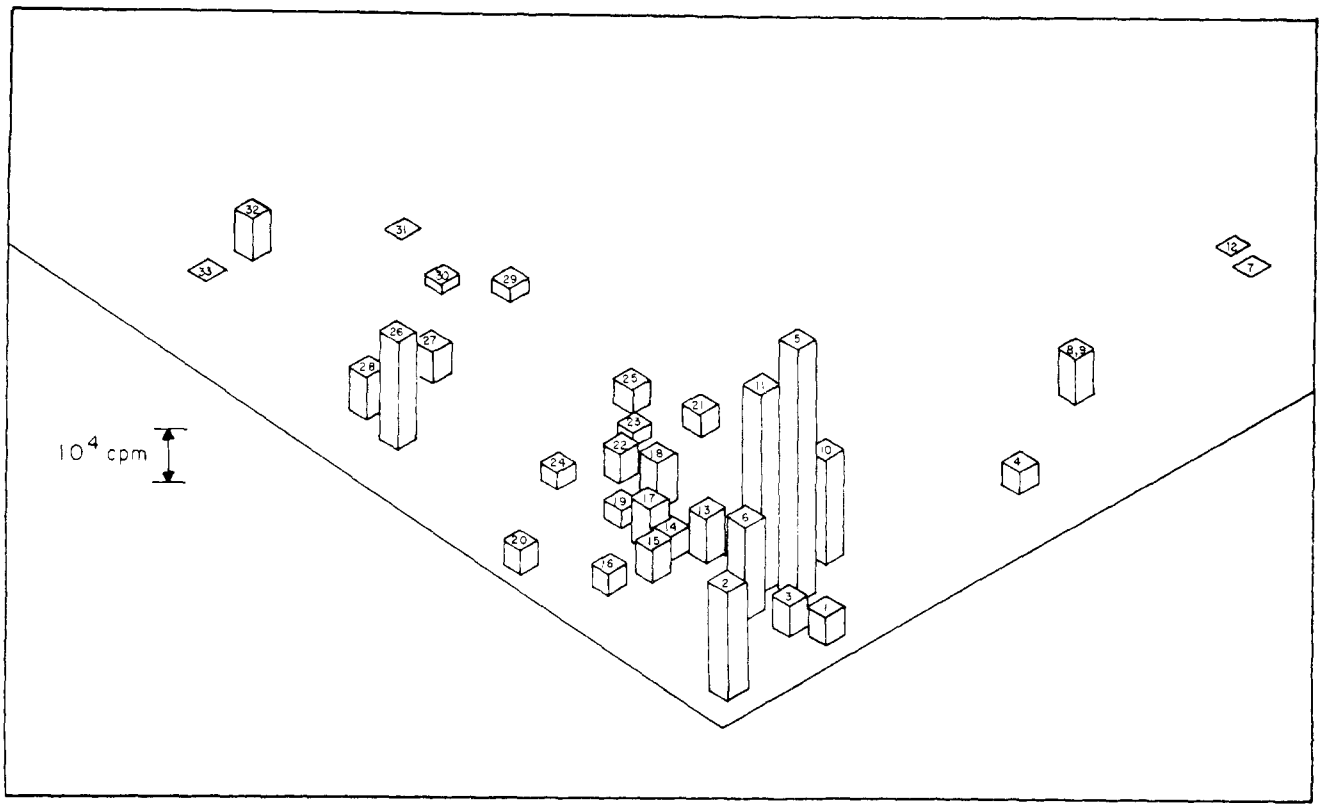

FIGURE 4: Projection drawing of iodinated 50S proteins. The blocks depict the spatial relationship between the 50S proteins on an average two-dimensional gel. The height of the blocks represents observed cpm.

The heavily labeled proteins do not constitute a special class of tyrosine rich proteins. It is useful to exclude other possible specificities of the enzymatic iodination that might account for the highly anisotropic pattern of iodination observed. The heavily labeled proteins vary greatly with respect to molecular weight from 28,000 for L2 to 12,500 for L26. The pK values also span a large range from pK > 12.0 for L2 to pK = 6.3 for L8 and L9 (Garrett and Wittmann, 1973). The variation in ionic charge of iodinated proteins is also evident in the two-dimensional gel patterns shown in Figure 4. Several of the heavily iodinated proteins are unitary, *e.g.*, L2, L6, L10, while others are fractional, *e.g.*, L8, L9, L26. There appears to be little evidence that lactoperoxidase is specifically selecting a class of proteins that share any common feature other than accessibility. It should be noted, however, that the absolute counts found in a protein spot will, certainly, be limited by the number of tyrosines in that protein. For that reason one would expect and we observe no appreciable counts in L7 and L12. These proteins have no tyrosine (Garrett and Wittmann, 1973).

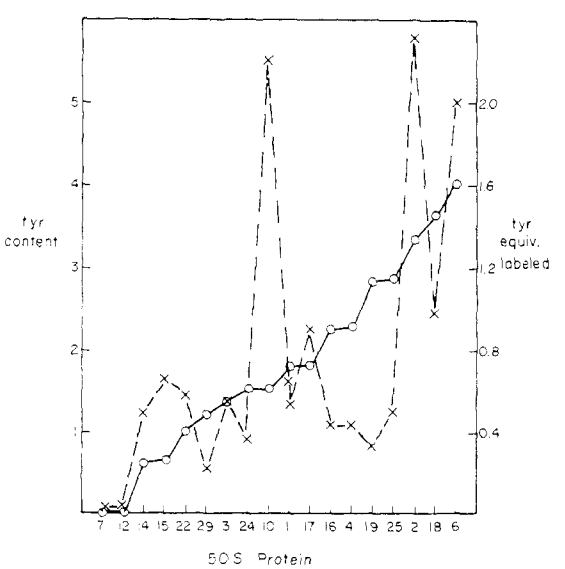

FIGURE 5: Illustration of the difference between the tyrosine content of selected 50S proteins and their iodine uptake, expressed as tyrosine equivalents labeled: (×---×) tyrosine equivalents from a 50S gel; (○—○) theoretical tyrosine content of the 50S proteins.

The results obtained from these experiments permit several general conclusions. The gross conformation of the 50S subunit changes very little when it complexes with the 30S subunit. Most of the proteins show similar degrees of labeling whether from the 50S subunit or the 50S moiety of the 70S ribosome. The changes that are observed, most evident in L2, and L26, are clear-cut and almost certainly reflect either the protection of the 50S proteins by the 30S subunit or conformation changes induced by this interaction. It is very probable that these proteins are near the interface of the two subunits. L2 has been shown to be near or at the peptidyl-tRNA binding site (Pellegrini *et al.*, 1972). Thus these results suggest that the 3′-end of peptidyl-tRNA is no more than one protein away from the 30S subunit. Since the shape of L2 is not yet known, the precise structural implication of this finding is hard to estimate. Less is known about L26, the other 50S protein markedly protected by the 30S subunit. Our finding should serve to foster additional interest in this protein.

It is interesting to compare our lactoperoxidase results with other previous studies of the surface of the 50S ribosomal subunit (Crichton and Wittmann, 1971; Kahan and Kalt-

TABLE III: Comparison of Iodine Uptake with Other Chemical Modifications.

50S Protein	Iodine Uptake 50S	FITC[a] Uptake	Trp[b] Digestion	Trp[c] Digestion	Glutaraldehyde[d] Uptake	*N*-Ethylmaleimide[e] Reaction
L1	+	++	++	+	−	NI
2	+++	++	+++	+	−	+++
3	+	++	−	−	−	++
4	−	+	+++	++	+	NI
5	+++	NI	++	+	+	+++
6	+++	NI	+++	−	+	−
7	NI	+	++	NI	++	NI
8,9	++	+	++	++	+	NI
10	+++	NI	+++	−	+	++
11	+++	NI	+++	−	+	−
12	NI	+	++	NI	++	NI
13	++	+	+	NI	−	NI
14	+	NI	++	NI	+	NI
15	+	NI	+	+	−	NI
16	−	NI	+++	+	−	++
17	++	NI	+	+	−	++
18	++	NI	−	+	+	++
19	−	+	−	++	+++	NI
20	−	−	++	++	−	+
21	−	++	++	NI	−	NI
22	+	NI	−	NI	+	−
23	−	+	+	NI	+	NI
24	−	NI	−	−	+	NI
25	+	NI	++	NI	++	NI
26	+++	−	+	NI	++	−
27	+	NI	−	++	+++	++
28	++	−	−	NI	−	NI
29	−	NI	+	NI	+	+
30	−	NI	−	NI	++	NI
32	++	+	−	NI	+	NI
33	−	−	++	NI	+++	NI

[a] Hsiung and Cantor (1973). [b] Crichton and Wittmann (1971). [c] Chang and Flaks (1971). [d] Kahan and Kaltschmidt (1972). [e] Moore (1971). Code: (+++) strong reaction, (++) moderate reaction, (+) weak reaction, (−) no reaction, (NI) no information.

schmidt, 1972; Chang and Flaks, 1971; Moore, 1971). This is reported in Table III. Taken together, data from a number of studies suggest that most of the proteins of the 50S particle are at least partially exposed. A close comparison of the data from different techniques shows many inconsistencies and a few similarities. L2, L5, and L10 appear to give strong indications of surface exposure in almost all cases. These proteins, therefore, are probably part of a class of highly exposed surface proteins on the 50S subunit. The general lack of consistency in comparing data from different studies is not surprising. There are vast differences in reagent specificity and size. Some modifications clearly proceed with disruption of the 50S particles. Compounding the problem is the heterogeneity of ribosomal particles. It seems clear that what will ultimately be needed is a careful statistical analysis of data obtained with different topographical probes. More work with different surface specific reagents will help to provide a clearer total picture of the surface topography of the ribosome.

The high reproducibility of the lactoperoxidase techniques encourages its use for factor binding site studies and other detailed attempts to map specific protein and nucleic acid contacts. Enzymatic iodination may also be attractive as a probe for conformation changes during protein synthesis.

Acknowledgments

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Oligonucleotide Composition of a Yeast Lysine Transfer Ribonucleic Acid†

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ABSTRACT: The purification of $tRNA_2^{Lys}$ from baker's yeast and the identification of the products of complete digestion with pancreatic ribonuclease and ribonuclease T1 are described. Seventeen modified nucleoside residues were found, including a 5′-terminal pseudouridine and 2-thio-5-carboxymethyluridine methyl ester in the first position of the anticodon. An alternative form of the latter nucleoside was found after purification of the tRNA. The alternative form of this nucleoside apparently was produced during isolation of the tRNA.

The nucleotide sequence of one of the two lysine transfer ribonucleic acids in baker's yeast has been reported (Madison *et al.*, 1972). The purification of the $tRNA^{Lys}$ and the isolation and determination of the nucleotide sequences of the oligonucleotides produced by complete digestion with RNase I and RNase T1 are reported here. The isolation of large fragments and reconstruction of the nucleotide sequence are described in the following paper (Madison and Boguslawski, 1974).

Materials and Methods

$tRNA_2^{Lys}$ Purification. Bulk yeast tRNA was extracted from commercial baker's yeast (Fleischmann's yeast) as described by Holley (1963). Countercurrent distribution of the crude tRNA was carried out with the ammonium sulfate system (Kirby, 1960) as described by Doctor (1967). Up to 12 g of bulk tRNA was dissolved in 800 ml of the ammonium sulfate system and distributed for 225 transfers at 25 ± 1°. The faster moving $tRNA^{Lys}$ peak was removed from the countercurrent system with $CetMe_3NBr$[1] (Eastman, technical) as previously described (Madison *et al.*, 1967), except that eight tubes from the countercurrent were combined (640 ml), and $CetMe_3NBr$ equal to about ten times the weight of RNA present was added. After the addition of diethyl ether, the gelatinous RNA–$CetMe_3NBr$ complex was collected in a 40-ml tube and centrifuged. As much as possible of the ether and aqueous layers was removed with a Pasteur pipet. The complex was dissociated with 10–20 ml 2 M NaCl and the RNA precipitated with 3 vol of 95% ethanol. The RNA was dissolved in water and reprecipitated with ethanol to remove residual NaCl and $CetMe_3NBr$.

$tNRA_2^{Lys}$ was further purified by reverse-phase chromatography (Weiss and Kelmers, 1967). About 250 mg of RNA from countercurrent distribution was absorbed on a 2.6 × 100 cm column of Chromosorb W (dimethylchlorosilane treated, acid washed, 100–120 mesh, obtained from Applied Science, Inc.[2]), treated with Aliquat 336. The RNA was eluted with a concave gradient made from 2 l. of 0.01 M $MgCl_2$, 0.01 M sodium acetate (pH 5.0), and 0.23 M NaCl in the mixing chamber and 2 l. of 0.4 M NaCl containing 0.01 M $MgCl_2$ and 0.01 M sodium acetate (pH 5). The columns were run at room temperature. The tubes could be assayed directly for lysine acceptor activity (Madison *et al.*, 1967). The peaks of $tRNA^{Lys}$ were combined, enough $CetMe_3NBr$ was added to make the $CetMe_3NBr$:RNA ratio about 150, and the RNA was isolated as above.

Analyses. Nuclease digestions, isolation of oligonucleotides, and analysis of nucleotide composition have all been described (Madison *et al.*, 1967) except that the chromatography on DEAE-cellulose in 7 M urea was done in the presence of 0.02 M morpholinopropanesulfonic acid (pH 7.0). The columns were run at room temperature with a flow rate of about 15 ml/hr. Two-dimensional thin-layer chromatography on Avicel plates (Analtech, Wilmington, Del.) using iso-

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[1] Abbreviations used are: $CetMe_3NBr$, hexadecyltrimethylammonium bromide; mcm^5S, 2-thio-5-carboxymethyluridine methyl ester (can also be named 2-thiouridine-5-acetic acid methyl ester); mcm^5S^*, modified form of mcm^5S; t^6A, *N*-[9-(β-D-ribofuranosyl)purin-6-ylcarbamoyl]-L-threonine (can also be named threonylcarbamoyladenosine); N*, unknown compound whose spectra is shown in Figure 8; RNase I, bovine pancreatic ribonuclease.

[2] Mention of a trademark or company name does not constitute a guarantee or warranty of the product by the U. S. Department of Agriculture.