Identification of Tyrosine Residues That Are Susceptible to Lactoperoxidase-Catalyzed Iodination on the Surface of *Escherichia coli* 50S Ribosomal Subunits or 70S Ribosomes†

Peter Maly, Jacek Wower,‡ Monica Zobawa, and Richard Brimacombe*

ABSTRACT: Further to our studies on the *Escherichia coli* 30S ribosomal subunit, the detailed surface topography of both 50S subunits and 70S ribosomes has been investigated by using iodination catalyzed by immobilized lactoperoxidase as the surface probe. In the 50S subunit, only proteins L2, L5, L10, and L11 were iodinated to a significant and reproducible extent. The targets of iodination were identified, after isolation of the individual iodinated proteins, and were as follows: in protein L2 (271 amino acids), tyrosine-102 and -160; in protein L5 (178 amino acids), tyrosine-142; in protein L10 (165 amino acids), tyrosine-132; in protein L11 (142 amino acids), tyrosine-7 and -61. In the 70S ribosome, only protein L5 was still iodinated to a significant extent from the 50S subunit, whereas in the 30S subunit the same spectrum of iodinated proteins was observed as that from iodinated isolated 30S subunits, with the exception that S21 was no longer present.

In a recent paper, we described the identification of a number of tyrosine residues that are exposed on the surface of the *Escherichia coli* 30S ribosomal subunit (Wower et al., 1983). The purpose of this investigation was to repeat some older topographical experiments with the lactoperoxidase-catalyzed iodination system [e.g., Michalski et al. (1973); Litman & Cantor (1974)] and to make an analysis not only of the proteins concerned in the reaction but also of the individual amino acids that are the targets of iodination. Our contention was (Wower et al., 1983) that detailed topographical information of this type will play an important part in building up structural models of the ribosome in the future, by helping to integrate results from crystallographic studies of isolated proteins on the one hand with data obtained from whole ribosomal subunits on the other hand. The latter type of data encompasses a variety of topographical techniques such as neutron scattering (Moore, 1980), immune electron microscopy (Stöffler et al., 1980; Lake, 1980), protein–protein cross-linking (Traut et al., 1980), or whole-subunit crystallographic (Wittmann et al., 1982; Clark et al., 1982) experiments.

In this paper, we describe a similar analysis of the tyrosine residues that are exposed to lactoperoxidase-catalyzed iodination on the surface of *E. coli* 50S ribosomal subunits. The principal proteins concerned in the reaction are proteins L2, L5, L10, and L11, in precise agreement with the data of Litman et al. (1976), and we show that six specific tyrosine residues in these four proteins are the targets of the iodination reaction. In addition, we describe a further set of iodination experiments with 70S ribosomes and compare the data with our results from the individual 30S and 50S subunits.

Materials and Methods

The procedures have already been described in detail (Wower et al., 1983) for isolation and ^{125}I-iodination of ribosomal subunits from *E. coli* strain MRE 600, extraction and separation by CM-cellulose chromatography of the iodinated proteins, tryptic digestion and chromatographic isolation of the radioactive peptides, and localization of the iodinated tyrosine residues by N-terminal sequence analysis. In addition, the following procedures were used:

Isolation of 70S Ribosomes. After disruption of the cells and removal of cell debris by centrifugation, the crude *E. coli* cytoplasmic extract was applied to a 10–30% sucrose gradient in 10 mM $MgCl_2$, 30 mM NH_4Cl, and 10 mM Tris-HCl,[1] pH 7.8 (buffer A), and was centrifuged for 22 h at 16000 rpm in a SW 27 rotor. The gradient fractions containing 70S ribosomes were diluted 2-fold with buffer A, and the ribosomes were pelleted by high-speed centrifugation and then resuspended in buffer A and dialyzed against 10 mM $MgCl_2$, 30 mM NH_4Cl, and 50 mM Hepes/KOH, pH 7.2. Aliquots of 20 A_{260} units were subjected to lactoperoxidase/glucose oxidase catalyzed iodination with 0.5 mCi of $Na^{125}I$, exactly as described previously (Wower et al., 1983). The iodinated 70S particles were pelleted by high-speed centrifugation and resuspended in buffer A. In order to demonstrate that no dissociation into subunits had occurred during the iodination procedure, this 70S suspension was applied again to a 10–30% sucrose gradient, and the ribosomes were isolated as described above. Finally, the 70S particles were suspended in 0.3 mM $MgCl_2$, 50 mM KCl, 6 mM 2-mercaptoethanol, and 10 mM Tris-HCl, pH 7.8, and the dissociated 30S and 50S subunits were separated as in our previous experiments (Wower et al., 1983).

Tryptic Digestion of Proteins from Iodinated 70S Ribosomes. 30S and 50S subunits from iodinated 70S ribosomes were treated with ribonucleases A and T_1 in dodecyl sulfate buffer, and the oligonucleotides released were removed by two ethanol precipitations (Ulmer et al., 1978). The ribosomal proteins were then separated by two-dimension gel electrophoresis as described (Wower et al., 1983), by using two or three gels for each sample. The ^{125}I-containing proteins were located by autoradiography, and the appropriate spots were cut out from the gel and dried under an infrared lamp. The dried gel pieces were soaked in 1 mL of trypsin (50 μg/mL) in 50 mM ammonium bicarbonate buffer, pH 8.0 [cf. Elder et al. (1977)]. When the gel pieces were fully rehydrated, they were cut into smaller pieces and incubated for 24 h at 37 °C.

† From the Max-Planck-Institut für Molekulare Genetik, Abteilung Wittmann, Berlin-Dahlem, West Germany. *Received December 13, 1982.* This work was in part supported by a grant from the Deutsche Forschungsgemeinschaft.

‡ Present address: Department of Biochemistry, University of Massachusetts, Amherst, MA.

[1] Abbreviations: Tris, tris(hydroxymethyl)aminomethane; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HPLC, high-pressure liquid chromatography; EDTA, ethylenediaminetetraacetic acid; CM, carboxymethyl.

0006-2960/83/0422-3157$01.50/0

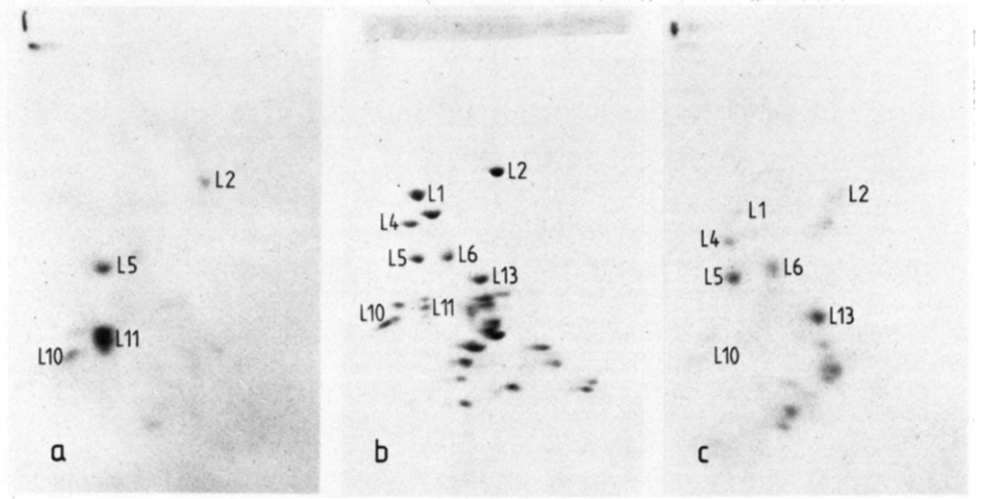

FIGURE 1: Two-dimensional polyacrylamide gels of proteins from iodinated 50S subunits. (a) Autoradiogram of the ^{125}I-labeled proteins, before separation by CM-cellulose chromatography. (b) Pattern of total 50S proteins after staining with Coomassie blue. Only those spots are named whose identities are unambiguously known in this gel system. L11 appears as a double or triple spot. (c) Autoradiogram of ^{125}I-labeled proteins from EDTA-treated 50S subunits. [Incorporation of iodine in the presence of EDTA was 3–4-fold higher than in the presence of magnesium (see text); the autoradiogram shown in panel c is a relatively light exposure, in comparison with panel a.] The gels were run from left to right (first dimension) and top to bottom (second dimension).

The polyacrylamide was then removed by centrifugation and filtration, and the supernatants containing the peptides were evaporated to dryness. Iodinated peptides obtained in this manner were separated by HPLC and "fingerprint" chromatography as before (Wower et al., 1983).

Amino Acid Analysis. In cases where N-terminal sequencing was not possible (see Results), amino acid analyses of the iodinated peptides were carried out, in a Durrum 500 amino acid analyzer, according to the procedure of Hitz et al. (1977).

Results and Discussion

Isolation of Iodinated Proteins from 50S Subunits. The same extremely mild conditions that we have described for the 30S subunit (Wower et al., 1983) were applied to the catalytic ^{125}I-iodination of 50S ribosomal subunits. Under these conditions, using the lactoperoxidase/glucose oxidase immobilized enzyme system (Hubbard & Cohn, 1972), the incorporation of iodine reached a level of approximately 1–2 iodine atoms per ribosomal subunit. As before (Wower et al., 1983), the enzyme system showed no detectable contaminating ribonuclease activity, the iodinated subunits retained their full activity in the poly(U) system (Homann & Nierhaus, 1971) as compared with untreated subunits, and control experiments showed no incorporation of ^{125}I into the subunits when any component of the iodination system was omitted.

Examples of the pattern of proteins from in situ iodinated 50S ribosomal subunits on two-dimensional polyacrylamide gels are illustrated in Figure 1. Figure 1a is an autoradiogram of the iodinated protein mixture prior to separation of the individual proteins by CM-cellulose chromatography [cf. Wower et al. (1983)], and Figure 1c gives the corresponding pattern of proteins from 50S subunits iodinated in the presence of EDTA. Figure 1b is a reference pattern of the total 50S protein, stained with Coomassie blue. Figure 1a shows that the primary targets of iodination are proteins L2, L5, L10, and L11, which, as noted in the introduction, is in precise agreement with the results of Litman et al. (1976). The same authors in an older paper (Litman & Cantor, 1974) had found some incorporation of iodine into a few additional proteins (in particular L6 and L26). Michalski & Sells (1975) on the other hand found comparable levels of iodination of a number of proteins, including L2, L5, L10, and L11, but in their hands, by far the most highly labeled protein was L27. The latter protein was not observed by Litman et al. (1976) and was not labeled in our experiments. Some faint radioactive spots in addition to proteins L2, L5, L10, and L11 can be seen in Figure 1a, but as before (Wower et al., 1983), we will consider only those proteins that were reproducibly and significantly labeled (cf. also the column separation of these proteins shown in Figure 2, below). For the four proteins concerned, the radioactivity distribution was approximately 7% (L2), 36% (L5), 10% (L10), and 45% (L11), taking the sum of the radioactivity values as 100%. Experiments in which the kinetics of the iodination were studied showed that this distribution was the same at very short reaction times [where the uptake of iodine was only ca. 25% of its value under the "standard" reaction conditions (see Materials and Methods)], which indicates that these radioactivity values reflect the different initial rates of iodination of the four proteins. The overall kinetics of incorporation were however precisely similar to those previously observed with the 30S particle (Wower et al., 1983).

When the reaction was carried out in the presence of EDTA, the incorporation of iodine was increased 3–4-fold [cf. Wower et al. (1983)], and a different pattern of iodination was observed (Figure 1c), in which most of the 50S proteins containing tyrosine (Wittmann et al., 1980) were now iodinated. Interestingly, the level of iodination of protein L11 was significantly reduced in the presence of EDTA; a similar situation was observed in the case of protein S21 in our experiments with the 30S subunit (Wower et al., 1983). The important point to note here is that the significantly increased iodination in the presence of EDTA supports our contention that the pattern of labeling observed with 50S subunits in their active conformation (Figure 1a) does indeed reflect a genuine surface labeling of the subunit.

^{125}I-Labeled proteins from 5000 A_{260} units of 50S ribosomal subunits were extracted with acetic acid (Hardy et al., 1969) and separated by chromatography on CM-cellulose (Hindennach et al., 1971), giving the radioactive profile illustrated in Figure 2. This pattern was extremely reproducible and showed four radioactive peaks marked A–D, corresponding to proteins L11, L10, L5, and L2, respectively. The identity of these proteins was unequivocal from the two-dimensional

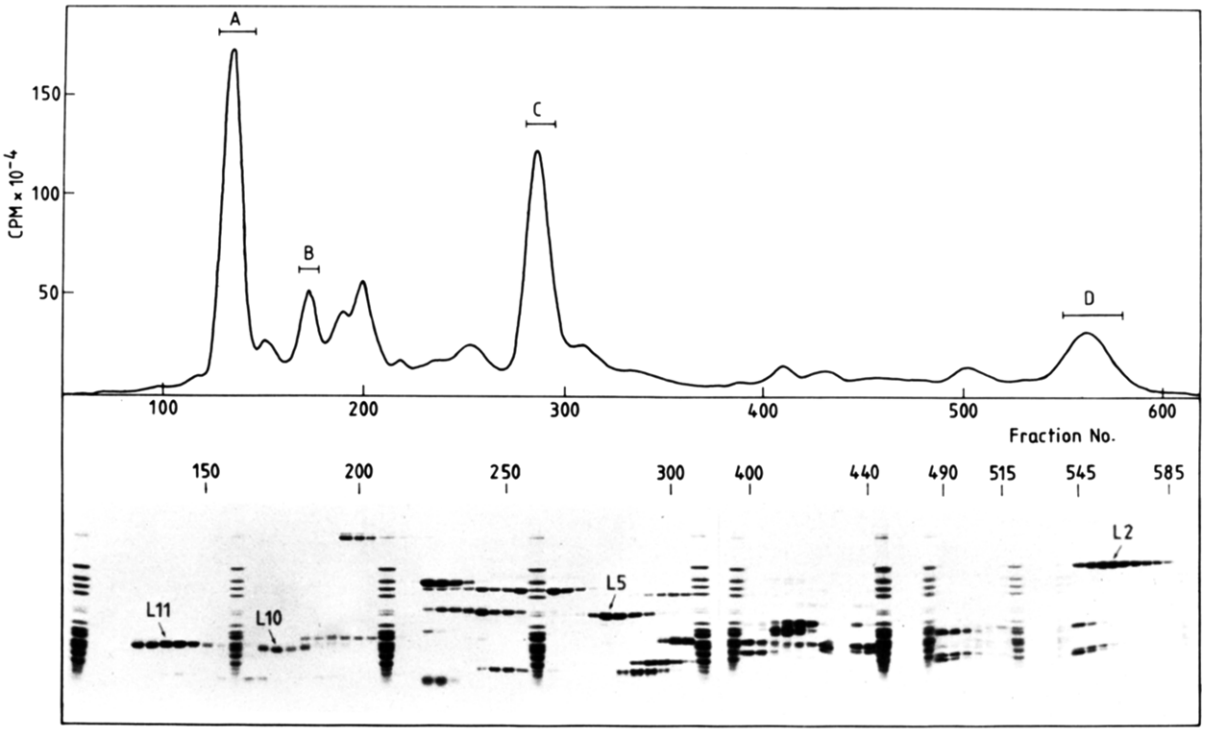

FIGURE 2: Separation of iodinated proteins by chromatography on CM-cellulose. The top part of the figure shows the ^{125}I radioactivity of the column fractions, the bars marked A–D indicating the fractions pooled for further analysis. The lower part of the figure shows the corresponding one-dimensional gel analyses of the column fractions [cf. Wower et al. (1983)], interspersed with control slots of total 50S protein. The gels were stained with Coomassie blue, and the bands that also contained ^{125}I radioactivity are indicated (L2, L5, etc.).

Protein	Ref.	Peptide	Sequence
L2	(a)	T19	Tyr*-Ile-Leu-Ala-Pro-Lys
		T24	Ser-Ala-Gly-Thr-Tyr*-Val-Gln-Ile-Val-Ala-Arg
L5	(b)	T22	Glu-Gln-Ile-Ile-Phe-Pro-Glu-Ile-Asp-Tyr-Asp-Lys-Val-Asp-Arg
L10	(c)	T3	Leu-Ala-Thr-Leu-Pro-Thr-Tyr*-Glu-Gln-Ala-Ile-Ala-Arg
L11	(d)	T1	TMA-Lys-TML-Val-Gln-Ala-Tyr*-Val-Lys
		T4	Gly-Leu-Pro-Ile-Pro-Val-Val-Ile-Thr-Val-Tyr*-Ala-Asp-Arg

FIGURE 3: Tryptic peptides from proteins L2, L5, L10, and L11 found to contain radioactive tyrosine. The peptides are numbered according to the sequence data in the appropriate literature reference: (a) Kimura et al., 1982; (b) Chen & Ehrke, 1976; (c) Heiland et al., 1976; (d) Dognin & Wittmann-Liebold, 1977. The radioactive tyrosine residues are indicated by asterisks, and the arrows indicate those amino acids that were positively identified in the N-terminal sequence analyses (see text).

gel electrophoresis pattern (Figure 1a). Further, the one-dimensional polyacrylamide gel analysis of the CM-cellulose column fractions (see lower half of Figure 2) showed that, in contrast to our findings with the iodinated 30S proteins (Wower et al., 1983), all four of the iodinated 50S proteins are eluted from the CM-cellulose column in an almost pure state, free from contaminating unlabeled protein. Some additional radioactivity was eluted from the column in between peaks B and C (around fraction 200, Figure 2), but this radioactivity ran as high molecular weight material in the corresponding gel analyses of the column fractions.

Analysis of Iodinated Tyrosine Residues. As stated in the introduction, the purpose of these experiments was to determine the precise targets of the iodination reaction, and for this purpose, the isolated iodinated proteins L2, L5, L10, and L11 were digested with trypsin, and the ^{125}I-containing peptides were isolated in pure form by successive HPLC and two-dimensional fingerprinting chromatography, as previously described (Wower et al., 1983). The identities of the peptides and the positions of the radioactive tyrosine residue within them were determined by N-terminal sequencing (Chang et al., 1978) or by amino acid analysis as described under Materials and Methods. The data were then fitted to the known sequences of the *E. coli* 50S ribosomal proteins [reviewed by Wittmann et al. (1980)]. Figure 3 gives the sequences of the peptides found to contain radioactive tyrosine from each of the four proteins (the labeled tyrosine residues being indicated

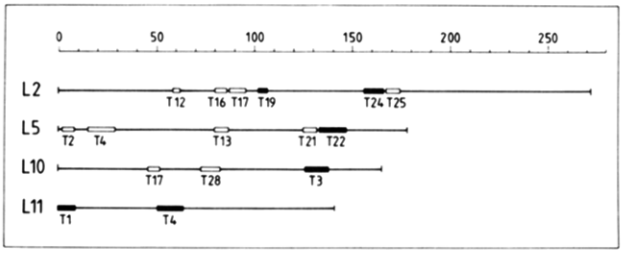

FIGURE 4: Summary of iodinated positions found in proteins L2, L5, L10, and L11. The top of the diagram gives a scale of amino acid residues, and in each protein the positions of tyrosine-containing peptides are indicated. The open boxes denote tryptic peptides with tyrosines that were not labeled; the filled boxes, peptides with tyrosines that were labeled.

by an asterisk), and Figure 4 shows the positions of these peptides within the complete amino acid sequences of the proteins [cf. Wower et al. (1983)].

Protein L11. L11 was the most highly labeled of the four iodinated proteins (peak A, Figure 2), and separation of the tryptic-digestion products from this protein in the HPLC and fingerprint systems enabled four distinct radioactive peptides to be isolated. Three of these peptides proved to be derived from the N-terminal tryptic peptide of L11 [T1, see Figure 3 and Dognin & Wittmann-Liebold (1977)], but since the N-terminus contains a trimethylated alanine in the first position and a trimethylated lysine in the third position, it was not possible to use the N-terminal sequencing procedure (Chang et al., 1978). However, the identities of the three radioactive peptides could be unequivocally established by amino acid analysis, and they were found to correspond to the three related tryptic-digestion products (T1a, T1b, and T1c, all partial further digestion products of peptide T1) that were observed in the original sequence determination of L11 (Dognin & Wittmann-Liebold, 1977). The peptide contains a single tyrosine residue, at the seventh position from the N terminus (Figure 3). The fourth radioactive peptide from L11 (which comprised approximately one-seventh of the total radioactivity present in the peptides from this protein) was on the other hand susceptible to N-terminal sequence analysis, and autoradiography of the sequencing chromatograms showed that the labeled tyrosine residue was at the 11th position of the peptide. This identifies the peptide clearly as T4 (Figure 3). Thus, both of the two tyrosine residues in L11 (Figure 4) are exposed to iodination. In the case of peptide T4, the N-terminal sequence analysis showed that only monoiodinated and not diiodinated tyrosine was present [cf. Wower et al. (1983) and Bruhns (1980)], whereas in peptide T1 these two possibilities could not be distinguished, since the N-terminal sequence method could not be applied (see above).

Protein L10. The tryptic digest of L10 proved very simple to analyze. Two radioactive peptides were found in the HPLC separation, and after final purification in the fingerprint system, both these peptides gave the N-terminal sequence Leu-Ala-Thr-Leu-Pro-Thr-Tyr*. The peptides therefore correspond to T3 [see Figures 3 and 4 and Heiland et al. (1976)], and the only difference between the two radioactive peptides was that one contained monoiodinated and the other diiodinated tyrosine. This was established from the mobilities of the iodinated tyrosine derivatives on the sequencing chromatograms (Bruhns, 1980; Chang et al., 1978), the relative radioactivities of the mono- and diiodinated species being in the ratio of approximately 3:1. A precisely analogous situation was found in the case of peptide T22 from protein S7 in our experiments with the 30S subunit (Wower et al., 1983).

Protein L5. Two radioactive peptides in the ratio of approximately 3:1 were also found in the tryptic-digestion products of protein L5. N-Terminal sequence analysis of the more radioactive of the two failed to identify the first two amino acids but gave the sequence Ile-Ile-Phe-Pro-Glu-Ile for the subsequent positions. This identifies the peptide as T22 from L5 (Chen & Ehrke, 1976), in which the tyrosine is at the tenth position (see Figure 3). We were not able to pursue the N-terminal sequence analysis beyond the isoleucine residue in the eighth position, and the autoradiograms of the sequencing chromatograms sometimes showed a "leakage" of ^{125}I-radioactivity as monoiodinated tyrosine throughout the degradation steps. This could be explained on the grounds that the aspartic acid at the ninth position of the peptide, immediately adjacent to the tyrosine residue, may be slightly labile under the acidic conditions of the N-terminal sequencing procedure. At all events, the identity of the peptide, and hence the position of the radioactive tyrosine residue, is unequivocal. The second (minor) radioactive peptide obtained from protein L5 could on the other hand not be identified in this series of experiments, but studies on protein L5 derived from iodinated 70S ribosomes showed that the peptide was in fact also T22, containing di- as opposed to monoiodinated tyrosine (see below).

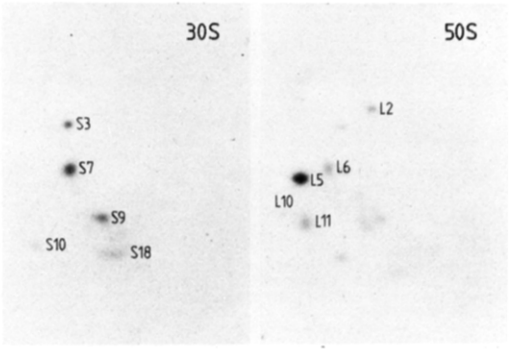

FIGURE 5: Two-dimensional polyacrylamide gels of proteins from 30S and 50S subunits derived from iodinated 70S ribosomes. The figures are autoradiograms of the 30S (left) and 50S (right) gels [cf. Figure 1a and Wower et al. (1983)]; the 50S autoradiogram was exposed for a longer time than that of the 30S. When comparing this autoradiogram with that shown in Figure 1a, it should be noted that the radioactivity measured in protein L5 (in terms of molar uptake of iodine) was similar in the 50S subunit and the 70S ribosome (see text). Note: The faint spot labeled as L10 in the right-hand panel is in fact not L10 but an unidentified different protein.

Protein L2. Protein L2 gave two iodinated peptides in the HPLC and fingerprint analysis, in the ratio of approximately 4:1. The major product gave the sequence Ala-Gly-Thr-Tyr* in the N-terminal sequence analysis, the alanine being the second amino acid of the peptide. This establishes the peptide as T24 (Kimura et al., 1982), in which the tyrosine is at the fifth position (Figure 3). In the case of the second (minor) radioactive peptide, autoradiography of the sequencing chromatograms showed that the radioactive tyrosine was in the first position, thereby identifying the peptide as T19 (Figure 3), which is the only tryptic peptide from L2 with a tyrosine as its N-terminal residue. The radioactive tyrosine residues in protein L2 were in both cases mono- and not diiodinated.

Iodination of 70S Ribosomes. Reports in the literature (Litman & Cantor, 1974; Litman et al., 1974, 1976; Michalski & Sells, 1975) have indicated that, when intact 70S ribosomes are subjected to lactoperoxidase-catalyzed iodination, the spectrum of modified 30S and 50S proteins obtained differs substantially from that obtained from iodinated isolated ribosomal subunits. In order to observe this effect under our reaction conditions, where as we have stressed only 1–2 atoms of iodine are incorporated per ribosome, a series of iodination experiments was carried out with intact 70S particles, as described under Materials and Methods. Sucrose-gradient centrifugation after the reaction showed that no dissociation into subunits occurred under these conditions, and as with the iodinated 30S and 50S subunits, the treated ribosomes were fully active in the poly(U) assay. In order to analyze the levels of iodine incorporation in the 30S and 50S moieties, the 70S particles were dissociated into subunits by a further sucrose density gradient centrifugation at low magnesium concentration. The proteins from the 30S and 50S subunits were then analyzed on two-dimensional polyacrylamide gels as before (cf. Figure 1a), and typical examples of the autoradiograms obtained are illustrated in Figure 5.

In the case of the 30S subunit, Figure 5 shows that the pattern of iodination is essentially the same as that found when isolated 30S subunits were the substrate (Wower et al., 1983), with the exception that the iodination of protein S21 is reduced to background level; as before (Wower et al., 1983), S7 is the main target of the reaction, with proteins S3, S9, and S18 also showing significant levels of iodination and S10 being modified to an extent that varied according to the individual ribosome preparation.

In the case of the 50S subunit, analysis of the sucrose gradient profiles showed that the overall amount of iodine incorporated was considerably reduced, in relation to that found when isolated 50S subunits were used for the iodination reaction, and this is reflected in the autoradiogram of Figure 5; the only protein iodinated to a significant extent is L5, with the other proteins (cf. Figure 1a) being present at background levels. Measurements of the radioactivity recovered in L5 confirmed that the pattern of 50S proteins in Figure 5 does indeed represent a reduction in the levels of iodination of L2, L10, and L11, as opposed to an enhancement in the level of L5. This raises the question as to whether the reduced iodination of the 50S proteins is simply a consequence of the available iodine being preferentially incorporated into the 30S moiety of the 70S particle. However, this argument can be discounted, since (as already noted above) the overall kinetics of iodine incorporation were the same for both 30S and 50S subunits in the isolated state.

As described under Materials and Methods, tryptic digestions of the isolated proteins derived from 70S ribosomes were made in situ in polyacrylamide slices cut from the gels, and after extraction from the gel, the tryptic peptides were analyzed by HPLC, fingerprinting, and N-terminal sequencing as before. The iodinated protein L5 again showed two radioactive peptides in these analyses, appearing at the same positions in the HPLC profile as before (cf. the foregoing discussion). However, the ratio of the two peptides was variable, and the peptide that was the minor product in iodinated L5 from isolated subunits now sometimes appeared as the predominant product. N-Terminal sequence analysis of the peptide showed that the radioactive tyrosine was released after the tenth sequencing step, which establishes the identity of the peptide as being again T22 (cf. Figure 3). The autoradiograms of the sequencing chromatograms showed however that the iodinated product was in this case diiodinated tyrosine, as opposed to the monoiodinated derivative.

Thus, in our hands, the iodination properties of 70S ribosomes, as compared with those of isolated 30S and 50S subunits, show only simple "shielding" effects. This shielding does not necessarily imply that the proteins concerned are at the subunit interface and could instead arise from conformational changes of the 30S and 50S subunits within the 70S particle. Our data are however in contrast to the results of Michalski & Sells (1975) and of Litman & Cantor (1974) and Litman et al. (1974), who found several proteins to be more accessible in 70S ribosomes than in the isolated subunits. These latter sets of data are however not in good agreement with one another and involve a number of proteins in both subunits that we do not consider to show significant levels of iodination in our own experiments. Of the nine proteins we have described in this and our previous paper (Wower et al., 1983), Litman et al. (1974) and Litman & Cantor (1974) found that S3, S7, and L5 were iodinated to similar extents both in 70S particles and isolated subunits and that S21 and L2 were labeled less in the 70S ribosome, in agreement with our findings. In contrast to our data, these authors found an increased exposure of proteins S9 and S18 in the 70S particle and no significant reduction in the exposure of L10 and L11. Similarly, Michalski & Sells (1975) found like us no change in S3, S9, S18, and L5 and a reduction in the exposure of L2 and L11, but on the other hand, they observed no change in S21 or L10 and a reduction in the exposure of S7. These differences almost certainly reflect the much higher level of iodine incorporation in the experiments reported by these latter two groups of authors; the data of Litman et al. (1974), for example, were derived from ribosomes containing on the order of 20 iodine atoms per particle.

Conclusions

In this and our previous paper (Wower et al., 1983), we have described the localization of 14 tyrosine residues that are exposed on the surface of 30S and 50S ribosomal subunits. In the 30S subunit, these tyrosines are in S3 (two sites), S7 (two sites), S9, S18, and S21 (two sites), whereas in the 50S subunit the tyrosines are in L2 (two sites), L5, L10, and L11 (two sites). In three cases (S7, L5, and L10), a diiodinated tyrosine is produced in addition to the monoiodinated adduct. As already discussed previously (Wower et al., 1983), the properties of the lactoperoxidase-catalyzed iodination system are such that the results can only be interpreted in terms of a labeling of tyrosine residues exposed on the particle surface. Again, however, it is important to remember that, in this type of experiment, only a positive result is significant; an absence of labeling may merely indicate low reactivity as opposed to topographical shielding of the tyrosine concerned.

When the 30S and 50S subunits are combined in a 70S ribosome, the labeling of S21, L2, L10, and L11 is reduced to background levels, and no new sites of iodination are observed. Thus, by working at extremely low levels of iodine incorporation and by considering only those proteins that are strongly and reproducibly labeled at this level of reaction, one can observe a clear picture of the shielding effects within the 70S particle. This is in marked contrast to the highly complex pattern of shielding and increased exposure seen by other authors (see the above discussion) working at higher levels of iodination. It is tempting to conclude that proteins S21, L2, L10, and L11 are at the subunit interface, although as already noted there are of course other explanations for their shielding in the 70S particle. More important, however, is the fact that the targets of iodination have been precisely identified, and as was discussed in detail for the case of the 30S subunit in our previous paper (Wower et al., 1983), all of these iodination sites—14 in the isolated subunits and 7 in the 70S ribosome—will contribute useful information for the eventual orientation of individual protein structures within the ribosomal particles.

Acknowledgments

We are grateful to Dr. H. G. Wittmann for his continued interest and support and for his critical reading of the manuscript.

Registry No. Tyrosine, 60-18-4.

References

Bruhns, J. (1980) Doctoral Thesis, Technical University, Berlin.

Chang, J. Y., Brauer, D., & Wittmann-Liebold, B. (1978) *FEBS Lett. 93*, 205–214.

Chen, R., & Ehrke, G. (1976) *FEBS Lett. 69*, 240–245.

Clark, M. W., Leonard, K., & Lake, J. A. (1982) *Science (Washington, D.C.) 216*, 999–1001.

Dognin, M. J., & Wittmann-Liebold, B. (1977) *FEBS Lett. 84*, 342-346.
Elder, J. H., Pickett, R. A., Hampton, J., & Lerner, R. A. (1977) *J. Biol. Chem. 252*, 6510-6515.
Hardy, S. J. S., Kurland, C. G., Voynow, P., & Mora, G. (1969) *Biochemistry 8*, 2897-2905.
Heiland, I., Brauer, D., & Wittmann-Liebold, B. (1976) *Hoppe-Seyler's Z. Physiol. Chem. 357*, 1751-1770.
Hindennach, I., Stöffler, G., & Wittmann, H. G. (1971) *Eur. J. Biochem. 23*, 7-11.
Hitz, H., Schäfer, D., & Wittmann-Liebold, B. (1977) *Eur. J. Biochem. 75*, 497-512.
Homann, H. E., & Nierhaus, K. H. (1971) *Eur. J. Biochem. 20*, 249-257.
Hubbard, A. L., & Cohn, Z. A. (1972) *J. Cell Biol. 55*, 390-405.
Kimura, M., Mende, L., & Wittmann-Liebold, B. (1982) *FEBS Lett. 149*, 304-312.
Lake, J. A. (1980) in *Ribosomes* (Chambliss, G., Craven, G. R., Davies, J., Davis, K., Kahan, L., & Nomura, M., Eds.) pp 207-236, University Park Press, Baltimore, MD.
Litman, D. J., & Cantor, C. R. (1974) *Biochemistry 13*, 512-516.
Litman, D. J., Lee, C. C., & Cantor, C. R. (1974) *FEBS Lett. 47*, 268-271.
Litman, D. J., Beekman, A., & Cantor, C. R. (1976) *Arch. Biochem. Biophys. 174*, 523-531.
Michalski, C. J., & Sells, B. H. (1975) *Eur. J. Biochem. 52*, 385-389.
Michalski, C. J., Sells, B. H., & Morrison, M. (1973) *Eur. J. Biochem. 33*, 481-485.
Moore, P. B. (1980) in *Ribosomes* (Chambliss, G., Craven, G. R., Davies, J., Davis, K., Kahan, L., & Nomura, M., Eds.) pp 111-133, University Park Press, Baltimore, MD.
Stöffler, G., Bald, R., Kastner, B., Lührmann, R., Stöffler-Meilicke, M., & Tischendorf, G. (1980) in *Ribosomes* (Chambliss, G., Craven, G. R., Davies, J., Davis, K., Kahan, L., & Nomura, M., Eds.) pp 171-205, University Park Press, Baltimore, MD.
Traut, R. R., Lambert, J. M., Boileau, G., & Kenny, J. W. (1980) in *Ribosomes* (Chambliss, G., Craven, G. R., Davies, J., Davis, K., Kahan, L., & Nomura, M., Eds.) pp 89-110, University Park Press, Baltimore, MD.
Ulmer, E., Meinke, M., Ross, A., Fink, G., & Brimacombe, R. (1978) *Mol. Gen. Genet. 160*, 183-193.
Wittmann, H. G., Littlechild, J. A., & Wittmann-Liebold, B. (1980) in *Ribosomes* (Chambliss, G., Craven, G. R., Davies, J., Davis, K., Kahan, L., & Nomura, M., Eds.) pp 51-88, University Park Press, Baltimore, MD.
Wittmann, H. G., Müssig, J., Piefke, J., Gewitz, H. S., Rheinberger, H. J., & Yonath, A. (1982) *FEBS Lett. 146*, 217-220.
Wower, J., Maly, P., Zobawa, M., & Brimacombe, R. (1983) *Biochemistry 22*, 2339-2346.

Direct Cross-Links between Initiation Factors 1, 2, and 3 and Ribosomal Proteins Promoted by 2-Iminothiolane†

Guy Boileau,‡ Peter Butler,§ John W. B. Hershey, and Robert R. Traut*

ABSTRACT: Complexes were prepared containing 30S ribosomal subunits from *Escherichia coli* and the three initiation factors IF1, IF2, and IF3. In different experiments, each of the factors was radiolabeled with the others unlabeled. The complexes were allowed to react with 2-iminothiolane and then oxidized to promote the formation of intermolecular disulfide bonds, some of which were between factors and ribosomal proteins. Each of the labeled factors becomes covalently cross-linked to the complex as judged by its failure to dissociate when centrifuged in a sucrose gradient containing a high salt concentration. Proteins from the complexes were extracted and analyzed on two-dimensional polyacrylamide gels by nonequilibrium isoelectric focusing and sodium dodecyl sulfate gel electrophoresis. Spots corresponding to cross-linked dimers that contained initiation factors, as indicated on autoradiographs, were cut out and analyzed further. The following cross-linked dimers between factors and ribosomal proteins were identified: IF1-S12, IF1-S18, IF2-S13, IF3-S7, IF3-S11, IF3-S13, and IF3-S19. Cross-links between factors IF1-IF2 and IF3-IF2 were also identified. A model integrating these findings with others on the protein topography of the ribosome is presented.

Initiation of protein synthesis in bacteria is promoted by three initiation factors: IF1 (M_r 8016), IF2 (M_r 118 000), and IF3 (M_r 20 668). The factors bind to 30S ribosomal subunits during initiation but are absent from 70S ribosomes during elongation [for a review, see Grunberg-Manago (1980)]. We have been interested in defining the ribosomal binding sites for the three initiation factors. The binding of radiolabeled factors to the 30S subunit has been studied by sucrose gradient centrifugation. IF3 binds tightly in the absence of the other components of initiation (Heimark et al., 1976b), whereas IF1 and IF2 bind best in the presence of all three factors (Fakunding et al., 1972; Langberg et al., 1977). The trifactor-ribosomal subunit complex has been postulated to be an intermediate in the initiation pathway (Fakunding et al., 1972; Langberg et al., 1977; Weiel & Hershey, 1981).

Earlier work in this laboratory employed bis(imido esters) to cross-link the three initiation factors to ribosomal proteins (Heimark et al., 1976b; Langberg et al., 1977; Bollen et al., 1975). Antibodies specific for individual ribosomal proteins were used for the identification of constituents in cross-linked complexes that contained initiation factors. Many of the same

† From the Department of Biological Chemistry, University of California, School of Medicine, Davis, California 95616. *Received February 2, 1983.* This work was supported by Grant GM 17924 from the National Institutes of Health. Dr. Boileau was supported in part by Le Conseil de la Recherche en Santé de Quebec.
‡ Present address: Département de Biochimie, Université de Montréal, Montréal, Quebec, Canada.
§ Present address: Microbiology Unit, Department of Biochemistry, University of Oxford, Oxford, OX1 3QV, U.K.

0006-2960/83/0422-3162$01.50/0