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Identification of Tyrosine Residues That Are Susceptible to Lactoperoxidase-Catalyzed Iodination on the Surface of *Escherichia coli* 30S Ribosomal Subunit†

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ABSTRACT: The detailed surface topography of the *Escherichia coli* 30S ribosomal subunit has been investigated, with iodination catalyzed by immobilized lactoperoxidase as the surface probe. Under mild conditions, only proteins S3, S7, S9, S18, and S21 were iodinated to a significant and reproducible extent. These proteins were isolated from the iodinated subunits, and in each case, the individual tyrosine residues that had reacted were identified by standard protein sequencing techniques. The targets of iodination that could be positively established were as follows: in protein S3 (232 amino acids), the tyrosines at positions 167 and 192; in S7 (153 amino acids), tyrosines 84 and 152; in S9 (128 amino acids), tyrosine 89; in S18 (74 amino acids), tyrosine 3 (tentative); in S21 (70 amino acids), tyrosines 37 and 70. The results represent part of a broader program to investigate ribosomal topography at the amino acid–nucleotide level.

Chemical modification of ribosomal proteins in situ is an approach that has been used in the past by many research groups in order to probe the surface topography of the *Escherichia coli* ribosome. In the earlier experiments [see, e.g., Benkov & Delihas (1974) and Brimacombe et al. (1976) for reviews] the objective was to determine which of the ribosomal proteins were buried in the structure and which of them were accessible on the surface, but as the data began to accumulate, it soon became clear that most if not all the proteins could be modified to a greater or lesser extent by one reagent or another. Interpretation of the data was very difficult, since each reagent showed a different spectrum of reactivity toward the individual proteins, and it was also impossible to determine to what extent some of the smaller reagents were able to penetrate into the structure. Errors and ambiguities in the identification of the modified proteins by gel electrophoresis undoubtedly added to the confusion. As a result, the chemical-modification approach has tended not to be favored in recent years.

However, chemical modification can yield useful and important data, provided that three preconditions are met. The

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first is that the reagent (or at least one of the components directly involved in the reaction) must be sufficiently large to preclude the possibility of significant penetration into the ribosome. Second, the level of reaction must be kept low, so as to minimize any distortion of the ribosomal particles resulting from the modification. Third, the analysis must be pursued to the level of determining which amino acid residues in the individual proteins are modified. In this way, a catalog of exposed residues for each protein could gradually be compiled, which would be of considerable help in integrating data from other sources. For example, as crystal structures of isolated ribosomal proteins become available (Leijonmark et al., 1980; Appelt et al., 1979, 1981, 1983), it will be important to test whether amino acid residues that are exposed in situ in the ribosome are also exposed in the isolated protein structure. If one assumes that the crystallographic and chemical modification data are indeed compatible, then the latter could be further used to orient each individual protein structure within the ribosomal subunit, in relation to topographical data from neutron scattering [e.g., Moore (1980)], immune electron microscopic [e.g., Stöffler et al. (1980) and Lake (1980)], or whole-subunit crystallographic (Yonath et al., 1980; Clark et al., 1982; Wittmann et al., 1982) experiments.

An obvious method to choose for this type of detailed chemical-modification study is lactoperoxidase-catalyzed iodination. This has been exploited previously by several authors (Miller & Sypherd, 1973; Michalski et al., 1973; Litman & Cantor, 1974; Michalski & Sells, 1975), using either soluble or immobilized lactoperoxidase. Particularly in the latter case, there is little chance of reagent penetration into the ribosome, and a well-defined and reproducible pattern of iodinated proteins is produced under very mild conditions. In this paper, we describe the iodination of *E. coli* 30S ribosomal subunits using the immobilized lactoperoxidase/glucose oxidase system (Hubbard & Cohn, 1972) and the subsequent identification of eight tyrosine residues (in proteins S3, S7, S9, S18, and S21) that are located on the surface of the 30S particle.

Experimental Procedures

Preparation and Iodination of Ribosomal Subunits. The 30S ribosomal subunits from *E. coli* strain MRE 600 were prepared by the usual procedure (Morgan & Brimacombe, 1972) and were activated by dialysis as described previously (Zwieb & Brimacombe, 1979). For the iodination reaction, 5000 A_{260} units of subunits were dialyzed against 50 mM KCl, 5 mM magnesium acetate, and 50 mM Hepes[1]/KOH, pH 7.2. The reaction was carried out in the presence of 20 μM NaI (unlabeled) and 1 mCi of $Na^{125}I$, together with eight vials of Enzymobead lactoperoxidase/glucose oxidase (Bio-Rad, catalog no. 170-6001) [cf. Hubbard & Cohn (1972)] in a total volume of 200 mL. The iodination was initiated by the addition of β-D-glucose to give a final concentration of 0.2%, and after an incubation at room temperature for 50 min, the reaction was terminated by addition of 2-mercaptoethanol to a concentration of 1%. The Enzymobeads were removed by centrifugation, and the iodinated subunits were precipitated with ethanol. The effect of the iodination conditions on the activity of the subunits was tested in trial experiments with nonradioactive iodide by the poly(U) system of Dohme & Nierhaus (1976). In some control experiments, 5 mM EDTA was substituted for 5 mM magnesium acetate in the dialysis and reaction buffers.

Isolation of Iodinated Proteins. The 30S proteins were extracted from the iodinated subunits by the magnesium/acetic acid procedure of Hardy et al. (1969), and the protein fraction was dialyzed successively against 10% acetic acid, against 2% acetic acid, and finally against a buffer containing (per liter) 0.8 mL of pyridine, 4.8 mL of formic acid, 1.0 mL of 2-mercaptoethanol, and 360 g of urea (Hindennach et al., 1971). The dialyzate was applied to a CM-cellulose column (30-cm length × 1-cm diameter) equilibrated in this latter buffer, and the proteins were eluted as described by Hindennach et al. (1971). Aliquots (15 μL) of every fifth column fraction were analyzed for protein content on 15% polyacrylamide slab gels in the system of Laemmli & Favre (1973), and after electrophoresis, the gels were both stained (with Coomassie blue) and autoradiographed. The fractions containing iodinated proteins were pooled (see Results), dialyzed against 2% acetic acid, and lyophilized. The protein content of the lyophilized fractions was analyzed by two-dimensional gel electrophoresis with a system in which the first dimension was that described by Mets & Bogorad (1974) and the second dimension a 15% polyacrylamide–dodecyl sulfate gel as described by Laemmli & Favre (1973). In this system, 0.1 cm thick gel slabs (20 cm long × 15 cm wide) were used in each dimension, 1 cm wide strips being cut from the first-dimension slabs for polymerizing into the second dimensions. In the case of protein pools containing S9 or S21, the two-dimensional gel system of Geyl et al. (1981) gave clearer identifications.

Iodinated proteins S3 and S7 were separated from each other (see Results) by a further chromatography step on a Sephadex G-100 superfine column (110-cm length × 1-cm diameter) in 10% acetic acid. In one series of experiments, so that one could obtain cleaner preparations of iodinated proteins S3, S9, and S18, the iodinated subunits were washed with 0.75 M LiCl as described by Homann & Nierhaus (1971), prior to extraction of the proteins. Both "core" and "split" protein fractions were then subjected to chromatography on CM-cellulose as above.

Analysis of Iodinated Peptides. The isolated proteins (ca. 50 nmol) were digested with TPCK-treated trypsin (Worthington) in 0.5–1.0 mL of 0.1 M *N*-methylmorpholineacetate buffer, pH 8.1, for 4 h at 37 °C, at an enzyme:substrate ratio of approximately 1:30. The tryptic peptides were separated by high-pressure liquid chromatography (HPLC) on an RP-18 column (5 μm, Merck, Darmstadt, West Germany), with a Beckman HPLC system (No. 420 and 110A). The column was eluted with a linear acetonitrile gradient (5–60%) in 20 mM ammonium acetate, pH 6.0, at a rate of 1 mL/min (see legend to Figure 4), and the ^{125}I radioactivity of the fractions (1 mL) was measured in a Beckman γ-counter. Fractions containing significant amounts of ^{125}I were further purified by electrophoresis and chromatography on two-dimensional thin-layer cellulose plates, in the "fingerprint" system of Hitz et al. (1977) [cf. Maly et al. (1980)]. After development, the plates were autoradiographed, and the labeled peptides were extracted with 50% acetic acid and lyophilized.

Aliquots of each isolated peptide were submitted to N-terminal sequence analysis, according to the procedure of Chang et al. (1978), and after development, the sequencing chromatograms were subjected to autoradiography. The data were fitted to the known sequences of the 30S ribosomal proteins [reviewed by Wittmann et al. (1980)].

Peptides isolated from protein S21, or undigested protein S21, were hydrolyzed with carboxypeptidase A (Worthington,

[1] Abbreviations: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EDTA, ethylenediaminetetraacetic acid; TPCK, L-1-(tosylamido)-2-phenylethyl chloromethyl ketone.

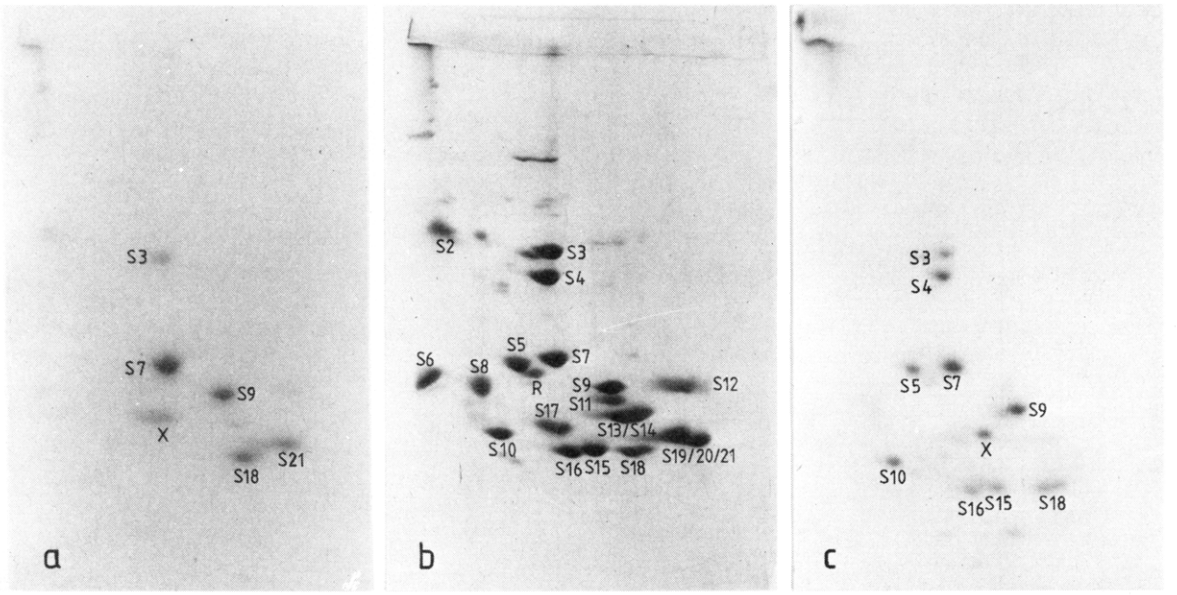

FIGURE 1: Two-dimensional polyacrylamide gels of iodinated 30S ribosomal proteins. (a) Autoradiogram of the ^{125}I-labeled proteins, before separation by CM-cellulose chromatography. The spot marked X is not a ribosomal protein (see text). (b) Stained pattern of total 30S proteins. R is ribonuclease, used in the preparation of the proteins for the gel [cf. Maly et al. (1980)]. (c) Autoradiogram of ^{125}I-labeled proteins from EDTA-treated 30S subunits. The gel system is described under Experimental Procedures, the first dimension running from left to right and the second from top to bottom.

0.5–1.0 unit of enzyme/nmol of peptide or intact protein) in 50 μL of 0.1 M *N*-methylmorpholineacetate buffer, pH 8.1, for 15 min at 37 °C. The amino acids released were derivatized with *o*-phthaldialdehyde (Umagat et al., 1982) and subjected to HPLC analysis. The column fractions were analyzed for ^{125}I-labeled tyrosine (both mono- and diiodinated adducts) by use of the corresponding unlabeled compounds (obtained from Sigma Chemical Co.) as standards.

Results and Discussion

Isolation of Iodinated Proteins. Iodination of 30S ribosomal subunits was carried out as described under Experimental Procedures with the combined lactoperoxidase/glucose oxidase immobilized enzyme system (Hubbard & Cohn, 1972). This is a particularly mild method, as the peroxide necessary for the iodination reaction is generated in situ. Preliminary trials indicated further that the enzyme system was essentially free of ribonuclease activity. Under our conditions, the incorporation of iodine reached a plateau level of 10–25% of the total ^{125}I after about a 30-min incubation, which corresponds to an incorporation of 1–2 atoms of iodine/30S subunit. This plateau level certainly does not imply that the ribosomes are saturated with iodine; Litman et al. (1976) have shown that, with the lactoperoxidase/H_2O_2 system, up to 20 atoms of iodine/30S subunit can be incorporated. The same authors (Litman & Cantor, 1974) showed that the level of incorporation is very sensitive to the amount of peroxide present in the reaction mixture, and our observed plateau, therefore, most likely reflects a limit imposed by the glucose oxidase/glucose part of the system. This relatively low level of reaction is however ideal for our purposes, since there is very little likelihood of distortions occurring as a result of multiple modifications within single subunits. No incorporation of ^{125}I took place when either the immobilized enzymes or glucose was omitted from the reaction mixture, so the observed reaction in the complete system should reflect a genuine labeling of residues exposed on the surface of the native 30S particle. Assays made with the poly(U) system showed that the iodinated 30S subunits retained their full activity, as compared with untreated subunits.

The pattern of iodinated ribosomal proteins obtained on two-dimensional gels is shown in Figure 1. Figure 1a is an autoradiogram of the iodinated protein mixture before application to the CM-cellulose column (see Experimental Procedures), and by comparison with the pattern of stained total 30S protein in Figure 1b, it can be seen that the principal products of reaction are proteins S3, S7, S9/S11, S18, and S19/S20/S21. Similar analyses on the gel system of Geyl et al. (1981) demonstrated that S21, and not S19 or S20, was the iodinated product, and the subsequent peptide analyses (see below) indicated that S9 was iodinated, and not S11. The spot in Figure 1a marked X does not correspond to a ribosomal protein, either from the 30S or the 50S subunit, and was not investigated further. A number of other faint spots can be seen in Figure 1a, which we do not consider to represent significant or sufficiently reproducible levels of iodination. In particular, proteins S10 and S12 were iodinated to a very variable extent, dependent on individual ribosome preparations. In contrast, the pattern of the five main protein products was extremely reproducible, the spots showing relative radioactivities of approximately 10 (S3), 35 (S7), 17 (S9), 21 (S18), and 15% (S21), the sum of the five radioactivity values being taken as 100%.

Figure 1c shows the corresponding pattern of products from 30S subunits iodinated in the presence of EDTA. Under these conditions, the level of iodine incorporation was increased by a factor of almost 4, and Figure 1c shows that a number of additional proteins (most notably S4, S5, S10, S15, and S16) were now involved in the reaction. A longer autoradiographic exposure indicated that almost all of the 30S proteins were iodinated to some extent in the presence of EDTA, although, interestingly, the level of reaction of S21 was significantly reduced. Since EDTA is known to cause free exchange of ribosomal proteins in addition to unfolding of the subunits (Newton et al., 1975), this result demonstrates that the majority of the proteins can become iodinated in this system, when the compact conformation of the 30S particle is destroyed. EDTA treatment would not be expected to cause serious denaturation of the proteins, and therefore, Figure 1c gives a good indication of the tyrosine residues that are normally

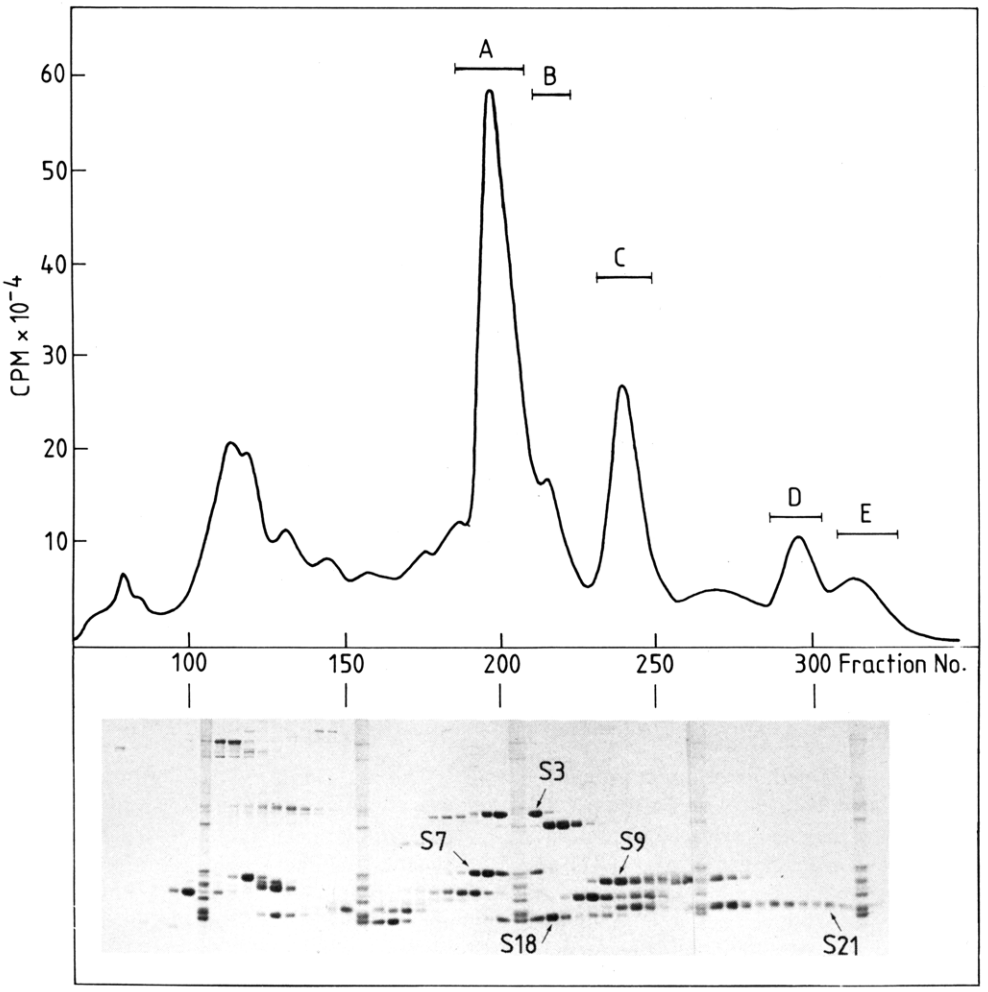

FIGURE 2: Separation of iodinated proteins by chromatography on CM-cellulose. The top part of the figure shows the ^{125}I radioactivity of the column fractions, the bars marked A–E indicating the fractions pooled for further analysis. The lower part of the figure shows the corresponding one-dimensional gel analyses of the column fractions (see Experimental Procedures), interspersed with control slots of total 30S protein. The gels were stained with Coomassie blue, and the bands that also contained ^{125}I radioactivity are indicated (S3, S7, etc.).

shielded by the structure of the 30S subunit, as opposed to those that are buried within the tertiary structures of the individual proteins.

The protein pattern in Figure 1a is in extremely close agreement with the chemical iodination data of Lam et al. (1979), with the interesting exception that these authors found S1 to be labeled, whereas we find S21. This may reflect a lower proportion of protein S1 in our ribosome preparations, which could be coupled with a concomitant exposure of S21; both these proteins have been implicated in interactions with the 3′ terminus of the 16S RNA within the 30S subunit [e.g., Czernilofsky et al. (1975) and Dahlberg & Dahlberg (1975)]. Litman et al. (1976) also found little labeling of S21, but again, the major products were proteins S3, S7, S9, and S18 (cf. Figure 1a). These authors also found significant labeling of proteins S4 and S5, possibly as a result of their more vigorous iodination conditions, which led to the incorporation of about 20 iodine atoms/subunit. Michalski & Sells (1974) did observe a significant labeling of S21, but they also found a number of other proteins to be highly labeled in addition to S3, S7, and S9. In contrast, S18 was rather weakly labeled, and surprisingly, Michalski & Sells found that EDTA treatment had very little effect on the labeling pattern (cf. Figure 1c). It seems likely that the very brief dialysis against EDTA that they performed was not sufficient to cause complete unfolding of the 30S subunits. Our results do not agree with those of Miller & Sypherd (1973), who obtained a very different spectrum of iodination of the 30S proteins. Since neither of these two older publications (Michalski & Sells, 1974; Miller & Sypherd, 1973) shows any photographs of the protein separations, it is not possible to assess how far the discrepancies can be ascribed to ambiguities in protein identification.

The separation of the iodinated proteins (cf. Figure 1a) on the CM-cellulose column is illustrated in Figure 2. The upper part of the diagram shows the radioactive profile of the column, and the lower part gives the corresponding polyacrylamide gel analysis of the column fractions. The five iodinated proteins appeared in the column eluate in well-defined and reproducible peaks, marked A–E in Figure 2. Peak A contained iodinated S3 and S7, the shoulder B was S18, and peak C contained S9. The iodinated S21 was present in both peaks D and E (see below). The first part of the elution profile (fractions 1–180) showed some rather heterogeneous radioactive peaks that ran as high molecular weight material in the corresponding gel analyses of the column fractions (Figure 2). This type of aggregated material is invariably seen in ribosomal protein preparations and was not analyzed further.

The iodinated proteins S3 and S7 could be separated from one another by a further chromatography step on Sephadex G-100 (see Experimental Procedures). The combined column procedures thus resulted in an acceptable separation of all five of the iodinated proteins. However, the CM-cellulose column eluate in the region of peaks A–C contained a number of

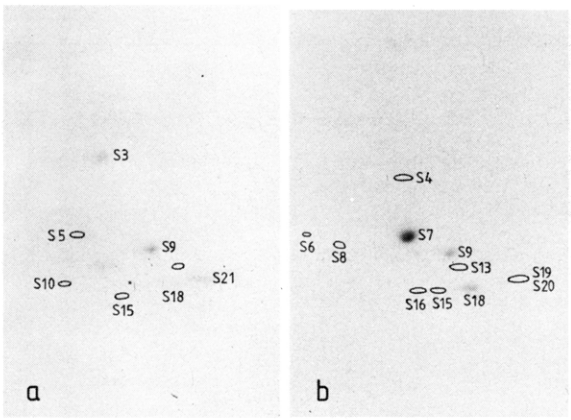

FIGURE 3: Autoradiograms of two-dimensional gels (cf. Figure 1) of iodinated proteins from 30S subunits after a washing with lithium chloride: (a) proteins from the split fraction; (b) proteins from the core fraction. The positions of stained unlabeled proteins are indicated by the circles.

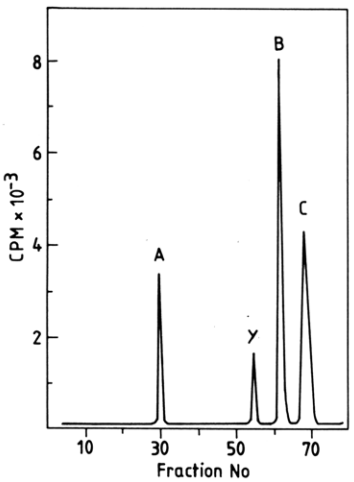

FIGURE 4: HPLC analysis of tryptic peptides from iodinated protein S7. The gradient of acetonitrile (5–60%, see Experimental Procedures) was applied between fractions 5 and 75. The figure shows the ^{125}I radioactivity in the column fractions, peaks A–C being derived from S7 and peak Y being a contaminant from S18 (see text).

nonlabeled 30S proteins (Figure 2), and these caused some difficulties in the subsequent peptide analyses (see below), since sometimes a tryptic peptide from a contaminating nonlabeled protein overlapped with the iodinated peptide under consideration in the HPLC or fingerprint separation system (see Experimental Procedures). For this reason, a batch of iodinated subunits was subjected to a lithium chloride wash (Homann & Nierhaus, 1971), and both the split and core protein fractions were separated on CM-cellulose columns, as described under Experimental Procedures (cf. Figure 2). In this case, the improved separation was adequate to resolve the remaining ambiguities in the analysis of the iodinated peptides. Radioactive gel patterns of the core and split protein fractions prior to CM-cellulose chromatography are given in Figure 3.

Analysis of Iodinated Tyrosine Residues. In order to identify the labeled tyrosine residues, it was necessary to digest the individual isolated proteins with trypsin, and the peptides released were isolated in a highly purified state (cf. the discussion above) by successive HPLC and "fingerprinting" chromatography, followed by N-terminal sequence analysis as described under Experimental Procedures.

Protein S7. The most highly iodinated protein (Figure 1) is S7, and the following section describes a typical analysis from this protein, as an example of the analytical procedures.

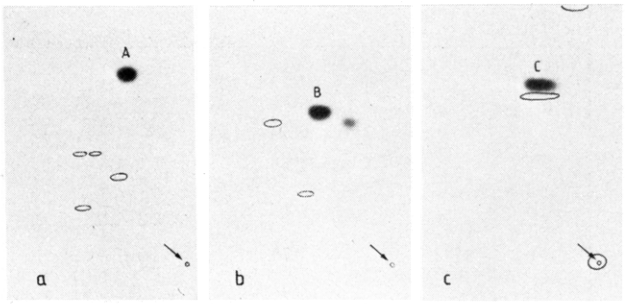

FIGURE 5: Two-dimensional fingerprints of peaks A–C from the HPLC analysis of Figure 4. The diagrams are autoradiograms of the thin-layer plates, with the positions of stained unlabeled peptides indicated by circles. The arrows mark the point of sample application. (a) Fingerprint of fraction A (Figure 4). (b) Fingerprint of fraction B; the minor labeled component to the right of the principal radioactive spot proved to be a variant of the radioactive peptide in (c). (c) Fingerprint of fraction C (Figure 4).

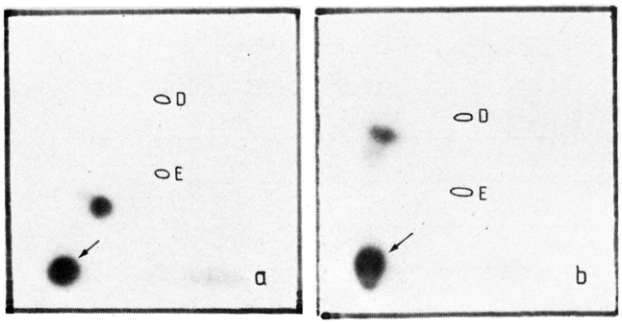

FIGURE 6: Autoradiograms of N-terminal sequence analysis plates, in the system of Chang et al. (1978). The arrows indicate the sample application point, and the circles marked D and E show the positions of the two derivatized diethylamine and ethanolamine markers (Chang et al., 1978). (a) Monoiodinated tyrosine, derived from the radioactive peptide of Figure 5b. (b) Diiodinated tyrosine, derived from the radioactive peptide of Figure 5c, [see text, and cf. Bruhns (1980)].

The peptides obtained by tryptic digestion of iodinated S7 were separated by HPLC, giving the radioactive profile illustrated in Figure 4. Three peaks were obtained, labeled A–C in Figure 4, together with a minor fourth peak Y, which proved to be derived from a slight contamination of the isolated S7 with protein S18 (cf. Figure 2, and see below). Pooled fractions containing peptides A–C were further purified by two-dimensional thin-layer electrophoresis and chromatography, and the chromatograms obtained were subjected to autoradiography and in some cases were also stained with ninhydrin or fluorescamine. Examples for each of the free fractions are shown in Figure 5, and it can be seen that in each case a single radioactive peptide was present, together with some unlabeled peptides arising either from S7 or from contaminating unlabeled proteins (cf. the foregoing discussion).

Sequence analyses of the radioactive peptides B and C both gave Ala-Phe-Ala-Z-Tyr*, Z being an amino acid that we could not unambiguously identify. The tyrosine at the fifth position contained the radioactive iodine, as denoted by the asterisk, and the only difference between the two peptides was that in peptide C this radioactive tyrosine residue was a diiodinated derivative, whereas in peptide B it was monoiodinated. Autoradiograms showing the different migration positions of the mono- and diiodinated tyrosines from these analyses on the two-dimensional sequencing chromatograms (Bruhns, 1980; Chang et al., 1978) are shown in Figure 6. The corresponding N-terminal sequence analysis of peptide A gave Val-Gly-Gly-Ser-Thr-Tyr*, with the tyrosine at the sixth position containing the radioactivity. In this case, as well

Protein	Ref.	Peptide	Sequence
S3	(a)	T24	Thr-Glu-Trp-Tyr*-Arg
		T27	Ala-Asp-Ile-Asp-Tyr-Asn-Thr-Ser-Glu-Ala-His-Thr-Thr-Tyr*
S7	(b)	T13	Val-Gly-Gly-Ser-Thr-Tyr*-Gln-Val-Pro-Val-Glu-Val-Pro-Val-Arg
		T22	Ala-Phe-Ala-His-Tyr*-Arg
S9	(c)	T14	Ala-Leu-Met-Glu-Tyr*-Asp-Glu-Ser-Leu-Arg
S18	(d)	T2	Tyr*-Phe-Arg
S21	(e)	T9	Glu-Phe-Tyr*-Gln-Lys-Pro-Thr-Thr-Glu-Arg
		T21	Leu-Tyr*-OH

FIGURE 7: Tryptic peptides from proteins S3, S7, S9, S18, and S21 found to contain radioactive tyrosine. The peptides are numbered according to the sequence data in the appropriate literature reference: (a) Brauer & Röming (1979); (b) Reinbolt et al. (1978); (c) Chen (1977); (d) Yaguchi (1977); (e) Vandekerckhove et al. (1975). The radioactive tyrosine residues are indicated by asterisks, and the arrows indicate those amino acids that were positively identified in the N-terminal sequence or carboxypeptidase analyses.

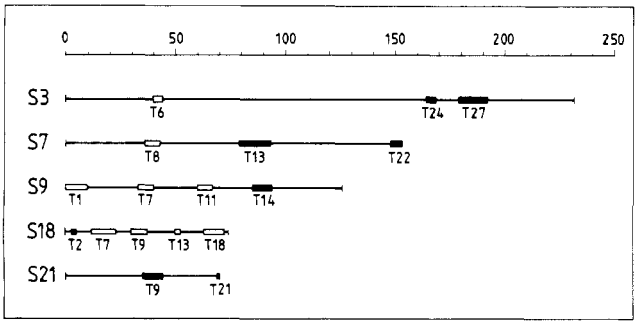

FIGURE 8: Summary of iodinated positions found in proteins S3, S7, S9, S18, and S21. The top of the diagram gives a scale of amino acid residues, and in each protein, the positions of tyrosine-containing peptides are indicated. The open boxes denote tryptic peptides with tyrosines that were not labeled; the filled boxes, peptide tyrosines that were labeled. In protein S3, peptide T27 contains two tyrosines, the one at position 192 being labeled. In protein S7, peptide T22 is the C-terminal peptide in *E. coli* strain MRE 600; in other strains, the protein is 24 amino acid residues longer. In protein S9, one more tyrosine, either in peptide T1 or T7, may also be labeled. In protein S18, the identification of T2 as the labeled peptide is tentative. See text for details.

as in all the peptide analyses from the other iodinated proteins (see below), the tyrosine residue was monoiodinated. The N-terminal sequence analyses of peptides A–C establish unequivocally that the radioactive tyrosine residues in protein S7 are in peptides T13 and T22 (Reinbolt et al., 1978). The sequences of these tryptic peptides, together with those found for the other iodinated proteins, are given in Figure 7, the radioactive tyrosine residues being indicated by an asterisk in each case, and the positions of the peptides in the complete amino acid sequences of the respective proteins are summarized in Figure 8. The corresponding peptide analyses of the iodinated proteins S3, S9, S18, and S21 were made in an analogous manner to that just described. In all cases, the results were entirely reproducible.

Protein S21. As already mentioned (Figure 2), the iodinated protein S21 appeared in two peaks from the CM-cellulose column, although the corresponding unlabeled protein (see the polyacrylamide gel fractions in Figure 2) showed a single broad peak. The reason for this separation into two peaks is not clear but does not appear to be directly related to the iodination reaction. Two-dimensional polyacrylamide gel analysis in the system of Geyl et al. (1981) established that both the radioactive peaks (Figure 2) did indeed correspond to protein S21, and HPLC analysis of the tryptic digests from both peaks showed in each case two radioactive peptides, which were present in approximately equal amounts. These peptides were further purified on thin-layer plates as in the case of protein S7. N-Terminal sequence analysis of the first peptide (cf. Figure 6) gave Glu-Phe-Tyr*-Gln, which corresponds to peptide T9 of protein S21 (see Figure 7). [It should be noted that Vandekerckhove et al. (1975) found glutamic acid instead of glutamine at the fourth position of this peptide. The S21 protein that these authors sequenced was however isolated from *E. coli* strain K12 instead of MRE 600.] The second iodinated peptide from S21 corresponded to peptide T21 (Figure 7), which is at the extreme C terminus of the protein. This posed a problem, since in the N-terminal sequencing procedure the radioactive tyrosine released after the first degradation step [cf. Chang et al. (1978)] was lost into the organic phase. So that this difficulty could be overcome, the peptide (and also an aliquot of the intact protein) was digested with carboxypeptidase A as described under Experimental Procedures, and the amino acids were derivatized with *o*-phthaldialdehyde and subjected to HPLC analysis (Umagat et al., 1982). This demonstrated that only leucine and radioactive monoiodinated tyrosine were present, thus establishing the identity of the peptide as T21.

Protein S3. HPLC analysis of the tryptic peptides derived from iodinated protein S3 also showed two radioactive peaks, present in approximately equal amounts. Again, the peptides were purified by thin-layer chromatography. One of them was a large peptide corresponding to T27 (Brauer & Röming, 1979) as shown by N-terminal sequence determination of the first three amino acids, Ala-Asp-Ile (Figure 7). This peptide contains two tyrosine residues at the fifth and fourteenth positions, respectively. The N-terminal sequence analysis established that the tyrosine at position 5 was definitely not labeled, and despite the large number of sequencing steps, we were able to demonstrate positively that the tyrosine at position 14 was the labeled residue, by autoradiographic analysis of the sequencing chromatograms (cf. Figure 6). The other radioactive peptide from protein S3 was readily identified, since N-terminal sequence analysis showed that the radioactive tyrosine was in the fourth position. S3 contains a total of four tyrosine residues (Brauer & Röming, 1979), two of which are in peptide T27 (above) and the other two being in peptides T6 (at the second position) and T24 (at the fourth position). The radioactive peptide must therefore be T24 (see Figure 7).

Protein S9. The tryptic peptides released from protein S9 gave two radioactive peaks in the HPLC analysis, which were

present in the ratio of approximately 3:1 (cf. Figure 4). After purification, N-terminal sequence analysis of the minor peak gave Ala-Leu-Z-Glu-Tyr*, which identifies the peptide as T14 from protein S9 (Chen, 1977), the unidentified amino acid at the third position being in this instance methionine (Figure 7). However, the corresponding N-terminal analysis of the major radioactive peptide consistently failed to identify the nonlabeled amino acids, although it was reproducibly observed that the labeled tyrosine was again at the fifth position of the peptide. Presumably, this peptide contained less material, despite being more strongly labeled. In both peptides derived from S9, the tyrosine was monoiodinated (cf. peptides B and C from S7 above), and the mobility of the major radioactive peptide in both the HPLC and thin-layer systems showed that it had a different composition to that of peptide T14. Protein S9 contains two other tryptic peptides (T1 and T7, see Figure 8) in which the tyrosine is at the fifth position. These peptides are rather similar to one another, and on the basis of our analysis, we were unable to establish whether one of these corresponded to the major radioactive peptide from S9 or whether this latter peptide arose from a variant of peptide T14, extended in the C-terminal direction. It should be noted that a contribution by iodinated peptides from protein S11 (which is known to run predominantly together with protein S9 in two-dimensional gel electrophoresis systems) can be excluded, since S11 contains only one tyrosine residue, and this occurs at the second position of the corresponding tryptic peptide (Kamp & Wittmann-Liebold, 1980).

Protein S18. The tryptic digest of protein S18 gave a single strong radioactive peak in HPLC and subsequent thin-layer chromatography, and N-terminal sequence analysis showed that the radioactive tyrosine was at the first position. However, as with the major iodinated product from protein S9, the sequence determination failed to identify the nonradioactive amino acids. In protein S18 there are no less than three tyrosine-containing tryptic peptides in which the tyrosine is at the first position (Yaguchi, 1977). Two of these (T2 and T13) are tripeptides, and one (T18) is a decapeptide. The position of the iodinated peptide on the fingerprint was consistent with that of the tripeptide T2 (Wittmann-Liebold, 1971), but this identification must be regarded as tentative in the absence of more positive evidence.

Conclusions

The results described in this paper clearly define a number of tyrosine residues that are accessible on the surface of the 30S ribosomal subunit, in proteins S3, S7, S9, S18, and S21. It is noteworthy that such a close agreement was found with the corresponding set of proteins found by chemical iodination (Lam et al., 1979), and this suggests that the ribosome may be rather inpenetrable, even to relatively small chemical reagents. The alternative explanation, namely, that reactive species formed on the surface of the immobilized enzyme beads could diffuse into the ribosome, can be discounted; Bayse & Morrison (1971) have provided good evidence that the reaction of lactoperoxidase with tyrosine takes place directly on the surface of the enzyme. The same authors (Bayse et al., 1972) have shown that the rate of lactoperoxidase-catalyzed iodination of tyrosine is highly dependent on the immediate amino acid environment of the tyrosine residue. At the very low level of iodination we have used, we are effectively observing initial rates of reaction, and it is therefore not surprising that the individual tyrosine-containing peptides are labeled to different extents (or that diiodotyrosine was also observed as well as the monoiodinated adduct). In this context, it is important to remember that, in this type of experiment, only a positive result has significance; an absence of labeling may merely be a result of low reactivity as opposed to topographical shielding of the tyrosine concerned.

As stated in the introduction, the results form part of a broad investigation of the detailed topology of RNA and protein in the ribosomal subunits, and in this context, proteins S7 and S21 are the most interesting of the five proteins. In the case of S7, it should be noted that the exposed tyrosine in peptide T22 (Figure 8) is the penultimate residue in S7 from *E. coli* strain MRE 600 (Reinbolt et al., 1978). This result suggests that the extra 24 amino acids at the C terminus of protein S7 from *E. coli* strain K (Reinbolt et al., 1978) are also at or near the surface of the subunit. Further, it is known from cross-linking experiments that the methionine residue at position 114 in protein S7 is in close contact with the uridine at position 1240 in the 16S RNA (Möller et al., 1978; Zwieb & Brimacombe, 1979). The cross-link site therefore lies in between the two iodinated tyrosines, which are at positions 84 and 152 in the protein (Figure 8). A second cross-link site between S7 and the 16S RNA at position 1377 or 1378 has recently been established (Wower & Brimacombe, 1983), although the corresponding site within the protein is not yet known. Thus, although each individual piece of data is in itself not very informative, when the data are considered together, a detailed picture of the topology of protein S7 and its interaction with ribosomal RNA begins to build up.

Similarly, in the case of protein S21, a number of studies have implicated this protein in interactions with the extreme 3′ terminus of the 16S RNA [e.g., Czernilofsky et al. (1975) and Backendorf et al. (1981)]. Our results show that both tyrosines in S21 are highly exposed, which is consistent with the fact that the 3′ terminus of the RNA is itself known to be readily accessible in the 30S subunit [e.g., Santer & Santer (1973) and Rinke et al. (1977)]. As already mentioned, however, other authors (Lam et al., 1979; Litman et al., 1976) have not observed strong iodination of protein S21, and this may reflect a rather flexible situation of this protein in the 30S subunit. In protein S18, the single cysteine residue at position 10 has been positively identified as the target of reaction with an affinity-labeling analogue of A-U-G (Yaguchi et al., 1978). This suggests that the N-terminal region of this protein is rather exposed, which is again consistent with our finding that the tyrosine at position 3 is labeled. Little is so far known at this level about the remaining two iodinated proteins (S3 and S9), but it is to be expected that the same type of detailed information will also slowly accumulate for these and other proteins. Similar studies with both 50S subunits and 70S ribosomes have been made (Maly et al., 1983), and all of these results will have to be taken into account in any detailed model-building studies of the ribosome in the future.

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