Structural Analysis of Ribosomal Protein L7/L12 by the Heterobifunctional Cross-Linker 4-(6-Formyl-3-azidophenoxy)butyrimidate†

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ABSTRACT: The structure of the dimeric form of the protein L7/L12 from ribosomes from *Escherichia coli* was studied by using the heterobifunctional cross-linker 4-(6-formyl-3-azidophenoxy)butyrimidate. The imidate group of the cross-linker reacts very specifically with Lys-51 of L7/L12. Subsequent cross-linking of this modified L7/L12 by reductive alkylation of the aldehyde group of the cross-linker results in the formation of a covalent cross-link between both polypeptide chains of the L7/L12 dimer. This covalently cross-linked dimer is fully active in reconstitution of elongation factor G dependent GTP hydrolysis of 50S cores lacking L7/L12, suggesting a conformation of the cross-linked protein similar to the conformation of native L7/L12. Analysis of the tryptic peptides of cross-linked L7/L12 shows the points of attachment of the cross-linker to be Lys-51 in one polypeptide chain and Lys-29 in the other. On the basis of a combination of this result with published data, a structure for the N-terminal region of L7/L12 dimers is proposed. The important feature of this model is a shifted parallel alignment of both polypeptide chains resulting in *one* free N-terminal stretch for each L7/L12 dimer which attaches the protein to the ribosome via protein L10.

During protein synthesis, the ribosome performs a number of discrete functions which are modulated by supernatant protein factors: the initiation–elongation and termination factors. In both prokaryotic and eukaryotic organisms, the acidic proteins from the large subunit are involved in the functioning of many of these factors (Matheson et al., 1980). The acidic proteins L7/L12 of ribosomes from *E. coli* are presently the most thoroughly studied ribosomal proteins. They have identical amino acid sequences, except for the presence of an acetylated amino-terminal serine in L7 (Terhorst et al., 1973). For this reason, they are referred to as a single protein termed L7/L12. The involvement of this protein in the functioning of the ribosome has been reviewed (Möller, 1974).

In order to understand its role during protein biosynthesis, better knowledge of the conformational state of this important protein is required. Physical studies show that in solution and probably also on the ribosome L7/L12 is present as a particle with a highly elongated shape. Besides, it contains as much as 76% α helix (Möller et al., 1972; Luer & Wong, 1979). On the basis of a combination of experimental data and theoretical considerations, two models for the L7/L12 dimer in solution have been proposed. In the model of Gudkov et al. (1977) an antiparallel alignment of the polypeptide chain is present whereas in the model of Luer & Wong (1979) both polypeptide chains are aligned in a parallel fashion.

In a previous paper, it has been shown that the heterobifunctional cross-linker 4-(6-formyl-3-azidophenoxy)butyrimidate (FAPB-imidate)[1] is able to cross-link both polypeptide chains of the L7/L12 dimer in a yield of about 50% (Maassen, 1979). Here, we report on the identification of the points of cross-linking between both polypeptide chains in the covalent L7/L12 dimer. On the basis of this result, combined with other experimental data, we propose a refined model for the N-terminal region of the L7/L12 dimer.

Materials and Methods

[^{3}H]$NaBH_4$ (15 Ci/mmol) and [γ-^{32}P]GTP (10 Ci/mmol) were obtained from Amersham. Trypsin treated with L-1-(tosylamido)-2-phenylethyl chloromethyl ketone was from Serva. Guanidinium chloride was from Pierce and Sequanal grade. The individual ribosomal proteins L7 and L12 and the cross-linker FAPB-imidate were prepared as described (Möller et al., 1972; Maassen, 1979).

Protein was determined according to Lowry et al. (1951). Amino acid analyses were performed on a Beckman Multichrom M amino acid analyzer after hydrolysis of the peptides for 22 h in 6 M HCl and 0.02% 2-mercaptoethanol.

Modification and Cross-Linking. Modification and cross-linking of L7/L12, via reductive alkylation, was done as described (Maassen, 1979). Typically, 10 mg of protein was treated 3 times with 0.3 mg of FAPB-imidate. Care was taken that the degree of modification ranged between 0.5 and 1 mol of cross-linker/mol of monomeric protein (the molar extinction coefficient of FAPB-imidate at 325 nm is 10 500 M^{-1} cm^{-1}).

Cross-linking was induced by reduction with [^{3}H]$NaBH_4$ at pH 8.5 in 50 mM sodium borate–hydrogen chloride.

Isolation of Cross-Linked L7/L12. After the protein mixture was cross-linked, it was dialyzed against 1 M acetic acid and lyophilized. The material was dissolved in a minimal volume (about 300 μL) of 6 M guanidinium chloride and applied on a column of Sephacryl S200 (0.9 × 100 cm) equilibrated with 6 M guanidinium chloride. The column was run at 1 drop/90 s, and fractions of 20 drops (about 0.9 mL) were collected. The absorbance at 280 nm (due to the presence of the reduced cross-linker) and the radioactivity were measured.

The elution profile is shown in Figure 1. Fractions 28–32, containing dimeric L7, were pooled, dialyzed against 1 M acetic acid, and lyophilized.

In order to obtain L7/L12, modified with FAPB-imidate and reduced with [^{3}H]$NaBH_4$ without the occurrence of cross-linking, the reduction was carried out in sodium borate–HCl, pH 8.5, in the presence of 0.1% $NaDodSO_4$. This material will be referred to as ^{3}H-labeled alcohol L7.

Trypic Digestion and Isolation of the Tryptic Peptide. Two milligrams of ^{3}H-labeled alcohol L7 or cross-linked ^{3}H-labeled

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[1] Abbreviations used: EF-G, elongation factor G; EF-Tu, elongation factor Tu; FAPB-imidate, 4-(6-formyl-3-azidophenoxy)butyrimidate; $NaDodSO_4$, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane.

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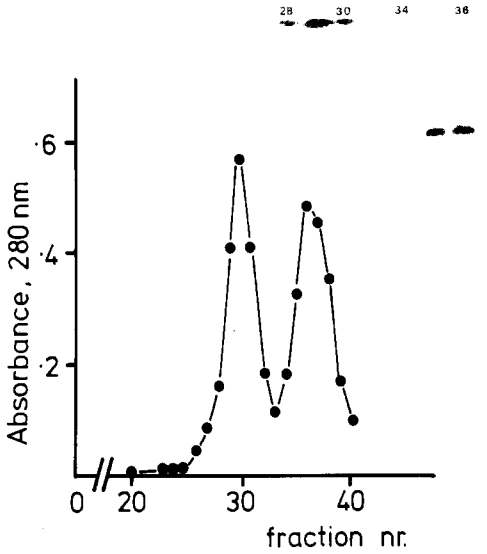


FIGURE 1: Separation of cross-linked and monomeric protein by gel filtration in the presence of 6 M guanidinium chloride. The insert shows the pattern of some of the fractions on $NaDodSO_4$–polyacrylamide gels.

L7 were dissolved in 1 mL of 1% ammonium carbonate and treated twice with 30 μL of trypsin (1 mg/mL in 1 mM HCl) for 1 h at 37 °C. The digest was lyophilized. About 20% of the digest was taken for peptide mapping; the remaining material was used for isolation of the tryptic peptides by gel filtration.

Peptide mapping was done on Whatman 3MM paper. The electrophoresis step was performed by using a Camag high-voltage electrophoresis unit at 60 V/cm for 45 min in pH 4.7 buffer (1.25% pyridine–1.25% acetic acid in water). For the descending chromatography, 1-butanol–acetic acid–water–pyridine (15:3:12:10 v/v) was used. Peptides were stained with ninhydrin. Spots were eluted from the paper with 25% pyridine in water.

Gel filtration of the tryptic digest was done on Sephacryl S200 (0.6 × 220 cm) equilibrated with 6 M guanidium chloride or 0.1% $NaDodSO_4$. The radioactive tryptic peptides were collected and used for amino acid analysis and end-group determination.

The purity of the tryptic peptides was checked by $NaDodSO_4$–polyacrylamide electrophoresis according to Laemmli (1970) by using 15% acrylamide–0.8% *N,N*-methylenebis-(acrylamide) in the separating gel. In case of elution of the peptides out of the gel for amino acid analysis, the gel was destained by using "pro analysis" reagents (25% ethanol–8% acetic acid in water). Bands containing the tryptic peptides were cut out, ground, and shaken for 72 h with 25% pyridine in water.

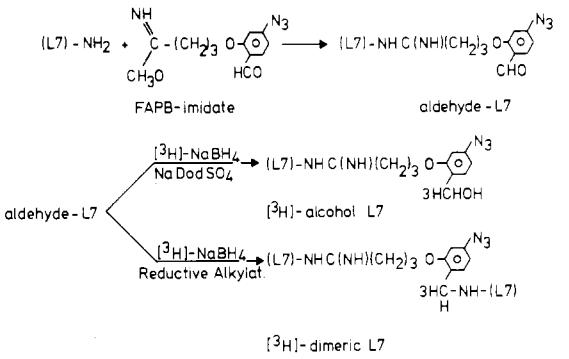
End-group analysis of peptides was carried out via the dansyl procedure (Bruton & Hartley, 1970) or by using a modified Edman degradation (Chang et al., 1978).

EF-G Dependent GTP Hydrolysis. Reconstitution of EF-G dependent GTP hydrolysis of P_0 cores with L7/L12, alcohol L7/L12, or cross-linked L7/L12 was performed as described (Schrier & Möller, 1975).

Results

The principle of cross-linking of L7/L12 with FAPB-imidate is outlined in Scheme I. Figure 1 shows the gel-filtration profile of aldehyde L7 after cross-linking and the analysis by $NaDodSO_4$–gel electrophoresis of the eluate. The molecular

Scheme I: Modification and Cross-Linking of L7



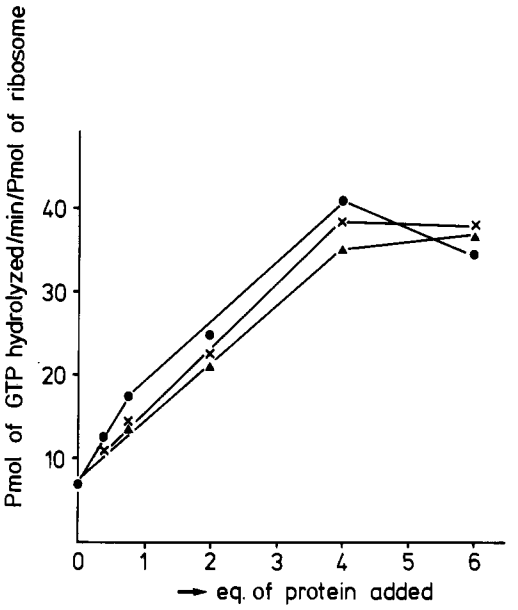


FIGURE 2: Reconstitution of EF-G dependent GTP hydrolysis of 50S cores, lacking L7/L12, with L7 (×) alcohol L7 (▲), and cross-linked L7 (●). Twenty-five picomoles of 50S cores was reconstituted with increasing amounts of L7 or modified L7. One equivalent corresponds to 0.3 μg of protein. GTPase was performed at a concentration of [γ-^{32}P]GTP of 0.5 mM. The activity of 50S ribosomes is 38 pmol of GTP hydrolyzed min^{-1} (pmol of ribosome)$^{-1}$.

weight of the cross-linked material is 26 000, in good agreement with the theoretical value of 2 × 12 200.

Reconstitution experiments were carried out in order to test the activity of cross-linked L7: 50S ribosomal subunits were deprived of L7/L12 by NH_4Cl–ethanol washings (Hamel et al., 1972), and reconstitution of GTPase activity was determined after back-addition of cross-linked L7, alcohol L7 (0.9 mol of cross-linker/mol of monomeric protein), or unmodified L7. Figure 2 shows that the cross-linked material is fully active in restoring GTPase activity, suggesting a structure for cross-linked L7 which is similar to that of non-cross-linked L7 dimers.

In order to get an impression of where the cross-link in the L7/L12 dimer is located, a comparison was made between the tryptic peptide maps of L7, ^{3}H-labeled alcohol L7, L7 being modified with FAPB-imidate to a degree of 0.9 mol of cross-linker/mol of protein and subsequently reduced by [^{3}H]$NaBH_4$ in the presence of $NaDodSO_4$, and ^{3}H-labeled dimeric L7. Peptide maps of ^{3}H-labeled alcohol L7 and ^{3}H-labeled dimeric L7 are shown in Figure 3. Both peptide maps are indentical, and they resemble closely that of unmodified L7 (cf. Terhorst et al., 1972).

Modification of lysine residues with FAPB-imidate abolishes the ability of trypsin to cleave after the modified residue (our

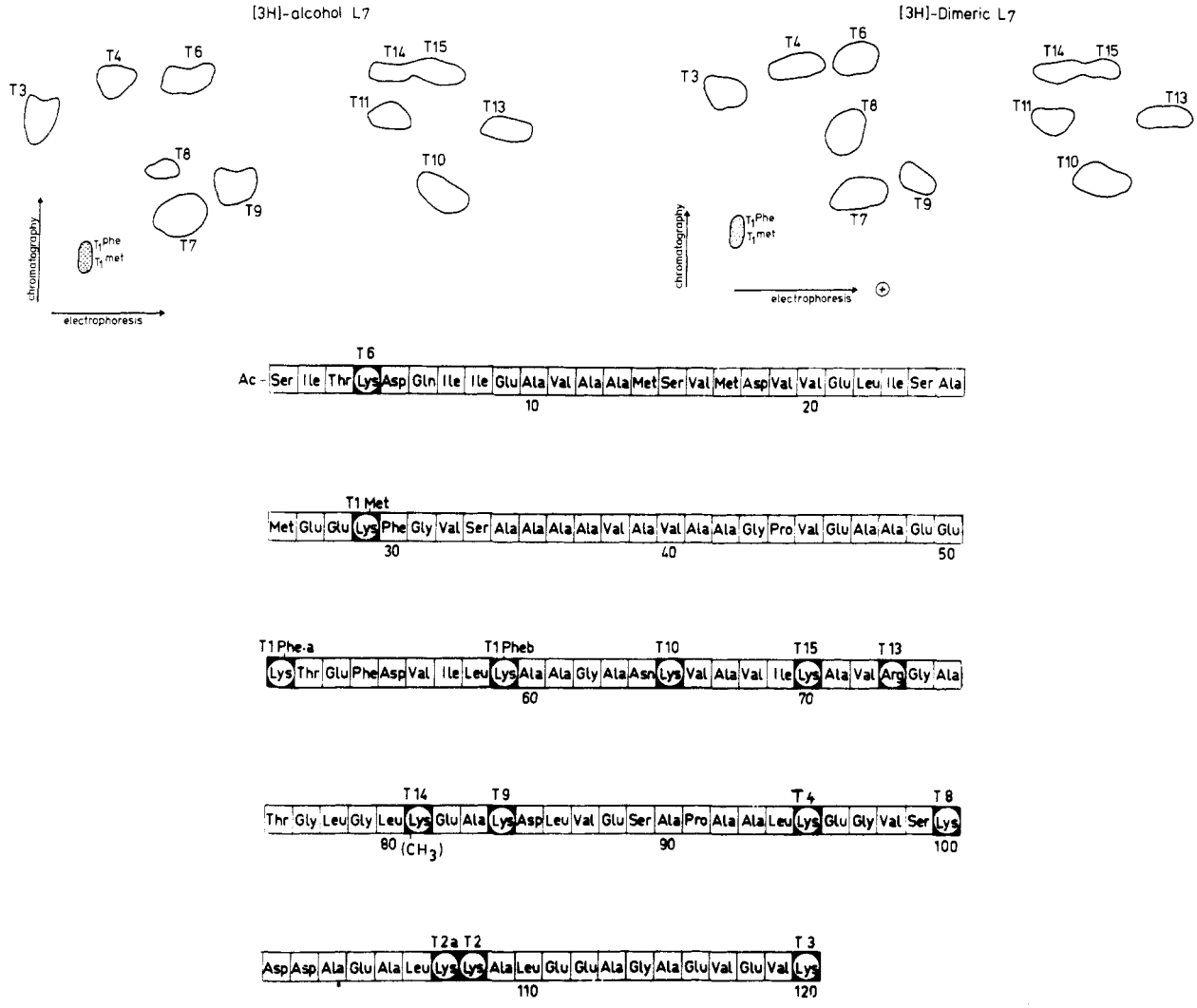


FIGURE 3: (Top) Tryptic peptide maps of ^{3}H-labeled alcohol L7 and ^{3}H-labeled dimeric L7. At least 80% of the applied radioactivity is present in the shaded spots. (Bottom) For comparison, the sequence of L7/L12 is also shown. T11 is composed of T14 + T9 and T7 of T8 + T2. Peptide T8 is only weakly visible.

unpublished observation; cf. for similar situations Allen et al., 1979). The identity of the peptide maps indicates that modification in alcohol L7 and cross-linked L7 as well has occurred at Lys-29 and/or -51. This conclusion is based on the following. Modification at Lys-29 results in the formation of peptide T_1^{Pheb} + T_1^{Met}. This peptide has no mobility on the peptide map (T_1^{Pheb} and T_1^{Met} also stay at the origin). Therefore, modification at Lys-29 does not change the pattern of the mobile peptides. The same holds for modification of Lys-51. Tryptic cleavage at this residue is very sluggish in unmodified L7 (cf. Terhorst et al., 1973), so modification of Lys-51 also does not affect significantly the pattern of the map.

In order to localize the position of the covalent bridge between both polypeptide chains in cross-linked L7 more precisely, the attachment point of the imidate group of FAPB-imidate to L7 was determined in ^{3}H-labeled alcohol L7. To this end, the protein was digested with trypsin, and the tryptic peptides were separated by gel filtration on Sephacryl S200 in the presence of $NaDodSO_4$. The elution profile is shown in Figure 4. $NaDodSO_4$–gel electrophoresis of the fractions containing the radioactivity showed the presence of one radioactive peptide with a mobility identical with that of T_1^{Pheb}. The peptide T_1^{Met} has a much higher mobility on the gel, as shown in Figure 5. It is seen that T_1^{Met} and T_1^{Pheb}, though having similar molecular weights, exhibit a large difference in electrophoretic mobility. Thus, on these gels, the mobility of these highly hydrophobic peptides does not reflect their true molecular weights. However, the behavior of these peptides on gel filtration on Sephadex G-75 in the presence of 6 M guanidinium chloride is in agreement with their molecular weights (not shown).

The amino acid composition of the radioactive peptide is shown in Table I. It is seen that it closely corresponds to the theoretical value of T_1^{Pheb}. Identification of the amino-terminal residue via both the dansyl method (Bruton & Hartley, 1970) and the modified Edman degradation (Chang et al., 1978) gave in both cases phenylalanine as N-terminal residues. L7/L12 contains only two of these residue, phenylalanine at position 30 being the only one after a lysine residue. Therefore the attachment of FAPB-imidate to L7 has taken place at Lys-51.

The other point of cross-linking in dimeric L7 was identified as follows: cross-linked ^{3}H-labeled L7 was digested with trypsin, and again about 80% of the applied radioactivity remained at the origin of the tryptic map. The components of the digest were also separated on Sephacryl S200 in the presence of $NaDodSO_4$ or guanidinium chloride. The elution profile is shown in Figure 4.

It is seen that there are two peaks containing the radioactivity. Analysis of the minor peak (fraction 99) on $NaDodSO_4$–polyacrylamide gels showed several bands with apparent molecular weights close to the starting material. The high molecular weight and the heterogeneity suggest an incomplete

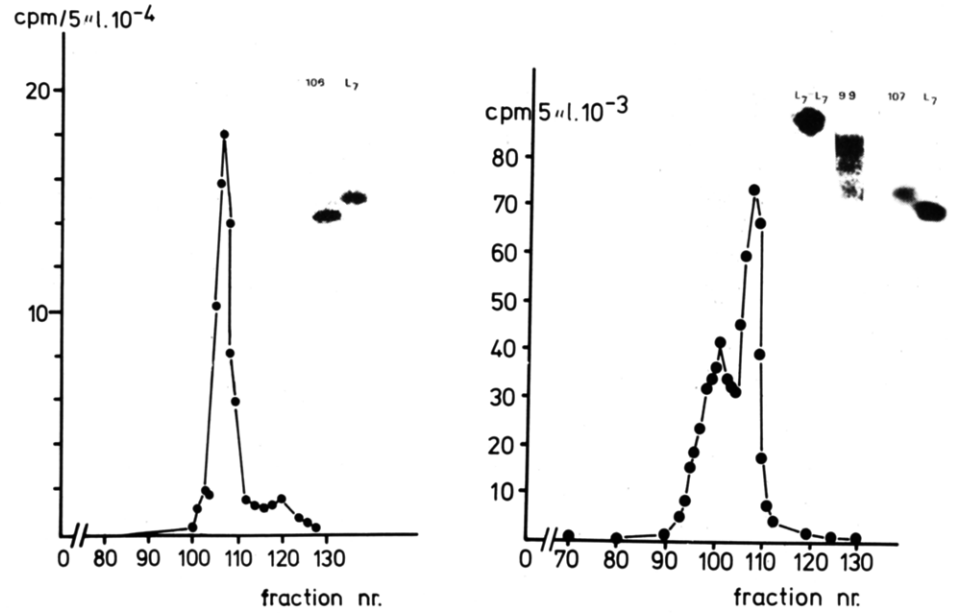


FIGURE 4: Gel-filtration profile of the tryptic digest of ^{3}H-labeled alcohol L7 (left) and dimeric L7 (right), performed in the presence of 0.1% $NaDodSO_4$. The insert shows the analysis of some of the fractions on $NaDodSO_4$–polyacrylamide gels.

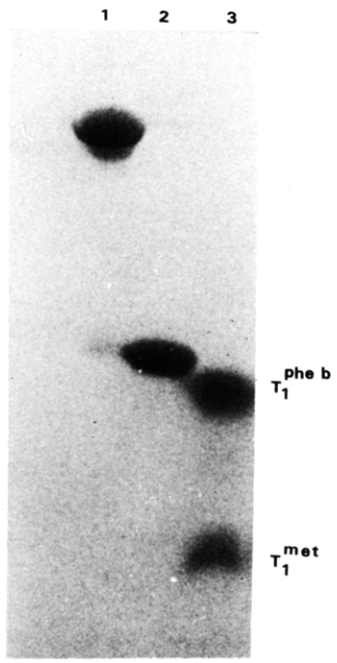


FIGURE 5: Gel electrophoresis on polyacrylamide gels containing $NaDodSO_4$ of cross-linked L7 (lane 1), L7 (lane 2), and a mixture of T_1^{Met} and T_1^{Pheb}. These peptide were prepd. according to Terhorst et al. (1973).

tryptic cleavage. The fraction containing the major amount of radioactivity gave on $NaDodSO_4$–polyacrylamide gels one band. The amino acid composition of this peptide is given in Table I and corresponds to the theoretical value of T_1^{Pheb} + (T_1^{Met} + T_1^{Pheb}). In this context, we would like to mention that T_1^{Met} contains all of the methionine residues present in L7. Due to partial tryptic cleavage at unmodified Lys-51 in the (T_1^{Met} + T_1^{Pheb}) moiety of T_1^{Pheb} + (T_1^{Met} + T_1^{Pheb}), it is likely that T_1^{Pheb} + (T_1^{met} + T_1^{Phea}) is also present, which may explain the somewhat low value for Phe.

Identification of the amino-terminal residues of this peptide was performed also via both the dansyl and modified Edman method, and it showed two amino acids, Phe and Asp, in agreement with the fact that the peptide is composed to T_1^{Pheb} and T_1^{Met} + T_1^{Pheb}. Therefore the cross-linker stretches from Lys-51 in T_1^{Phe} to Lys-29 in T_1^{Met} + T_1^{Pheb}.

Table I: Amino Acid Composition of the Radioactive Tryptic Peptides[a]

amino acid	^{3}H-labeled alcohol L7	theoretical value of T_1^{Pheb}	cross-linked ^{3}H-labeled L7	theoretical value of T_1^{Pheb} + (T_1^{Met} + T_1^{Phe})
Asp	1.2	1	3.8	4 (3)[b]
Thr	1.0	1	2.4	2 (1)
Ser	1.3	1	4.6	4 (4)
Glu	4.2	4	12.5	13 (12)
Pro	1.2	1	1.5	2 (2)
Gly	2.6	2	4.6	4 (4)
Ala	9.5	9	20.7	21 (21)
Val	4.8	5	12.2	14 (13)
Met	0.2	0	2.7	3 (3)
Ile	1.1	1	5.1	5 (4)
Leu	1.8	1	3.9	3 (2)
Phe	1.5	2	3.2	4 (3)
Lys	2.1	2	4.9	5 (4)

[a] Amino acid composition of the radioactive tryptic peptides obtained by digestion of ^{3}H-labeled alcohol L7 and cross-linked ^{3}H-labeled L7 (cf. Figure 4). Values are averages of three independent experiments. [b] Values in parentheses represent the peptide T_1^{Pheb} + (T_1^{Met} + T_1^{Phea})T_1^{Phea} ranging from 30 to 52, due to tryptic cleavage at unmodified Lys-51 in T_1^{Met} + T_1^{Pheb}.

Identical cross-linking results were obtained with L12 instead of L7.

Discussion

It has been clearly demonstrated that protein L7/L12 is present in four copies on the 50S subunit (Subramanian, 1975; Hardy, 1975) and that the strong dimerization in solution (Möller et al., 1972) suggests two L7/L12 dimers on the ribosome. Each dimer is attached to the ribosomal particle via protein L10 (Schrier et al., 1973; Petterson et al., 1976). Recently two models of the structure of the L7/L12 dimer have been proposed. Both models show a highly elongated structure in which the two polypeptide chains are aligned side by side, parallel (Luer & Wong, 1979) or antiparallel (Gudkov, 1977). In the parallel model of Luer and Wong, Lys-51 in one polypeptide chain is located near Lys-51 in the other

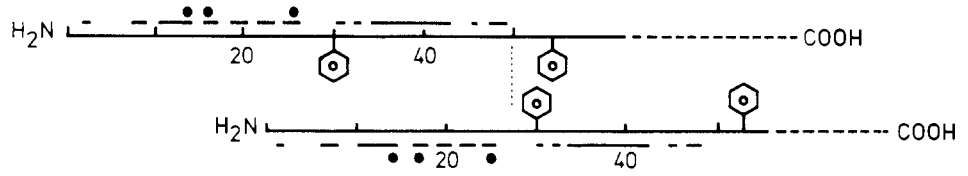


FIGURE 6: Schematic representation of the possible structure of the N-terminal region of L7/L12. The dotted line indicates the position where cross-linking takes place. Hydrophobic amino acid residues are indicated by a solid line. The position of methionine residues is reflected by circles.

chain. The antiparallel model of Gudkov shows a close neighborship between Lys-51 and Lys-4. However, our cross-linking data show that the amino groups of Lys-51 and Lys-29 of both polypeptide chains are located close to each other.

In order to position Lys-51 and Lys-29 near each other, a different alignment of both polypeptide chains is required. On the basis of experimental data, discussed below, we prefer a shifted parallel alignment rather than a shifted antiparallel alignment. In the shifted parallel alignment, each L7/L12 dimer has one free N-terminal region of approximately 20 amino acid residues in length. The structure of the N-terminal region of such a shifted parallel L7/L12 dimer is depicted in Figure 6. The representation of this region of the polypeptide chain by a straight line seems to be justified because this region is almost completely in an α-helical form. When the lengths of the side chains of both lysine residues (2×0.6 nm) and the length of the cross-linker (0.9 nm) are taken into account, the maximum distance between Lys-51 and Lys-29 in the two polypeptide backbones can be 2.1 nm. The value corresponds with four α-helical turns or about 14 amino acid residues. Thus, in principle, the staggering between the two α helixes may range from 36 amino acid residues [i.e., $(51 - 29) + 14$] in one extreme situation to eight amino acid residues [i.e., $(51 - 29) - 14$] in the other extreme. In the first case, Lys-51 in one chain is located opposite to amino acid residue 15 in the other chain, and therefore the nearest lysine residue (Lys-4) is located at a distance corresponding to 11 amino acid residues from Lys-51. In the other case, Lys-51 is located opposite to amino acid residue 43, so that the distance between Lys-51 on one chain and the nearest lysine (Lys-51) on the other chain corresponds to eight amino acid residues. In both extreme cases, the distance between the real cross-linked lysine residues 29 and 51 corresponds to 14 amino acid residues. Since the cross-linker can maximally span a distance corresponding to 14 amino acid residues and because both Lys-51 and Lys-4 are easily modified by reductive alkylation (Amons & Möller, 1974), one would expect to find cross-linking between Lys-51 on one chain and Lys-4 or Lys-51 on the other chain. Experimentally, we have found no indications for significant cross-linking other than between Lys-51 and Lys-29. This makes a shift between both polypeptide chains which is intermediate between both extremes, a more likely situation. Therefore, a staggering of the two α helixes by 20 residues seems to be a reasonable assumption.

As mentioned before, in our model, each L7/L12 dimer possesses one free N-terminal region. In contrast, the models of both Luer and Wong and Gudkov do not show such a feature. The attractiveness of our model is that it agrees with the following observations.

(a) An N-terminal fragment of L7/L12 containing the amino acid residues 1–73 binds strongly to L7/L12 deprived ribosomes whereas the C-terminal fragment (74–120) does not bind at all (Van Agthoven et al., 1975). In addition, fragment 28–120 of L7/L12 also fails in binding to the 50S subunit (Stöffler, 1974; Gudkov & Behlke, 1978). This supports a direct role of the N-terminal part in the attachment of L7/L12 to the 50S subunit via protein L10.

(b) NMR data show that the positions of the phenylalanine resonances in L7/L12 and in an 1–73 N-terminal fragment are different (Gudkov et al., 1978), suggesting that in the N-terminal fragment the region where the phenylalanine residues are located (30 and 54) has another conformation than intact L7/L12. Still, fragment 1–73 binds normally to L10, possibly indicating that indeed the region 1–30, lacking the Phe residues, is involved in binding to L10.

(c) On the ribosome, all of the amino groups of L12 are susceptible for chemical modification with the exception of the amino groups of the N terminus. This has been attributed to an involvement of this part of the protein in the physical interaction between L7/L12 and L10 (Hasnain et al., 1977).

(d) Fluorescence data of L7/L12 are interpreted in a sense that the phenylalanine chromophores from both polypeptide chains interact with each other (Luer & Wong, 1979). In our model, Phe-30 of one polypeptide chain is located close to Phe-54 in the other chain, making a Phe–Phe interaction possible.

(e) Oxidation of the methione residues, located at positions 14, 17, and 26 in L7/L12, results in both a loss of binding of L7/L12 to L10 and a monomerization of the protein (Gudkov & Behlke, 1978; Caldwell et al., 1978). Oxidation makes the very hydrophobic region 14–27 more polar due to conversion of methionine into the sulfoxide and sulfone. In our model, this region is both involved in attachment of L7/L12 to L10 and in dimerization of L7/L12 via interaction with the hydrophobic stretch 30–42. The structure we propose for L7/L12 is based on a combination of various types of experimental data. Future experimental findings will be needed to test whether our model reflects the true in vivo situation of L7/L12 on the ribosome.

Acknowledgments

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Role of the Carbohydrate Moiety in the Antigenic Site(s) of Human Serum Low-Density Lipoprotein[†,‡]

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ABSTRACT: Radioimmunoassay techniques have been used to evaluate the contribution of the carbohydrate moiety to the immunological reactivity of human serum low-density lipoprotein (LDL). Low-density lipoprotein (d = 1.024–1.045 g/mL) was isolated from normolipidemic serum by ultracentrifugal flotation. Radioimmunoassay was performed with ^{125}I-labeled LDL and several homologous antisera, each corresponding to different periods (1–18 weeks) of immunization and thus containing various antibody populations. Unlabeled LDL and different monosaccharides characteristic to this particle, i.e., mannose, sialic acid, glucose, *N*-acetylglucosamine, galactose, *N*-acetylgalactosamine, and fucose, were used as competitors in the binding of the labeled antigen with antibody. In the reaction with antisera corresponding to the highest antibody titer, unlabeled LDL, sialic acid, and mannose inhibited the binding of labeled LDL up to 62%, 25%, and 16%, respectively; a low degree of inhibition (some 13%) was occasionally obtained with glucose. Galactose, galactosamine, glucosamine, and fucose failed to compete with labeled LDL. Studies with antisera corresponding to different periods of immunization (2, 4, and 8 weeks) indicated that antibodies reacting with mannose appeared early (maximum 31% inhibition at 2 weeks), disappearing at 6–8 weeks; in contrast, antibodies reacting with sialic acid augmented progressively (10% inhibition at 2 weeks, 20% at 4 weeks, and 35% at the end of the immunization). These data are consistent with the conclusion that sialic acid and mannose, the terminal residues of LDL glycopeptides I and II [Swaminathan, N., & Aladjem, F. (1976) *Biochemistry 15*, 1516–1521], are implicated in the antigenic site(s) of LDL.

The nature of the carbohydrate moiety of serum low-density lipoprotein is incompletely known, although several structural studies were described in the early sixties (Schultze & Heide, 1960; Ayrault-Jarrier, 1961; Marshall & Kummerov, 1962), and a renewal of interest has occurred in recent years (Kwiterovich et al., 1974; McConathy & Alaupovic, 1974; Swaminathan & Aladjem, 1976; Dawson et al., 1976; Chatterjee & Kwiterovich, 1976).

The carbohydrate components bound to the protein moiety of LDL[1] amount to 8–10% of its dry weight and are essentially mannose (4.8%), galactose (2.1%), sialic acid (1.7%), and glucosamine (0.9%) (Swaminathan & Aladjem, 1976). The latter authors succeeded in the separation and structural characterization of two types of glycopeptides, with sialic acid and mannose, respectively, as terminal residues and which together represented some 50% of the carbohydrate moiety of LDL.

Several monosaccharides are also present in the glycolipid fraction of LDL; they amount to ~10 μmol of glucose/g of phospholipid and are principally glucose, galactose, galactosamine, and sialic acid (Chatterjee & Kwiterovich, 1976; Dawson et al., 1976). In addition, Marcus & Cass (1969) have reported the presence of fucose and glucosamine in certain LDL glycosphingolipids apparently related to Lewis blood activity.

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[1] Abbreviations used: LDL, low-density lipoproteins of density 1.024–1.045 g/mL; Apo-B, apolipoprotein B; NANA, *N*-acetylneuraminic acid; Man, mannose; Fuc, fucose; Glu, glucose; Gal, galactose; GluNAc, *N*-acetylglucosamine; GalNAc, *N*-acetylgalactosamine; ID, immunodiffusion; IEP, immunoelectrophoresis; RIA, radioimmunoassay; $NaDodSO_4$, sodium dodecyl sulfate; Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol; EDTA, ethylenediaminetetraacetic acid.

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