MOLECULAR MORPHOLOGY OF RIBOSOMES: STRUCTURAL ALTERATION OF 50S SUBUNITS FOLLOWING THE REMOVAL OF PROTEINS L_7 AND L_{12}

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1. Introduction

Ribosomal proteins L7 and L12 are involved in the initiation, elongation and termination of polypeptide synthesis [1–6]. These acidic proteins do not function independently, but act in concert with the ribosome to perform the above steps. These observations suggest that L7 and L12 are necessary to maintain the conformational integrity of the ribosome, and that loss of ribosomal activity following the removal of L7 and L12 results from a structural alteration of the large subunit. The present investigation is an attempt to determine whether L7 and L12 are involved in preserving the structural conformation of the 50S subunit.

Lactoperoxidase-catalyzed iodination of ribosomal proteins has been utilized as a probe to study ribosome structure [7–10]. Although this probe was originally designed to investigate the surface topography of ribosomes, it has recently been employed to detect conformational alterations of ribosomal subunits accompanying changes in ionic strength, temperature and isolation conditions [11–13].

To determine whether structural alterations of the 50S subunit occur following the removal of L7 and L12 from the ribosome, we have again used the lactoperoxidase-catalyzed iodination system. Investigations have been designed to detect changes in reactivity of individual ribosomal proteins following the removal and readdition of L7 and L12 as measured by enzymic iodination.

2. Materials and methods

2.1. *Materials*

E. coli MRE-600, RNase-I-less was used throughout these studies. Growth conditions, labelling of r-proteins with [^{3}H]Tyrosine, ribosome preparations, lactoperoxidase-catalyzed iodination and extraction of r-proteins were as previously described [7,11,13–15].

2.2. *Two-dimensional electrophoresis*

Two-dimensional polyacrylamide electrophoresis was performed according to the method of Kaltschmidt and Wittmann [16]. Individual r-proteins were removed from the gel slabs and prepared for liquid scintillation counting as previously described [7,11,13].

2.3. *Ethanol–NH_4Cl extraction of 50S subunits*

L7 and L12 were extracted from the 50S subunits in the presence of 1 M NH_4Cl and 40% ethanol at 0°C. Precipitated subunits were collected by centrifugation at 15 000 *g* for 15 min. Pellets were resuspended in buffer containing 10 mM Tris, pH 4.7, 10 mM Magnesium acetate 30 mM NH_4Cl and retained for iodination. All protein measurements were made using the method of Groves et al. [15].

2.4. *G-Factor-Dependent GTPase Activity*

G-factor activity was determined by measuring ribosome dependent GTPase hydrolysis of [γ-^{32}P]-GTP. Reaction mixtures contained per 250 μl: 48 pmol 30S ribosomes, 16 pmol 50S ribosomes, 4 μg

purified G-factor, 50 mM Tris–HCl buffer, pH 7.9, 160 mM NH_4Cl, 10 mM $MgCl_2$, 1 mM dithiothreitol and 20 mM [γ-^{32}P] GTP. The [γ-^{32}P] GTP was added following a 5 min preincubation and the reaction was allowed to proceed for 10 min at 30°C. Inorganic [^{32}P] phosphate was isolated according to the method of Nishizuki et al. [17] and prepared for counting by the addition of 1.0 ml of Protosol and 10 ml of toluene-omnifluor.

2.5. *Sedimentation analysis*

Samples were analyzed in a Beckman Model E analtyical ultracentrifuge at 48 000 rev/min and 20°C using schlieren optics. Ribosome concentrations were 2.5–3.0 mg/ml. Sedimentation constants were corrected for solvent properties and are expressed as $S_{20,w}$.

3. Results

3.1. *Influence of removal of L7 and L12 upon iodination of 50S subunit proteins*

To determine the influence of L7 and L12 upon the reactivity of 50S subunits to enzymic iodination, the effect of their removal upon the protein iodination pattern was required. The 50S tritiated subunits were treated as described in the Methods section, to remove L7 and L12. The resultant particles were then iodinated enzymatically in the presence of ^{131}I, pelleted, resuspended in buffer and the proteins extracted. Following two-dimensional gel electrophoresis, the protein spots were removed from the gels and their ^{131}I/^{3}H ratios determined. A comparison of the normalized ratios of ^{131}I/^{3}H of the ribosomal proteins from intact and treated 50S ribosomes is outlined in table 1.

These data indicate that the normalized ratios of the majority of ribosomal proteins do not change significantly following extraction of L7 and L12. However, proteins L2, L6, L8, L10 and L11 become more susceptible to enzymatic iodination as indicated by the considerable increase in their normalized ratios. Proteins L27, L29 and L30, on the other hand, appear less susceptible to iodination under the same conditions, when compared to those of untreated ribosomes. When L7 and L12 are added to subunits from which L7 and L12 previously removed, the normalized ratios of the individual ribosomal proteins are essentially the same as those observed for untreated ribosomes.

Table 1

Isotope content (normalized ratios of ^{131}I/^{3}H) of 30S and 50S ribosomal protein from exponentially growing cells incubated for three generations with [^{3}H] tyrosine and iodinated with Na^{131}I in the presence of soluble lactoperoxidase. The mean (^{131}I/^{3}H) ratio was calculated and the normalized ratios determined by dividing the appropriate mean (^{131}I/^{3}H) ratio into the individual (^{131}I/^{3}H) ratio

	Normalized ratios of ^{131}I/^{3}H for 50S r-proteins		
Protein	Untreated subunits	Extracted subunits (–L7, –L12)	Extracted subunits (+L7, +L12)[a]
L1	0.11	0.14	0.12
L2	1.09 (± 0.10)[b]	1.44 (± 0.16)	1.35 (± 0.12)
L3	0.33	0.29	0.29
L4	0.29	0.31	0.28
L5	2.16	2.36	2.09
L6	0.41 (± 0.14)	0.85 (± 0.27)	0.49 (± 0.09)
L8	0.97 (± 0.16)	1.75 (± 0.20)	0.91 (± 0.22)
L9	0.49	0.56	0.46
L10	1.80 (± 0.28)	2.69 (± 0.31)	1.89 (± 0.13)
L11	1.56 (± 0.37)	2.29 (± 0.27)	1.68 (± 0.29)
L13	0.28	0.28	0.23
L14	0.30	0.30	0.32
L15	0.72	0.79	0.77
L16	0.79	0.90	0.85
L17	0.32	0.38	0.35
L18	0.18	0.20	0.22
L19	0.14	0.17	0.18
L21	0.20	0.23	0.23
L22	0.38	0.42	0.44
L23	0.25	0.26	0.27
L24	1.56	1.36	1.44
L25	0.27	0.33	0.31
L26	2.47	2.26	2.41
L27	3.26 (± 0.30)	2.36 (± 0.09)	3.06 (± 0.34)
L28	1.08	0.93	1.06
L29	3.58 (± 1.22)	2.67 (± 0.91)	3.83 (± 1.1)
L30	3.56 (± 0.93)	2.51 (± 1.09)	3.67 (± 0.87)
L32	0.22	0.25	0.30
L33	0.06	0.08	0.07

[a] Purified L7 and L12 were added in a 4 molar excess to the extracted ribosomes and the preparation incubated for 15 min at 30°C prior to iodination.

[b] Results based on at least four experiments. Standard deviation included for those r-proteins whose normalized ratios changed significantly after L7 and L12 extraction and reconstitution.

Table 2
G-factor dependent GTPase activity

Additions	pmoles of GTP Hydrolyzed	% Activity
1. Untreated 50S ribosomes	31.9	100
2. Extracted 50S ribosomes	0.3	3
3. Extracted 50S ribosomes and L7 and L12	29.2	91

Reactions conditions were as described in Materials and methods. Where indicated, 48 pmol of L7 and L12 were added to extracted 50S ribosomes and the preparation incubated at 30°C for 10 min prior to the assay.

3.2. *G-Factor dependent GTPase activity of ribosomes*

To determine whether the biological activity of the ribosome preparations was affected by removal of proteins L7 and L12 and their restoration to the extracted particles, G-factor dependent GTPase activity was determined for the preparations used for the iodination experiments. As indicated in table 2, although the extracted ribosomes were almost completely inactive, at least 90% of the activity was restored following the addition of L7 and L12.

3.3. *Sedimentation analysis of 50S subunits*

Ribosome preparations used in the iodination experiments were subjected to sedimentation analysis in the Model E ultracentrifuge. As indicated in table 3, the $S_{20,w}$ values for untreated, extracted and L7 and L12 reconstituted 50S ribosomes were found to be constant. These studies indicate that conformational changes, if they occur, are not extensive following removal of L7 and L12. In combination with the iodination data, the results suggest only a localized alteration.

Table 3
Sedimentation coefficients for 50S ribosomes

	Untreated 50S	Extracted 50S	Extracted 50S (+L7, L12)
$S_{20,w}$	49.46	50.08	50.13

4. Discussion

The data obtained from enzymic iodination suggest that a structural alternation of the 50S subunits occurs following the removal of L7 and L12. Extensive conformational changes, such as unfolding, have been shown to significantly alter the sedimentation characteristics [18] and the susceptibility of individual ribosomal proteins to enzymatic iodination [7,11]. Since no difference in the sedimentation behavior was observed and relatively few ribosomal proteins were significantly modified by enzymatic iodination following the extraction of L7 and L12, the structural alteration in the large subunit may be a localized one.

In comparing the iodination patterns of the individual ribosomal proteins of untreated 50S ribosomes to those devoid of L7 and L12, it is observed that the normalized ratios of L2, L6, L8, L10 and L11 increase from 32 to 107%. One interpretation of this observation is that the above proteins are located in close proximity to L7 and L12 on the ribosome and that removal of L7 and L12 results in unmasking and increased exposure of proteins L2, L6, L8, L10 and L11 to enzymatic iodination. On the other hand, proteins L27, L29 and L30 become iodinated to a lesser degree. These results imply that the loss of L7 and L12 may also produce a localized conformational change in the 50S subunit.

Recent studies have demonstrated that proteins L2, L6, L8, L10, L11, L27 and L30 are structurally and/or functionally related. Affinity labelling experiments utilizing analogs of puromycin revealed that proteins L2 and L6 are in the neighborhood of the ribosomal A-site of the peptidyl transferase center [1,9]. L2 and L27 were concluded to be part of the peptidyl t-RNA binding site (P-site) based on affinity labelling experiments with an analog of natural initiator t-RNA [20,21]. Crosslinking experiments with dimethyl suberimidate indicate that proteins L8, L9, L10 and L11 occupy neighboring sites on the ribosome [22]. Peptidyl transferase activity of the 50S subunit has been linked to protein L11 [23] and reconstitution experiments have shown that L6 stimulates peptidyl transferase activity [24].

In addition to initiation and termination [3–5], L7 and L12 are necessary for both EF-G and EF-Tu dependent functions [1,2,6]. Cross-linking experi-

ments have revealed that L7 and L12 are located at the EF-G binding site [25]. Antibody and reconstitution studies have also demonstrated that L6 and L10 are important for EF-G dependent GTP hydrolysis [26,27]. Affinity labelling [28] studies revealed L11 and L30 to be involved in GDP binding and reconstitution experiments have also shown L30 to bind to 5S RNA which is important for ribosomal functions linked to the A-site [29,30]. Furthermore, Highland and Howard [31] have presented evidence suggesting that L11 is necessary for the binding of L10 which in turn is required for L7 and L12 binding.

The data presented in this communication suggest several possibilities with respect to ribosome structure and function. Since L7 and L12 are not active as isolated proteins but are required together with the 50S subunit to restore EF-G and EF-Tu dependent functions, these two proteins appear necessary for both the structural integrity and biological activity of the ribosome. This postulate is substantiated by the apparent conformational change which occurs in the 50S subunit following removal of L7 and L12 and restoration to its original state upon addition of L7 and L12 to the 'core' particle. That the original conformation is restored is indicated by the iodination patterns of the individual ribosomal proteins. The ribosomal proteins altered as measured by enzymatic iodination (L2, L6, L8, L10, L11, L17, L29 and L30) appear to be structurally and/or functionally related. It is, therefore, suggested that L7 and L12 are located in proximity to the above proteins. In a recent review, Pongs et al. [32] proposed a scheme of the functional arrangement of components in the active sites on *E. coli* ribosomal subunits and suggested that the A-site, P-site and GTPase centers are in close proximity on the large subunit. Based on the results presented here, we further suggest that L7 and L12 and their binding sites are in close proximity to the above functional sites.

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