ACTIVITIES OF RIBOSOMAL CORES DEPRIVED OF PROTEINS L7, L10, L11 AND L12

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1. Introduction

The protein L11 of the large ribosomal subunit from *Escherichia coli* has been reported to be involved in the peptidyl transferase activity of the ribosome [1]. Thus protein L11 was postulated as either a part of the active center or even as the actual peptidyl transferase itself [2].

Recently Highland and Howard [3] have described the preparation of 50S subunit derived core particles deprived of proteins L7, L10, L11 and L12 following basically the method of Hamel et al. [4]. Because of the low number of proteins separated in the treatment, these particles are very useful for studies on the role of the split proteins on the ribosomal activities.

We have investigated the role of protein L11 on the activities of the peptidyl transferase centre using the L11-deprived cores and the results are reported in this communication.

2. Materials and methods

2.1. *Ribosomes and ribosomal cores*

Ribosomes, prepared from log phase *Escherichia coli* strain B or D10 by alumina grinding, were washed 5–6 times with 20 mM Tris–HCl buffer, pH 7.4, containing 1 M NH_4Cl and 40 mM Mg acetate [5]. Ribosome subunits were prepared in a zonal rotor by centrifugation using isokinetic sucrose gradients.

Ribosome- and 50S-derived cores were obtained respectively from ribosomes and 50S subunits by treatment with 1 M NH_4Cl in the presence of ethanol as previously described [4]. The only deviation in our method from that previously described was that we finally dissolved the core precipitate in 10 mM Tris–HCl buffer, pH 7.4, containing 50 mM NH_4Cl, 10 mM $MgCl_2$, 1 mM 2-mercaptoethanol and 50% glycerol. For our cores we have followed the nomenclature of Highland and Howard [3]. The P_0 cores and split protein fraction SP_0 (either 70S ribosome or 50S cores) were obtained when the saltethanol treatment was carried out at 0°C. P_{37} cores and split protein fraction SP_{37} were resulting from the direct treatment at 37°C, whereas P_{0-37} cores and split protein fraction SP_{0-37} were obtained by the salt–ethanol treatment first at 0°C and finally at 37°C. All our core preparations were stored at −20°C when not immediately used.

2.2. *Other methods*

The peptidyl transferase activity was studied following the fragment reaction assay [6,7]. Conditions for polyphenylalanine synthesis and EF G-dependent GTPase activity are described in the text. Two-dimensional polyacrylamide gel electrophoresis of the ribosomal proteins was carried out as described [8]. Binding of antibiotics was measured in the presence of 33% ethanol at 0°C as described [9,10].

2.3. *Materials*

CACCA–[^{3}H]Leu–Ac (5.05 Ci/mmol) was prepared by RNAase T_1 treatment of *N*–acetyl–leucyl–tRNA as described elsewhere [11]. [γ-^{32}P]GTP was obtained following the method of Glynn and Chappell [12]. EF G was prepared as described [13]. [^{14}C]Phe–tRNA was prepared by charging batch tRNA (General Biochemicals) with [^{14}C]phenylalanine (513 mCi/mmol). All the radioactive materials were obtained from the Radiochemical Center, Amersham, England.

Source of methyl-[^{14}C]chloramphenicol (10.2 mCi/mmol), *N*-methyl-[^{14}C]lincomycin (4.7 mCi/mmol) and

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Table 1
Activity of NH_4Cl–ethanol particles in polyphenylalanine synthesis and EF G-dependent GTP hydrolysis

Particle	Addition of split protein fraction	Polyphenylalanine synthesis mol/ribosome	GTP hydrolysis mol/ribosome
50S	–	0.36	67.1
50S-P_0	–	0.01	2.0
50S-P_0	50S-SP_0	0.43	49.2
50S-P_{0-37}	–	0.00	1.4
50S-P_{0-37}	50S-SP_{0-37}	0.02	1.7
50S-P_{0-37}	50S-SP_0+50S-SP_{0-37}	0.30	44.0
50S-P_{0-37}	50S-SP_{37}	0.27	48.3
50S-P_{37}	–	0.00	0.6
50S-P_{37}	50S-SP_{37}	0.39	52.1
50S-P_{37}	50S-SP_0+50S-SP_{0-37}	0.43	54.4

The particles were preincubated with the indicated split protein fractions at 37°C for 30 min in 40 mM Tris–HCl buffer, pH 7.8, containing 200 mM NH_4Cl, 20 mM $MgCl_2$ and 2 mM 2-mercaptoethanol. The ionic conditions in the activity assays were 20 mM Tris–HCl buffer, pH 7.8 containing 100 mM NH_4Cl, 10 mM $MgCl_2$ and 1 mM 2-mercaptoethanol. The reactions were carried out in 0.05 ml volumes containing 0.200 mg/ml 50S or their derived particles, 0.1 mg/ml 30S ribosomes, 0.1 mg/ml poly(U), 0.3 μM [^{14}C]phenylalaninyl-tRNA, 2 mM GTP and 5 μl of S-100 supernatant in the polyphenylalanine synthesis assay and 0.5 mg/ml ribosomes, 100 μM [γ-^{32}P]GTP, 0.02 mg/ml EF G in the EF G-dependent GTPase assay. Incubation was carried out at 30°C for 5 min for the GTPase assay and 20 min for polyphenylalanine synthesis.

Table 2
Peptidyl transferase activity of 50S- and ribosomal-derived cores obtained by NH_4Cl–ethanol treatment

Particles	mmol Ac-Leu-tRNA fragment reacted/mol ribosomes		
	50S subunits *E. coli* D10	70S ribosomes *E. coli* B	50S subunits *E. coli* B
Control particles	–	–	20.1
P_0 cores	14.1	21.8	20.3
P_{0-37} cores	13.1	20.8	18.4
P_{37} cores	12.4	22.8	19.1

Peptidyl transferase activity was tested by the fragment reaction as previously described [6,7] using 1 mg/ml of ribosomes in the reaction mixture.

Table 3
Binding of radioactive antibiotics to ribosomal particles

Particles	[^{14}C]chloramphenicol	[^{14}C]lincomycin	[^{14}C]erythromycin
	cpm bound	cpm bound	cpm bound
50S	668	259	316
30S	0	61	0
50S-P_0	629	238	220
50S-P_{0-37}	625	250	339

The particles were obtained from *E. coli* D10. The binding assays were carried out as described in section 2.2 using 3 mg/ml of ribosomes and 10^{-6} M of antibiotic.

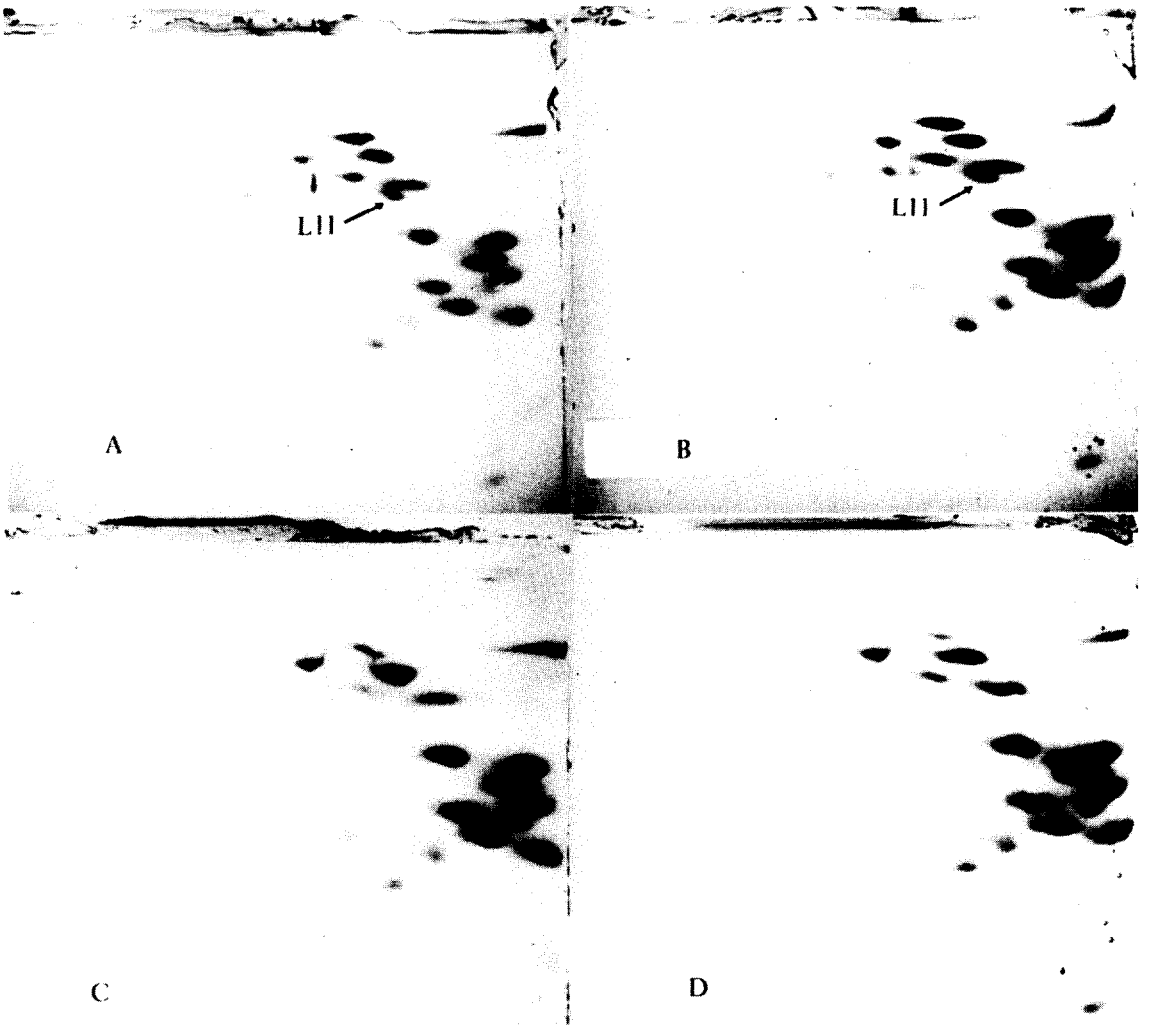

Fig. 1. Two-dimensional polyacrylamide gel electrophoresis of proteins extracted from: a) Control 50S subunits; b) 50S-P_0; c) 50S-P_{0-37} and d) 50S-P_{37}.

N-methyl-[^{14}C]erythromycin (5.1 mCi/mmol) were previously indicated [10].

3. Results

3.1. *Polyphenylalanine synthesis and EF G-dependent GTP hydrolysis*

All our 50S-derived cores require reassociation with the pertinent split protein fraction to reconstitute the 50S subunit completely in order to recover either the EF G-dependent GTPase activity or their capacity to synthesise polyphenylalanine when complemented with 30S subunits (table 1). From the functional point of view the 50S-P_{37} and the 50S-P_{0-37} cores are very similar since they are cross-reactivated with their split protein fractions.

3.2. *Peptidyl transferase activity*

All the different core preparations from either ribosomes or 50S subunits from *E. coli* B are active for peptide bond formation in the fragment reaction assay (table 2). 50S-derived cores from *E. coli* D10 were also tested and found active in the same assay (table 2).

3.3. *Binding of antibiotics*

Ribosomal subunits as well as core preparations derived from *E. coli* D10 were tested for their activity in binding different radioactive antibiotics known to interact with the peptidyl transferase center of the ribosome [8]. As it is shown in table 3 the ribosomal cores are able to bind as much drug as the control 50S subunit.

3.4. *Protein composition of the 50S-derived cores*

The proteins of the 50S-derived cores were resolved by two-dimensional gel electrophoresis. The times of the electrophoresis in some cases were longer than in the standard conditions in order to obtain a better resolution in the region of the gels in which differences might possibly be observed. Therefore some basic proteins which are present in all our cores are not shown in fig. 1. It is shown that 50S-P_0 cores lack proteins L7 and L12 (fig. 1b) when their ribosomal pattern is compared with that of the control 50S subunits (fig. 1a). On the other hand the protein patterns of our 50S-P_{37} (fig. 1c) and 50S-P_{0-37} (fig. 1d) cores are indistinguishable; both totally lacking of proteins L7, L10, L11 and L12 and showing weaker spots than the control for proteins L1, L5 and L8/9.

4. Discussion

It has been reported by Nierhaus and Montejo [1] that protein L11 is required for reconstitution of the peptidyl transferase activity of ribosomal cores prepared by 0.8 M LiCl treatment. We were therefore rather surprised to observe that our 50S-P_{37} and 50S-P_{0-37} cores which are devoid of protein L11 are fully active in the fragment reaction assay (table 2). These apparently conflicting results, can be easily explained if protein L11 is required by the 0.8 M LiCl cores (lacking many other proteins) to get the correct ribosomal conformation in order to express the peptidyl transferase activity. On the other hand, the 50S-P_{37} and 50S-P_{0-37} cores (which have 29 of the 33 proteins of the 50S subunit) might have the adequate conformation for the peptidyl transferase activity to be expressed. It is obvious from our results that protein L11 by itself is neither the peptidyl transferase nor the peptidyl transferase centre. These results emphasise that caution should be taken in the interpretation of results when identifying the proteins involved in some ribosomal functions. Thus a protein (L11) which is required for a function in some cores [1] is not required for the same function with other cores (table 2).

The results presented in this work clearly show that our 50S-P_{37} and 50S-P_{0-37} cores are identical in their functional proportion and protein patterns. We have no obvious explanation at the present time for the difference between our 50S-P_{37} cores (lacking proteins L7, L10, L11 and L12) and those cores obtained by Highland and Howard [3] (lacking of proteins L7, L10 and L12) following apparently the same or very similar method. The slight differences observed might be indeed due to small differences in the bacterial strain and in the storage of either the bacteria or the ribosomes. Indeed differences in the protein pattern of 2 M LiCl-treated ribosomes from the same bacterial strain have been obtained by different workers [14,15]. However it is important to note that similar results were obtained when studying the role of protein L11 in the peptidyl transferase activity using the cores of

Highland and Howard [3] as shown by Howard and Gordon [16] in independent experiments.

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