PEPTIDYL TRANSFERASE ACTIVITY OF RIBOSOMAL PARTICLES LACKING PROTEIN L11

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1. Introduction

Thiostrepton and the related antibiotic siomycin do not inhibit the peptidyl transferase reaction catalyzed by *E. coli* ribosomes [1 review]. Further, we have recently shown that the actual binding of thiostrepton to the ribosomes is dependent on the presence of protein L11 [2]. It has also been reported that protein L11 is involved in the peptidyl transferase active site [3]. If this is the case, then thiostrepton may bind to protein L11 without affecting its peptidyl transferase function. Alternatively, protein L11 may not be absolutely required for the peptidyl transferase catalytic activity.

We have recently reported on a procedure to prepare *E. coli* 50S ribosomal particles lacking only proteins L7/L12, L10 and L11 [4]. It thus seemed possible to test for the necessity of these particular proteins in the peptidyl transferase reaction. The results of these experiments are reported below.

2. Experimental

2.1. *Materials*

E. coli ribosomes and subunits were prepared as previously reported [5]. 50S ribosomal subunit core particles and split proteins were prepared by the NH_4Cl–ethanol procedure of Hamel et al. [6], as recently described [4]. Since the P_{0-37} particles described still contained (in some instances) a small but detectable amount of protein L11 [4], we slightly modified the procedure by treating the 50S subunits a second time at 37°C with 1 M NH_4Cl–50% ethanol. In keeping with our previous designations [4], this

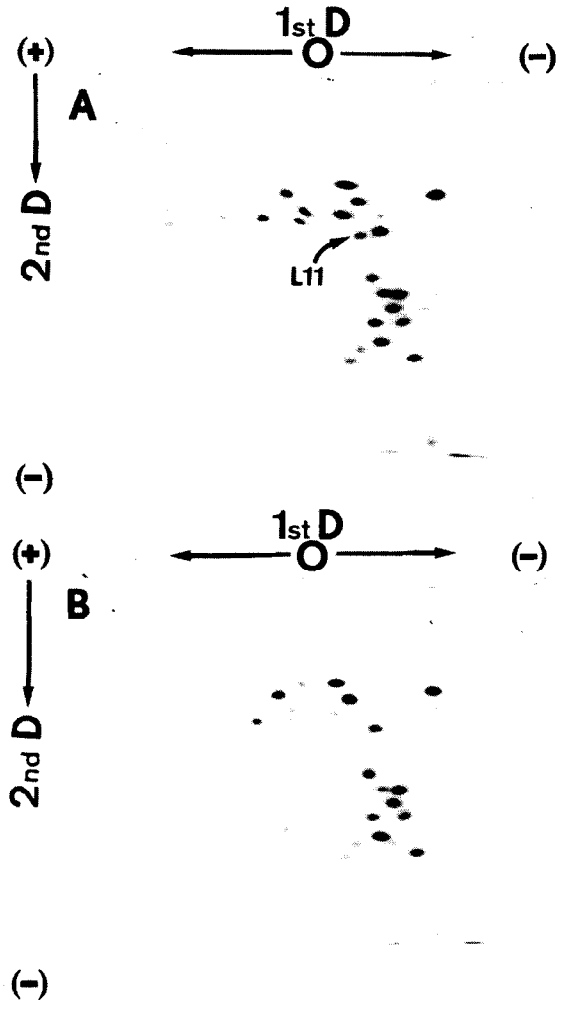

Fig. 1. Two-dimensional gel electropherogram of *E. coli* ribosomal proteins. Proteins were from (A) 50S subunits and (B) $P_{0-37-37}$ core particles.

particle is referred to as a $P_{0-37-37}$ core. It has no trace of protein L11 (fig. 1).

2.2. *Two-dimensional electrophoresis*

Total protein from 50S ribosomal particles was prepared by a simplification [7] of the LiCl–urea procedure [8], and separated via two-dimensional polyacrylamide gel electrophoresis as previously described [9], with minor modifications [10]. See the legend to fig. 1 for details of the electrophoresis conditions.

2.3. *Assay conditions*

CACCA–[^{3}H]Leu-Ac (approx. 1000 cpm/0.15 ml reaction mix. spec. act. approx. 5.05 Ci/mmol) was a generous gift of Dr J. Ballesta and was prepared as described elsewhere [11]. The binding of this tRNA fragment to ribosomes in the presence of 50% ethanol was assayed as described elsewhere [12]. Refer to the legend to table 1 for specific incubation conditions used here. The 'fragment reaction' or actual transfer of Ac–Leu from the tRNA fragment to puromycin, catalyzed by ribosomes in the presence of 30% methanol, was assayed basically as described elsewhere [11,13]. Refer to the table and figure legends for the experimental details in each case.

Table 1
Binding of CACCA–Leu–Ac to ribosomal particles

Particle	% of control
70S (control)	–
50S	100.0
P_0	104.0
P_{37}	85.0
P_{0-37}	99.0
$P_{0-37-37}$	98.6

Assays were incubated at 0°C for 30 min as described [12]. The activity was determined by taking the difference between available counts and those remaining in the supernatant after the ribosome. CACCA–Leu–Ac complex was formed and removed by centrifugation at 50 000 rev/min, 3 hr in a Beckman Ti 50 rotor. All assays were pre-incubated at 37°C for 5 min before addition of the ethanol. All ribosomal particles were present at 15 A_{260}/ml. Refer to Highland and Howard [4] for core particle nomenclature: P_0 lack proteins L7/L12; P_{37} lack L7/L12, L10; P_{0-37} and $P_{0-37-37}$ lack L7/L12, L10, L11. The control (100%) represents 44.8 cpm/pmol ribosomes.

3. Results

A necessary first step in the peptidyl transferase reaction as assayed here is the binding of the aminoacyl tRNA analogue CACCA–Leu–Ac, to the ribosomes before the actual peptide transfer can take place. We thus tested the ability of various protein-depleted 50S ribosomal particles for their ability to bind the fragment. The $P_{0-37-37}$ cores, totally lacking protein L11 as judged by their two-dimensional electrophoresis pattern (fig. 1), have essentially the same ability to bind the tRNA fragment as do ribosomal particles with more proteins present (P_{37} and P_0 particles) or even intact 50S subunits, as can be seen by the results shown in table 1.

Since the various ribosomal particles can thus bind the fragment, the next obvious question is whether or not they are capable of effecting the peptidyl transferase reaction. The ribosomal core particles as well as untreated 50S subunits were assayed for this activity under the standard conditions [12] and the results are shown in table 2. Not only can ribosomal core particles lacking proteins L7, L12 and L10 (P_0 and P_{37} particles) fully catalyze the reaction as well as untreated 50S subunits, but most important the $P_{0-37-37}$ ribosomal particles completely lacking protein L11 can also effect the Ac–Leu transfer to puromycin. If a very minute amount of protein L11 remained on the $P_{0-37-37}$ core particles, possibly undetected by our two-dimensional gel procedure, the reaction might be catalyzed at a much slower rate, yet reach the same plateau level of Ac–Leu transferred

Table 2
Transfer of Ac–Leu from CACCA–Leu–Ac to puromycin by ribosomal particles ('fragment reaction')

Particle	% of control
50S (control)	–
P_0	97.2
P_{37}	95.0
P_{0-37}	99.7
$P_{0-37-37}$	94.6

All particles were present at 15 A_{260}/ml. Assays were first pre-incubated at 37°C for 5 min before adding the methanol, then incubated for 30 min at 0°C as previously described [11,13]. The control (100%) represents 63.0 cpm/pmol ribosomes.

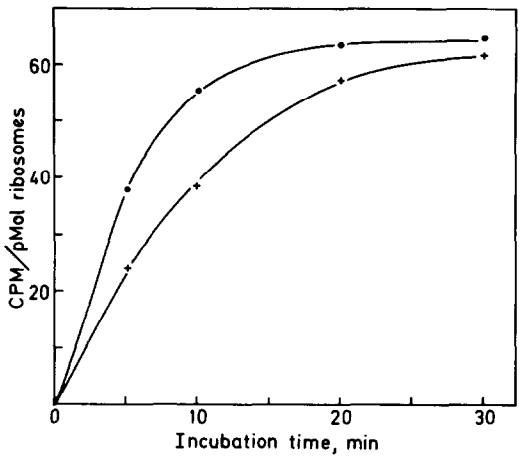

Fig. 2. Time dependence of peptidyl transferase assay ('fragment reaction'). Assays were done as described in the legend to table 2, except for the variation in incubation time at 0°C. Both the 50S subunits (●–●–●) and the $P_{0-37-37}$ particles (X–X–X) were present at 15 A_{260}/ml, final concentration. The ordinate depicts the amount of [^{3}H]Ac–Leu · puromycin formed.

Table 3
Peptidyl transferase assay ('fragment reaction'): effect of adding split proteins to ribosome particles

Particle	Split proteins	% of control
1. 50S (control)	–	100.0
2. $P_{0-37-37}$	–	68.5
3. $P_{0-37-37}$	P_{0-37}	65.9
4. P_{0-37}	–	69.9
5. P_{0-37}	P_{0-37}	71.0
6. P_{0-37}	$P_0 + P_{0-37}$	97.3

Assays were carried out as previously described [11,13], except the incubations were only 10 min at 0°C. All particles were present at 15 A_{260}/ml. The split protein fraction P_{0-37} contains mainly protein L11, along with L10 and some L1, L5, L6; split fraction P_0 contains only proteins L7/L12 [4]. The split proteins were added in a 4:1 molar excess to the particles. Refer to the legend to table 2 and to the text for details of assay conditions. The control (100%) represents 56.7 cpm/pmol ribosomes.

during the relatively long incubation times (30 min) of the standard assay conditions. To test this possibility we assayed both the untreated 50S subunits and the $P_{0\_37\_37}$ ribosomal core particles at shorter incubation times to determine the extent of transfer being catalyzed in each case. The results are shown in fig. 2. There does appear to be a slightly slower rate to transfer by the $P_{0\_37\_37}$ particles than the 50S subunits.

If the decrease in the transfer rate shown in fig. 2 is due to the depletion of protein L11, then the activity should be restored by the quantitative rebinding of protein L11. We have previously established conditions for this [4]. The results of such an experiment are shown in table 3: the addition of a split protein fraction containing protein L11 at a molar ratio of 4:1 was ineffective in raising the rate of Ac–Leu transferred by the P_{0_37} (line 5) or $P_{0\_37\_37}$ (line 3) particles during the 10 min incubations of the assay. We have shown previously [4] that the split proteins, L11 and L10, do in fact bind to the core particles under the conditions described in table 3, lines 3 and 5. Thus the slower rate of transfer with these core particles is not accounted for by the depletion of protein L11 per se. Readdition of all the split proteins (i.e. L7/L12, L10, L11) reconstitutes the P_{0_37} particles both in terms of protein content and of elongation factor EF-G catalyzed GTP hydrolysis [4,14]. Moreover, these fully reconstituted particles are now capable of catalyzing the peptidyl transferase reaction at the same rate as untreated 50S subunits (table 3, line 6). The only difference between the reconstituted particles on lines 5 and 6 is the presence or absence of proteins L7/L12. Thus we would conclude that the slightly slower rate of catalyzing the 'fragment reaction' by core particles as opposed to 50S subunits is probably due to a slight change in the ribosome when proteins L7/L12 are missing.

4. Discussion

We have shown that $P_{0\_37\_37}$ ribosomal core particles lacking protein L11 are fully capable of carrying out the peptidyl transferase reaction to the same total extent (albeit at a slightly slower rate) as intact, untreated 50S subunits.

Granted these results do not rule out the possibility that protein L11 is involved in the overall peptidyl transferase reaction, but they do appear to eliminate ribosomal protein L11 as being absolutely required for peptidyl transferase catalytic activity.

Independent experiments by Dr J. Ballesta have given essentially the same results [14].

Acknowledgements

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References

[1] Pestka, S. and Bodley, J. W. (1974) in: Antibiotics (Gottlieb, D. and Shaw, P. D., eds.) Springer-Verlag, Berlin, in press.
[2] Highland, J. H., Howard, G. A., Ochsner, E., Stöffler, G., Hasenbank, R. and Gordon, G. (1975) J. Biol. Chem., in press.
[3] Nierhaus, K. and Montejo, V. (1973) Proc. Natl. Acad. Sci. U.S. 70, 1931–1935.
[4] Highland, J. H. and Howard, G. A. (1974) J. Biol. Chem., in press.
[5] Gordon, J. and Highland, J. H. (1974) Eur. J. Biochem., in press.
[6] Hamel, E., Koka, M. and Nakamoto, T. (1973) J. Biol. Chem. 247, 805–814.
[7] Howard, G. A., Smith, R. L. and Gordon, J., in preparation.
[8] Spitnik-Elson, P. (1965) Biochem. Biophys. Res. Commun. 18, 557–562.
[9] Howard, G. A. and Traut, R. R. (1973) FEBS Lett. 29, 177–180.
[10] Howard, G. A. and Traut, R. R., submitted for publication.
[11] Monro, R. E. (1971) Meth. Enzymol. 20, 472–481.
[12] Celma, M. L., Monro, R. E. and Vazquez, D. (1970) FEBS Letters 6, 273–277.
[13] Monro, R. E., Cerna, J. and Marcker, K. A. (1968) Proc. Natl. Acad. Sci. U.S.A. 61, 1042–1049.
[14] Ballesta, J. P. and Vazquez, D. (1974) FEBS Lett. preceeding report.