RECONSTITUTION OF THE 50 S RIBOSOME SUBUNIT. LOCALIZATION OF ACTIVITIES RELATED TO THE PEPTIDYL TRANSFERASE CENTRE

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1. Introduction

The peptidyl transferase centre, responsible for the catalytic activity required for the peptide bond formation step in protein synthesis, is an integral part of the larger ribosomal subunit in procaryotic [1] as well as eucaryotic [2] cells. To investigate this active centre we have developed adequate systems to study: (a) peptidyl transferase catalytic activity, (b) substrate interaction at the P- and A-sites of the centre and (c) interaction of a number of antibiotics. These assays are particularly useful to investigate the above activities in the reconstitution of 50 S ribosome subunits in order to know their requirements and localization. The results obtained are reported in this work.

2. Materials and methods

2.1. *Ribosomes and derived particles*

Ribosome subunits were prepared from log phase *E. coli* D-10 by centrifugation in sucrose gradients using either conventional rotors [3] or zonal centrifugation [4].

Core nucleoprotein particles were obtained from 50 S ribosome subunits (with less than 4% contamination with 30 S particles) by isopycnic centrifugation in CsCl gradients [5]. By this method we obtained the nucleoprotein cores α, β and γ, and two sets of split proteins, $SP_{50-\beta}$ and $SP_{\beta-\gamma}$, released in the 50 S to β-core conversion and in the β-core to γ-core conversion respectively. Reconstitution of active particles from the nucleoprotein cores and split proteins was carried out by activation of the reaction mixtures at 50° for 90 min [5].

2.2. *Activities of the preparations*

The peptidyl transferase activity of the different particles in peptide bond formation was studied by following the "fragment reaction" assay [6]. Binding of CACCA–Leu–Ac to the donor site of the peptidyl transferase centre was carried out as previously described [7]. Binding of antibiotics was measured in the presence of 33% ($^{v}/_{v}$) ethanol at 0° as described elsewhere [8, 9].

2.3. *Materials*

CACCA–^{3}H–Leu–Ac was prepared by RNase–T_1 treatment of N′-acetylleucyl–tRNA as described elsewhere [10]. Sources of methylene-^{14}C-chloramphenicol (10.2 mCi/mmole), N-methyl-^{14}C-lincomycin (4.7 mCi/mmole) and N-methyl-^{14}C-erythromycin (5.1 mCi/mmole) were as previously indicated [9].

3. Results

3.1. *Activities of 50 S subunits, cores and reconstituted particles*

The activities of various protein deficient ribosomal cores and reconstituted particles were examined and the results are summarized in table 1. As previously reported [11, 12] the loss of the 7 proteins ($SP_{50-\beta}$) involved in the conversion of 50 S

Table 1
Activities of 50 S ribosomes and derived particles.

Conditions	Experiment 1				Experiment 2	
	Peptidyl transferase (fragment reaction)	CACCA–Leu–Ac binding	Erythromycin binding	Lincomycin binding	Peptidyl transferase	Chloramphenicol binding
50 S	100	100	100	100	100	100
α	118	–	124	106	121	98
$\beta+SP_{50-\beta}$	95	100	97	60	–	–
β	96	95	80	31	89	63
$\gamma+SP_{\beta-\gamma}$	90	97	93	49	84	66
$*\gamma+SP_{\beta-\gamma}$	2	0	2	2	–	–
γ	0	0	1	3	2	5

Experiments 1 and 2 were carried out with different core preparations. Particles were reconstituted under conditions previously described [5], except in one case ($*\gamma+SP_{\beta-\gamma}$) in which the reaction mixture was kept at 0°. The assays for activity were carried out as described in (sect. 2.2) using 1 mg/ml of particles in the "fragment reaction" and 3 mg/ml of particles and 10^{-6} M of antibiotics in the binding assays. In all cases data presented are percentage of the activities of the 50 S subunit preparations and represent extent of the reactions rather than rates.

Table 2
Activities of particles reconstituted in the presence of sparsomycin and N-ethyl-maleimide.

Additions in:				
Reconstitution mixture	Assay for activity	Peptidyl transferase (fragment reaction)	Erythromycin binding	Lincomycin binding
$\gamma+SP_{\beta-\gamma}$	None	100	100	100
$\gamma+SP_{\beta-\gamma}$ + sparsomycin	None	98	94	60
$\gamma+SP_{\beta-\gamma}$	Sparsomycin	27	95	95
$\gamma+SP_{\beta-\gamma}$	None	92	61	43
$\gamma+SP_{\beta-\gamma}$	NEM	88	85	95

Reconstitution of the samples was carried out as described elsewhere [5] in the presence of either 10^{-4} M sparsomycin or 10^{-2} M N-ethyl-maleimide (NEM) when indicated. After the incubation of the reconstitution mixture the particles were precipited by ethanol, redissolved in fresh buffer and precipited again, this process being repeated 3 times. Sparsomycin (10^{-4}) and NEM (10^{-2}) M were added when indicated in the assays for the fragment reaction, erythromycin binding and lincomycin binding.

subunits into β-cores does not significantly affect the peptidyl transferase activity of the resulting particles. The capacity for binding of CACCA–Leu–Ac induced by sparsomycin is also preserved; however, the lincomycin and chloramphenicol binding sites seem to be partially impaired in the β-cores. Our previous work has shown that the number of binding sites per particle is the same in the reconstituted particles as in the original 50 S subunits [12]. It is interesting to note that α-cores, which

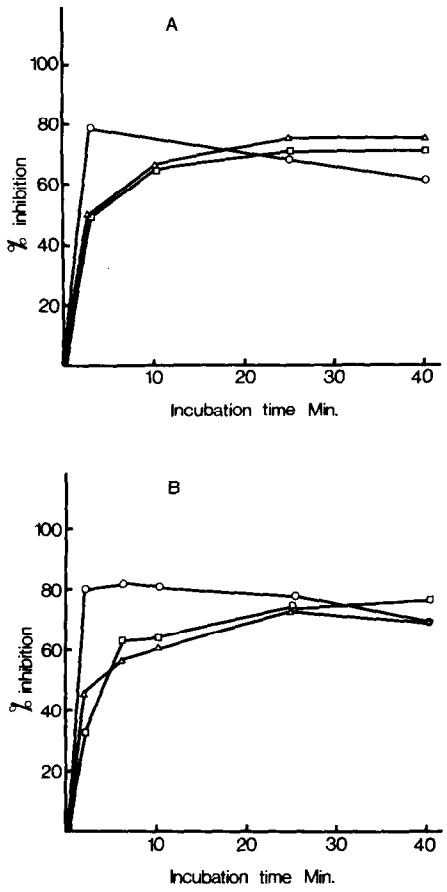

Fig. 1. Inhibition of peptidyl transferase activity (fragment reaction) by (A) lincomycin and (B) chloramphenicol. (○—○) 50 S particles; (△—△) β-cores; (□—□) γ + $SP_{\beta-\gamma}$. The reaction mixture in a final volume of 0.5 ml contained 1 mg/ml of particles and either 10^{-4} M lincomycin or 10^{-5} M chloramphenicol. Aliquots were taken at different times and processed as described [6]. Particles obtained as described in (sect. 2.1).

lack at least two proteins, show a higher activity than the 50 S subunits not only for the peptidyl transferase [11] but also in the binding of antibiotics (table 1). All the activities tested in this work are completely abolished in the γ-cores, resulting from the loss of seven additional proteins ($SP_{\beta-\gamma}$) from the β-cores.

Active particles were obtained by mixing the $SP_{\beta-\gamma}$ split proteins and the γ-cores followed by heat reactivation. The reconstituted particles show identical characteristics with the original β-cores.

The lower affinity of the β-nucleoprotein derived particles (obtained either from 50 S subunits or reconstituted from γ-cores) for chloramphenicol and lincomycin (table 1) is reflected in the rate of their inhibition on the fragment reaction catalysed by the cores. Fig. 1 shows that the initial rate of inhibition by both drugs is slower in the case of β-cores and reconstituted particles than in 50 S subunits although the extent of the final inhibition is the same in both cases.

3.2. *Inhibitors of reconstitution*

We were able to show differences in the structural characteristics of the nucleoprotein cores in the presence of compounds such as antibiotics and –SH reagents known to act on the ribosomes. The activity of the particles reconstituted in the presence of sparsomycin and N-ethyl-maleimide is shown in table 2.

Sparsomycin is known to bind to the 50 S subunits [7]. Cores reconstituted in its presence recover only about half of the lincomycin binding capacity while the peptidyl transferase and the erythromycin binding activities are reconstituted to the same extent as in the controls.

As indicated by the sensitivity of the active centers to the N-ethyl-maleimide, the –SH groups seem to play a role in the reconstitution of the lincomycin and erythromycin binding centres although they are not required for the binding of the drugs. The peptidyl transferase activity and the reconstitution of the active centre are insensitive to N-ethyl-maleimide.

3.3. *Release activity*

Different workers have pointed out the probable role of the peptidyl transferase in termination of the polypeptide synthesis [13, 14]. We have used the release assay developed by Caskey and co-workers [13] to compare behaviour of the nucleoprotein cores in (a) the termination process and (b) peptide bond formation activity. Table 3 shows that the release activity of the cores is also lost in the γ-cores and recovered in the reconstituted particles. Similar results have been obtained by Caskey (personal communication).

Table 3
Peptidyl transferase and release of f-met activities of 50 S and derived particles.

Particles	Peptidyl transferase	Release of f-methionine
γ	2	12
$\gamma + SP_{\beta-\gamma}$	64	44
β	95	64
50 S	100	100

Peptidyl transferase activity was tested by the fragment reaction [6]. Release activity was assayed using partially purified released factors (fraction IV of Milman et al. [15]) by the codon independent f-met release assay [11]. Particles were reconstituted as in (sect. 2.1).

4. Discussion

It is shown in this work the importance of the proteins released in the conversion from β-cores to γ-cores ($SP_{\beta-\gamma}$) for a number of activities of the 50 S ribosome subunit. The γ-cores are depleted of all the 50 S subunit activities so far tested by us and these activities are totally or partially recovered upon the addition of the $SP_{\beta-\gamma}$ split proteins.

In addition we have shown that not all the active centres studied have similar structural characteristics. For instance it has been possible to alter the reconstitution of one of the binding sites (lincomycin) without affecting some others (erythromycin and CACCA–Leu–Ac). These results confirm our previous conclusion [16] suggesting a different location for the erythromycin and lincomycin binding sites.

Lincomycin and chloramphenicol binding sites seem specially sensitive to the manipulation of the ribosomes while peptidyl transferase shows minimal structural requirements for activity. The lower affinity of the β-cores for lincomycin and chloramphenicol points to a more important role of the $SP_{50-\beta}$ in determining the correct conformation of the binding centres for these drugs than in the case of peptide bond formation catalytic activity. Our data are compatible with the involvement of the peptidyl transferase in termination of the polypeptide synthesis [13, 14] although the activity of the particles in releasing f-methionine appears to be more difficult to reconstitute quantitatively than the peptide bond formation activity.

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