PROTEIN L16 INDUCES A CONFORMATIONAL CHANGE WHEN INCORPORATED INTO AN L16-DEFICIENT CORE DERIVED FROM *ESCHERICHIA COLI* RIBOSOMES

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1. Introduction

The protein L16 from the large ribosomal subunit is involved in several functions of this subunit. Affinity label and reconstitution experiments pointed to L16 as the chloramphenicol-binding protein [1,2]. The binding of other antibiotics also depends on this protein; this is true for virginiamycin S [3] and erythromycin [4]. The latter antibiotic binds to the large subunit only if proteins L15 and L16 are present [4,5]. Furthermore, L16 is important [6] or essential [7] for the peptidyltransferase activity. Finally, L16 influences the association of the ribosomal subunits [8].

It is possible, although not very likely, that L16 itself exerts all these activities. Another possibility is that L16 controls the conformation of the large subunit and, therefore, is related indirectly to many functions of this subunit. We show here that L16 indeed changes the conformation of a core particle lacking L16.

2. Materials and methods

Cells of *Escherichia coli* K12, strain A19, were harvested in the early log phase, and ribosomes and their subunits were isolated as in [9]. Subunits, 50 S, were heat-activated (15 min, 40°C, in 10 mM Tris–HCl, pH 7.5, 10 mM Mg acetate, 60 mM NH_4Cl, 4 mM β-mercaptoethanol buffer) before being treated with 1.3 M LiCl to obtain 1.3c cores and the complementary split proteins SP1.3 [10]. The split proteins SP1.3 were extracted with acetic acid and passed through a Sephadex G-100 column. Fractions containing L16 were pooled and subjected to CM-cellulose column chromatography [11]. Partial reconstitution and the measurement of the peptidyltransferase activity followed [12]. The description of [^{14}C]erythromycin binding (a generous gift from Dr Tanaka, Shionogi Research Laboratory, Osaka) to the reconstituted particles in equilibrium dialysis cells is in [5].

3. Results and discussion

The 1.3c core is inactive with respect to binding of various antibiotics and the peptidyltransferase activity. L16 can reactivate the 1.3c core when both L16 and the core are incubated under partial reconstitution conditions [2,3,5,7]. A 5-fold molar excess of this protein over the 1.3c core does not lead to optimal activity; e.g., a 10-fold molar excess gives about twice the activity as a 5-fold excess. This effect is not due to partially inactive L16 but to weak binding of L16 to the 1.3c core (Schulze and K.H.N., unpublished).

When L16 is reconstituted into the 1.3c core which lacks this protein [10] we did not detect a reproducible and significant *S*-value shift of the core by sucrose gradient analysis. Therefore, we tried another approach. In order to analyze the effect of L16 on the conformation of the 1.3c core, we determined the incubation requirements for two L16-

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dependent activities, i.e., the peptidyltransferase activity in the fragment reaction and the binding of [^{14}C]erythromycin (erythromycin does not inhibit the fragment reaction). If L16 is able to activate the core in the cold, then a drastic conformational change is unlikely to have taken place. However, if the activation is possible only after heating the core with L16, a conformational change is probable and this could then be verified by kinetic analyses.

Accordingly, 5-fold molar excess of L16 was added to the 1.3c core under reconstitution conditions at 0°C and at 50°C (400 mM NH_4^+ and 20 mM Mg^{2+}). No active particles were formed from the sample at 0°C, in contrast to the sample incubated at 50°C (exp. 1, table 1). However, when the (1.3c core + L16) combination was first centrifuged (4 h, 135 000 × *g*) and the pelleted particle then incubated at 50°C, no activity was detected (exp. 2). The same amount of L16 must be added to the pelleted particles at 50°C in order to obtain the same activity as that in exp. 1 (cf. exp. 2 and exp. 1, respectively). We conclude that L16 does not bind to the 1.3c core in the cold. Preincubation of either 1.3c core or L16 at 50°C has no effect (exp. 3). Thus, the simultaneous incubation of L16 with the core is essential for the formation of active particles, indicating an energy-consuming conformational change of the 1.3c core and/or L16.

Before we can analyze further the L16-dependent reaction we have to test whether the preincubation of the 1.3c core at 50°C influences the L16-dependent activation. The preincubated core was incubated with L16 at various temperatures and the peptidyltransferase activities were compared to those of a non-preincubated core (fig.1). Preincubation of the core at 50°C shifts the curve towards lower temperatures. Thus, a preincubated 1.3c core must be taken in order to analyze the L16-dependent reaction. Next, kinetic analyses of the incubation of preincubated 1.3c core and L16 were performed at various temperatures (fig.2A) and the data were plotted assuming a first-order law (fig.2B). The experimental points fall on straight lines demonstrating a first-order reaction.

Table 1
Incubation requirements for the L16-dependent activities

Exp. no.	Components	Treatment	Peptidyltransferase activity (cpm)	[^{14}C]erythromycin binding (cpm)
1	1.3c + L16	0°C	13	9
		90 min/50°C	1202	298
		4 h/45 000 rev/min		
2	$(1.3 + L16)_p$	0°C	0	2
		90 min/50°C	10	13
		+ L16 (90 min/50°C)	1167	328
3	1.3c	90 min/50°C + L16 (0°C)	0	16
	L16	90 min/50°C + 1.3c (0°C)	0	11
	1.3c + L16	90 min/50°C → 0°C	0	7
	50 S		7918	980

A 5-fold molar excess of L16 was added to the 1.3c core under reconstitution conditions. One aliquot was incubated at 0°C, and a second one at 50°C, before testing for peptidyltransferase activity and [^{14}C]erythromycin binding (exp. 1). A third aliquot was centrifuged and the resulting pellet $(1.3c + L16)_p$ was resuspended in a buffer containing 20 mM Tris–HCl, pH 7.5, 400 mM NH_4Cl and 20 mM Mg acetate. The solutions were incubated either at 0°C or at 50°C, or at 50°C after the addition of a 5-fold molar excess of L16 (exp. 2). Details of exp. 3 are described in the text

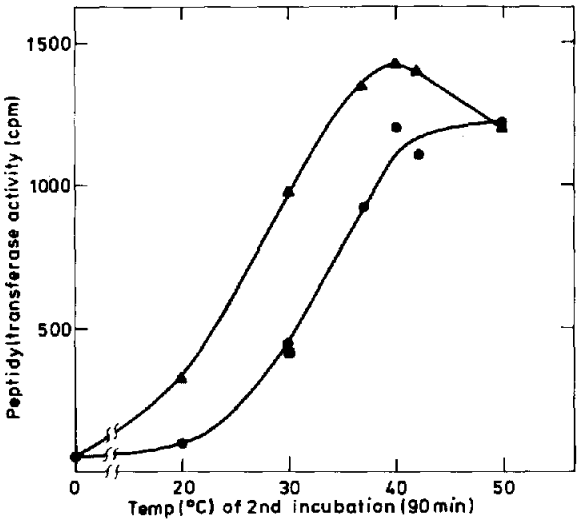

Fig.1. Peptidyltransferase activity of (1.3c core + L16) particles heated for 90 min at various temperatures under reconstitution conditions (400 mM NH_4^+ and 20 mM Mg^{2+}). (▲——▲) 1.3c core preincubated (90 min at 50°C) before incubation with L16; (●——●) non-preincubated 1.3c core; (■) L16 preincubated (90 min at 50°C) before incubation with the non-treated 1.3c core.

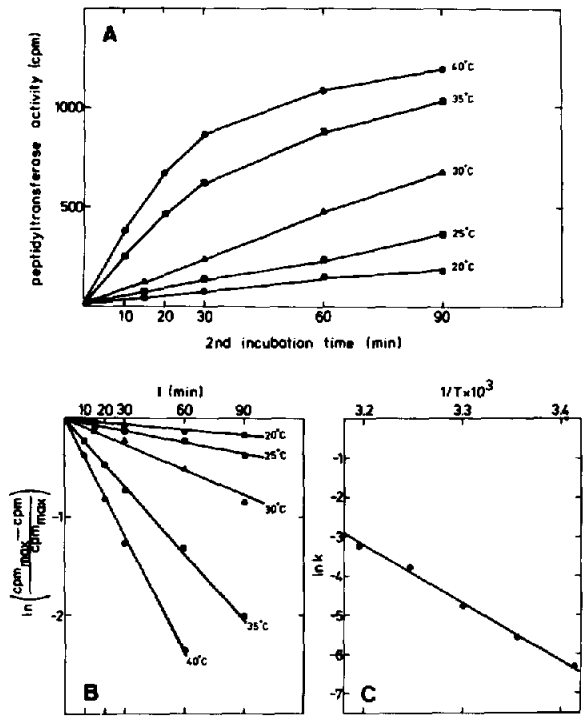

Fig.2. Kinetics of the formation of (1.3c core + L16) particles. (A) 1.3c cores were preincubated for 90 min at 50°C. Then kinetic analyses were performed at various temperatures in the presence of a 5-fold molar excess of L16, and the activity was measured by the fragment assay at 0°C. The maximum activity was 1200 cpm. (B) Plot of the data from fig.2A, according to the first order law. The rate constants k are given by the slope of the straight lines. The following values were obtained: $k_{20} = 0.0018\ min^{-1}$; $k_{25} = 0.0039\ min^{-1}$; $k_{30} = 0.0086\ min^{-1}$; $k_{35} = 0.023\ min^{-1}$; $k_{40} = 0.0396\ min^{-1}$. (C) Arrhenius plot of the logarithm of the rate constants obtained from fig.2B against the reciprocal of absolute temperature, $1/T$. The slope is equivalent to $-E_a/R$, from which the activation energy E_a was calculated as 123 kJ/mol (29.5 kcal/mol).

Therefore, the rate-limiting step of the L16-dependent reaction during reconstitution appears to be an unimolecular reaction, i.e., a conformational change. Using the Arrhenius plot (fig.2C) we obtained 123 kJ/mol (29.5 kcal/mol) as the activation energy for the L16-dependent conformational change.

The obtained value for the activation energy is higher than those known for enzyme-catalyzed reactions (4–100 kJ/mol or 1–25 kcal/mol, [13]) and is comparable to that obtained for the total reconstitution of the small ribosomal subunit (160 kJ/mol or 38 kcal/mol, [14]). Obviously, L16 triggers an extensive reorientation of the core.

L16 belongs to the late protein group assembled during the in vitro 50 S reconstitution [9]. Therefore, it is likely that L16 plays an important role for the conformational change of the last assembly step (RI_{50} (2) → 50 S, [9,15]). However, the temperature optimum (40°C) observed in the L16-dependent reaction (fig.1) is different from that of the second incubation (50°C, [16]). Furthermore, the activation energy of the latter reaction (225 kJ/mol, [15]) considerably exceeds that of the L16-induced effect (160 kJ/mol). Therefore, the L16-dependent reaction might be part of, but is not identical with, the conformational change occurring during the second incubation of the total reconstitution procedure for the large ribosomal subunit.

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