EFFECTS OF TRIS AND OLIGOAMINES ON THE RIBOSOMAL SIZE DISTRIBUTION IN LYSATES OF *B. LICHENIFORMIS*

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1. Introduction

The size distribution of ribosomes in bacterial lysates depends on the cationic composition of the lysis buffer [1–4]. Little or no 70 S particles are present when lysis is carried out in 5 mM tris-buffer containing 40 to 60 mM Na^+ [3–6] or a low concentration of Mg^{2+} ions [1, 2]. In other cases a prominent peak of 70 S particles is observed in the sedimentation pattern [7–11].

The principal cationic composition of the cellular fluid of bacteria consists of K^+, Mg^{2+} [11, 12] and oligoamines [13–15]. Cations like tris and Na^+ are non-physiological in the concentrations in which they are normally used. In order to obtain some information about the situation *in vivo,* we studied the effect of physiological concentrations of oligoamines, as well as the possible artifactual effects of the tris ion on the ribosomal size distribution in *B. licheniformis.*

The present results indicate that the 70 S particles in the lysates of *B. licheniformis* are not artifacts, but instead consist mainly of run-off products and, in part of, initiation complexes. The physiological oligoamines were found to be necessary for the integrity of the run-off monomers.

2. Methods

B. licheniformis was continuously cultured in Brain Heart Infusion broth at 37° at the dilution rate of 3 hr^{-1}. The cell density was kept at 1.1×10^8 cells/ml. For other details and preparation of the radioactive cells see [11].

The cells were converted to spheroplasts and lysed as previously described [11], except that the lysozyme incubation was carried out in 25 mM (rather than 51 mM) tris-buffer, and the spheroplasts were collected by centrifugation, resuspended in lysis buffer and lysed by adding Brij-58 (final concentration 0.5%). The lysis was carried out in buffers containing tris-HCl (10, 5 or 1 mM), 10 mM magnesium acetate, 60 mM monovalent cation (K^+, NH_4^+, Na^+ or tris) as chloride, 100 μg/ml chloramphenicol, 1 mM dithiothreitol, and in some indicated cases 0.5 mM spermine *plus* 0.75 mM spermidine all with a pH 7.6 (at 0°).

In our method of lysis about 90% of the ribosomes, including all polysomes, remain attached to the membranes [11]. These ribosomes were solubilized by a combined treatment with lipase (65 μg/ml) and deoxyribonuclease (2 μg/ml) in the presence of the non-ionic detergent Brij-58 and analysed by sucrose gradient centrifugation (figs. 1–4, A). The soluble ribosome fraction was analysed separately (figs. 1–4, B). Sucrose gradients were prepared in lysis buffers (see above) from which dithiothreitol was omitted.

3. Results

3.1. *Interplay of monovalent cations with oligoamines*

If oligoamines are present in a physiological concentration (1.25 mM) in 10 mM tris-buffer during the lysis and in sucrose gradients, the resulting ribosomal size distribution appears to be independent on the nature of the monovalent cation employed (K^+ or NH_4^+ or Na^+, see table 1). In all cases the membrane-

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Table 1

Effect of monovalent cations and oligoamines on the size distribution of ribosomes in lysates of *B. licheniformis*.

Lysis buffer				
Conc. of tris (mM)	Monovalent cation used (60 mM)	Gradient centrifugation buffer	Oligoamines* (1.25 mM)	Profile in fig.
1, 5 or 10	K^+ or NH_4^+	as lysis buffer	present	1
			absent	2
10	Na^+	as lysis buffer	present	1
			absent	3
1, 5 or 10	Na^+	1 or 5 mM tris with 60 mM Na^+	present	4 (——)
			absent	4 (- - -)
65 or 70	none	as lysis buffer or 10 mM tris with 60 mM K^+ or NH_4^+	present	1
			absent	2

Exponential-phase cells of *B. licheniformis* were lysed and the ribosomes analysed on sucrose gradients. The lysis and the gradient centrifugation were carried out in buffers varying in the tris concentration, the monovalent cation used and in the presence or absence of the phyiological oligoamines (0.5 mM spermine and 0.75 mM spermidine). The Mg^{2+} concentration was in all buffers 10 mM. The different sedimentation profiles are shown in figs. 1–4.

* Present: oligoamines added to either the lysis buffer or the gradient buffer or both.

bound ribosomes consist for about 75% of polyribosomes, 22% of 70 S particles and 3% of subunits (fig. 1A). In the soluble fraction only 70 S particles and subunits are present (fig. 1B).

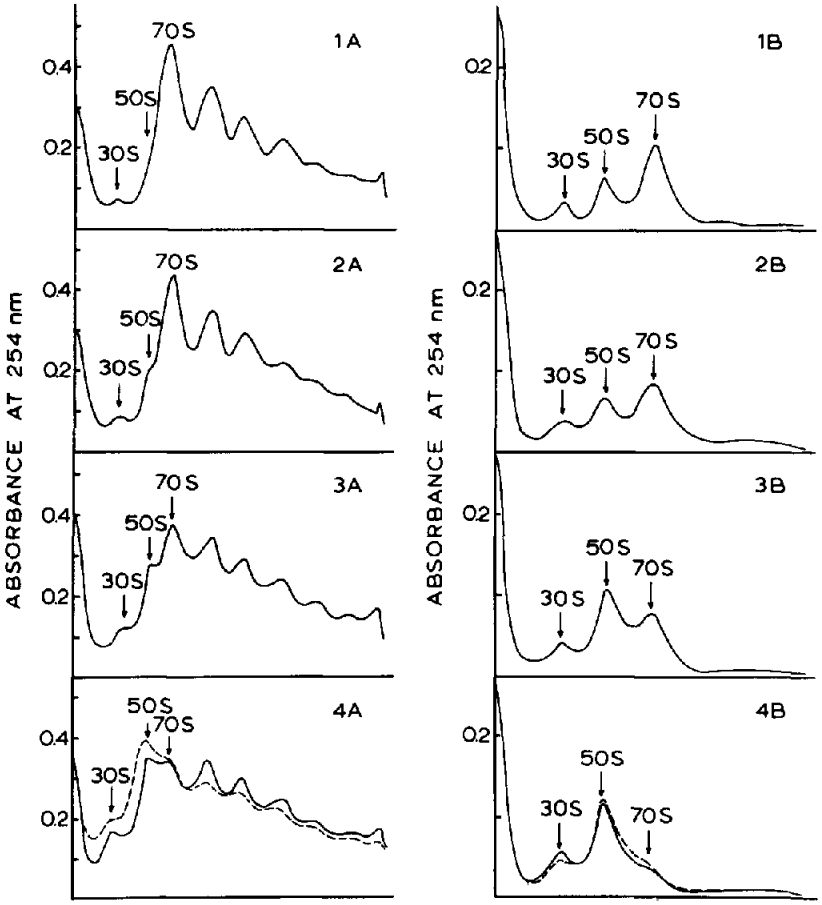
But if oligoamines are omitted, a part of the 70 S particles appear to become labile. The relative quantity of 70 S particles then depends on the nature and concentration of the monovalent cations used, including the tris-ion (table 1). In buffers prepared with 60 mM K^+or NH_4^+ most of the 70 S particles remain intact (fig. 2). On the other hand, in Na^+ media the 70 S particles are found to be largely dissociated, particularly if at the same time the tris concentration is low.

Thus the use of Na^+ as the main monovalent cation leads to dissociation of 70 S particles. The tris ion seems to overcome this effect by stabilizing the ribosomal structure in a K^+-like manner (as does NH_4^+). We checked this "K^+-like" action of the tris ion by lysing bacteria in buffer in which tris was used as the sole monovalent cation. Indeed, the sedimentation profiles of the ribosomes prepared in 65 or 70 mM tris were indistinguishable from those prepared with 60 mM K^+ or NH_4^+ in 5 or 10 mM tris-buffer (table 1). This is not an exclusive property of tris, for another commonly used buffer substance, triethanolamine, gave very similar results.

3.2. *Heterogeneity of the 70 S fraction*

A small amount of 70 S particles persists in the membrane fraction of the lysates prepared in Na^+ media, even if the concentration of tris is decreased to 1 mM (table 1). In contrast to this, the soluble fraction of the same lysates contains practically no 70 S particles. Therefore *B. licheniformis* might possess two types of 70 S particles, of which only one is stable in Na^+ media with a low concentration of tris.

We tested the membrane-bound and soluble 70 S particles for their ability to bind aminoacyl- or peptidyl-tRNA *in vivo*. ^{14}C-Pre-labelled, exponential-phase cells were pulse labelled for 2 min with ^{3}H-amino acids. The culture was subsequently poured on liquid N_2, thawed, washed and lysed in buffer containing K^+ and oligoamines. The radioactivity patterns of the sucrose gradients of the membrane-bound and soluble fractions are shown in fig. 5A and B. As can be seen in this figure, the 70 S particles of the soluble fraction (fig. 5B) do not carry labelled peptidyl residues, while a part of the membrane-bound 70 S particles obviously does (fig. 5A).



Figs. 1–4. 400 mg of wet cells were lysed [11] in buffers which varied in tris concentration, in monovalent cations used and the presence of oligoamines (cf. table 1). The solubilized membranes (0.1 ml) were analysed on exponential sucrose gradients (15 to 31.8%), centrifuged in the Spinco SW 41 rotor for 2 hr at 35 000 rpm (figs. 1–4, A). The soluble fractions (1 ml) were analysed on linear 15–30% sucrose gradients, centrifuged in the Spinco SW 25:1 rotor for 9 hr at 22 500 rpm (figs. 1–4, B). The absorbance was continuously recorded at 254 nm with the Uvicord photometer connected to a Heath recorder.

4. Discussion

The cationic composition of the intracellular fluid of exponential-phase bacteria appears to be about 60 mM K^+, 10 mM Mg^{2+} and 1 to 2 mM of oligoamines [4, 11–15]. Other cations are usually present in much lower concentrations. Therefore the ribosomal distribution obtained after lysis in a medium of the aforesaid composition most probably reflects the situation *in vivo*.

The physiological oligoamines appear to play a role in preserving the integrity of the 70 S ribosomes. This is especially manifest if either the concentration or the type of the monovalent cations in the lysis buffer is suboptimal (figs. 1–4).

A good buffering is necessary during the lysis, especially during the lysozyme digestion. For this purpose tris is commonly employed. However, tris is known to interact with *E. coli* ribosomes [16]. The results presented here show that if the bacteria are lysed in buffer containing a physiological concentration of K^+ and Mg^{2+}, tris has no effect on the ri-

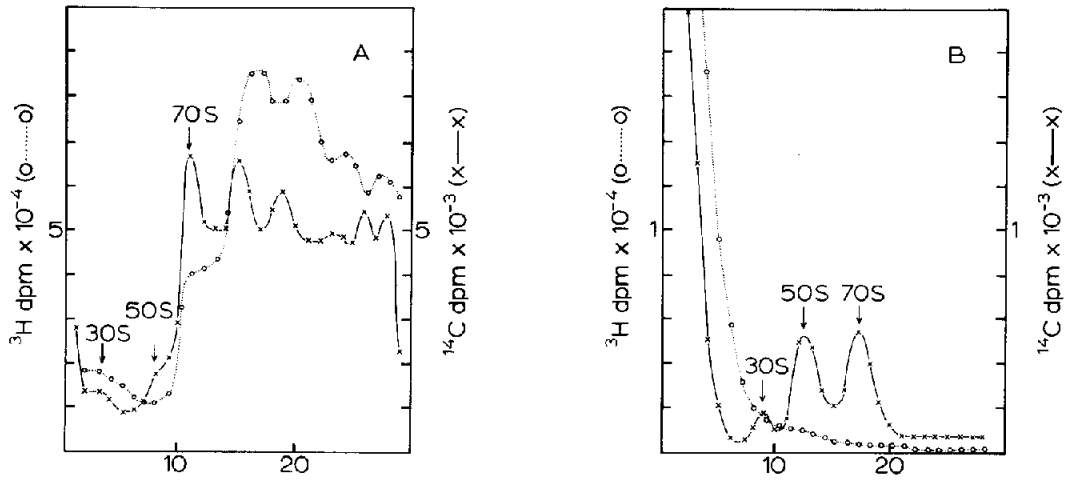


Fig. 5. *B. licheniformis* was grown in synthetic medium [11] containing ^{14}C-uridine (0.057 μCi/ml) for 4 hr and then pulse labelled for 2 min with ^{3}H-amino acids (1.6 μCi/ml) at a cell density of 1.5 X 10^8 cells/ml. The culture (100 ml) was poured in liquid N_2. After thawing, 250 mg of unlabeled carrier cells were added, the cells were collected by low-speed centrifugation, washed and lysed in a buffer containing 60 mM K^+, 10 mM tris, 10 mM Mg^{2+} and 1.25 mM of the physiological oligoamines. The solubilized membranes (A) and soluble fraction (B) were analysed on linear 5–20% (A) or 15–30% (B) sucrose gradients made in the same buffer as used for lysis. The gradients were centrifuged for 3–½ hr (A) or 9 hr (B) in the Spinco SW 25:1 rotor at 22,500 rpm .
The radioactivities were measured in each of the 10-drop fractions in a Nuclear Chicago Scintillation Counter (Mark I) [11].

bosomal size distribution, irrespective whether the buffer also contains oligoamines or not. We therefore believe that the ribosomal distribution shown in fig. 1A and B is not distorted by artifactual effects of tris but rather reflects the situation *in vivo*.

It was found that in Na^+ media a strong dissociation of the 70 S particles occurs: the 70 S particles of the soluble fraction dissociate completely and the membrane-bound 70 S particles to a large extent (fig. 4). The tris ion partly counteracts this effect of the Na^+ ion so that the sedimentation profile of the ribosomes in Na^+ media is essentially dependent on the concentration of tris employed (figs. 2–4). 10 mM tris combined with oligoamines (1.25 mM) is sufficient to prevent dissociation of the 70 S particles entirely (compare fig. 4 to fig. 1). The "Na-labile" 70 S ribosomes may be identical to the "run-off monomers" suggested by Kohler et al. [17] because we obtained evidence that these 70 S particles carry not nascent peptide chains (fig. 5). The remaining part of the membrane-bound 70 S particles might be considered as "initiating monomers" [18].

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