ASSOCIATION OF RIBOSOMAL SUBUNITS: STUDIES ON THE BINDING OF POLYAMINES TO 30S AND 50S PARTICLES*

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Received 12 April 1976

1. Introduction

Polyamines are present in all cells and have been implicated in a great number of reactions [1–4]. These organic cations are preferently bound to nucleic acids and ribosomes [5,6], suggesting their participation in the biosynthesis of DNA and RNA, as well as in the transformations occurring in ribosomal particles during translation [7,8].

Our studies on the association of ribosomal subunits promoted by an association factor (AF) extracted from ribosomes of *Bacillus stearothermophilus* [9], have indicated that AF could bind functionally to 30S but not to 50S particles [10]. These findings and the fact that polyamines seem to be the active components of AF [11] led us to investigate the correlation between the binding of the above mentioned organic cations to each one of the ribosomal subparticles and the association reaction.

In this paper we describe the interaction between spermidine and ribosomal subunits from *B. stearothermophilus*. The results indicate that 30S particles have at least two different types of binding sites for the polyamine, and that only one of them is involved in the association reaction. On the other hand 50S subunits contain one class of site for the binding of spermidine, which is not functional in the formation of 30S-50S couples.

*This is paper No. 5 of the series 'Association of Ribosomal Subunits'.

2. Materials and methods

RNase-free sucrose (density gradient grade) was obtained from Schwarz-Mann, spermidine trihydrochloride from Sigma and [^{3}H]spermidine trihydrochloride (spec. act. 510 Ci/mol) from New England Nuclear Corp.

2.1. *Bacterial cultures and preparation of ribosomes*

Bacillus stearothermophilus 1503-4R was grown and cells were harvested and disrupted as previously reported [12,13].

Purified ribosomal subunits were obtained according to the procedure described earlier [11], concentrated by precipitation with ammonium sulphate as reported by Gavrilova and Spirin [14] and de-salted by filtration through Bio-Gel A-0.5m.

2.2. *Binding of [^{3}H]spermidine to 30S or 50S subunit*

The mixtures contained 5–20 A_{260} units of 30S or 50S particles and 20 μCi of [^{3}H]spermidine at the indicated concentration in 0.3 ml of buffer A (10 mM Tris-HCl, pH 7.8, 5 mM magnesium acetate, 50 mM KCl and 2.5 mM dithiothreitol). The incubation was performed for 5 min at the temperature specified in each case. After chilling to 0°C each sample was submitted to filtration through a Bio-Gel A-0.5m column (0.5 × 12 cm) equilibrated with buffer A. The elution was carried out with the same buffer solution and fractions containing ribosomes were detected by the absorbance at 260 nm and pooled. Different aliquots were used to measure the radioactivity bound by the ribosomes and to perform the association assay under

the standard conditions after adding the other ribosomal subparticle at 0°C.

In some experiments the binding of radioactive spermidine to ribosomes was measured by equilibrium dialysis followed by filtration through Bio-Gel.

2.3. *Standard assay*

The reaction mixture used to measure association contained in 0.2 ml of buffer A either [30S-spermidine] or [50S-spermidine] complexes obtained as described above and the other free subparticle (a total amount of 0.4–0.6 A_{260} units of 30S and 50S particles in the ratio 1:2 was used). After incubation for 5 min at 0°C the samples were analyzed by centrifugation in 4.6 ml of 5–20% linear sucrose gradients as previously reported [11]. Percentages of association were calculated as detailed earlier [9].

When the association of subunits was performed in a single step, the reaction was carried out as previously described [9], but using spermidine instead of AF.

3. Results and discussion

Fig.1 shows the association of ribosomal subunits promoted in a single step reaction by different concentrations of spermidine. The reaction reaches a plateau of about 50% association at 0.5 mM spermidine. We could not attain a higher percentage of association probably due to the fact that this value may correspond to the equilibrium between monomers and subparticles in our preparations; some subunits might be damaged and therefore could not undergo association. On the other hand we did not use spermidine levels higher than 1 mM in order to avoid aggregation or precipitation of ribosomes.

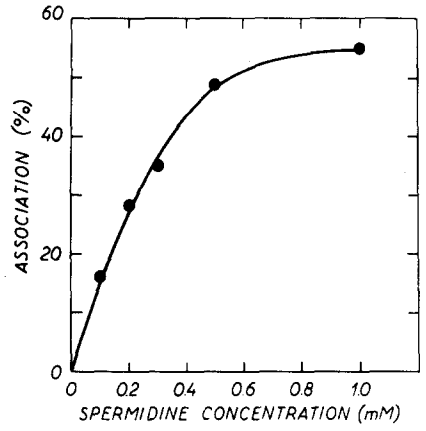

Fig.1. Association of ribosomal subunits induced by spermidine. The reaction mixtures containing about 0.5 A_{260} units of 30S and 50S particles (ratio 1:2) in buffer A were incubated for 5 min at 65°C in the presence of the indicated spermidine concentrations. After chilling to 0°C the samples were analyzed by sucrose gradient centrifugation. Percentages of association were calculated as already described [9].

The association of subunits can be performed in vitro either in a single reaction or in two steps as we have shown previously [10,11]. During the first step the associating agent binds to the 30S particles giving a complex which is able to form 30S–50S couples when 50S subunits are added in the subsequent association step.

The possibility of using radioactive spermidine as associating agent, allowed us to investigate several aspects of the binding sites for polyamines in the ribosomal particles, as well as whether the spermidine had to be bound to a special site in order to promote the association. For this purpose 30S and 50S particles were separately incubated at different temperatures with [^{3}H]spermidine and the resulting mixtures were submitted to filtration through Bio-Gel A-0.5m as described in Materials and methods. The excluded fractions contained ribosomal subunits with or without spermidine attached to them. Only the polyamine molecules more tightly bound should remain on the particles and be excluded, since free spermidine is retarded by the Bio-Gel column and therefore the equilibrium between bound and free polyamine is shifted towards the latter. It should be mentioned, in this respect, that after equilibrium dialysis about 120 molecules of spermidine were found per 30S particle whereas this number is reduced to a few molecules when the binding is measured after filtration through Bio-Gel.

Table 1 gives the relationship between the number of spermidine molecules attached to each one of the subunits after the binding step and the percentage of association obtained when the other subparticle was added in the second step of the reaction. It can be seen that although more spermidine could be bound to 50S particles, the resulting [50S-polyamine] complex was not able to produce couples as we have already

Table 1
Binding of spermidine to each of the ribosomal subunits and formation of 30S–50S couples

Experimental conditions in the binding step of the reaction			Spermidine molecules bound per particle	Association (%)
Ribosomal subunits	Spermidine concentration (mM)	Incubation temperature (°C)		
30S	0.5	25	0.8	0
	2.0	25	2.4	0
	0.5	65	1.2	48
	2.0	65	3.3	46
50S	0.5	25	1.7	0
	2.0	25	4.2	0
	0.5	65	3.6	0
	2.0	65	8.3	0

The binding step under the conditions indicated in each case and the subsequent association assay were performed as described in Materials and methods.

shown with AF [10,11]. The same results were obtained independently of the number of spermidine molecules bound per 50S subunit. On the other hand the 30S particles gave a complex active for association only when the binding step was carried out at high temperature. We have used 65°C because this is the optimal temperature for bacterial growth. At 2 mM spermidine and 25°C more polyamine was bound than at 0.5 mM spermidine and 65°C, but nevertheless no association could be obtained under the former conditions. These facts strongly suggest that two different types of binding sites for polyamine are present in the 30S subunit: site A that binds spermidine active in association, and site B which binds polyamine in a form non-active for inducing the formation of 30S–50S couples. The polyamine cannot reach the site A unless the binding reaction is supplied with enough energy, which is probably spent in some conformational change of the small subunit. If the 30S particle or polyamine are preincubated at 65°C and the subsequent reactions are carried out at 25°C the association does not occur.

It is interesting to point out that by increasing the spermidine concentration during the binding step at 65°C it was possible to attach a higher number of polyamine molecules per 30S particle, but the percentage of association remained unchanged. This result might indicate that the site A was already saturated.

Almost the same percentages of association were obtained whether or not the reaction mixtures containing 30S particles and polyamine were submitted to gel filtration before the addition of 50S subunits. This result supports the idea that spermidine bound to site A is firmly attached to the ribosomal particles and cannot be released easily.

We have found some variability in the number of polyamine molecules bound and the corresponding percentage of association values obtained with different ribosomal preparations. In a series of fifteen experiments the average values were 0.7 molecules of spermidine bound per 30S subunit and 35% association. Considering these results we can calculate that a maximum of 2 molecules of spermidine per 30S particle are required to saturate the site A.

In order to know if the translocation of polyamine from site B to site A of the small subparticle can occur we have carried out the following experiment: two identical aliquots of a 30S subunits preparation were separately incubated at 25°C in the presence of spermidine. After filtration through Bio-Gel one of the mixtures was incubated at 65°C for 5 min and then chilled to 0°C, whereas the other was maintained at low temperature. Afterwards 50S subunits were added

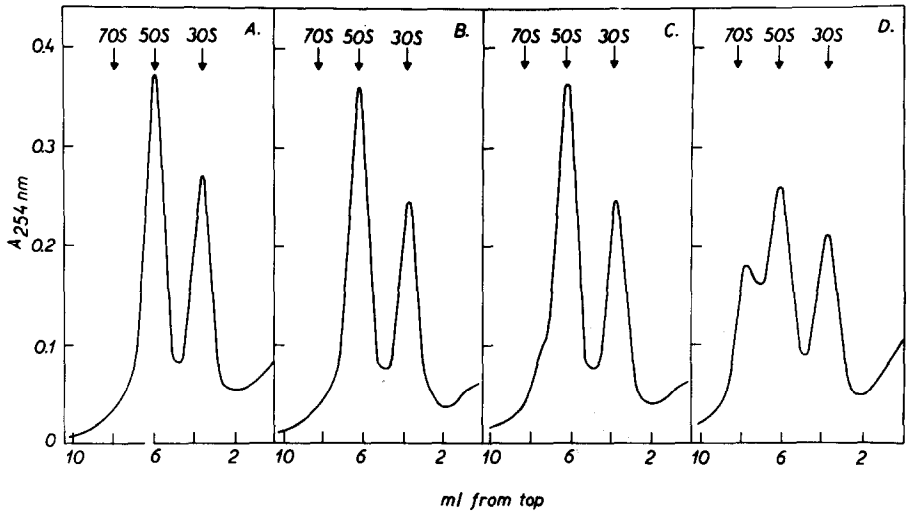

Fig.2. Effect of heating during or after the binding step on the association reaction. Sedimentation profiles of samples incubated in two steps under the following conditions: (A) and (B) contained 30S subunits incubated at 25°C in the absence and in the presence of 2 mM spermidine, respectively. After chilling at 0°C and filtration through Bio-Gel columns 50S subunits were added and samples were analyzed by sucrose gradient centrifugation. (C) same as (B), but after filtration through Bio-Gel the mixture was incubated at 65°C for 5 min and then cooled at 0°C; 50S particles were added and samples were analyzed. (D) 30S particles were incubated at 65°C with spermidine and then cooled to 0°C. After gel filtration the assay was carried out as in (A) and (B). All other details as indicated in Materials and methods.

to both mixtures in the cold and the association was assayed by sucrose gradient centrifugation. Fig.2 shows that in both cases there was no association, indicating that spermidine attached to site B did not translocate to site A by incubation at high temperature. On the other hand when 30S particles were incubated with spermidine at 65°C before the filtration through Bio-Gel the association occurred normally (fig.2D). Similar results were obtained when equilibrium dialysis was used in the binding step.

Other preliminary experiments seem to indicate that the spermidine bound to 30S subunits is not released by formation of 30S–50S couples.

From our results we conclude that it is possible to distinguish at least two classes of binding sites for polyamines in ribosomal 30S subparticles: sites A, which are involved in the formation of 30S–50S couples and can be functional only at high temperature, and sites B, which do not participate in the reaction of association. On the other hand the binding sites of 50S subunits are non-active to induce association.

The present studies may contribute to a further understanding on the role of polyamines in the interactions occurring between ribosomal particles.

Acknowledgements

We thank Professor L. F. Leloir, Dr S. H. Goldemberg, Dr B. Fridlender and all members of the Instituto de Investigaciones Bioquímicas for valuable discussions. This work was supported in part by grants from the Ministerio de Bienestar Social, O.E.A. (Programa Regional de Desarrollo Cientifico y Tecnológico) and Consejo Nacional de Investigaciones Cientificas y Técnicas (Argentina). I.D.A. is a Career Investigator of the latter institution.

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