TEMPERATURE DEPENDENCE FOR PHYSICAL AND FUNCTIONAL RECONSTITUTION OF 30S RIBOSOMES OF E. COLI

by

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SUMMARY

Employing the reconstitution system of Traub and Nomura, the temperature dependence of physical and function reconstitution was studied. The data show that the physical formation of 30S particles occurs at temperatures 15° below the maximum temperature for functional reconstitution. This may indicate that the high activation energy required for functional reconstitution is directed toward arrangement of active sites rather than the interaction of proteins with their cognate sites on RNA.

INTRODUCTION

The reconstitution of functional 30S ribosomes from purified 16S RNA and total proteins derived from 30S ribosomes has been studied in detail. The in vitro assembly requires only those components which are an integral part of the ribosome structure (1,2), and this has led to the view that the assembly of ribosomes proceeds by a self-assembly mechanism. One of the significant requirements for in vitro assembly of bacterial ribosomes is high temperature. In the case of the reconstitution of E. coli 30S ribosomes optimum temperatures between 40° and 50° have been reported (3). Although 40° is at the upper extreme of the permissive growth temperature for E. coli, it is not unreasonable to assume that the high temperature requirement is a reflection of an energy barrier which is overcome by other mechanisms in vivo. For example, certain entropy

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changes which come about by the interaction of the macromolecular components with membranes or other cellular components could be coupled with the assembly process.

Because ribosome assembly in vivo is initiated on RNA molecules that have not yet undergone their complete post-transcriptional modification (4, 5, 6, 7), we recently studied the in vitro assembly of ribosomes employing a natural precurser of 16S RNA termed p16 RNA, that is found in relaxed mutants that have been starved for a required amino acid (8, 9). However, employing this natural precurser did not result in lowering the temperature optimum for the reconstitution reaction (8). In general, successful reconstitution in vitro is measured by the acquisition of ribosome function as determined by its ability to participate in protein synthesis. Reconstitution of a 30S ribosomal particle with the precurser to 16S RNA results in a particle which is inactive (9). Because of this, it was necessary for us to moniter the success of reconstitution with 16S RNA by the acquisition of the correct sedimentation value. During the course of these experiments we discovered that there was a disparity between the temperature requirement for physical reconstitution of 30S particles and the acquisition of activity when reconstitution is carried out with mature 16S RNA. The data reported here will show that the physical reconstitution of 30S particles occurs 15° lower than the formation of active particles.

MATERIALS AND METHODS

The preparation of RNA, reconstitution conditions and assay for protein synthesizing capacity have been described previously (8, 9). In some cases (i. e., Figure 1) ribosomes reconstituted from 16S RNA and total proteins were compared, in their polyphenylalanine synthesizing capacity, to 30S ribosomes which were also incubated with total 30S proteins. The native ribosomes were incubated at 40° with proteins under the conditions described (9) at the ratio of 44 μg of 30S ribosomal protein per A_{260} unit of RNA.

RESULTS AND DISCUSSION

Particles which were formed by reconstitution with mature 16S RNA and the total 30S ribosomal proteins were tested for activity in a polyuridylic acid-polyphenylalanine incorporating system. The nature of the experiments required that the systems be responsive to increasing amounts of ribosomes.

The data in Figure 1 (A and B) show that over the range of concentrations of ribosomes employed, there was a linear response in the amount of polyphenylalanine formed for the 30S ribosomes put into the reaction mixture. The differences between Figure 1A and 1B, which show a greater activity of native 30S ribosomes in Figure 1B, result from the stimulation of native 30S activity by their prior exposure to 30S ribosomal proteins under reconstitution conditions. This stimulation has been reported by Kurland and his associates (10). It should be noted that the reconstitution reactions which we carried out were done in protein excess. It is probable that the more accurate comparison for total activity of reconstituted particles therefore, comes from a comparison similar to that in

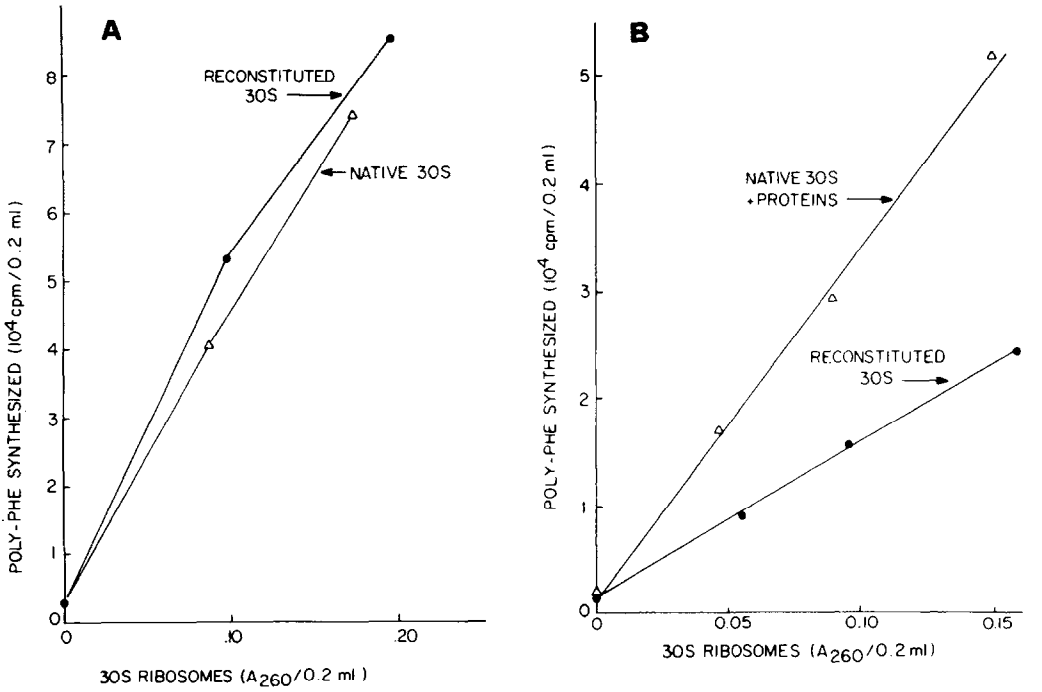

Figure 1. Polyuridylic acid-directed poly-phenylalanine synthesis by native and reconstituted 30S ribosomes. Samples of 30S ribosomes were added to a complete incorporating system containing excess native 50S ribosomes. (A): comparison of reconstituted 30S particles with native particles from which the total 30S proteins were derived (B): comparison of reconstituted 30S particles with native 30S ribosomes that had been incubated under reconstituting conditions with total 30S proteins. Ribosomes were recovered from sucrose gradients before being tested for poly-phenylalanine synthesizing capacity.

Figure 1B. In some experiments, not reported here, we have found that the differences between native 30S ribosomes and reconstituted 30S ribosomes often depends on the preparation of ribosomal proteins used in the reconstitution and that in some preparations, the differences shown in Figure 1B are not evident.

Reconstitution as a function of temperature of the reconstitution mixture was followed in two ways. First, after the incubation period of 20 minutes at a given temperature, the mixture was layered on a sucrose gradient and analyzed for the percentage of the input RNA which resided in 30S particles in the gradient. Secondly, portions of the reconstitution mixture were transfered immediately to a polyuridylic acid-polyphenylalanine incorporating system. The data in Figure 2 show the results of the sucrose gradient analyses of the reconstitution mixtures held at various temperatures. As expected, reaction mixtures held at lower temperatures gave particles of intermediate sedimentation value and as the temperature approached the maximum (50°) more of the RNA was included in 30S particles.

Figure 3 is a plot of the percentage of 30S particles formed at various temperatures, as determined by integrating the peaks shown in Figure 2, and of the polyphenylalanine synthesizing activity determined for reconstitution mixtures held at each temperature. In this case we used a maximum incubation temperature of 50°. It is clear that the half maximal point on the curve for physical reconstitution is 15° less than the half maximal acquisition of protein synthesizing activity. We are not aware of studies which show the differences between the temperature dependence of physical reconstitution and temperature dependence of the reconstitution of active particles. Nomura et. al. (11) have previously shown that physical reconstitution of active particles are closely related when compared at various times at 35°. In the experiments reported here, we have examined the temperature dependence after the reconstitution at each temperature had apparently gone to completion. However, we have not examined the temperature dependence at times beyond 20 minutes.

There are several interpretations which can be placed on the experiments

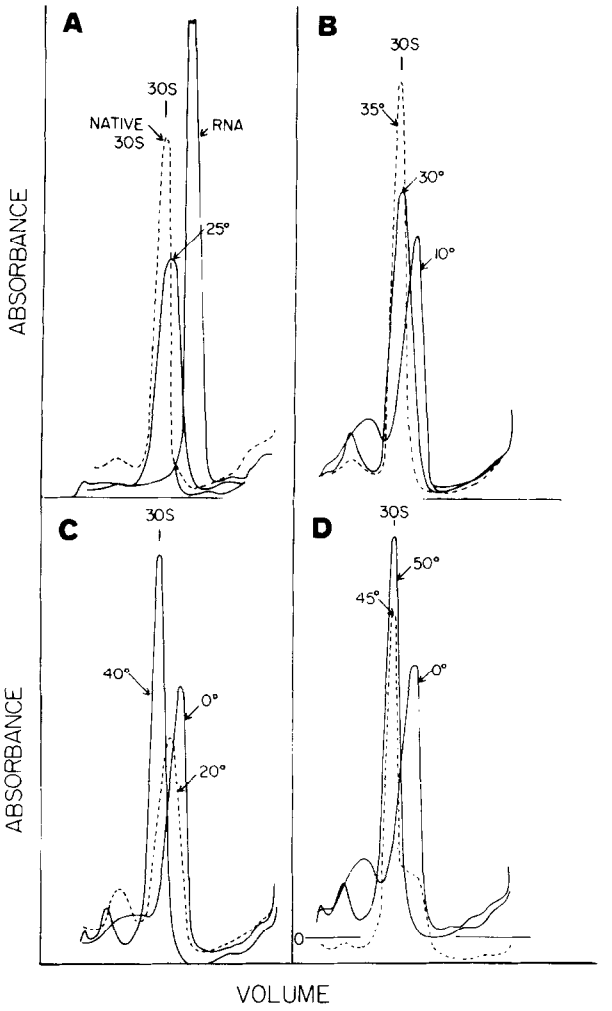

Figure 2. Sedimentation profiles of particles formed at different temperatures. Samples were removed from reconstitution mixtures which were held for 20 minutes at the temperatures indicated, and layered on sucrose gradients in 0.01M Tris pH 7.4, 0.001M Mg++ and 0.1M KCl in a Beckman SW50.1 rotor. After 90 minutes at 50,000 rpm, the tube contents were pumped through an ISCO UV moniter and the absorbance was recorded. The location of the 30S marker was determined by including suitable labelled native 30S ribosomes. The base line for the 0° and 50° samples in panel D was adjusted up for clarity.

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reported here. For example, it is possible that although particles with sedimentation coefficients of 30S are formed at lower temperatures the particles lack some critical proteins. Schulte and Garrett (12) have shown that

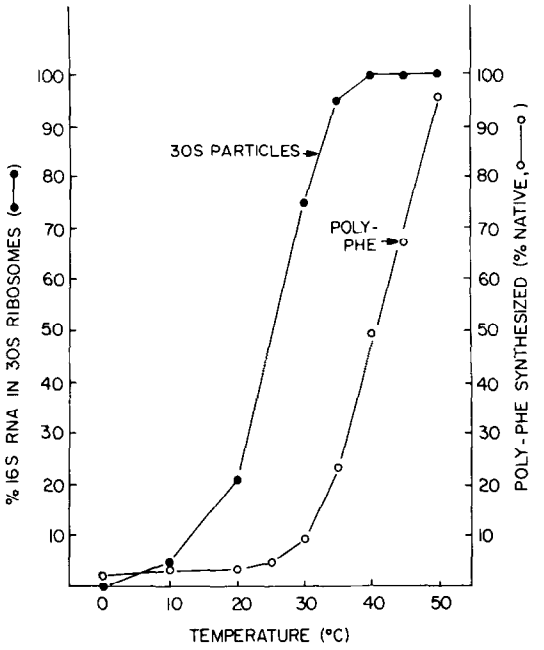

Figure 3. The temperature dependence of the physical reconstitution of 30S particles and the recovery of active ribosomes. Physical reconstitution was determined by integrating the material in the peaks shown in Figure 2. Polyphenylalanine synthesizing capacity was determined on paired samples which were withdrawn from the reconstitution mixtures and put directly into the polyuridylic acid directed assay. Synthetic capacity was compared with native 30S ribosomes which had been incubated under reconstitution conditions with 30S proteins as shown in Figure 1B.

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protein S8 reacts with RNA optimally at 42°. However, particles formed without this particular protein do not sediment at 30S (13). In previous work Nomura et. al. (11) found that the high temperature required for the reconstitution of active 30S ribosomes was due to a rate limiting reaction which possibly was dependent upon a structural rearrangement of one of the intermediate particle forms and perhaps of the RNA itself.

It is possible that the difference in temperature for the formation of 30S ribosomes and for the formation of active particles is explained by the requirement for a higher activation energy for the precise configuration required for the expression of the full range of ribosome functions, and that the assembly

process itself, that is, the interaction of proteins with their cognate sites on the RNA, requires a lower activation energy. The differences between these two activation energies may be related to recent indications (14,15,16) that the cell may employ "assembly factors" which could serve to lower the activation energy of the assembly of active particles. Along these lines it should be noted that some of the ribosomal proteins themselves play a kinetic role in ribosome assembly, and may indeed serve no further role in the activity of the particle (17).

The available data which suggest a role for extrinsic factors in the assembly of ribosomal particles exists only for the 50S ribosome. However, it is possible that the differences shown here provide a system for searching for factors which are able to "fine tune" the assembly process at temperatures within the growth range of E. coli in order to produce active 30S ribosomes from 30S particles which lack full activity. We are in the process of searching for such factors.

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