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THE NUCLEATION OF CRYSTALS OF THE LARGE RIBOSOMAL SUBUNITS FROM BACILLUS STEAROTHERMOPHILUS

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SUMMARY

Several steps in the formation of crystals of the large ribosomal subunit from Bacillus stearothermophilus have been visualized by electron microscopy. It was observed that the process of growth starts with the formation of aggregates. These, in turn, undergo a rearrangement toward the creation of nucleation centers. The nature and the quality of the expected crystals are dictated by the shape and the degree of order of the nuclei.

INTRODUCTION

The primary structures of the 53 proteins and of the three RNA chains of the E. coli ribosome have recently been determined (1-3). Also, various chemical, physical and immunological techniques have been used to gain insight into the architecture of the ribosomal subunits (4,5).

Diffraction techniques are powerful direct methods with the potential to yield a reliable model of the ribosome. Use of these methods is dependent on the availability of three-dimensional crystals or, if these cannot be obtained, of two-dimensional ordered arrays.

The process of crystal growth is initiated by nucleation. Although many biological molecules and complicated particles have been crystallized, little is known about the mechanism of nucleation. Theoretical models have been developed for the nucleation of crystals of small molecules (6,7) but only a few of them apply to biological macromolecules. Most of the data currently available concerning the process of nucleation of crystals of biological systems are based on rather indirect evidence, such as

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use of scattering techniques for monitoring the size of the readily formed aggregates under the right crystallization conditions (8).

Recently, three-dimensional crystals of the large ribosomal subunits from Bacillus stearothermophilus were obtained (9). These crystals provide an excellent system for direct investigation of nucleation since the individual particles are large enough to be seen by electron microscopy. Once the conditions for crystal growth were determined and refined (9,10), the crystallization process was interrupted before the formation of mature crystals, and the crystallization medium was examined by electron microscopy. As described here, these studies led to the visualization of nucleation. It is suggested that nucleation centers are created from previously formed aggregates and develop to crystalline objects of varying degrees of order.

MATERIALS AND METHODS

The large ribosomal subunits from B. stearothermophilus (strain 799) were obtained, as described in (9). The crystallization procedure is described in detail in (10). The crystallization medium which contained the ribosomal subunits in their storage buffer (10), 6 mM spermidine and 100 mM Mes buffer, pH = 6.3 - 6.9, was equilibrated with either 10% ethanediol (using the hanging drop technique) or with 30% methanol (in capillaries) in the presence of 0.5 M NaCl in the reservoir. Several identical crystallization experiments were carried out simultaneously. Each of these was terminated at a different time, at intervals of 4 - 6 days, starting from the fifth day.

Upon termination, the content of each drop was collected, and either used for conventional negative-staining (with 1% uranyl acetate) experiments or fixed (in 0.6% glutaraldehyde), embedded (in resin ERL 4206), and sectioned (9). The sections were successively stained with lead citrate, 2% uranyl acetate, and again with lead citrate (9,11).

RESULTS AND DISCUSSION

A typical electron micrograph of a section (about 50 nm thickness) of a two months old, well ordered crystal of the ribosomal 50S subunit is shown in Fig. 1, whereas Fig. 2 shows the early steps of crystallization.

During the first few days, the subunits aggregate and form clusters which show no clear order (Fig. 2a-c). The nucleation of

Fig. 1: An electron micrograph of a thin section through a crystal grown in 10% ethanediol, pH = 6.6.

the crystals occurs within the first 15 days of crystallization (Fig. 2d). Since hardly any isolated particles could be detected in the micrographs of the initial period regardless of glutaraldehyde fixation (Fig. 2a-c) and since there are some small aggregates close to most of the nucleation centers (Fig. 2d), it is likely that nucleation starts within the aggregates. These are formed during the early period of the crystallization experiment and later undergo a rearrangement toward the formation of nucleation centers. The significance of the dynamic processes that take place in the initial stages of crystallization has already been discussed elsewhere (10). It has been observed that during this period even a slight expansion of the volume of the crystallization drop may prohibit the production of crystals.

A few of the nucleation centers have the same morphology as a proper crystal (Fig. 2d) whereas others seem to develop anisotropically, forming star-shaped crystallites. The initiation of growth of proper crystals is generally independent of that of the star-like crystallites. Thus, during the initial stage of nucleation, there is not only competition between the formation of crystals and of the precipitate but also between the formation of proper crystals and of star-like crystallites. It has been observed earlier that in most of the successful experiments crystal growth is accompanied by the appearance of a fine precipitate (10),

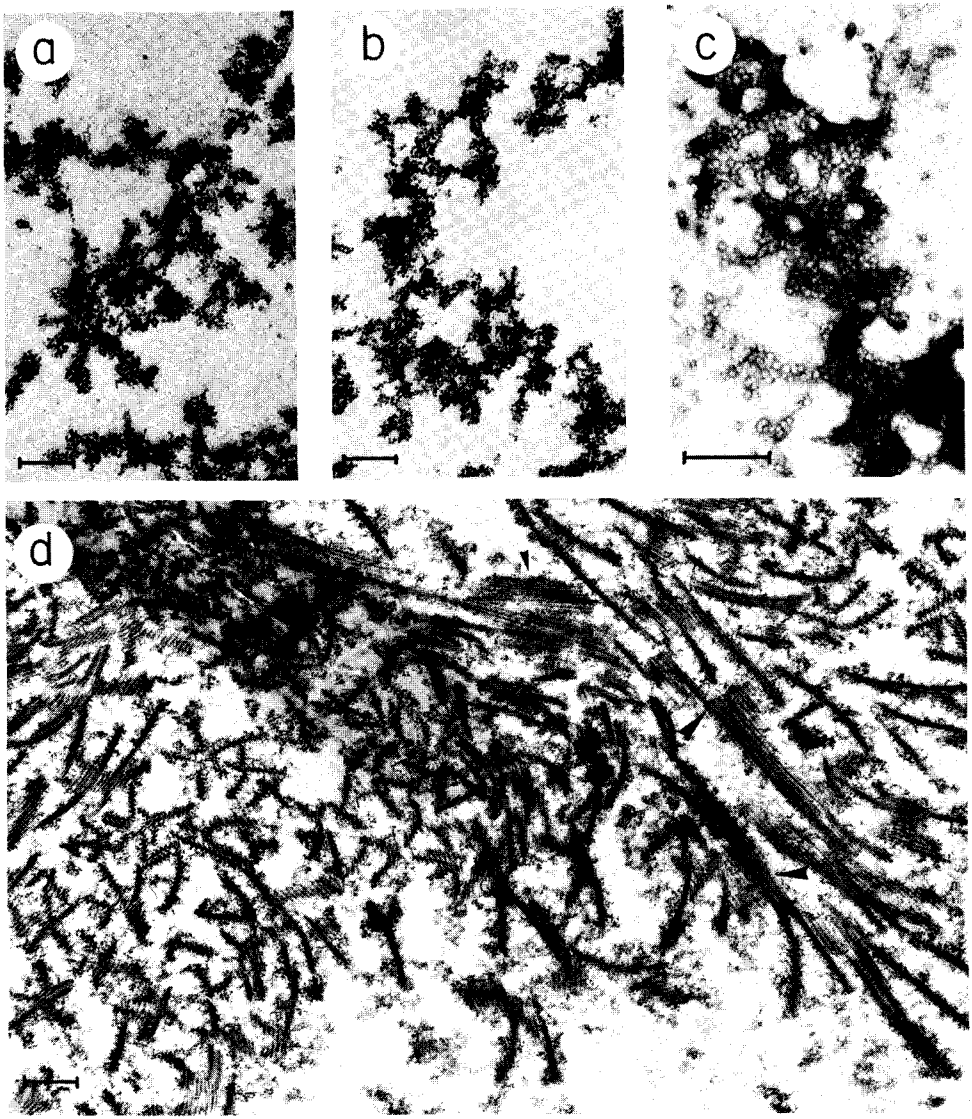

Fig. 2: Electron micrographs of thin sections through embedded droplets which were equilibrated with 10% ethanediol, pH = 6.6. The experiments were interrupted after 5 days (a), 9 days (b), and 15 days (d). "Proper" crystals are marked by arrows. (c) An electron micrograph of the content of a droplet which was applied to a grid and negatively stained with 1% uranyl acetate. Growth condition as in 2a. Bar length = 500 nm.

and the existence of a precipitate seems to inhibit crystal growth but does not seem to affect, or even to enhance, the number of nucleation centers which are produced in a droplet.

The nucleation centers of both the proper crystals and the arms of the star-like crystallites appear to be reasonably well-formed. However, not all the arrays exhibit the same pattern and repetition. Their periodic spacings range between 20 nm and 33 nm for one axis and 13 nm and 27 nm for the second, whereas the "proper" nucleus is 25 ± 2 nm x 15 ± 2 nm.

Since only projected images are seen by electron microscopy the observed variations in the apparent cell constants of the arms may stem either from the production of another crystal form or from different projected views of the same form. For both cases, the branching is due to a multiplicity of specific interactions rather than to a repetition of the same contacts. A complex and asymmetric particle such as the ribosomal 50S subunit has a large number of potential attachment sites, some of which may have relatively small binding energies and a tendency toward conformational flexibility. Thus, ribosomes are able to form crystalline sheets (12-15), but only rarely are well-ordered three-dimensional crystals of ribosomal particles produced (9,16,17).

A high degree of polymorphism was also observed for the nucleosome cores and was correlated to their large conformational variability (18). Growth along different directions has also been observed while aiming to obtain other crystal forms. An example is shown in Fig. 3, where the proper crystals are of a = 28.8 ± 3 nm and b = 31.6 ± 3 nm (Form III in ref. 16). Although it is not expected that the star-like crystallites will grow further, occasionally small microcrystals with a similar morphology have been seen in the light microscope (Fig. 4). If indeed these formations are the products of the star-like nuclei, it may indicate that multidirectional growth is an inherent characteristic of the system and is expressed not only during nucleation but also during crystal growth. This might also be the cause for the unusual fragility of the proper crystals and for their extreme sensitivity to small changes in external conditions. It may also be one of the reasons for the difficulties encountered so far in obtaining larger crystals. It is of interest that most of the arms of the star-like

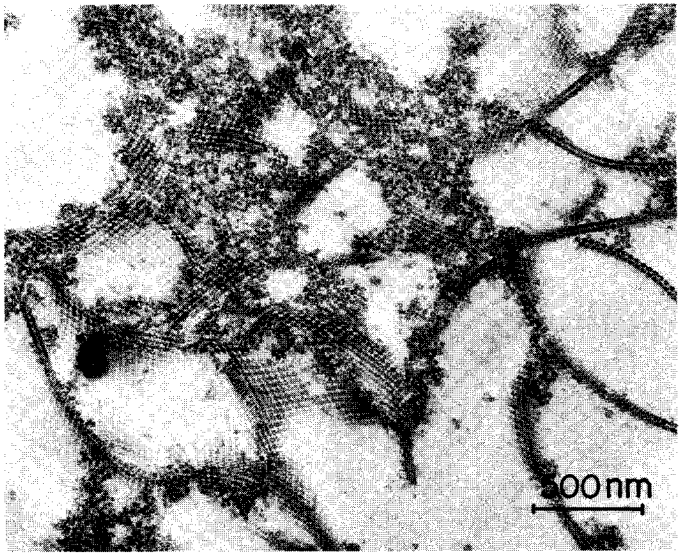

Fig. 3: An electron micrograph of a thin section through an embedded droplet. Crystallization conditions: 30% methanol, pH = 6.3.

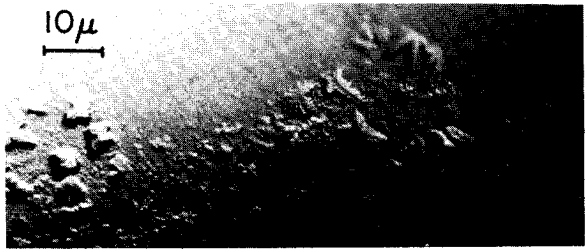

Fig. 4: Crystals grown in a capillary as seen by light microscopy (30% methanol, pH = 6.9).

crystallites are short and straight, whereas most of the proper crystals nucleate as thin, relatively long and somewhat bent laths. During post-nucleation growth these laths become thicker and less curved, as if crystal growth forces a higher degree of order.

CONCLUSIONS

We have shown that, despite the high degree of complexity and the lack of internal symmetry, the large ribosomal subunits can be crystallized *in vitro*. The crystals nucleate within pre-formed aggregates and are not a result of collision between single particles. Due to its complexity, the surface of the ribosomal subunit is a composite of a variety of potential interaction sites some of which are used for interparticle contacts, that may lead to the production of various periodic structures. Thus, it is not surprising to find that different crystal forms are developed under the same crystallization conditions. Although it is still not possible to predict the nature of the product, we do hope that it will be possible to direct the pattern of crystal growth in early stages.

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REFERENCES

1. Wittmann, H.G., Littlechild, J.A. and Wittmann-Liebold, B. (1979) "Ribosomes: Structure, Function and Genetics" (Chambliss, G. et al., Eds.) pp 51-88, Univ. Park Press, Baltimore.
2. Noller, H.F. (1979) "Ribosomes: Structure, Function and Genetics" (Chambliss, G. et al., Eds.) pp 3-22, Univ. Park Press, Baltimore.
3. Wittmann, H.G. (1982) Ann. Rev. Biochem. 51, 155-183.
4. Chambliss, G., Craven, G.R., Davies, J., Davis, K., Kahan, L. and Nomura, M., Eds. (1979) "Ribosomes, Structure, Function and Genetics", Univ. Park Press, Baltimore.
5. Brimacombe, R., Stöffler, G. and Wittmann, H.G. (1978) Ann. Rev. Biochem. 47, 217-249.
6. Gibbs, J.W. (1928) "Collected works of J.W. Gibbs", pp 55-353, Longmas Green, New York.

7. Zettlemover, A.C., Ed. (1969) Nucleation, Marcel Dekker, New York.
8. Kam, Z., Shore, H.B. and Feher, G. (1978) J. Mol. Biol. 123, 539-554.
9. Yonath, A.E., Müssig, J., Tesche, B., Lorenz, S., Erdmann, V.A. and Wittmann, H.G. (1980) Biochem. Inter. 1, 428-435.
10. Yonath, A., Müssig. J. and Wittmann, H.G. (1982) J. Cell. Biochem., in press.
11. Reynolds, E.S. (1963) J. Cell Biol. 17, 208.
12. Clark, M.W., Leonard, K.R. and Lake, J.A. (1982) Science 126, 999-1002.
13. Barbieri, M. (1979) J. Supramol. Struct. 10, 349-357.
14. Kühlbrandt, W. and Unwin, P.N.T. (1982) J. Mol. Biol. 156, 431-448.
15. O'Brien, L., Shelley, K., Towfighi, J. and McPherson, A. (1980) Proc. Natl. Acad. Sci. USA 77, 2260-2264.
16. Leonard, K.R., Arad, T., Tesche, B., Erdmann, V.A., Wittmann, H.G. and Yonath, A. (1982) in Proc. 10th Inter. Cong. E.M., Hamburg. Vol. 3, pp. 9-15.
17. Wittmann, H.G., Müssig, J., Piefke, J., Gewitz, H.S., Rheinberger, H.J. and Yonath, A. (1982) FEBS Letters, in press.
18. Dubochet, J. and Noll, M. (1978) Science 202, 280-286.