Short Communications

Analysis, by Two-Dimensional Gel Electrophoresis, of Ribosome Crystal Proteins

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A ribosome crystal is an aggregate of ribosomes which are packed in a regular array. Preliminary experiments analysing the proteins from ribosome crystals by two-dimensional gel electrophoresis show that, although most proteins appear similar to those from polyribosomes, four extra proteins also seem to be characteristic of ribosome crystals.

The structure and formation of ribosome crystals in several eukaryotic organisms has been studied extensively by electron microscopy (for review, see Nanninga, 1973). A ribosome crystal is an aggregate of ribonucleoprotein particles which are packed in a regular array. Unlike the other polymeric forms of ribosomes, the polyribosome, crystal bodies contain many more ribosomes and yet appear to be inactive in protein synthesis.

Slow cooling of fertilized chicken eggs causes disaggregation of polyribosomes, which in turn leads to the formation of a pool of ribosomes prone to crystallization (Morimoto *et al.*, 1972). The recent isolation of a fraction of intact crystals from such embryos has made it possible to study these structures biochemically (Dondi & Barker, 1974). A result of these investigations was the finding that crystals contain the same complement of ribosomal RNA molecules as normal post-embryonic chick ribosomes. The present communication describes the continuation of such studies, in which proteins from crystals and polyribosomes were compared by two-dimensional polyacrylamide-gel electrophoresis.

For polyribosomes, approximately 150 embryos, after 5 days' incubation at 37°C, were isolated from their eggs at room temperature (22°C). These embryos were washed in cold (approx. 4°C) TKM₁ buffer (50 mM-Tris-HCl, pH 7.5, 50 mM-KCl, 5 mM-MgCl₂) and then homogenized in approx. 3 vol. of TKM₁ buffer, by using six to eight strokes of a Teflon-glass homogenizer, driven by electric motor at approx. 500 rev./min. The homogenate was centrifuged in the MSE 8×50 rotor of the MSE High-Speed 18 centrifuge for 10 min at 4°C and 20000g_{av.}. The supernatant fluid was then carefully layered over a discontinuous sucrose gradient

consisting of 6 ml of 2.0 M-sucrose in TKM₁ buffer and 4 ml of 0.5 M-sucrose in TKM₁ buffer in tubes of the MSE 8×25 rotor and centrifuged at 180000g_{av.} in the MSE Superspeed 65 for 5-6 h at 4°C. Pellets obtained in this way could be resuspended in a small volume of TKM₁ buffer and gave an E_{260}/E_{280} ratio of approx. 1.75. Sucrose-density-gradient analysis was carried out on a linear 15-45% (w/v) sucrose gradient in TKM₁ buffer centrifuged at 132000g_{av.} for 80 min at 4°C in the Beckman SW 36 rotor. The gradient was generated and fractionated by using the equipment previously described (Dondi & Barker, 1974) and an E_{254} profile was obtained which indicated that polyribosomes containing up to 14 ribosomes had been isolated.

Crystal fractions were obtained from embryos subjected to hypothermia, by centrifugation through a high-density discontinuous sucrose gradient, as previously described (Dondi & Barker, 1974). Polyribosome and crystal suspensions were subjected to the same protein extraction procedure, which used 67% (v/v) acetic acid and 0.2 M-MgCl₂ (Kalt-schmidt & Wittmann, 1972). Proteins obtained in this way were analysed by two-dimensional gel electrophoresis in a specially prepared apparatus, which gave gel slabs in the second dimension measuring 10 cm×20 cm (Dondi, 1975). Proteins extracted from polyribosomes and analysed by using this apparatus were resolved into 68-71 cationic proteins and two to eight anionic proteins. After washing of the polyribosomes under conditions of high salt (Chatterjee *et al.*, 1973) the cationic protein pattern remained unchanged, whereas practically all the anionic proteins, apart from two strongly stained spots, were removed.

The overall two-dimensional pattern obtained for crystal proteins appeared to be very similar to that of polyribosomes. The anionic protein pattern for crystals (not shown) exhibited no major difference from that for polyribosomes. However, when the cationic proteins of crystals were analysed, four extra

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protein spots were observed (Plate 1). Plate 1 is a photograph of two polyacrylamide-gel slabs containing the cationic proteins of crystals (Plate 1a) and polyribosomes (Plate 1b). Proteins C₁ and C₂ are separated by charge in the first dimension but migrate to the same level in the second dimension, indicating a similar shape and size. Protein spots C₃ and C₄, however, appear to have a similar charge in the first dimension but migrate to different levels in the second dimension. After high-salt washing these four protein spots are still observed, although their staining intensity may sometimes be decreased.

In considering the overall gel pattern obtained from the crystal fraction it is obvious that, for the majority of proteins, the particles in crystals contain the same proteins as the ribosomes of polyribosomes. This result, when taken in conjunction with the previously determined RNA content of crystals (Dondi & Barker, 1974), shows, in terms of biochemical parameters, that crystal structures do indeed consist of ribosomes.

However, the occurrence of four extra proteins in crystals is also of interest. Although the crystal fraction cannot be said to be completely pure it is doubtful that these proteins are due to contamination, for several reasons.

For example, they migrate electrophoretically well

within the general pattern formed by the other ribosomal proteins. They also appear to take up stain to the same extent as the other proteins. Finally, general contamination would not be expected to give the regular appearance of only these specific proteins, and yet they are always observed when crystal proteins are analysed.

These extra proteins may be involved in the bonding of ribosomes within the crystal structure and may also have some functional role in protein synthesis. Any further studies will require purification of these crystal-specific proteins.

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EXPLANATION OF PLATE I

Two-dimensional gel-electrophoresis patterns of crystal and polyribosomal proteins

Photograph of polyacrylamide gels showing cationic proteins from crystals (a) and polyribosomes (b). Crystal-specific proteins C₁, C₂, C₃ and C₄ are arrowed. Conditions of electrophoresis: load of approx. 500 μ g of protein per gel, electrophoresed at 5 mA/gel for 16–18 h in the first dimension and 30 mA/gel for 20–22 h in the second dimension.