RIBOSOMAL AGGREGATES IN CHICK EMBRYO TISSUES AFTER EXPOSURE TO LOW TEMPERATURES

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1. Introduction

In 1967 Byers showed that exposure of chick embryo cells to hypothermia led to the aggregation of ribosomes in crystalline sheets in which the lattice was of the P_4 plane group [1]. The smallest repeating unit was a group of four ribosomes. This observation provided an explanation [2] of some earlier results of Bell et al. [3, 4] and led to the conclusion that the tetramer unit is stable to procedures for cell fractionation.

A hypothesis for the origin of these aggregates is that they form from ribosomes which have detached from the messenger after completing the round of synthesis and which are undergoing a process of activation prior to starting the next round. Low temperature may either arrest the activation process at a stage in which the ribosomes are capable of aggregating or directly facilitate the aggregation. Further investigations of the aggregates may therefore throw some light on the mechanism of initiation of protein synthesis in animal cells. An early finding was that the pattern of peaks in sucrose gradients obtained with ribosome preparations from cold-treated embryos was more complex than was shown in the previous studies, and this communication describes this observation.

2. Materials and methods

Chick embryos were produced by incubating fertile eggs at 38°C for seven days. They were exposed to 7–10°C for 20–28 hr and ribosomes were prepared from the whole embryo minus the head. The tissues were homogenised in 2 volumes of 0.25 M sucrose/TKM (0.05 M tris-HCl pH 7.5, 0.025 KCl, 0.005 M $MgCl_2$) and centrifuged at 10,000 X *g* for 20 min. The supernatant was layered over 3 ml 2 M sucrose/TKM and centrifuged at 105,000 X *g* overnight. The pellet surface and tube were washed with TKM and the pellet re-suspended in TKM. 0.1 ml of suspension containing 2 A_{260} units was layered over a 11.5 ml isokinetic gradient [5] containing TKM and a lowest sucrose concentration of 10% w/w, and was centrifuged at 2° for 1.5 hr at 40,000 rpm in the Spinco SW 40 rotor. It was aspirated via a metal capillary driven to the bottom of the tube,

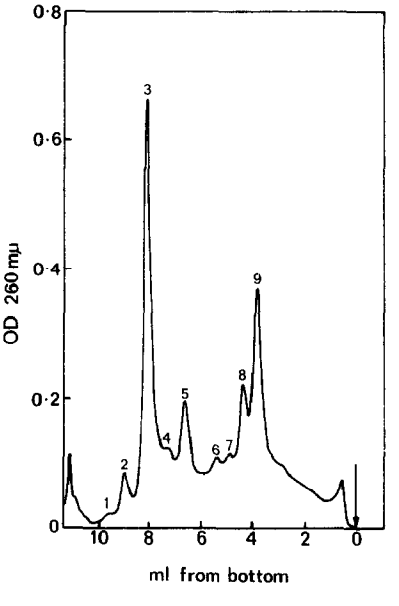

Fig. 1. Sucrose gradient profile of ribosomes from cold-treated chick embryos.

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Table 1
Sedimentation constants of the peaks shown in fig. 1.

Peak number	Probable identity	Sedimentation constant
1	Small subunit	48
2	Large subunit	63
3	Monomer	84
4	Unknown	106
5	Dimer	122
6	Trimer	157
7	Polyribosome trimer	170
8	Tetramer	184
9	Tetramer	197

through a flow cell (volume 0.1 ml, path length 1.0 cm) in a Cary 14 spectrophotometer (Applied Physics Corp., Monrovia, California) and optical density at 260 mμ was recorded. Ribosomes from sucrose gradient fractions were fixed for electron microscopy by a method similar to that of Haschemeyer and Gross [6] and negatively stained [7].

3. Results and discussion

Fig. 1 shows the distribution of peaks obtained. This gradient was well enough resolved to allow an assessment of the sedimentation constant of even minor peaks and these are shown in table 1. The major peak is the monomer (confirmed by electron microscope observation (fig. 2) on material from other gradients) with a sedimentation constant of 84 *S*. On the light side are two small peaks of subunits at 48 *S* and 63 *S*. Peak 5 at 122 *S* is presumed to be a dimer but this has not yet been confirmed by electron microscope observation.

Bell et al. observed a peak in a region ascribed to tetramers. In this study material in this region has been resolved into two peaks (8 and 9) with sedimentation constants of 184 *S* and 197 *S*. Electron microsope observation has shown tetramers in this region (fig. 3) and evidence is available to suggest that both of these peaks are composed of tetramers. The sedimentation constants given above are all higher than those observed by Bell who found 72 *S* for the

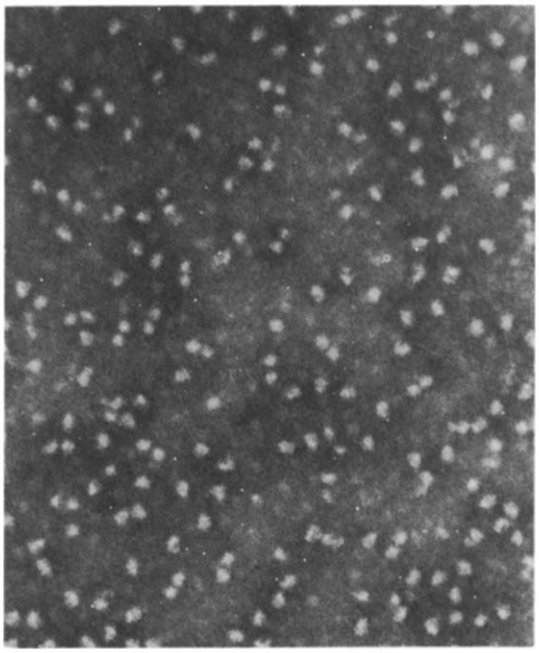

Fig. 2. Ribosome monomers from peak 3 of a sucrose gradient. X 100,000.

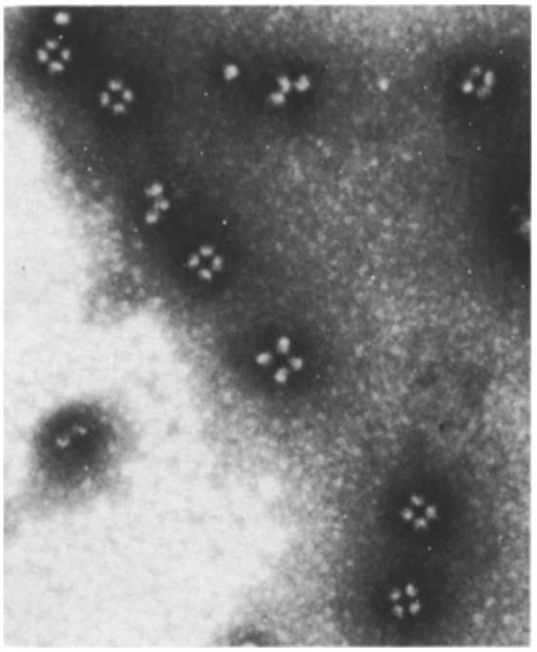

Fig. 3. Ribosome tetrameters from peaks 8 and 9 of a sucrose gradient. X 100,000.

monomer and 158 *S* for the tetramer, but similar to those of mouse liver polyribosomes measured by Noll [5].

In other experiments ribosome preparations have been treated with ribonuclease either by the addition of 0.1 μg/ml of the enzyme to the final suspension or by placing a layer of 1.0 M/TKM sucrose containing 0.5 μg/ml ribonuclease above the 2.0 M sucrose/TKM of the final centrifugation. These treatments convert polyribosomes from normal chick embryos or mouse liver to monomers, but the peaks described above were unaltered by them (cf. ref. [4]). Two other ribonuclease-stable peaks were observed, peak 4, 106 *S*, between the monomer and dimer, and peak 6, 157 *S*, between the dimer and tetramer. Peak 7, 170 *S*, was probably a polyribosome since it disappeared after ribonuclease treatment, as did much of the poorly resolved material to the heavy side of the tetramer. Peak 6 may be a trimer, composed of part of the tetramer, but the nature of peak 4 is unknown.

These results indicate that the association of ribosomes into tetramers observed in electron micrographs of cold-treated chick embryo cells *in vivo* is part of a more complicated pattern of ribosome interactions. In a recent report, Friedman, Lu and Rich [8] showed that incubation of *E. coli* cells at 6–8° resulted in the cessation of protein synthesis and the conversion of ribosomes into subunits. They suggested that this was due to an interruption by low temperature of the process of reinitiation of synthesis. They also quoted a private communication from Vournakis which suggested that cold treatment of chick embryo led to the accumulation of ribosomal subunits. No such result has been found in this study although it was observed that chick embryo ribosomes were much more sensitive to dissociation by low magnesium or high potassium ion concentrations than mammalian ribosomes (unpublished observations).

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