Short Communications

The Location of the Ribosomal Small Subunit in the Structure of Hypothermic Ribosome Tetramers

By N. H. CAREY

Searle Research Laboratories, Lane End Road, High Wycombe, Bucks., U.K.

(Received 26 July 1971)

It has been observed that cooling the tissues of some organisms causes the ribosomes to crystallize *in vivo* (Byers, 1967; Taddei & Piscitelli, 1970). The smallest repeating unit of the crystal is a group of four ribosomes, and these tetramers are found on sucrose density gradients of ribosome preparations from cooled chick-embryo tissues (Humphreys & Bell, 1967; Carey, 1970). It was shown by Carey & Read (1971) that the tetramer can exist in at least three forms, depending on the potassium chloride concentration, one of these forms being stable under conditions that dissociate normal ribosome monomers almost completely to subunits. It was proposed that the tetramer that is stable at high potassium chloride concentrations is composed of large subunits only. The present paper presents further evidence, based on the RNA content of the tetramers stable at high potassium chloride concentrations, that supports this hypothesis.

Ribosomes were prepared without ribonuclease treatment in sucrose solutions in TKM buffer (50 mM-tris-25 mM-KCl-5 mM-MgCl₂) by methods described previously (Carey & Read, 1971). Tetramers were found to be unstable in high potassium chloride concentrations if new cellulose nitrate centrifuge tubes (Beckman Spinco) were used that had not been adequately washed as described below. Traces of some detergents left on centrifuge tubes also rendered the tetramers unstable in high potassium chloride concentrations. Satisfactory results have been obtained by scrubbing with 2% (v/v) Decon 75 (Medical Pharmaceutical Developments Ltd., Brighton, U.K.) in distilled water followed by thorough rinsing in distilled water.

Fig. 1(a)(i) shows the normal pattern of distribution of ribosomes on a sucrose density gradient when resuspended in TKM buffer. There is a large peak of monomer [peak 3 in Fig. 1(a)(i)], a smaller peak of dimer (peak 4) and the double peak of tetramer (peaks 5 and 6). Smaller peaks of ribosome subunits are also observed (peaks 1 and 2). The proportion of tetramer to monomer in this material has been increased compared with previous work by shortening the period of the last preparative centrifugation from 16 to 2 h.

Fig. 1(a)(ii) shows the same material that had been resuspended and centrifuged in solutions containing 0.35 M-potassium chloride. The trace consists mainly of the small subunit (peak 7) and large subunit (peak 8) together with the tetramer stable in high potassium chloride concentrations (peak 9).

Fig. 1(b) shows the u.v.-absorption traces of polyacrylamide-gel separations of the RNA from some of the sucrose-density-gradient peaks. The monomer from peak 3 [Fig. 1(b)(i)] and the tetramer obtained at low potassium chloride concentrations [peaks 5 plus 6 in Fig. 1(b)(ii)] showed the normal ribosome content of 18S and 28S RNA. In these experiments no attempts were made to resolve peak 4 (the dimer) completely from the monomer, but gels from this peak showed no differences in RNA content from those of the monomer.

Peak 7 was found to contain only 18S RNA, confirming that this peak contained the ribosomal small subunit. However, the amount of material recovered in this experiment was low. Peak 8 contained predominantly 28S RNA. In three experiments out of six this was the major RNA peak, but in the remaining experiments there was some 18S RNA present, presumably owing either to contamination from the adjacent small-subunit material on the sucrose density gradient or to the presence of some undegraded monomers. From the appearance of the sucrose density gradients the former seems the more likely explanation.

Peak 9, the tetramer stable in high potassium chloride concentrations, was found [Fig. 1(b)(iii)] to contain 28S RNA, but no 18S RNA, confirming the hypothesis that this particle is composed of ribosomal large subunits only.

The results illustrated here have been confirmed in six separate experiments.

These results clearly eliminate any model for the structure of the tetramer in which the small subunit participates significantly in the bonds of the association of the intact tetramer. The first three models examined by Carey & Read (1971) can thus be eliminated. The structure of the tetramer must be formed by bonds between the large subunit only of the constituent monomers, and the small

Fig. 1. (a) Separation of hypothermic chick-embryo ribosomes. Ribosomes prepared as described in the text were resuspended either in TKM buffer or in a similar buffer containing 0.35M-KCl. Samples containing 4.5 E_{260} units of ribosomes in TKM buffer or 3.4 E_{260} units in the similar buffer containing 0.35M-KCl were layered over 11.5 ml isokinetic sucrose density gradients (Noll, 1967) containing the same salts, and centrifuged for 100 min at 40000 rev./min in the Spinco SW41 rotor. (i) Ribosomes in TKM buffer; (ii) ribosomes in a similar buffer containing 0.35M-KCl. (b) Polyacrylamide-gel electrophoresis of RNA from ribosome peaks separated in (a). Peaks from four to six gradients were pooled and centrifuged at 150000g for 2 h. The pellet was resuspended in 1 mM-EDTA-36 mM-tris-30 mM- NaH_2PO_4 buffer, pH 7.8, containing sodium dodecyl sulphate (1%, w/v) for electrophoresis by the method of Loening (1967). (i) Peak 3 (monomer); (ii) peaks 5+6 (tetramer obtained at low KCl concentrations); (iii) peak 9 (tetramer stable at high KCl concentrations).

subunits must be located in a position that would allow them to dissociate easily from the tetramer, perhaps above the plane of the four large subunits. This is the fourth model examined by Carey & Read (1971). Byers (1971) has referred to findings that allow the same conclusion to be drawn. The monomer in this model of the tetramer is very similar to that proposed by Nonomura, Blobel & Sabatini (1970) for the structure of the rat liver ribosomes.

A point that may now be considered is the nature of the bond that joins the tetramer to form the microcrystalline sheets seen in the cells of the hypothermic chick embryos (Byers, 1967). It is possible that, whereas bonds between the large subunits are responsible for holding the monomer in the tetramer, it is the bonds between the small subunits that are necessary for sheet formation. If each small subunit has only one site for such bond formation, then the model for the tetramer proposed

by Carey & Read (1971) readily gives rise to the crystal lattice observed *in vivo* when a composite model is constructed.

The author thanks Mr M. Colley for skilful technical assistance and Dr A. J. Hale for providing excellent research facilities.

Byers, B. (1967). *J. molec. Biol.* **26**, 155.

Byers, B. (1971). *Proc. natn. Acad. Sci. U.S.A.* **68**, 440.

Carey, N. H. (1970). *FEBS Lett.* **6**, 128.

Carey, N. H. & Read, G. S. (1971). *Biochem. J.* **121**, 511

Humphreys, T. & Bell, E. (1967). *Biochem. biophys.*

Res. Commun. **27**, 443.

Loening, U. E. (1967). *Biochem. J.* **102**, 251.

Noll, H. (1967). *Nature, Lond.*, **215**, 360.

Nonomura, U., Blobel, G. & Sabatini, D. (1970). *Proc. 7th int. Cong. Electron Microscopy, Grenoble*, vol. 3, p. 67.

Taddei, C. & Piscitelli, M. (1970). *Proc. 7th int. Cong. Electron Microscopy, Grenoble*, vol. 3, p. 77.