A LOW BUOYANT DENSITY ACTIVE COMPLEX FOR PROTEOSYNTHESIS IN MOUSE PLASMOCYTOMA CELLS

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1. Introduction

Protein synthesis is carried out by polysomal structures, consisting of ribosomal particles attached to single stranded messenger RNA. These particles have a CsCl-buoyant density of about 1.55 g/cm^3. Lower density cytoplasmic ribonucleoprotein (RNP) complexes, called informosomes, have been recently described by Spirin et al. [1, 2] and by others [3–9]. The biological significance of these low density RNP complexes has not yet been completely elucidated in mammalian systems. In previous communications we described the existence of low CsCl-buoyant density-RNP particles in rapidly growing plasmocytoma cells [10, 11]. We suggested a more complex structure for these particles than that proposed by Spirin. In this paper we report some properties of this low density cytoplasmic RNP fraction, showing that this macromolecular association has a role in the protein synthesis, both *in vitro* and *in vivo*.

2. Material and methods

Transplantable mouse plasmocytoma cells RPC-5 were used in all experiments. For protein labelling *in vitro*, the cell-free amino acid incorporating system was prepared as described [12] with following modifications. TEA-salt buffer (0.02 M triethanolamine-HCl buffer pH 7.6; 0.05 M KCl; 0.008 M Mg-acetate; 0.006 M β-mercaptoethanol) was used in this procedure instead of tris-salt buffer. Microsomes were pelleted by centrifugation at 200,000 *g* for 1 hr. The microsomal pellet was suspended either in TEA-salt buffer 0.1% (v/v), Triton X-100 (procedure A), or in TEA-salt buffer 1.0% DOC (procedure B). To remove the detergents, both fractions were sedimented at 200,000 *g* for 1 hr, resuspended in TEA-salt buffer and resedimented as described. After incubation, the cell-free amino acid incorporation mixture was centrifuged at 200,000 *g* for 1 hr. Pelleted RNP particles were suspended in TEA-buffer and "fixed" with 5% formaldehyde.

For protein labelling in living cells, plasmocytoma cells were dispersed by gentle agitation of solid tumor fragments. The cells were suspended in Hank's balanced salt medium containing leucine-^{14}C and phenylalanine-^{14}C and incubated as described in the legends. After incubation the cells were cooled, washed with TEA buffer and collected by centrifugation at 1,000 *g* for 3–4 min. The cells were lysed with 0.5% (v/v) Triton X-100 in TEA-buffer 10 min. Nuclei, cell debris, and mitochondria were sedimented by centrifugation (15 min at 10,000 *g*). The supernatant fraction was fixed with formaldehyde.

CsCl equilibrium centrifugation was conducted as described by Henshaw [4]. Drop fractions were collected on filter papers as described by Mans and Novelli [13]. Radioactivity was determined by liquid scintillation counting.

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3. Results

Fig. 1 shows the CsCl buoyant density distribution of the hot TCA-insoluble ^{14}C-label obtained in the cell-free system. It appears that when Triton X-100 was used for the isolation of RNP particles, the radioactivity was detected in the region of $\rho \sim 1.54$ as well as of $\rho \sim 1.37$ (fig. 1A). With the DOC treatment, however, the radioactivity was detected only in the zone about $\rho = 1.55$ (fig. 1b).

Fig. 2 shows the CsCl-buoyant density distribution of the ^{14}C-amino acids incorporated in the cytoplasmic RNP particles when the cell suspension was used. Again we found that the radioactivity incorporated in the cytoplasmic RNP particles isolated with Triton X-100 banded in two peaks of material with $\rho \sim 1.54$ and $\rho \sim 1.42$.

After either 5, 10 or 15 min labelling time, the same amount of radioactivity was found in the particles banding at $\rho \sim 1.54$ and $\rho \sim 1.43$, whereas the radioactivity of soluble proteins increased, accumulating in the density-zone of $\rho \sim 1.3$ (fig. 3).

4. Discussion

These results suggest that two classes of cytoplasmic RNP particles, able to take part in the proteosynthesis in a cell-free system as well as in a cell suspension, exist in mouse plasmocytoma. Amino acids are incorporated not only in sedimentable structures of $\rho \sim 1.52-1.55$, the classical messenger-ribosome association [3, 4], but also in lower density structures with $\rho \sim 1.37-1.44$. Thus this lower density active complex in proteosynthesis probably contains ribosomes and mRNA, as well as material that lowers the density of the complex. Since this complex is found in the cell-free system as well as in the cell suspension, we can assume that it is not an artefact. It is noteworthy to recall that in the latter case these particles

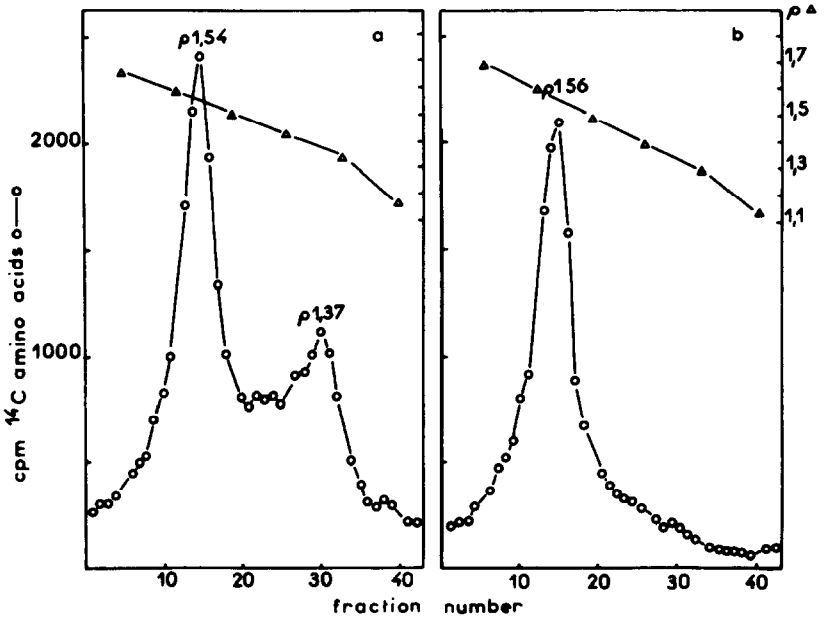

Fig. 1. The cytoplasmic fraction, sedimentable in TEA-salt buffer at 200,000 *g* is incubated at 37° in a cell-free system for protein synthesis with the following ^{14}C-amino acids: Leu, Phe, Val, Arg, Lys, each with an activity of 0.5 μCi/ml. After 30 min incubation, the particulate fraction was resedimented at 200,000 *g* and further analysed by isopycnic centrifugation in a CsCl gradient. The 200,000 *g* fraction was treated with 0.1% Triton X-100 (a) or with DOC (b) before incubation in the cell-free system.
-o-o-o- Radioactivity of the hot TCA insoluble ^{14}C-amino acids incorporating material.
-△-△-△- Buoyant density of the fractions as determined by pycnometry.

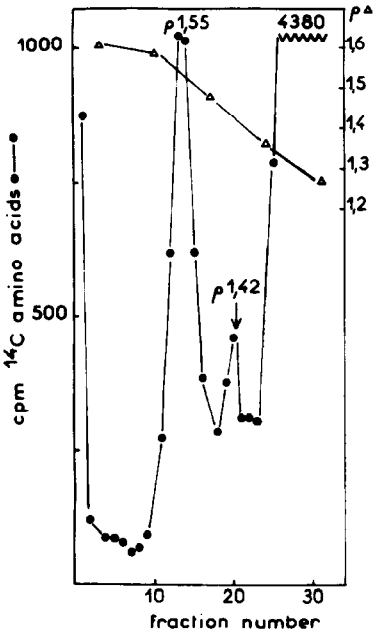

Fig. 2. A tumour cell suspension in Hank's balanced salt solution was incubated with ^{14}C-amino acids: Leu, 1 μCi/ml; Phe, 1 μCi/ml, at 37° during 15 min. After incubation, cells were lysed with 0.1% Triton X-100 and analysed by isopycnic CsCl centrifugation.
-●-●-●- Hot TCA insoluble ^{14}C-label.
-△-△-△- Buoyant density of the fractions as determined by pycnometry.

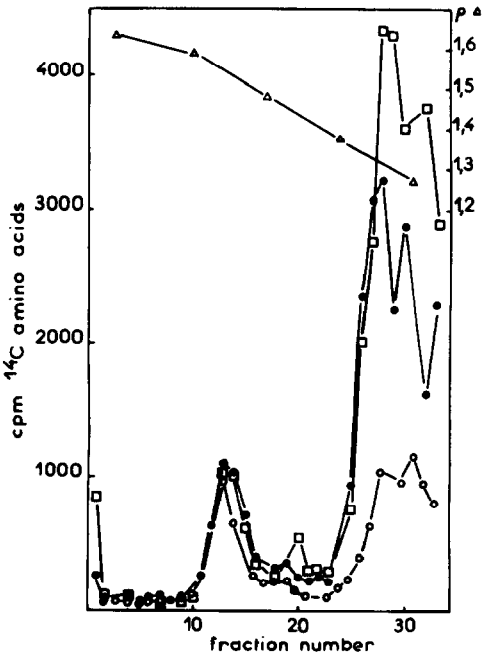

Fig. 3. Living tumoral cells, suspended in Hank's balanced salt solution, were incubated and analysed in the same conditions as in fig. 2, but with varying incubation times. Radioactivity of hot TCA insoluble ^{14}C-amino acids:
-○-○-○- Incubation during 5 min
-●-●-●- Incubation during 10 min
-□-□-□- Incubation during 15 min
-△-△-△- Buoyant density of the fractions as determined by pycnometry.

were obtained after mild detergent treatment of an incubated cell suspension.

The time-dependant labelling of the two particle populations in the cell suspension shows that the particle-bound labelled peptides have reached a steady-state within 5 min. Actually the radioactivity of these two RNP particle populations is at a plateau after this time. By extending the time of incubation an increase of only the soluble radioactive proteins can be observed.

No membranes are found in these 1.4 density structures, as shown elsewhere by electron microscopy [14]. Nevertheless, the low density active complex is sensitive to DOC treatment which suggests a tightly bound lipoprotein complex not dissociated by the simple Triton treatment.

These results on a neoplastic tissue, differ from those of Spirin [1, 2] and others [3–8] who found that the 1.4 density particles did not contain ribosomal structures.

Acknowledgements

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