PREFERENTIAL SYNTHESIS OF β LACTOGLOBULIN BY THE BOUND POLYRIBOSOMES OF THE MAMMARY GLAND

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SUMMARY - The free polyribosomes of the mammary gland of the ewe in lactation, in spite of being very active in incorporating amino-acids into proteins, nevertheless have potentialities different from those of the bound polyribosomes for the elaboration in vitro of βlactoglobulin. The latter protein is almost exclusively synthetized by the forms bound to the membranes of the endoplasmic reticulum.

The polyribosomes of the mammary epithelial cells are localized in two distinct topographic situations, some in a free state in the cytoplasmic matrix, others bound to the membranes of the endoplasmic reticulum (1). The induction of the synthesis of proteins and milk at the moment of lactogenesis corresponds to very great changes in the intracellular distribution of these particles. Thus, the free forms which are more abundant during the periods of gestation and pseudo-gestation, corresponding to the growth of the gland, represent only a small percentage of the total polyribosomal contents during the phase of natural secretion or after experimental induction of lactogenesis by means of prolactin (2). This situation suggests that the free polyribosomes and the bound ones have distinct functional roles in the formation of the proteins elaborated in the mammary gland.

In this communication we compare the abilities of the free and the bound polyribosomes from ewe mammary gland to synthesize a secretory protein, βlactoglobulin in a cell-free system. We find that protein is preferentially synthesized by the polyribosomes bound to the membranes of the endoplasmic reticulum.

MATERIALS AND METHODS

The radioactive amino-acids ^{14}C lysine (101 mC/mM), ^{14}C leucine (129 mC/mM) and ^{14}C glutamic acid (114 mC/mM) used in these experiments come from the C.E.A. Saclay (France). The mammary gland taken from primiparous ewes (30th to 50th day of lactation) are manually emptied of milk, following an intravenous injection of ocytocin (Syntocinon, Sandoz). The bound polyribosomes

are prepared from the isolated microsomal fraction in 0,88 M sucrose according to MOULE and DELHUMEAU de ONGAY (3). Prior to treatment with sodium deoxycholate (final concentration of 1 %), the microsomes are suspended in the 105.000 g supernatant from the mammary gland (4). The polyribosomes detached from the membrane are then purified by centrifugation in a discontinuous sucrose gradient (0,5 M-2 M) according to WETTSTEIN and al. (5).

The free polyribosomes are obtained by centrifuging post-mitochondrial supernatant obtained by centrifuging the tissue homogenate for 10 min. at 15.000g, on a discontinuous sucrose gradient (0,5 M-2 M) for 17 hours at 105.000 g according to BLOEMENDAL and al. (6).

The cell free system utilized in our experiments has already been described (1) and the incubations (one hour at 37°C) are carried out in the presence of either 105.000 g supernatant previously filtered through Sephadex G-25 (7) or a "pH 5 enzyme fraction". A carrier composed of β lactoglobulin-^{3}H (synthesized by slices of mammary gland maintained in a culture medium containing tritiated amino acids) and of native proteins, is added at the end of incubation, and the ribosomes are sedimented by centrifugation for 3 hours at 105.000 g.

The β lactoglobulin synthesized in vitro is isolated from the post-ribosomal supernatant by precipitation with 3,3 M $(NH_4)_2 SO_4$ after precipitation of the greater part of the tissue proteins with 2,2 M $(NH_4)_2 SO_4$. The redissolved precipitate is desalted on Bio-Gel P-30. The protein fraction is adsorbed to a column of CM-cellulose previously equilibrated at pH 4,9. The β lactoglobulin is eluted by a linear gradient of NaCl maintained at the same pH. Further purification of this protein is carried out by gel filtration on Sephadex G-100.

RESULTS

- Characteristics of the free and bound polyribosomes used in the cell-free systems

The free and bound polyribosomes show different sedimentation profiles in a sucrose gradient (Fig. 1a and 1b). The two types of polyribosomes are very active in incorporating amino acids into proteins, but the bound polyribosomes possess higher synthetic capacities per mg of RNA (Table I). About 40 % of the total radioactivity of the protein is liberated in the medium after 60 min. of incubation. This proportion approaches that reported for a cell-free liver system (8).

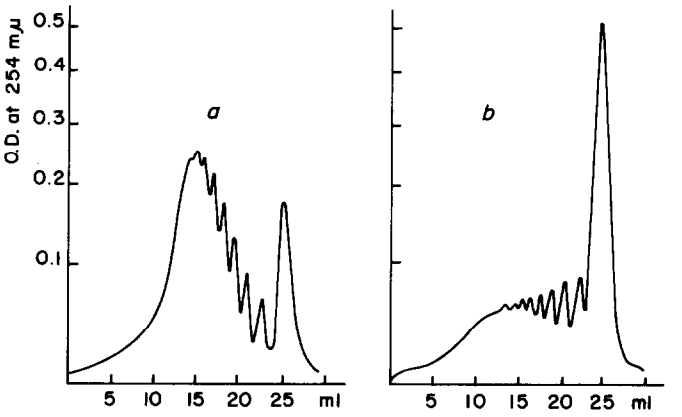

Fig. 1 - Distribution of polyribosomes from ewe mammary gland. Aliquots containing 1 mg of ribosomes were layered on an 0,3 M - 1 M exponential sucrose gradient in TKM and centrifuged for 150 min. at 25.000 rpm in the SW 25 Spinco Rotor. Direction of sedimentation is right to left.
a) Bound polyribosomes b) Free polyribosomes

TABLE I

Incorporation of leucine into protein by free and bound polyribosomes

	Bound polyribosomes	Free polyribosomes
Experiment I	344 *	288
Experiment II	320	258

* cpm x 10^{-3}/mg of ribosomal RNA

- Synthesis of β lactoglobulin by a cell-free system containing bound polyribosomes from lactating mammary gland

Figure IIa illustrates a fractionation of β lactoglobulin-^{14}C obtained from an incubation of bound polyribosomes and prepared by an ammonium sulphate precipitation. A fair similarity is observed between the elution profile of the ^{14}C radioactivity and that of the β lactoglobulin-^{3}H added at the end of incubation or the absorption at 280 nm of the native protein. The fraction corresponding to the β lactoglobulin after chromatography on CM-cellulose is

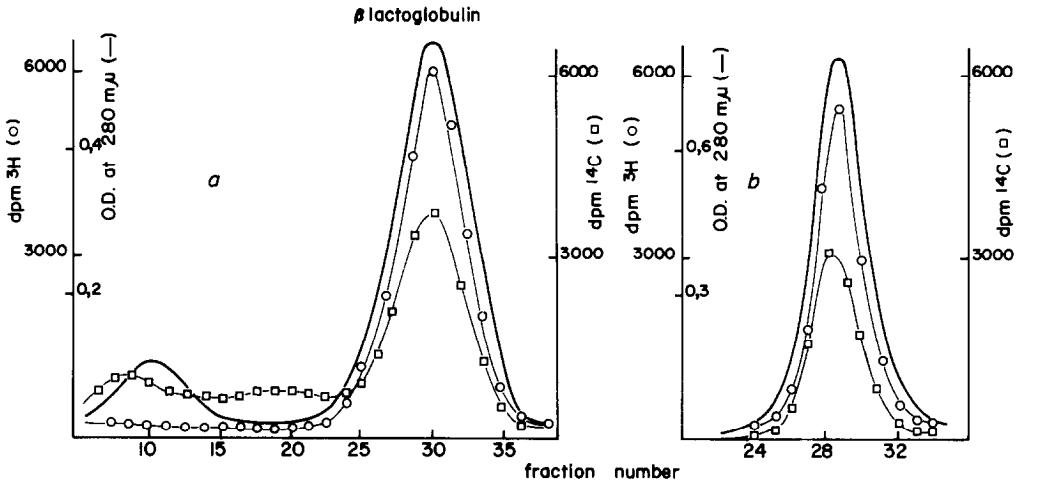

Fig. II - a) Chromatography on CM-cellulose of 3,3 M $(NH_4)_2SO_4$ precipitate obtained after incubation of bound polyribosomes. The desalted fraction was applied to a 1 x 8 cm column equilibrated with 0,02 M acetate buffer pH 4,9 and eluted with NaCl gradient (in the same buffer) up to 0,4 M.

b) The purification of the β lactoglobulin from (a) by gel filtration on Sephadex G-100. Freeze-dried β lactoglobulin was applied to a 1,4 x 70 cm column equilibrated with 0,05 M tris-HCl pH 7,6 , KCl 0,2 M and eluted with the same buffer.

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dialysed, lyophilised, then filtered on a column of Sephadex G-100. The elution of the ^{14}C - ^{3}H radioactive and native (absorption at 280 nm) proteins is quite comparable (Fig. IIb). The ^{14}C protein synthesized in vitro possesses moreover immunological properties similar to native β lactoglobulin, and the distribution in electrochromatography of the peptides resulting from its trypsic hydrolysis is similar to that obtained with native protein (9).

- Comparison of the abilities of the two classes of polyribosomes to synthesize lactoglobulin

The isolation and the purification of the ^{14}C- β lactoglobulin obtained from the incubation of a quantity of free polyribosomes by means identical with those previously used for protein from the bound polyribosomes, are illustrated by Fig. IIIa and IIIb. The ^{14}C-radioactivity is very low in the position of ^{3}H - labelled or native β lactoglobulin and the low level of incorporation of ^{14}C-amino acids into β lactoglobulin is emphasized after its purification on Sephadex G-100. The incorporation is too small to allow a more elaborate identification. It represents only 100-250 dpm/mg of the RNA of the free polyribosomes while the

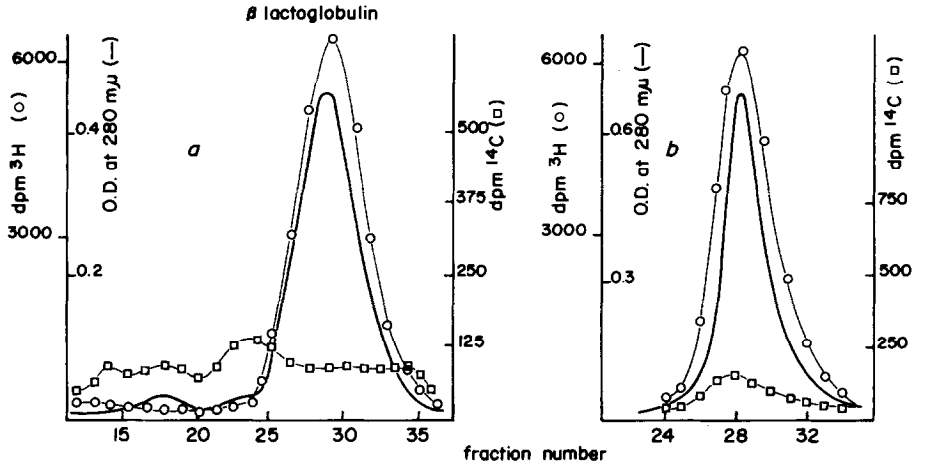

Fig. III - a) Chromatography on CM-cellulose of 3,3 M $(NH_4)_2 SO_4$ precipitate obtained after incubation of free polyribosomes. The desalted fraction was applied to a 1 x 8 cm column equilibrated with 0,02 M acetate buffer pH 4,9 and eluted with NaCl gradient (in the same buffer) up to 0,4 M.

b) The purification of the β lactoglobulin from (a) by gel filtration on Sephadex G-100. Freeze-dried β lactoglobulin was applied to a 1,4 x 70 cm column equilibrated with 0,05 M tris-HCl pH 7,6 , KCl 0,2 M and eluted with the same buffer.

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same experimental designs results in an incorporation into β lactoglobulin of an average of 5.000 dpm/mg of the RNA of the bound polyribosomes.

In a second series of experiments, equal quantities of free and bound polyribosomes are incubated separately and respectively with ^{14}C and ^{3}H - amino acids (their specific activities having been previously adjusted). At the end of incubation, the post-ribosomal supernatants diluted with native β lactoglobulin are mixed and fractionated by the method previously described. Under these conditions ^{14}C-radioactivity corresponding to β lactoglobulin synthesized by the free polyribosomes is also very low. In fact, the ratio of radioactivities ^{3}H/mg of ribosomal RNA (representing the β lactoglobulin synthesized by the bound polyribosomes) on the radioactivities ^{14}C/mg of the RNA is about 20.

DISCUSSION

Although very active in carrying out in vitro protein synthesis, the free polyribosomes of the mammary gland, in contrast to the bound polyribosomes, elaborate only very small quantities of β lactoglobulin. Since the bound polyribosomes represent 80 % of the total polyribosomes during the secretory phase,

these particles are most lokely responsible for the synthesis of the far greater part of the β lactoglobulin secreted in vivo by mammary gland. Our observations are supported by the recently proved existence of functional differences between the two types of hepatic polyribosomes for the synthesis of the serum albumin. Thus, the latter takes place almost exclusively on the polyribosomes attached to the membranes (10, 11, 12, 13) whilst the synthesis of ferritin (constitutive protein) takes place essentially on the free polyribosomal forms (14, 15).

These results do not however exclude the possibility that certain non-secreted proteins are synthesized on the bound polyribosomes and vice-versa. In this respect, it is interesting to notice that cytochrome cNADH-reductase (a constitutive protein of the membranes) (16) and certain immunoglobulins (secretory proteins) (17) may be elaborated by the two types of particles indiscriminately.

Whatever factors may be involved at the polyribosomal level in reading the genetic message, membrane systems seem to play a major role in animal cells, but the mechanism by which the separation of the two categories of polyribosomes (free and bound) is brought about is still obscure. It is difficult at the present stage of our knowledge to say whether the association of ribosomes and membranes is the consequence of a special properties of ribosomal proteins (18) or whether it is determined by the nature of the messenger RNA.

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