The Detection of the Messenger Ribonucleic Acid for the α -Lactalbumin of Guinea-Pig Milk

By P. N. CAMPBELL, DIANA McILREAVY and D. TARIN

Departments of Biochemistry and Anatomy, University of Leeds, Leeds LS2 9LS, U.K.

(Received 23 February 1973)

RNA was extracted from the polyribosomes isolated from the mammary glands of a lactating guinea pig and injected into *Xenopus* oocytes. On incubation the oocytes effected the biosynthesis of α -lactalbumin.

In this laboratory Fairhurst *et al.* (1971) have previously demonstrated that a polyribosome-containing fraction from the mammary glands of a lactating guinea pig on incubation will effect the synthesis of the milk protein α -lactalbumin. The polyribosomes were incubated in the presence of [3 H]leucine, and after the addition of carrier α -lactalbumin the radioactive protein was purified by means of chromatography on Sephadex G-100, CM-cellulose and DEAE-cellulose. Following the work of Brew (1972), we treated the isolated α -lactalbumin with CNBr and separated the *N*-terminal and *C*-terminal fragments from the undegraded protein by gel chromatography. The material containing [3 H]leucine followed closely the curves for the absorption (E_{280}) of the peptides. Assuming equivalent recoveries of the two peptides the radioactivity/mol of leucine of the *C*-terminal fragment was 9 times that of the *N*-terminal fragment. This was consistent with the incorporation of [3 H]leucine being mainly due to chain elongation rather than the initiation of new chains.

We have further demonstrated that, when slices of mammary tissue from lactating guinea pigs are incubated with [3 H]leucine under the conditions described by Brew & Campbell (1967) and the α -lactalbumin is isolated and treated with CNBr as described above, the radioactivities/mol of leucine in the *N*- and the *C*-terminal peptides were virtually identical. This is consistent with the synthesis of α -lactalbumin *in toto* in the slice system.

In view of the activity of the isolated polyribosomes for the synthesis of α -lactalbumin, we concluded that the fraction must have present the mRNA for this protein. Accordingly we have been seeking ways of identifying this mRNA. The present communication describes the results of our current experiments with *Xenopus laevis* oocytes as an assay system.

Experimental

Materials. The materials were obtained from commercial sources as indicated previously (Fairhurst *et al.*, 1971). L-[4,5- 3 H]Leucine (sp. radioactivity 38 Ci/mmol) was obtained from The Radiochemical Centre, Amersham, Bucks., U.K.

Methods. Polyribosomes were prepared from the mammary glands of lactating guinea pigs as described previously (Fairhurst *et al.*, 1971). The polyribosomes were resuspended in 10mM-Tris-HCl buffer, pH 7.6, containing 1mM-EDTA, 100mM-NaCl and 0.5% (w/v) sodium dodecyl sulphate. The RNA was extracted by using the chloroform-phenol method of Perry *et al.* (1972). The RNA was precipitated by the addition of 2 vol. of ethanol and stored at -10°C in 75% (v/v) ethanol containing 2% (w/v) sodium acetate. When required, the RNA was centrifuged into a pellet, the supernatant decanted and excess of ethanol removed by placing the tube in a vacuum desiccator. The RNA was redissolved in 'injection medium' containing 88mM-NaCl, 1.0mM-KCl and 15mM-Tris-HCl buffer, pH 7.6, to a concentration of 10mg/ml (Lane *et al.*, 1971).

Xenopus laevis females used to provide oocytes had been induced by hormone treatment to lay eggs 3 weeks before the time when their oocytes were taken for RNA injection. The oocytes were removed and maintained in a culture medium as described by Gurdon (1968). At the time of injection each oocyte was placed on a dry microscope slide. After injection (with the micrometer-syringe device described by Tarin, 1973) with 50nl of RNA solution the oocytes were incubated with [3 H]leucine. The latter, containing 1 mCi in 1 ml of solution, was dried down *in vacuo* and taken up in 1 ml of injection medium. The oocytes, after the injection, were incubated in batches of 30 in approx. 70 μ l of the [3 H]leucine-containing medium for 8 h at room temperature before being deep-frozen.

For extraction of radioactive protein the oocytes were carefully transferred to a small Potter-type hand-operated homogenizer and disrupted with 1 ml of 50mM-NH₄HCO₃ containing 25mg of carrier α -lactalbumin, which had been purified from guinea-pig milk as described by Fairhurst *et al.* (1971). After disruption, the oocyte suspension was centrifuged at 3000g for 15 min at room temperature. The supernatant was removed with a Pasteur pipette, care being taken to avoid the fat layer at the top, and loaded on to a Sephadex G-100 column (2.0cm $\times$ 140cm) equilibrated with 50mM-NH₄HCO₃ and eluted with the

Fig. 1. Isolation of α -lactalbumin from the soluble medium after disruption of oocytes that had been incubated with [3H]leucine after their injection with RNA from the polyribosomes of the mammary gland of lactating guinea pigs

(a) Fractionation on a column of Sephadex G-100 equilibrated and eluted with 50mM- NH_4HCO_3 . Fractions 45–56 were pooled. (b) Fractionation of the α -lactalbumin-containing peak on a column of CM-cellulose equilibrated in 15mM-sodium acetate buffer, pH 5.0. The column was eluted with a linear gradient of 0–60mM-NaCl in acetate buffer. Fractions 78–98 were pooled and treated with CNBr. (c) Fractionation of fragments obtained by CNBr cleavage of α -lactalbumin on a column of Sephadex G-50 eluted with 5% formic acid. O, E_{280} ; ●, radioactivity. For details please see the text.

same buffer. Fractions (6.5 ml) were collected, the E_{280} of each was measured and the radioactivity determined on 0.2 ml samples after the addition of 5 ml of a scintillation fluid containing 100 g of naphthalene and 5 g of 2,5-diphenyloxazole in 1 litre of dioxan. A Beckman LS230 scintillation spectrometer was used for the counting of radioactivity. The fractions containing α -lactalbumin were pooled, freeze-dried and dissolved in 2 ml of 15 mM-sodium acetate buffer, pH 5.0, dialysed for 2 h against the acetate buffer, loaded on to a column (1.8 cm $\times$ 20 cm) of CM-cellulose (Whatman grade CM52) equilibrated with 15 mM-sodium acetate buffer, pH 5.0, and eluted with a linear gradient of 0–60 mM-NaCl in the acetate buffer. Fractions (5.6 ml) were collected, the E_{280} of each was measured and the radioactivity determined on 0.4 ml samples in 10 ml of scintillation fluid. The samples containing α -lactalbumin were pooled and de-salted on a Sephadex G-25 column, after which the fractions containing the protein were freeze-dried. The protein was aminoethylated and cleaved with CNBr as described by Brew & Hill (1970). After cleavage the solution was diluted fivefold with water and freeze-dried. The CNBr-treated freeze-dried protein was dissolved in 1 ml of 5% (v/v) formic acid and loaded on to a Sephadex G-50 column (1.8 cm $\times$ 100 cm), then eluted with 5% formic acid, 3.5 ml fractions being collected. The E_{280} of each was measured and the radioactivity determined. In this case 1 ml of each fraction was placed in a scintillation vial, freeze-dried, 0.2 ml of water added to dissolve the protein and 5 ml of scintillation fluid added.

Results and discussion

The results of the determination of the radioactivity on the proteins from the columns of Sephadex G-100, CM-cellulose and Sephadex G-50 are shown in Figs. 1(a), 1(b) and 1(c) respectively. In Fig. 1(a) the peak containing the carrier α -lactalbumin is well separated from the radioactive oocyte protein to the left and the free [^3H]leucine to the right. In Fig. 1(b) it is evident that much contaminating radioactive material is eliminated and there is a reasonable coincidence between the radioactive profile and that due to α -lactalbumin. In Fig. 1(c) there is a good coincidence between the C-terminal fragment and the E_{280} pro-

file. Unfortunately in this particular column the N-terminal fragment and the undegraded α -lactalbumin are not well separated, but again the two profiles coincide. An approximate calculation of the radioactivity/mol of leucine in the C-terminal and the N-terminal fragments, assuming equivalent recoveries in the peaks and knowing the numbers of leucine residues in the two fragments, indicates that they were closely similar. This would be expected if the α -lactalbumin had been synthesized *in toto*.

In earlier experiments *Xenopus* oocytes were injected with buffer alone and incubated in the presence of [^3H]leucine. Although radioactive protein was recovered, indicating the viability of the oocytes, the carrier α -lactalbumin, which was subsequently isolated by the methods described above, contained no significant radioactivity. These experiments enabled the methods of fractionation now used to be worked out and demonstrated their soundness for the elimination of contaminating radioactive material. Moreover, they showed that oocytes do not synthesize a protein with the characteristics of α -lactalbumin. We are grateful to Dr. C. D. Lane for his help with these experiments.

We conclude that we have demonstrated that the oocyte system is able to effect the synthesis of guinea-pig α -lactalbumin and that the mRNA for this protein may be isolated from the polyribosomes from the mammary gland of lactating guinea pigs.

We are grateful to Dr. Keith Brew for his advice. We are also grateful to the Medical Research Council for their continued financial support.

- Brew, K. (1972) *Eur. J. Biochem.* **27**, 341–353
Brew, K. & Campbell, P. N. (1967) *Biochem. J.* **102**, 265–274
Brew, K. & Hill, R. L. (1970) *J. Biol. Chem.* **245**, 4559–4569
Fairhurst, E., McIlreavy, D. & Campbell, P. N. (1971) *Biochem. J.* **123**, 865–874
Gurdon, J. B. (1968) *J. Embryol. Exp. Morphol.* **20**, 401–414
Lane, C. D., Marbaix, G. & Gurdon, J. B. (1971) *J. Mol. Biol.* **61**, 73–91
Perry, R. P., La Torre, J., Kelley, D. E. & Greenberg, J. R. (1972) *Biochim. Biophys. Acta* **262**, 220–226
Tarin, D. (1973) *Differentiation* **1**, in the press