SYNTHESIS AND CORE GLYCOSYLATION OF THE α SUBUNIT OF HUMAN CHORIONIC GONADOTROPIN IN *XENOPUS* OOCYTES

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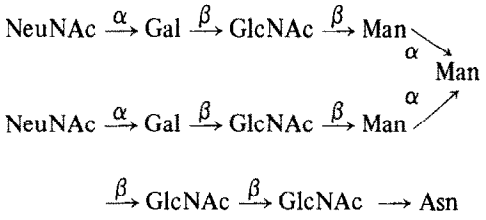
1. Introduction

The major hormone secreted by the human placenta in early pregnancy is human chorionic gonadotropin (hCG). This polypeptide hormone is composed of two non-identical subunits, α and β. In placenta the α subunit is the predominant subunit synthesized and far exceeds the production of β subunit [1–2]. Recent studies using cell-free translation of mRNA isolated from first trimester placentae have shown that the α subunit is synthesized as a pre-protein like many other secretory proteins [3–5]. The α subunit of hCG is a glycoprotein containing two identical oligosaccharide chains which represent 30% of its molecular weight [6]. The carbohydrate chains are linked through an N-glycosidic bond to asparagine residues located at positions 52 and 78 in the polypeptide chain. The basic structure of these *N*-glycosidic carbohydrate units, determined in [7], is:



The *Xenopus* oocyte translation system is frequently used for its capacity to modify many of the newly synthesized polypeptides after translation just as the cell from which the mRNA originated [8]. In this respect the oocytes are known to glycosylate newly synthesized heterologous proteins [9,10] but to what extent this process reflects the in vivo situation remains to be elucidated. Here, we present evidence that microinjection of mid-term placental RNA directs the synthesis of a glycosylated form of hCG-α, which has a different carbohydrate composition as compared with the authentic hormone. No mature α subunit could be detected in the oocytes.

2. Materials and methods

The preparation of total RNA from human term and mid-term placentae and the subsequent separation of mRNA from ribosomal RNA by affinity chromatography on oligo(dT)-cellulose was done essentially as in [11]. The poly(A)-containing RNA obtained was dissolved in sterile water and stored at −80°C. Batches of 15–20 oocytes of *Xenopus laevis* were microinjected with 50 nl poly(A)-containing RNA (0.3 mg/ml). Each set of oocytes was then incubated in the presence of 200 μCi [^{3}H]proline (117 Ci/mmol, Radiochemical Centre, Amersham) or [^{3}H]mannose (22 Ci/mmol, New England Nuclear) [11]. After incubation, the oocytes were homogenized in 500 μl phosphate-buffered saline. Debris were removed by centrifugation (60 min at 105 000 × *g*) prior to analysis of the peptides synthesized. Translation of the different RNA preparations was also assayed in the wheat germ cell-free system as outlined in [12].

The synthesized proteins were analyzed by immunoprecipitation with a specific antiserum generated against the purified reduced and carboxymethylated α subunit of hCG. Aliquots (25 μl) of the cell-free translation mixture or the oocyte supernatant were incubated for 1 h at 37°C in a total volume of 200 μl phosphate-buffered saline containing 1% Triton X-100, 1% sodium deoxycholate and 1 μl hCG-α

antiserum. Then 10 μl goat anti-rabbit antiserum (Calbiochem AG, Buchs) were added and the incubation was continued for an additional 1 h at 37°C and 16 h at 4°C. The washed immunoprecipitates were dissolved either in 1 ml Lumasolve for counting or in gel sample buffer for analysis by SDS–polyacrylamide gel electrophoresis on 15% slab gels [12].

To study the effect of glycosidase treatment on the oocyte products, 50 μl aliquots of the supernatant of mRNA-injected oocytes were made 50 mM in sodium citrate (pH 5.5); then 0.01 unit endoglycosidase H (Miles) was added and the mixture was incubated for 6 h at 37°C. After incubation, the mixture was adjusted to pH 7 and immunoprecipitated as above. Treatment of the oocyte products with α-mannosidase (EC 3.2.1.24; Boehringer, Mannheim) was as in [10].

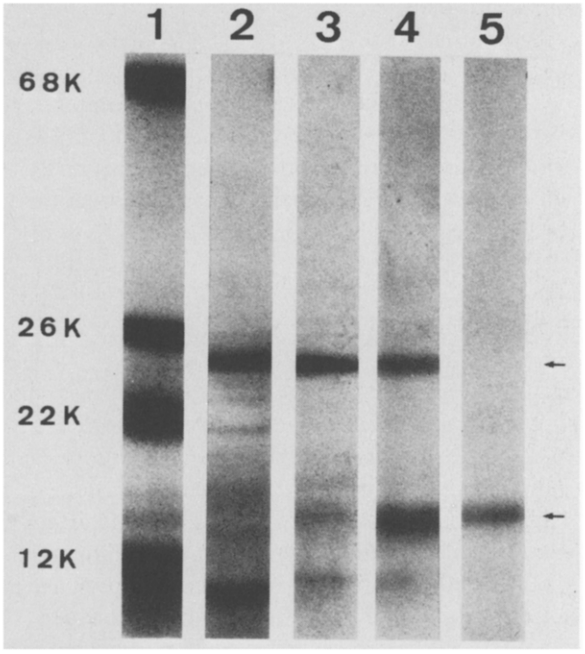


Fig.1. Sodium dodecylsulfate polyacrylamide gel electrophoresis of proteins synthesized in a wheat germ system programmed with placental mRNA. Poly(A)-containing RNA isolated from term or mid-term placenta was translated in the cell-free system in the presence of [^{3}H]leucine or [^{3}H]-proline. Synthesized peptides were either precipitated with trichloroacetic acid (2–4) or immunoprecipitated with hCG-α antiserum (5). The resulting precipitates were dissolved in SDS buffer and examined on 15% SDS–polyacrylamide slab gels. (1) ^{14}C-Labeled M_r markers: bovine serum albumin (68 000); chymotrypsinogen A (25 700); hPL (22 000); cytochrome *c* (12 400); (2) + term placenta mRNA in the presence of [^{3}H]leucine; (3) + mid-term placenta mRNA in the presence of [^{3}H]leucine; (4) + mid-term placenta mRNA in the presence of [^{3}H]proline; (5) products of (4) immunoprecipitated with hCG-α antiserum. The gel was exposed to fluorography. The arrows indicate the positions of pre-hPL and pre-hCG-α. The same amount of radioactivity was applied into (3,4); the pre-hCG-α band in (3) is weak because this protein contains 4-times less leucine than pre-hPL [5,13,14].

3. Results

Poly(A)-containing RNA was prepared from normal term placenta and from mid-term placenta (20 weeks) made available to us after a therapeutic abortion. As a routine the different RNA preparations were tested in the wheat germ cell-free protein synthesizing system and the translation products were analyzed on SDS–polyacrylamide gels (fig.1). Term placental mRNA directed the synthesis of a major product with app. M_r 25 000 (fig.1 (2)) which was identified as the precursor of human placental lactogen (pre-hPL; [12]). Analysis of the products synthesized in response to mRNA from a mid-term placenta revealed the presence of an additional distinct radioactive polypeptide with app. M_r 14 000 (fig.1 (3,4)).

Immunoprecipitation was utilized to identify this polypeptide. As seen in fig.1 (5) a specific antiserum generated against the reduced carboxymethylated α subunit of human chorionic gonadotropin (hCG) successfully precipitated the 14 000 M_r product. This result gives presumptive evidence that the labeled 14 000 M_r product contains hCG-α sequences and represents the precursor of the hCG-α apoprotein in [3–5]. Quantitation of the labeled α protein by immunoprecipitation revealed that it contained 2–3% of the total radioactivity incorporated as [^{3}H]proline into protein by the wheat germ system programmed with mRNA from a mid-term placenta, and 0.5% when the system was programmed with term placental mRNA.

Consequently, to study the synthesis of hCG-α in a whole cell system, the *Xenopus* oocyte, the poly(A)-containing RNA from mid-term placenta was utilized. Full-grown oocytes were microinjected with either placental mRNA or with injection buffer alone and incubated in the presence of [^{3}H]proline. Since multiple labeled acid-precipitable proteins were synthesized both in the absence or in the presence of added mRNA, immunoprecipitation was essential to dif-

ferentiate synthesized placental peptides from endogenous oocyte peptides. Accordingly, aliquots of the oocyte supernatant were immunoprecipitated with the specific hCG-α antiserum. Analysis by SDS–polyacrylamide gel electrophoresis of the immunoprecipitated proteins demonstrated a prominent [^{3}H]proline-containing polypeptide with app. M_r 20 000 (fig.2, (3)). No radiolabeled peptides were precipitable from the extracts of the oocytes injected with buffer (fig.2 (6)). Because *Xenopus* oocytes are able to perform several post-translational modifications on newly synthesized heterologous proteins [8,11], we anticipated that the 20 000 M_r polypeptide represented a processed and glycosylated form of hCG-α. However, the migration of the immunoreactive oocyte product was obviously not identical to that of native hCG-α, since the mature glycosylated α subunit has app. M_r 22 500 on SDS gels (fig.2).

Batches of oocytes microinjected with placental mRNA were also incubated in the presence of [^{3}H]-

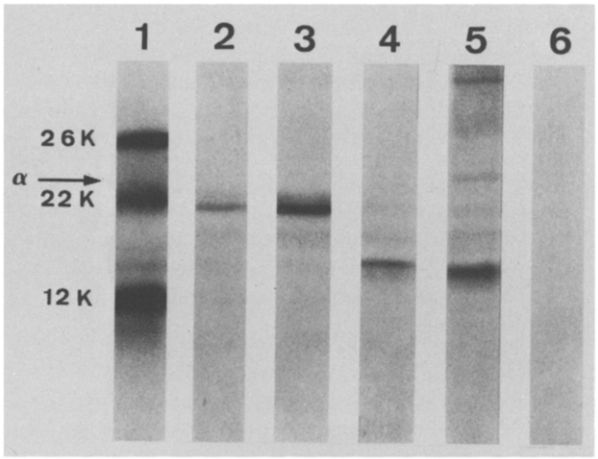


Fig.2. Fluorograph on the hCG-α related polypeptide synthesized in *Xenopus* oocytes. The oocytes were microinjected with mRNA isolated from a mid-term placenta and incubated in the presence of [^{3}H]proline or [^{3}H]mannose. Following incubation the synthesized proteins were immunoprecipitated with hCG-α antiserum and analyzed on SDS–polyacrylamide slab gels. Digestion of the immunoreactive oocyte products with the glycosidases was as in section 2. (1) ^{14}C-Labeled M_r markers: chymotrypsinogen A (25 700); hPL (22 200); cytochrome *c* (12 400); (2) [^{3}H]mannose-labeled hCG-α synthesized in oocytes; (3) [^{3}H]proline-labeled hCG-α synthesized in oocytes; (4) as in lane 3 but after treatment with α-mannosidase; (5) after treatment with endoglycosidase H; (6) immunoprecipitate obtained from buffer-injected oocytes. The arrow indicates the position of authentic hCG-α.

mannose. As can be seen in fig.2 (2) [^{3}H]mannose was incorporated into one polypeptide immunoprecipitable with the hCG-α antiserum and comigrating with the [^{3}H]proline-labeled immunoreactive product. These data suggest that the 20 000 M_r polypeptide synthesized in oocytes in response to microinjected placental mRNA is glycosylated and contains mannose residues.

To investigate the sugar composition of the hCG-α related polypeptide, we treated it with different glycosidases. α-Mannosidase apparently removed a considerable amount of sugar from the glycosylated product as shown by its shift in migration (fig.2 (4)). The product of this exoglycosidase treatment appeared to have app. M_r 15 000 on SDS gels. This finding suggests the presence of mannose residues at the non-reducing ends of the attached glycocores. Furthermore, the marked increase in mobility of the oocyte product after α-mannosidase treatment indicates that it contains several mannose residues linked together in α-anomeric configuration. Native hCG-α, of which the carbohydrate chains end with sialic acid [7], was insensitive to the action of α-mannosidase (not shown).

Further evidence that the 20 000 M_r polypeptide contains high-mannose core carbohydrate units was obtained by digestion of aliquots of oocyte supernatant with endoglycosidase H. This enzyme splits the asparagine-bound di-*N*-acetyl-chitobiose when linked to a high-mannose type glycocore [15]. Immunoprecipitation of the digested oocyte extracts with hCG-α antiserum resulted in the appearance of a band with app. M_r 14 800 (fig.2 (5)). Treatment of authentic hCG-α with endoglycosidase H had no effect, because the native α subunit has two complex type carbohydrate chains attached to asparagine residues [7]. These data are consistent with the idea that the hCG-α related oocyte product (20 000 M_r) contains immature high-mannose core oligosaccharide units which are not processed up to the carbohydrate composition found in native hCG-α.

4. Discussion

Microinjection of the mRNA isolated from a mid-term placenta into *Xenopus* oocytes results in the formation of a glycosylated polypeptide immunoprecipitable with hCG-α antiserum and having app. M_r 20 000. Evidence for glycosylation was: (i) [^{3}H]-mannose was specifically incorporated into this

oocyte product; and (ii) the migration of this protein on SDS gels was sensitive to the action of different glycosidases.

Part of the popularity of the *Xenopus* oocyte system for the translation of heterologous mRNAs is related to its capacity to modify many of the newly synthesized polypeptide chains just as the cell from which the mRNA originated [8]. One of the post-synthetic modifications that is known to occur in the oocytes is glycosylation [9,10], but it was not yet examined whether this process parallels the in vivo events. These results indicate that the glycosylated hCG-α related polypeptide synthesized in the oocytes is not identical to the mature α subunit. The mobility of the immunoreactive oocyte product on SDS gels is somewhat greater than that of the authentic subunit. Since it has been demonstrated that the processing of so called pre-proteins is carried out in oocytes in a correct way [11,16], it is likely that the observed difference in mobility is due to differences in the composition of the carbohydrate chains attached. Indeed, our experiments with the different glycosidases indicate that the hCG-α related polypeptide synthesized in the oocytes contains, in contrast to the native α subunit, high-mannose core oligosaccharides attached to asparagine residues. These results suggest that the glycosylation of the oocyte α protein is limited to the initial addition of a preformed oligomannosyl core [17] and some but incomplete processing. We plan to study whether comparable results are obtained with the β subunit of hCG, which apart from 2 asparagine-linked oligosaccharide units also contains 4 serine-linked carbohydrate chains [18].

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