SYNTHESIS AND GLYCOSYLATION OF RAT PROSTATIC BINDING PROTEIN IN *XENOPUS LAEVIS* OOCYTES

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1. Introduction

Prostatic binding protein (PBP) is a quantitatively important steroid-binding protein present in rat ventral prostate [1]. Its synthesis is positively affected by the action of androgen hormones [2]. Native PBP is a complex glycoprotein composed of two subunits, F and S. Upon reduction of the disulfide bridges subunit F dissociates into two smaller polypeptide chains C_1 and C_3, while subunit S dissociates into components C_2 and C_3. Component C_3, occurring in both subunits, is glycosylated [3]. Translation of prostatic mRNA in *Xenopus* oocytes yields three polypeptides which are electrophoretically indistinguishable from these of native PBP [4].

Here we have studied the glycosylation of the oocyte-synthesized polypeptides as one of the post-translational modifications which lead to the formation of a native protein.

2. Materials and methods

The isolation of total RNA from rat ventral prostate was done essentially as in [2]. Poly(A)-containing RNA was purified on oligo(dT)cellulose (type T-2, Collaborative Res. Walthman, MA) as outlined [5] and dissolved in injection medium consisting of 88 mM NaCl, 1 mM KCl, 15 mM Tris–HCl (pH 7.6). Batches of 10–15 oocytes of *Xenopus laevis* were microinjected with 50 nl poly(A)-containing RNA (0.2 mg/ml) [4]. Each set of oocytes was then incubated for 24 h at 20°C in 100 μl modified Barth's medium [6] in the presence of 0.5 mCi/ml [^{3}H]leucine (60 Ci/mmol, The Radiochemical Centre, Amersham) or in the presence of 1 mCi/ml [^{3}H]mannose (13.2 Ci/mmol; New England Nuclear, Boston, MA). After incubation the oocytes were homogenized in 0.5 ml phosphate-buffered saline and centrifuged for 1 h at 35 000 rev./min in the Beckman rotor SW 65 Ti. The supernatant was removed and stored at –80°C until analyzed.

Highly specific antisera to PBP and to the three different components of PBP were prepared by immunization of rabbits with the respective purified antigens [7]. Aliquots of oocyte supernatant were immunoprecipitated using one of these antisera followed by excess goat antirabbit immunoglobulin antiserum. The washed immunoprecipitates were dissolved in gel sample buffer and analyzed by SDS–polyacrylamide gel electrophoresis using a discontinuous 15% acrylamide slab gel [8]. After electrophoresis the protein bands were identified by Coomassie blue staining and the radioactivity was localized by autofluorography [9].

To study the possible effect of glycosidase treatment of the oocyte products, the immunoprecipitates were dissolved in 50 mM sodium acetate (pH 5.0), 0.1 M NaCl, 0.1 mM $ZnSO_4$, 2% SDS, 5% 2-mercaptoethanol; this mixture was heated for 2 min at 100°C. After cooling, 25 μg α-mannosidase (EC 3.2.1.24; Boehringer, Mannheim) was added and the mixture was incubated for 24 h at room temperature. The proteins were then precipitated with 10% trichloroacetic acid and prepared for SDS–polyacrylamide gel electrophoresis as described.

3. Results

Microinjection of *Xenopus* oocytes with poly(A)-containing RNA isolated from rat ventral prostate results in the synthesis of polypeptides immunoprecipitable with an anti-PBP antiserum. Analysis by SDS–polyacrylamide gel electrophoresis of the immunoprecipitates demonstrates the identity of the three polypeptides made in oocytes and the components of native PBP (fig.1, slot 1,2). We have noticed [4] that in oocytes these polypeptides apparently associate into two higher molecular weight complexes which correspond in mobility with the native F and S subunits. In order to confirm this observation and to verify the correctness of the post-translational arrangement of the three polypeptides, the oocyte-synthesized products were immunoprecipitated with different, very specific antisera to each of the three components of PBP. As shown in fig.1, slot 3, the antiserum to component C_1, which is characteristic for subunit F, precipitates both components of subunit F, C_1 and C_3. On the other hand, the antiserum to component C_2 precipitates both components of subunit S, C_2 and C_3 (fig.1, slot 4), , whereas the antiserum to component C_3 precipitates all three components, that is both subunits (fig.1, slot 5). Thus, the oocyte is able to perform the appropriate post-translational modifications of the PBP-mRNA-directed polypeptides resulting in their association into native-like F and S subunits.

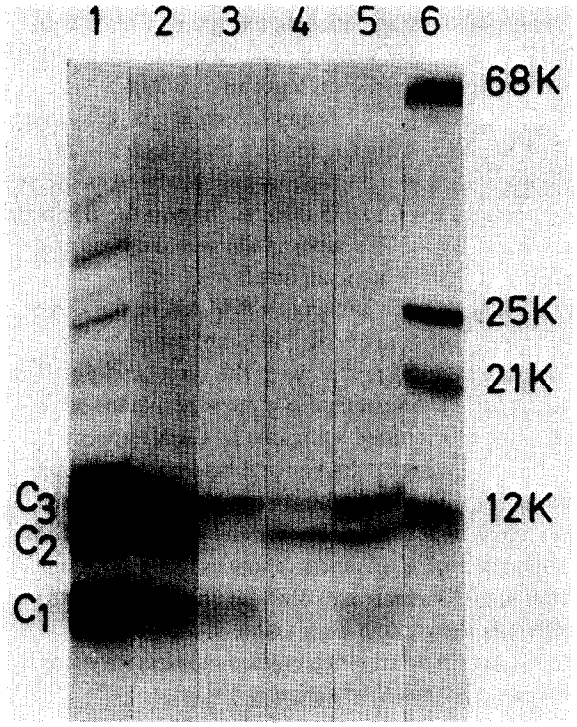

Fig.1. Analysis by SDS–polyacrylamide gel electrophoresis of prostatic binding protein (PBP) synthesized in *Xenopus* oocytes. Oocytes were microinjected with poly(A)-containing RNA from rat ventral prostate and incubated in the presence of [^{3}H]leucine. Immunoprecipitations were then performed with various specific antisera as in section 2. (1) ^{14}C-labelled native PBP; (2) PBP synthesized in oocytes; (3) anti-component C_1 immunoprecipitate; (4) anti-C_2 immunoprecipitate; (5) anti-C_3 immunoprecipitate; (6) ^{14}C-labelled marker proteins (mol. wt): BSA, 68 000; chymotrypsinogen A, 25 000; human placental lactogen, 21 000; cytochrome *c*, 12 300.

Since PBP is a glycoprotein (it contains 3.2% of carbohydrate [3]) glycosylation is one of the expected modifications. Hence, oocytes injected with prostatic mRNA were incubated in the presence of [^{3}H]mannose. This sugar was chosen because it is frequently found in glycoproteins and because it is easily taken up by the oocytes. As can been seen in fig.2, slot 3, RNA-injected oocytes incorporate [^{3}H]mannose into one polypeptide immunoprecipitable with anti-PBP antibody and comigrating with component C_3. This component is the only peptide of PBP which produces a positive Schiff staining [3]. An insignificant amount of label from [^{3}H]mannose was incorporated in some other proteins of higher molecular weight. We conclude that oocytes can glycosylate specific proteins which are synthesized in response to exogenous mRNA.

To investigate the extent of glycosylation, we treated the immunoprecipitable proteins with α-mannosidase. As a result of this treatment, the [^{3}H]mannose-derived radioactivity which was incorporated into component C_3, completely disappears (fig.2, slot 4). This result indicates that the possible drift of label from [^{3}H]mannose into amino acids as a result of oocyte metabolism was minimal. On the other hand, treatment of a [^{3}H]leucine-labelled immunoprecipitate with the enzyme leads to an increase in mobility on SDS–gels of component C_3 (fig.2, slot 2). The product of the glycosidase treatment now appears to migrate in the region of component C_2. This shift in migration suggests that

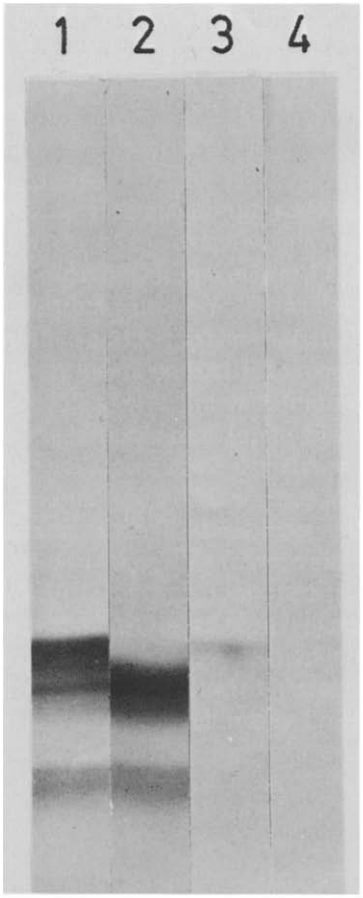

Fig.2. Incorporation of [^{3}H]mannose into PBP synthesized in oocytes and the effect of α-mannosidase. RNA-injected oocytes were incubated in the presence of [^{3}H]leucine (slots 1,2) or [^{3}H]mannose (slots 3,4). Following incubation aliquots of oocyte supernatant were immunoprecipitated with anti-PBP antibody. (1) [^{3}H]leucine-labelled PBP synthesized in oocytes; (2) idem, after treatment with α-mannosidase; (3) [^{3}H]mannose-labelled PBP synthesized in oocytes; (4) idem after treatment with α-mannosidase.

α-mannosidase removed a considerable amount of sugar from the glycosylated polypeptide. The enzymatic treatment did not alter the migration of the two other components of PBP, which suggests that the enzymatic changes of the glycosylated product were not related to possible proteolytic action.

4. Discussion

In order to explain the observed identity of native cytosolic PBP and the immunoprecipitable product synthesized in *Xenopus* oocytes in response to injected prostatic mRNA, we studied the incorporation of carbohydrate as one of the necessary post-translational modifications of the primary translation product. *Xenopus* oocytes have been shown [10,11] to glycosylate immunoglobulin chains programmed by exogenous mRNA isolated from rat spleen and MOPC-46 B plasmacytoma. The data presented here show that one of three polypeptide components of PBP, component C_3 which normally contains carbohydrate, is specifically glycosylated in the oocytes. Evidence for glycosylation was:

(i) [^{3}H]Mannose was specifically incorporated into component C_3;
(ii) Treatment with α-mannosidase removed all of the incorporated [^{3}H]mannose-derived radioactivity;
(iii) The migration of component C_3 on SDS–gels was sensitive to the action of α-mannosidase.

This marked sensitivity of the oocyte-synthesized component C_3 to the action of α-mannosidase suggests the presence of several α-mannose residues. However, as the sugar composition of native PBP is at present largely unknown, further studies are planned to evaluate the correctness of the carbohydrate attachment observed in the oocytes.

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