Vol. 117, No. 1, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 30, 1983 Pages 306-312

THE PRESENCE OF THE MILK PROTEIN, α-LACTALBUMIN AND ITS mRNA IN THE RAT EPIDIDYMIS

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Received October 7, 1983

SUMMARY: α-Lactalbumin, a modifier protein that changes the substrate specificity of galactosyltransferase, to promote the synthesis of lactose, is found in the mammary glands of lactating mammals and in milk. Molecules similar to mammary gland α-lactalbumin but distinct in their modifier activity have been found in rat epididymal fluid. We report here, using a rat mammary gland α-lactalbumin cDNA clone as a hybridization probe, RNA sequences homologous to α-lactalbumin mRNA were detected in total RNA from the rat epididymis. This finding suggests that α-lactalbumin or similar molecules, in adddition to regulating lactose synthesis in the mammary gland, may have other important functions in mammalian reproduction.

α-Lactalbumin (α-LA) is a protein synthesized by the mammary gland of lactating mammals and is secreted in milk. This protein has no known enzymatic function of its own but acts as a modifier (1) for galactosyltransferase, an enzyme that is normally involved in the biosynthesis of glycoproteins in many tissues (2). Specifically α-LA promotes the binding of glucose, otherwise a poor substrate, to galactosyltransferase, thereby permitting synthesis of lactose. α-LA appears during the functional differentiation of the mammary gland (3) and its expression has been shown to be regulated by insulin, prolactin and hydrocortisone (4).

Recently, a protein having α-LA-like activity in the lactose synthase system has been detected in fluid from the rat epididymis (5). However, this activity has been shown to differ from mammary gland α-LA activity in that it transfers galactose from UDP-galactose to either glucose or myo-inositol with equal

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0006-291X/83 $1.50

efficiency. In the present report we have confirmed these findings and further demonstrate the presence of RNA sequences homologous to mammary gland α-LA mRNA in the rat epididymis.

MATERIALS AND METHODS

Preparation of Tissue Extracts and Assay of α-LA-like Activity: About 5g of rat epididymis or mammary gland tissue were homogenized in 15 ml buffer (20 mM Tris-HCl pH 7.4, 10 mM $MgCl_2$ and 1% Triton-X). The 40-60% saturated ammonium sulfate fraction of the homogenate was dialyzed overnight against 1-liter of 20 mM Tris pH 7.4 containing 10 mM $MgCl_2$. The dialyzed material (1-1.2 ml) was passed through an (1x3 cm) N-acetyl glucosamine (NAG) Sepharose column to remove endogenous galactosyltransferase activity (6). After washing the column with 2 ml of buffer, the eluate was immediately assayed for α-LA activity. Five grams of rat epididymis gave 4 mg of total protein in the ammonium sulfate fraction and about 1 mg in the effluent from the NAG-Sepharose column as determined by the method of Lowry et al (7). The enzymatic assay of α-LA like activity was carried out essentially as described earlier (8).

Hybridization of total RNA from Epididymis or from Mammary Gland with α-LA cDNA probe: RNA was isolated from the tissue by the guanidinium thiocyanate method (9). Plasmid DNA, p-α-LA35, a full length α-LA-cDNA clone, was purified as described earlier (10). A) For Dot-blot analysis, samples containing epididymal total RNA or 5-day lactating mammary gland total RNA were dissolved in 20 µl of 10 X SSC (1 X SSC is 0.15M NaCl, 0.15M sodium citrate). Each sample was adjusted with yeast t-RNA to 50 µg of final RNA concentration and then bound to nitrocellulose paper by slow filtration and washing with 0.5 ml of 10 X SSC. B) For gel electrophoresis RNA samples were adjusted with yeast tRNA to 100 µg of final RNA concentration and then electrophoresed through 2% agarose gels containing 6% formaldehyde at 30 volts for 20 hours (11). Gels were soaked in 20 X SSC, and the RNA transfered to nitrocellulose paper (12). Filters from Dot-blot analysis and gel electrophoresis were then baked, prehybridized, and hybridized with heat denatured ^{32}P-labeled p-α-LA-35 probe (1×10^6 cpm/ml) and subsequently washed and exposed to X-ray film as detailed elsewhere (13).

RESULTS

α-LA-Like Activity in the Epididymal extract. For the present studies, we have concentrated the proteins from the epididymal homogenate by ammonium sulfate precipitation (40-60%) and tested for α-LA-like activity after removing the endogenous galactosyltransferase by passing the extract through an N-Acetyl glucosamine-Sepharose column (6). Figure 1 compares this activity in extracts from the epididymis and from the 5 day lactating mammary gland prepared under identical conditions, and with pure rat mammary gland α-LA. These transfer reactions showed absolute dependence on exogenous bovine milk galactosyltransferase and linearity with increasing concentrations of the extract. Lactose synthase activity in the epididymal extracts was about 30-50 times less than in the comparable extracts from 5-day lactating mammary gland.

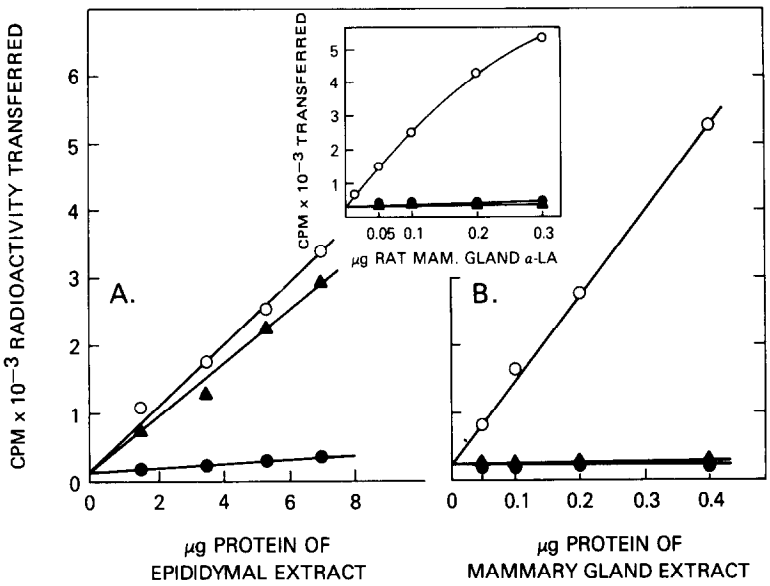

Figure 1: Transfer of galactose from UDP-[^{3}H]-galactose to glucose (o) and myo-inositol (▲) by epididymal extract (A), 5-day lactating rat mammary gland extract (B) and purified rat mammary gland α-LA (insert) in the presence of bovine milk galactosyltransferase. Values obtained without added sugar are represented by solid circles.

Furthermore, epididymal extract, in contrast to rat mammary gland extract or pure α-LA, promotes the transfer of galactose from UDP-galactose to either glucose or myo-inositol with nearly equal efficiency. Even using higher concentrations of myo-inositol (20-100 mM), mammary gland extracts failed to transfer galactose to myo-inositol. Neither epididymal extract nor bovine milk galactosyltransferase alone catalyse this reaction. The products of these reactions, lactose and galactinol were further characterised by paper chromatography (results not shown) (14).

Detection of homologous α-LA mRNA Sequences in Total RNA from Rat Epididymis. The availability of cloned rat mammary gland α-LA complementary DNA (cDNA) (10) allowed us to determine the α-LA mRNA or the homologous RNA sequences in epididymal total RNA. The levels of these sequences were therefore determined by hybridizing the ^{32}P-labeled full length cDNA probe, p-α-LA 35, with the total RNA either from whole epididymis or from 5-day lactating rat mammary gland. First we determined the degree of homology between the epididymal RNA sequences and the mammary gland α-LA cDNA probe by comparing hybridization with the labeled probe under different stringent conditions. These results showed

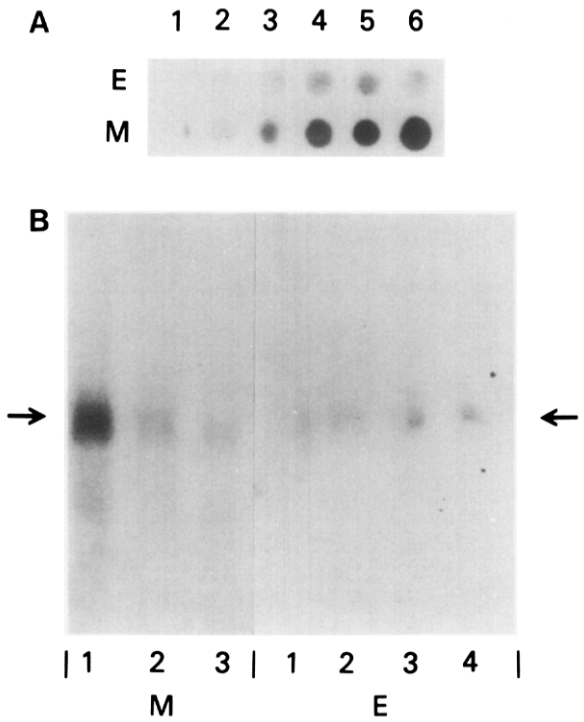

Figure 2. Detection of α-LA mRNA homologous sequence in the total RNA from the epididymis by hybridization with ^{32}P-labeled rat mammary gland specific α-LA cDNA probe, p-α-LA 35. (A) Autoradiogram of Dot-blot analysis: 1,2,3,4,5,6, respectively, represent samples containing 1, 5, 10, 20, 30 and 50 µg of epididymal total RNA (E) and 0.1, 0.2, 0.5, 1, 2 and 5 µg of 5-day lactating mammary gland total RNA (M). (B) Autoradiogram of the hybridization of total RNA after gel-electrophoresis and transfer to nitro-cellulose filters: Samples containing 50, 60, 75 and 100 µg of epididymal total RNA (E) were electrophoresed in lanes 4, 3, 2, 1, respectively and 1, 2 and 5 µg of 5-day lactating mammary gland total RNA (M) in lanes 3, 2, 1, respectively.

that epididymal RNA hybridized with the probe with efficiency equal to that of mammary gland α-LA mRNA. Under the stringent conditions of both hybridization (50% formamide, 5 X SSC at 42°C) and subsequent washing of the filter (15) (0.1 X SSC, 0.1 % SDS at 60°C), homologous RNA sequences could be detected by Dot-blot analysis (12) in 5-10 µg of total epididymal RNA (Fig 2A). Electrophoresis of the RNA through agarose gels containing formaldehyde (11) and subsequent analysis on Northern blots (Fig 2B)(12) showed that a) at least 50 µg of the total epididymal RNA were required to detect the homologous RNA sequences and b) the size of this RNA is very similar to the mammary gland α-LA mRNA. Both these methods, however, showed that the epididymal total RNA contained about fifty to seventyfive times less homologous α-LA RNA sequences than in 5-day lactating rat mammary gland total RNA. Under similar conditions

of hybridization, mRNA sequences homologous to other mammary specific cDNA clones, such as whey-phosphoprotein and caseins clones (16), could not be detected in the epididymal RNA.

DISCUSSION

We have immunocytochemically detected the presence of α-LA-like molecules in the rat epididymis (17) using antibody against pure mammary gland α-LA (8). This immunological similarity between rat epididymal α-LA-like protein and rat mammary gland α-LA, together with the apparent homology of their mRNAs suggests that the two proteins are similar molecules. In addition, both proteins modify the activity of galactosyltransferase in a way which permits the synthesis of lactose. However, rat epididymal α-LA-like protein, in contrast to the rat mammary gland or bovine protein modifies the activity of galactosyltransferase in such a way that it also allows the transfer of galactose from UDP-galactose to myo-inositol. The molecular differences between the rat epididymal and mammary gland proteins which give rise to these changes in their modifier activity remain to be determined. Rat mammary gland α-LA unlike the major fraction of bovine α-LA or human α-LA, is known to be a glycoprotein (8,18) and differs from α-LAs isolated from other species, in that it has an additional 17-residue C-terminal hydrophobic extension (10,18). It is possible that the rat epididymal α-LA-like protein differs from the rat mammary gland α-LA in carbohydrate content or in some amino acid residues, differences which cannot be easily detected by conventional immunological methods or by hybridization with cDNA probes.

The presence of homologous α-LA mRNA sequences and an α-LA-like protein in the epididymis raises many interesting questions about the role of this protein in mammalian reproduction. In the rat mammary gland, α-LA appears for a short period during the first few days of gestation and then reappears during late gestation or early lactation (3). The absence of any detectable lactose production in the mammary gland during early gestation, led us to suggest that α-LA may have functions other than in lactose synthesis, such as in priming other cells of the mammary gland for cellular differentiation or in priming the

cells of other organs involved in the maintenance of the reproductive status of the animal (3). In the epididymis, where lactose is absent and free glucose levels are barely detectable (5), a role of an α-LA-like protein in lactose biosynthesis is unlikely. However, as myo-inositol is present in high amounts (19), α-LA-like protein may interact with galactosyltransferase in either transfering galactose to myo-inositol linked to membrane bound glycoproteins, or to some other sugars resulting in a specific oligosaccharide sequence on sperm surface glycoproteins which are recognized as new antigenic determinants or specific differentiation antigens. The initial binding of sperm to the egg zona pellucida has recently been shown to involve sperm surface galactosyltransferase (20) and α-LA specifically inhibits this binding. In addition it is known that both sperm capacitation and the acrosome reaction are Ca^{++} dependent phenomena in many animals (21). In this regard it is interesting to note that α-LA is a heat stable protein with two binding sites for Ca^{++} with a Kd of 0.3 μM and 30 μM, respectively (22). Whatever the role of α-LA and galactosyltransferase in the epididymis, the results show that α-LA can no longer be regarded as unique to the mammary gland. They also indicate that α-LA and perhaps other galactosyltransferase modifiers may exist in other tissues e.g., the testis (17) and function in the modification of cell surface carbohydrates involved in cell-cell interactions and cell differentiation.

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