Biochimie xxx (2014) 1–8

Contents lists available at ScienceDirect

Biochimie

journal homepage: www.elsevier.com/locate/biochi

Research paper

Expression of microsomal triglyceride transfer protein in lipoprotein-synthesizing tissues of the developing chicken embryo☆

Christine Eresheim, Julia Plieschnig, N. Erwin Ivessa, Wolfgang J. Schneider, Marcela Hermann*

Max F. Perutz Laboratories, Department of Medical Biochemistry, Medical University of Vienna, Dr. Bohr-Gasse 9/2, 1030 Vienna, Austria

ARTICLE INFO

Article history:
Received 25 November 2013
Accepted 21 December 2013
Available online xxx

Keywords:
Microsomal triglyceride transfer protein
Apolipoprotein B
Yolk sac
Lipoprotein
chicken
Development
Embryo

ABSTRACT

In contrast to mammals, in the chicken major sites of lipoprotein synthesis and secretion are not only the liver and intestine, but also the kidney and the embryonic yolk sac. Two key components in the assembly of triglyceride-rich lipoproteins are the microsomal triglyceride transfer protein (MTP) and apolipoprotein B (apoB). We have analyzed the expression of MTP in the embryonic liver, small intestine, and kidney, and have studied the expression of MTP in, and the secretion of apoB from, the developing yolk sac (YS). Transcript and protein levels of MTP increase during embryogenesis in YS, liver, kidney, and small intestine, and decrease in YS, embryonic liver, and kidney after hatching. In small intestine, the MTP mRNA level rises sharply during the last trimester of embryo development (after day 15), while MTP protein is detectable only after hatching (day 21). In the YS of 15- and 20-day old embryos, apoB secretion was detected by pulse-chase metabolic radiolabeling experiments and subsequent immunoprecipitation. Taken together, our data reveal the importance of coordinated production of MTP and apoB in chicken tissues capable of secreting triglyceride-rich lipoproteins even before hatching.

1. Introduction

The major structural protein of triglyceride-rich lipoproteins secreted by the liver and intestine is apolipoprotein B (apoB). The microsomal triglyceride transfer protein (MTP) plays a major role in the synthesis and secretion of apoB-containing lipoproteins [1]. MTP is located in the lumen of the endoplasmic reticulum (ER) and is involved in the initial loading of nascent lipoproteins with neutral lipids, particularly triglycerides and cholesterol esters. Alternatively, or additionally, MTP may participate in the intricate process of apoB translocation across the ER membrane [2]. In humans, MTP has been shown to be involved in the biogenesis of lipoproteins containing apolipoprotein B100 in the liver and apolipoprotein B48 in the small intestine [3–5]; for review see Ref. [6].

Studies of human embryos showed expression of apoB RNA in the fetal liver, fetal intestine, and in the yolk sac (YS), but not in other embryonic tissues [7–9]. MTP and apoB are also expressed in the human placenta, suggesting that this organ can also synthesize and secrete apoB-containing lipoproteins [10]. During mouse embryonic development, MTP is expressed in the YS as well as in liver and small intestine [11,12], but not in cardiac and renal tissues. In the laying hen, it has been shown that MTP is expressed in tissues involved in lipid metabolism, i.e., the liver, small intestine, and the kidney [13]. Chicken liver synthesizes and secretes apoB100-containing lipoproteins, but apoB48 production is totally absent in any organ of chickens. Interestingly, chicken small intestine secretes so-called portomicrons, which are apoB100-containing lipoproteins [14,15].

Genetic deficiency of MTP in humans results in the autosomal recessive disease, abetalipoproteinemia [16,17]. The pathophysiology of this inherited condition may be attributed, at least in part, to the impaired absorption of the fat-soluble vitamins A and E [18,19]. Gene defects leading to structurally altered apoB secreted from the liver or intestinal mucosa results in hypobetalipoproteinemia [20–24], which also is associated with fat-soluble vitamin deficiencies in humans. In liver and intestine of heterozygous MTP knockout mice, the MTP mRNA and protein levels were reduced by 50%, and a marked reduction in total plasma cholesterol levels was observed. Studies of MTP knockout mouse embryos showed an impaired capacity of the YS to export lipids to the developing embryo and demonstrated an accumulation of cytosolic lipid droplets in the endodermal cells of the yolk sac, followed by lethal developmental abnormalities [25].

* Corresponding author. Tel.: +43 1 4277 61820; fax: +43 1 4277 9618.
E-mail address: marcela.hermann@meduniwien.ac.at (M. Hermann).

0300-9084/$ – see front matter © 2014 The Authors. Published by Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.biochi.2013.12.020

The chicken YS plays a crucial role in embryo growth, as it supplies the growing embryo with nutrients taken up from the yolk. Acquisition of this function is a process which is coordinated with that of vascularization of the YS [26]. MTP has been identified in chicken YS [26,27], but the expression of MTP in fetal tissues has not been studied in detail yet. We now show that MTP is expressed not only in the YS of the developing chicken embryo, but also in fetal liver, intestine, and kidney. Secretion of newly synthesized apoB in ex-vivo cultured YS tissue, which also synthesizes MTP, is compatible with the notion that this tissue exports triglyceride-rich lipoproteins to the embryonic circulation.

2. Material and methods

2.1. Animals

Sexually mature Derco brown (TETRA-SL) laying hens and roosters were purchased from Diglas Co. (Feuersbrunn, Austria) and maintained on open floor space with free access to water and feed (standard diet, Ssniff, Germany) under a daily light period of 16 h. For fertilized eggs, hens and roosters were housed together in flocks in our animal facility. Freshly fertilized eggs available in house were incubated at 37.5 °C and 60–70% humidity to maintain normal embryonic development. Pre-hatch eggs were removed from the incubator after 5, 7, 9, 10, 11, 13, 15, 17, 20 days; 1-day-old and 3-day-old chicks were also utilized. For tissue and organ retrieval, the chicks were euthanized by decapitation. The shell of eggs incubated for up to 20 days was cut open to expose the embryo for tissue preparation (liver, kidney, and small intestine); the YS membranes were excised, washed with ice-cold phosphate-buffered saline (PBS), and protease inhibitors (Complete, Roche, Manheim, Germany) were added to PBS buffer prior to the preparation of Triton X-100 protein extracts. For tissue and organ retrieval, the laying hens were euthanized by decapitation. All animal procedures were approved by the "Animal Care and Use Committee" of the Medical University of Vienna.

2.2. Preparation of antibodies

Polyclonal antibodies directed against a chicken MTP C-terminal fragment [13], and against chicken apoB100 [28] were raised by a standard procedure in adult female New Zeeland White rabbits in our laboratory. Antisera were tested by Western blotting.

2.3. Preparation of yolk sac layers and tissue culture

Chicken eggs were incubated to embryonic day (E) 9. YS tissue from E9 embryos was washed in PBS. Separation of layers was performed in PBS using watchmakers' forceps. Starting from the mesodermal layer containing blood vessels, the endodermal epithelial cell (EEC) layer and the mesoderm were peeled apart. The separated layers were immediately used for protein isolation.

For ex-vivo culture, yolk sacs were isolated from 15 or 20 days old embryos, and small pieces of 0.5–0.7 cm^2 were cut off and incubated in DMEM D-Valine medium supplemented with 10% fetal calf serum, 2% horse serum, 100 units penicillin/ml, 0.1 mg/ml streptomycin, 1% fungizone and 2 mM L-glutamine, which was changed every day. The tissue fragments were incubated two days to remove the adherent yolk before use in experiments.

2.4. Metabolic labeling and immunoprecipitation

Cultured YS tissue specimens were washed in PBS and incubated in starvation medium (RPMI 1640 without L-glutamine, L-methionine, L-cysteine and L-cystine) for 1 h at 37 °C and 5% CO_2. Thereafter, the medium was removed and the YS fragments were metabolically labeled in starvation medium with Met-label ([^{35}S] methionine and [^{35}S]cysteine) for 1 h. After the pulse phase, the labeling medium was removed and the YS tissue-pieces were incubated in medium as described above, supplemented with 200 mM L-glutamine, 400 mM L-methionine, 400 mM L-cysteine and L-cystine. After the indicated chase times, the supernatant was processed as follows. For immunoprecipitation, 500 μl of the supernatant were incubated with the anti-ApoB100 IgG (1:100) and protein A-Sepharose beads (40 μl wet volume) for 16 h at 4 °C. The protein A-Sepharose beads were washed 5 times with PBS, suspended in Laemmli sample buffer containing 150 mM 2-mercaptoethanol and heated to 95 °C for 5 min. After electrophoresis on a denaturing 10% polyacrylamide gel, the gel was fixed (10% acetic acid, 30% methanol), treated for fluorography with EN^{3}HANCE solution, dried, and X-ray films were exposed at −80 °C for 24 h to 8 days and quantifications were performed with ImageJ analysis program.

2.5. Preparation of membrane protein extracts and determination of protein concentrations

Triton X-100 extracts from various tissues prepared from chicken embryos of different developmental stages, from hatched chicks, or from laying hens were prepared as described previously [29]. These samples were used immediately or were quick-frozen in liquid nitrogen and stored at −80 °C until use. Protein concentrations were determined by the method of Bradford (BioRad, Hercules, California, USA) with bovine serum albumin as standard.

2.6. SDS-polyacrylamide gel electrophoresis and immunoblotting

For Western blotting, aliquots of extracts were subjected to 10% SDS-PAGE under reducing or non-reducing conditions, and the separated proteins were electrophoretically transferred to nitrocellulose membranes (Hybond-C Extra, Amersham Biosciences, Little Chalfont, UK). The amounts loaded were monitored by Ponceau-S staining of the membranes. Nonspecific binding sites were blocked with TBS (20 mM Tris–HCl, pH 7.4, and 137 mM NaCl) containing 5% (w/v) nonfat dry milk and 0.1% Tween-20 (blocking buffer) for 1 h at room temperature. GgMTP was detected with rabbit anti-ggMTP antiserum (1:5000) followed by incubation with HRP-conjugated goat anti-rabbit IgG (1:50,000, Sigma) and development with the enhanced chemiluminescence protocol (Pierce, Rockford, Illinois, USA). Quantifications were performed with AlphaEaseFC analysis program. The sizes of proteins were estimated with a Precision Plus Protein molecular mass standard (10–250 kDa) from Bio-Rad (Hercules, California, USA).

2.7. Preparation of total RNA, cDNA synthesis, PCR, DNA cloning, and sequencing

Total RNA from the indicated galline tissues was extracted using the NucleoSpin RNA II kit (Macherey-Nagel, Dueren, Germany) following the manufacturer's instructions. These samples were used immediately or stored at −80 °C until use. Single-stranded cDNA was synthesized using SuperScript II reverse transcriptase (Invitrogen, Carlsbad, California, USA) and oligo(dT)18-primer. PCR amplification was performed with a T3000 Thermocycler (Biometra, Goettingen, Germany) with a touch-down program using the High Fidelity PCR Enzyme Mix (Fermentas, St. Leon-Rot, Germany). The amplified products were subjected to 1% agarose gel electrophoresis and stained with ethidium bromide. Subsequently, the PCR product was excised from the gel, and DNA was purified with Xact Extraction Kit (GenXpress, Wiener Neudorf, Austria). The

Please cite this article in press as: C. Eresheim, et al., Expression of microsomal triglyceride transfer protein in lipoprotein-synthesizing tissues of the developing chicken embryo, Biochimie (2014), http://dx.doi.org/10.1016/j.biochi.2013.12.020