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Biochimica et Biophysica Acta 1760 (2006) 1380–1385
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
www.elsevier.com/locate/bbagen

Perchloric acid-soluble protein is expressed in enterocytes and goblet cells in the intestine and upregulated by dietary lipid

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Received 20 December 2005; received in revised form 24 March 2006; accepted 24 March 2006
Available online 19 April 2006

Abstract

We previously identified perchloric acid-soluble protein (PSP) in the rat liver, kidney, brain and lung, and reported that it appeared to be related to repression of cell proliferation. In the present study, we clarified that PSP was expressed in the intestine, and found that the amino acid sequence of the intestinal PSP was consistent with those of other PSPs present in other tissues. An immunohistochemical study revealed that PSP was expressed in enterocytes and goblet cells, but not in other cell types among the lamina propria epithelial cells. A comparison of the expressions of PSP and proliferating cell nuclear antigen demonstrated that the proliferating cells did not express PSP. Intestinal PSP expression was induced by ~3-fold by oral administration of dietary fat. These findings indicate that the proliferation repression activity may be related to renewal of the intestinal epithelium, and that PSP is one of the fatty acid-inducible proteins.

Keywords: Perchloric acid-soluble protein; Rat intestine; Fatty acid-binding protein; Cell proliferation

1. Introduction

Perchloric acid-soluble protein (PSP), which was originally identified in the rat liver [1], is one of the highly conserved proteins throughout evolution [2]. However, the central role of PSP has not yet been revealed. There are many reported studies on PSP function, for example, its ribonuclease activity [3], fatty acid-binding activity [4], mitochondrial DNA maintenance activity [5], purine repressor association [6] and isoleucine synthesis co-factor activity [7]. However, we have focused on the PSP functions associated with cell proliferation and differentiation, since PSP expression is not recognized in immature cells but appears in mature cells in the kidney, brain and lung [8–10]. Using cell lines that overexpress PSP, we previously revealed that PSP repressed cell proliferation [11]. Furthermore, by the suppression of PSP by an anti-PSP antibody injection into chick embryos, the neuroepithelial cells excessively proliferated in the embryonic spinal cord [12]. Considering these results, we hypothesized that PSP is related to the control of cell proliferation during development. In the present report, we examined PSP expression in the intestine, since the adult rat intestinal epithelium undergoes continuous and rapid renewal and provides an attractive model system for examining the processes of proliferation and differentiation.

Epithelial cell renewal in the adult intestine is supplied by multipotent stem cells located at, or near, the base of flask-shaped mucosal invaginations known as crypts of Lieberkühn [13–15]. These crypts are dynamic structures, with an average lifespan of ~110 days. Maintenance of the crypts is dependent upon their stem cell population. The committed daughters of the stem cells undergo several rounds of division, forming a rapidly cycling transit cell population located in the mid-portion of each adult crypt. The stem cells ultimately give rise to four cell types in the intestine: enterocytes (representing >90% of all epithelial cells), goblet cells, enteroendocrine cells and Paneth cells. The first three lineages complete their terminal differentiation and migrate in an orderly manner from the crypt up to an adjacent villus. As the mature epithelial cells approach the villus tip, they are

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doi:10.1016/j.bbagen.2006.03.023

removed by apoptosis or exfoliation. The Paneth cell lineage differentiates during a downward migration to the crypt base [16].

The intracellular fatty acid-binding proteins (FABPs) belong to a superfamily of lipid-binding proteins and are abundantly expressed in vertebrate tissues [17]. It has been suggested that the functions of FABPs are not only to solubilize fatty acids but also to modulate microsomal phospholipid synthesis, peroxisomal β-oxidation and so on [18,19]. PSP has also fatty acid-binding activity despite its lack of homology with common FABPs [4]. Liver PSP was reported to be induced by oral injection of bezafibrate, a known PPARα ligand [4]. PPARα plays a key role in several processes, including lipid metabolism [20], and binds peroxisome proliferators such as fatty acids or eicosanoids. Once activated by a ligand, the receptor undergoes a conformational change that enables it to form heterodimers with the retinoid X receptor (R X R). The heterodimers then bind to PPAR-responsive elements (PPREs), which are located in the promoter elements of PPAR-responsive genes, such as those encoding fatty acid transport protein (FATP) and apolipoproteins. In the present study, we tested whether intestinal PSP was induced by oral injection of bezafibrate and dietary fat.

2. Materials and methods

2.1. Materials and rats

General chemicals were purchased from Nacalai Tesque (Kyoto, Japan). The anti-PSP rabbit serum and purified anti-PSP IgG were prepared as described previously [1]. The anti-proliferating cell nuclear antigen (PCNA) antibody was purchased from Sigma (St. Louis, MO). The ECL western blotting analysis system was purchased from Amersham (Little Chalfont, UK). Nine-week-old Wister rats were purchased from SLC (Shizuoka, Japan). The rat was fed a AIN93G diet [21] and tap water ad libitum, and kept on a 12-h light/12-h dark cycle at 20 °C. All experimental procedures were examined and approved by the Animal Research Ethics Committee at Kagoshima University.

2.2. Identification of intestinal PSP

Total RNA was obtained from the intestine and liver of 9-week-old male rats using TRIzol (Invitrogen, Tokyo, Japan). cDNAs were synthesized from 5 μg of the total RNA using a commercial kit (Amersham, Tokyo, Japan), according to the manufacturer's instructions. The full-length PSP mRNA registered as GenBank Accession No. NM_031714 was amplified by polymerase chain reaction (PCR) using specific primers (PSP-F primer: 5′-cacgaggttgttactgccgtttcttctctgga-3′; PSP-R primer: 5′-ctcgagttttttttttttttttttacacatc-3′), and 200 ng of cDNA from each tissue as a template. The reaction mixtures contained 0.3 μM of each primer, 0.2 μM of a deoxy-NTP mixture, 1 mM $MgSO_4$, 1× buffer and 1 unit of KOD plus DNA polymerase (TOYOBO, Tokyo, Japan). The PCR conditions for PSP were as follows: 30 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s and extension at 72 °C for 30 s. The amplified DNA was subjected to gel electrophoresis in a 1% agarose gel to check the sizes of the PCR products, and purified using a PCR purification kit (Qiagen, Tokyo, Japan) for sequencing. The sequencing reaction was performed using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Tokyo, Japan) and the PSP-F primer or PSP-R primer according to the manufacturer's instructions. Intestine was dissected from rat and equally cut three parts from proximal to distal. PSP protein in the three parts of intestine was detected by immunoblotting according to a previous report [1]. Briefly, a rat intestinal mucosa sample was obtained by scratching the intestinal inner surface with the edge of a glass slide and homogenized in homogenization buffer (250 mM sucrose, 50 mM Tris–HCl pH 6.5, 25 mM KCl and 10 mM $MgCl_2$) in a potter type homogenizer. A rat liver homogenate was prepared from whole liver. The homogenate was filtered through nylon mesh and centrifuged at 12,000×*g* for 30 min. After the measurement of protein concentrations using a BCA protein assay kit (Pierce, Rockford, IL), the supernatant was used for SDS-PAGE and immunoblotting analysis, The proteins were separated in SDS-polyacrylamide gels (15%) and blotted onto nitrocellulose membranes (Amersham). Immunoblotting was performed using an anti-rat PSP rabbit serum and a horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG, and visualized by enhanced chemiluminescence (ECL). Images were analyzed with a Fuji LAS-1000 Luminescent Image Analyzer (Fuji Film, Tokyo, Japan).

2.3. Immunohistochemistry

Rats were anesthetized with ethyl ether, and sacrificed by cervical dislocation. The intestine was dissected out, and its inner surface was fixed with 4% paraformaldehyde (PFA). Next, the fixed intestine was embedded in paraffin, and serially sectioned at 3 μm thickness. The sections were deparaffinized and soaked in 0.3% H_2O_2 in 100% ethanol for 30 min at room temperature to block endogenous peroxidase activity. After rehydration and rinsing in phosphate-buffered saline (PBS; 10 mM sodium phosphate buffer, pH 7.2, containing 0.85% NaCl), the sections were blocked by incubation in 2.5% normal horse serum (Vector Laboratories, Burlingame, CA) for 20 min at room temperature to reduce nonspecific staining, and then incubated with two types of primary antibody at 4 °C overnight in a moist chamber. One of these was an anti-PSP polyclonal antibody diluted 1:1600 in PBS containing 0.1% BSA, while the other was an anti-PCNA monoclonal antibody diluted 1:500 in the same solution. The section was washed extensively with PBS and then incubated with the ImmPress reagent (Vector Laboratories) for 30 min at room temperature. After washing with PBS, the section was incubated with a DAB Substrate Kit for Peroxidase (Vector Laboratories) until a suitable level of staining had developed. After washing with PBS, the section was counterstained with hematoxylin for 20 s, and then dehydrated and mounted. Negative controls were prepared using non-immunized rabbit IgG as the primary antibody.

2.4. Effects of bezafibrate and dietary fat on intestinal PSP

Bezafibrate (Sigma) was dissolved in sunflower oil, and 500 μl of dissolved bezafibrate was administered to 9-week-old male Wister rats (at 400 mg/kg body weight) via an oral zonde every day for 3 or 7 days. Five hundred μl of sunflower (SF) oil without bezafibrate was administered to the sunflower oil group. Control group of rats were not fed SF nor bezafibrate, and sacrificed at 7 days. All groups of rats were fed ad libitum AIN93G diet ($n=3$). We prepared experimental diets containing 0 wt.%, 12.5 wt.% and 25 wt.% corn oil were prepared according to the AIN-93G type diet. Nine-week-old male Wister rats were fed each experimental diet for 2 weeks ($n=3$). After the 2-week period, all the above rats were sacrificed and their intestines were dissected. The intestinal epithelium was homogenized in homogenization buffer, and the protein concentration of each homogenate was measured. The homogenate was subjected to immunoblot analysis using an anti-PSP antibody, and the detected bands were quantified using a computer program (Fuji Film).

2.5. Statistical analysis

Data were analyzed by one-way ANOVA and Turkey–Kramer post hoc multiple comparisons test when one-way ANOVA tests yielded $P<0.05$. All data are presented as means±S.E.M.

3. Results

3.1. PSP mRNA and protein expression in the intestine

Using an RT-PCR method, we detected the expression of PSP mRNA in the rat intestine. The molecular size of the rat