HISTOCHEMICAL STUDY OF EXPRESSION OF GALECTIN–1 AND ITS REACTIVE CARBOHYDRATE EPITOPES IN NORMAL BOVINE EMBRYONAL AND ADULT PANCREAS

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Summary

The galectins are family of ß-galactoside-binding lectins, which are found in species ranging from sponges to humans. Despite the high degree of structural conservation among the members of this family, and broad distribution of these molecules in a variety of tissues, their precise function in any specific tissue is not fully understood.

The mammalian pancreas in the developmental stage provide an excellent system for studying the role(s) of cell surface glycoproteins and lectins in embryogenesis because the three cell types constitute the stroma of this organ (acinar, endocrine, and centroacinar) while each is involved in different aspects of exocrine gland function. Since no information is available on the level of presence of galectin-1 and galectin-1-reactive glycoconjugates in development of bovine pancreas, we have focused on this protein. To determine whether gelactin-1 was present in bovine pancreas or not, we used polyclonal rabbit antiserum raised against galectin-1 for histochemical analysis. Likewise biotin labeled galectin-1 was applied to visualize the galectin-1 reactive carbohydrate epitopes. We have used affinity chromatography and SDS-PAGE for the separation of galectin-1. Galectin-1 was identified by electrophoresis in extracts of adult and fetal pancreas. A single 14-kD band comigrated with a standard marker of the molecule. Histochemically, galectin-1 expression was localized mainly in the blood vessels at earlier stages, and found abundantly in the connective tissue cells. Furthermore, the cytoplasm of acinar cells of the adult pancreas was diffusely labeled. Histochemistry with biotin-labeled galectin-1 revealed only sporadic staining in the acinar cells of adult pancreas.

Key words: Galectin, pancreas, bovine, electrophoresis.

Introduction

Mammalian ß-galactoside-binding lectins (galectins), which are Ca^{2+}- independent (1,2) and require thiol reagents for maintenance of their sugar binding activity (3,4), are distinct from the Ca^{2+}-dependent (C-type) lectin family, and thought to be closely associated with development, differentiation, neoplastic transformation, and metastatic progression (5-9). They recognize certain sugar structures (N-acetyllactosamine, ß-D-galactose) and carry out appropriate tasks in given circumstances (10). Galectin-1 is a homodimer of 14.5 kDa subunits (11-13). It binds to desialylated glycoconjugates (glycoproteins, glycolipids, proteoglycans and lipopolysaccharids) containing clustered glycosides with terminal ß 1,4-linked galactosyl residues and binds to N-acetyllactosamine better than lactose (14). Like other members of the family, galactin-1 does not possess a signal peptide or transmembrane domain (15,16).

Materials and methods

Specimens of adult bovine pancreas and at three different stages of fetal development, were freshly obtained from a slaughterhouse and grouped according to crown-rump length into distinct age categories; F1=7-12 cm (week 7-11), F2=13-23 cm (week 12-16) and F3=24-38 cm (week 17-21). Specimens were fixed in 4% paraformaldehyde for 24-72 h at 4 C^{0}, and embedded in paraffin.

Isolation of galectin-1 from bovine heart

Fresh adult bovine heart tissue (total wet weight 110g) was cut into small pieces and homogenized with a Braun-Multimix MX32 homogenizer in cold (4°C) phosphate-buffered saline (diluted 1:10)/0.01M 2-mercaptoethanol/0.1 M lactose [PBS (1:10)/ME/Lac] containing 0,1mM phenylmethylsulfonyl fluoride. Then the homogenate was centrifuged at 27,000xg for 1h at 4°C, and the clear supernatant was mixed with DEAE-Sepharose (10ml supernatant/1ml resin), pre-equilibrated with PBS (1:10)/ME. After a gentle mix for 1h at 4°C, the slurry was transferred to a fritted glass funnel, and the resin was washed with 10 bed volumes of cold PBS (1:10)/ME to remove lactose and unbound protein. The bound proteins were eluted with 500 ml PBS/0.002M EDTA/0.5 M NaCl. The eluate was adsorbed on a column of lactosyl-sepharose pre-equilibrated with PBS/0.002M EDTA/0.5 M NaCl. The column was washed until the absorbance reached the baseline with equilibrating buffer, then with 5 bed volumes of PBS (1:10)/ME and finally, the bound protein was eluated with 0,1M lactose in PBS (1:10)/ME. The fractions were pooled and aliquoted and were then absorbed on DEAE-Sepharose columns (0.5 ml bed volume) and overlaid with 50 % glycerol in eluting buffer and stored at -20°C.

Analysis of lectin on SDS-PAGE

Following extraction with diluted PBS, the galectin was first absorbed on DEAE-Sepharose, washed and then eluted with a

high salt buffer. The high salt eluate from DEAE-Sepharose was immediately loaded on a lactosyl-Sepharose column, the resin was washed until the absorbance reached the baseline, and the bound protein was eluted with lactose. The presence of the eluted galectin-1 was confirmed by SDS-PAGE using standard molecular weight markers.

Histochemical processing

The paraplast-embedded sections were processed by rehydration, followed by treatment with 1 % hydrogen peroxide solution for 30 minutes to block endogenous peroxidase activity, incubation with 0.3 % normal-goat serum to saturate unspecific protein binding sites, incubation with IgG (1/400), containing 0.1 % BSA, raised against galectin-1, for overnight at 4°C. After several washes for complete removal of unbound marker, sections were incubated with biotin-labeled second antibody for 1 h, and subsequently with ABC (Avidin-peroxidase complex) for 1h. The bound antibody was detected by the addition of the chromogenic substrate DAB (3l -3l - Diaminobenzidine).

Biotinylation of the lectin

To protect the carbohydrate-binding site of the purified lectin during biotinylation, 500 µg lectin in 8 mL of 2 mM phosphate-buffered saline, pH 8.0, was dialyzed for 12 h against the same buffer containing 20 mM lactose. Biotinylation was initiated by slow addition of 3 mg biotinyl-N-hydroxysuccinimide ester, dissolved in 0.6 mL N,NI –dimethylformamide. After 14 h at 4°C, the mixture was dialyzed against 2 mM phosphate buffer, pH 7.2. The biotin content comprised 13 moieties per subunit.

Antibody preparation

Polyclonal antibodies against galectin-1 were raised in rabbit by intradermal injection of the nitrocellulose-bound lectin. The IgG fraction of serum was purified using an A-Sepharose 4B (Pharmacia, Freiburg, FRG) chromatography and specificity of the controls were determined by Ouchterlony double immuno diffusion. Immunospotting and immunoblotting were similarly performed with the IgG fractions from pre-immune serum and the antibody-containing serum, as described previously.

Results

Affinity chromatography with immobilized lactose yielded galectin-1 with no indication of the presence of further galectin-like-galactoside-binding proteins on gel electrophoretic analysis from adult and embryonic pancreas (Fig. 1).

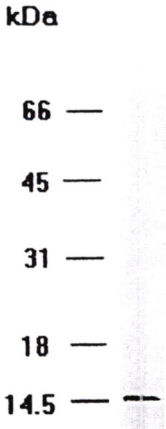
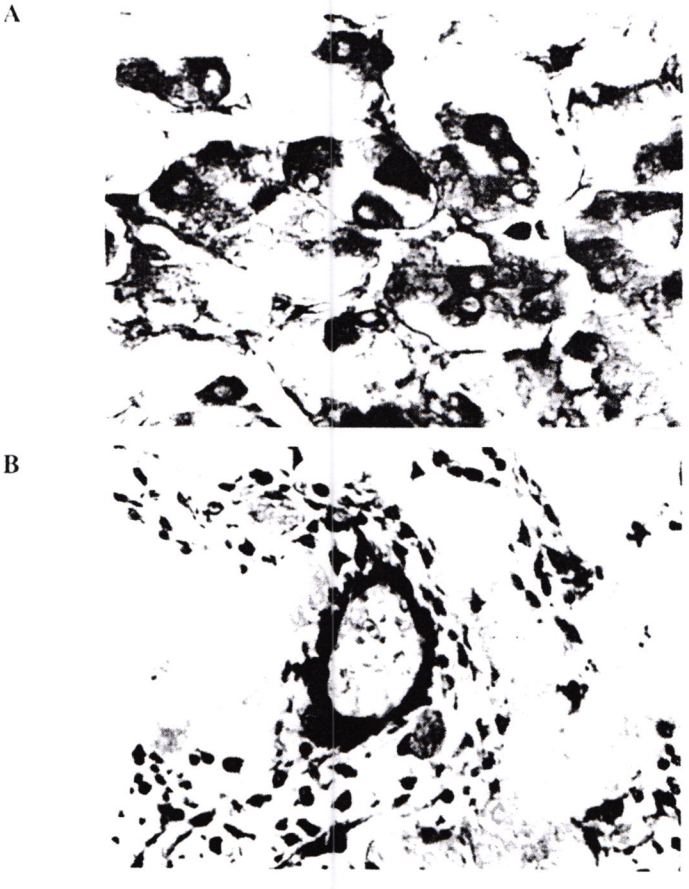


Fig. 1: Visualization of purified galectin-1 (200 ng protein, 14.5 kDa) from bovine heart by silver staining after reducing SDS polyacrylamide gel electrophoresis on a 15 % running gel

Immunhistochemical study revealed a specific staining pattern for all three developmental and adult stages. Immunstaining for IgG raised against galectin-1 was positive in the muscle of blood vessels. The staining was not restricted in the cytoplasm, the nucleus also exhibited staining for galectin-1 (Fig. 2 B). An obvious difference between adult and embryonal stages was the cytoplasmic staining on the acinar cells at the adult stage. In contrast to adult stage, no reactivity was seen on the acinar cells of embryonal pancreas (Fig. 2 B). Moreover, some connective tissue cells of all the embryonal stages were positive for the galectin-1. The reactive acinar cells of adult stage exhibited a diffuse cytoplasmic reactivity (Fig. 2 A), while the acinar cells of embryonal stage remain unstained. At the embryonal stage, the nuclei of smooth muscle cells around the corpus glanduleae (acinus) were strongly stained. Biotin-labeled galectin-1 located only at the adult stage with a weak staining in the acinar cells of corpus glanduleae. The nuclear membranes of acinar cells exhibited a positive reaction (Fig. 2 C). However the embryonal stages were not stained with biotinylated galectin-1 (Fig. 2 D). The islets of Langerhans were not stained with IgG raised against galectin-1 nor with biotinylated protein at all three embryonal stages and adult.

When the primary antibody was omitted or replaced with unimmunized rabbit serum, no positive staining was observed. Furthermore, specific staining with the primary antibody was completely inhibited by the addition of the antigen.



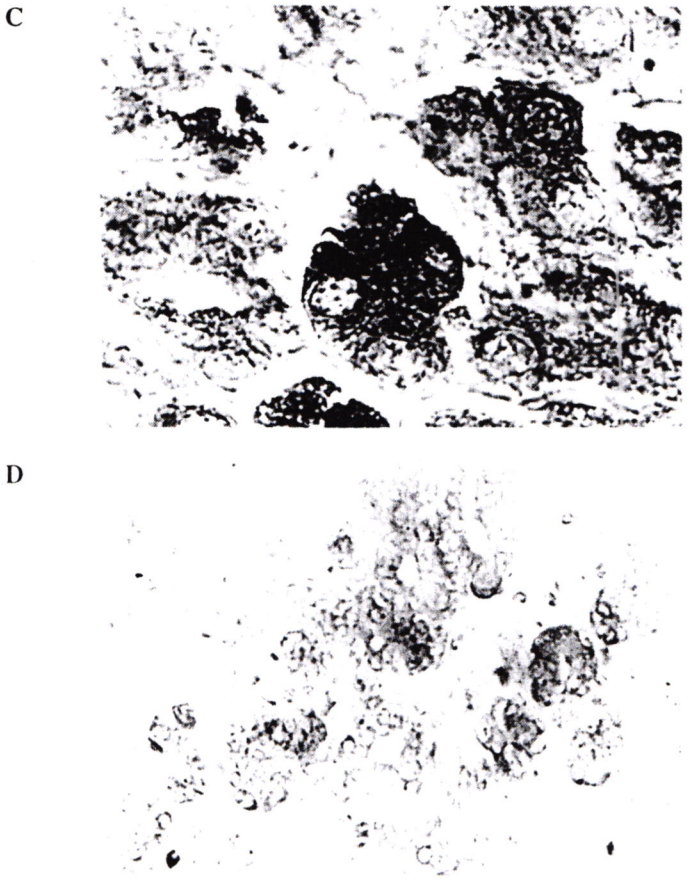


Fig. 2.: Immunohistochemical localization of bovine galectin-1 in paraffin-embedded sections of adult (A) and fetal (B) pancreas (x540 and x340) and visualization of galectin-1 epitopes in adult (C) and fetal (D) pancreas (x1360 and x440).

Discussion

Despite extensive studies on the structure, carbohydrate binding-site specificity and immunolocalization of galectin-1, the in vivo function of this lectin is unknown (17,18). Focusing on ß-galactosides, especially on galectin-1 as a constituent of oligosaccharide chains and the predominant members of the family of galectins in mammals, we addressed the question whether expression of galectin-1 and galectin-1 reactive glycoconjugates exhibits alterations in three morphologically defined fetal stages and in adult bovine pancreas. This report emphasizes the importance of combined monitoring of the galectin-1 and its possible in vivo ligands to eventually unravel organ-related functions of tissue lectin.

A number of investigators have performed biochemical and immunohistochemical analyses of animal and human tissues to detect of galectin-1. However, there is no information available on the level of presence of galectin-1 and galectin-1-reactive glycoconjugates, e.g. poly-N-acetyllactosamines and galectins, in development of bovine pancreas. In the present study, we examined the localization of galectin-1 and showed its expression in embryonal and adult pancreas by immunohistochemistry. We also confirmed the occurrence of galectin-1 on adult and fetal bovine pancreas by electrophoretic separation.

Wasano et al. have identified galectin-1 in rabbits only in smooth muscle layer of large vessels such as arteries and veins (19). Similar to the study reported by Wasano et al. we found that the 14.5 kDa galectin exists abundantly in the smooth muscle cells of blood vessels. Corpus glanduleae of pancreas also contains galectin-1. However, there was a slight difference between the stage F3 and the other two (F1, F2), namely the Corpus glanduleae of F3 stage were stained weakly, whereas the acinus cells of the other embryonal stages seemed to be free from staining. There was a significant difference in the staining patterns between the adult and embryonal stages. The acinar cells of adult pancreas were cytoplasmicly reactive, whereas none of the embryonal stages were reactive. Biotin-labeled galectin-1 exhibited no staining at adult and embryonal stages. Since the sample processing in histochemistry can affect ligand presentation we fixed the samples in other fixatives (4% para-formaldehyde, Bouin and Methanol/glacial acetic acid) and compared their effects. There was no significant difference in staining patterns among fixatives. For proper interpretation, however, it should be recalled that endogenous lectin in tissues may mask crucial binding sites. To establish a suitable technique to solve this problem, a tissue-proper type needs to be selected, where only the dissociation of lectin-carbohyrate complexes will give access to the tissue ligand.

According to our results, the expression of galectin-1 is developmentally regulated in the pancreas leading to a speculation that this protein may be important in the development of smooth muscle cells of blood vessels and the corpus glanduleae. Since almost all known glycoconjugates are extracellular molecules, it needs to be elucidated further why proteins supposed to interact with glycoconjugates are localized intracellularly.

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