Characterization of the binding specificity of *Anguilla anguilla* agglutinin (AAA) in comparison to *Ulex europaeus* agglutinin I (UEA-I)

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Using immunochemical and immunohistochemical methods, the binding site of *Anguilla anguilla* agglutinin (AAA) was characterized and compared with the related fucose-specific lectin from *Ulex europaeus* (UEA-I). In solid-phase enzyme-linked immunoassays, the two lectins recognized Fucα1-2Galβ3HSA. AAA additionally cross-reacted with neoglycolipids bearing lacto-N-fucopentaose (LNFP) I [H type 1] and II [Leα] and lactodifucotetraose (LDFT) as glycan moieties. UEA-I, on the other hand, bound to a LDFT-derived neoglycolipid but not to the other neoglycolipids tested. Binding of AAA to gastric mucin was competitively neutralized by Leα-specific monoclonal antibodies. UEA-I binding, on the other hand, was reduced after co-incubation with H type 2- and Leα-specific monoclonal antibodies. According to our results, AAA reacts with fucosylated type 1 chain antigens, whereas UEA-I binds only to the α1-2-fucosylated LDFT-derived neoglycolipid. In immunohistochemical studies, the reactivity of AAA and UEA-I in normal pyloric mucosa from individuals with known Lewis and secretor status was analysed. AAA showed a broad reaction in the superficial pyloric mucosa from secretors and non-secretors, but AAA reactivity was more pronounced in Le(a+b-) individuals. On the other hand, UEA-I stained the superficial pyloric mucosa only from secretor individuals. A staining of deep mucous glands by the lectins was found in all specimens. Both reacted with most human carcinomas of different origin. Slight differences in their binding pattern were observed and may be explained by the different fine-specificities of the lectins.

Keywords: lectin, *Anguilla anguilla* agglutinin, carbohydrate antigen, tumour-associated antigen

Introduction

Various ‘fucose-specific’ lectins derived from different sources are important reagents in glycobiology, histo- and cytochemistry [1–3]. In 1952, Watkins and Morgan demonstrated [4] that the agglutination of blood group O and A₂ erythrocytes exerted by a substance derived from the serum of *Anguilla anguilla* can be inhibited by α-methyl-L-fucopyranoside and L-fucose. Contrasting this finding, other monosaccharides, especially D- and L-glucose, D- and L-galactose and N-acetyl-D-galactosa-
2\text{Gal\beta}1-4\text{GlcNAc} (H type 2 blood group antigen) with a cross-reactivity to Fuc\text{\alpha}1-2\text{Gal\beta}1-4(\text{Fuc\text{\alpha}1-3})\text{GlcNAc\beta} (Lewis\text{\alpha} antigen), but not to internal \text{\alpha}-L-fucose units. LTA, on the other hand, is most strongly inhibited by Fuc\text{\alpha}1-6\text{GlcNAc}, followed by Fuc\text{\alpha}1-2\text{Gal\beta}1-4(\text{Fuc\text{\alpha}1-3})\text{GlcNAc\beta}. We recently observed striking differences in the cyto- and histochemical reaction to human megakaryocytes exerted by UEA-I on the one side and AAA and LTA on the other hand [15]. Similar discrepancies were also reported by Ito et al. in their study [16] on lectin binding to ABH antigens in other human tissues. Therefore, the purpose of this study was: (1) to characterize the binding specificity of AAA by immunochemical analysis in comparison to the well-investigated lectin UEA-I; and (2) to evaluate their immunohistochemical staining pattern in gastric tissues defined by secretor and Lewis status as well as carcinomas originating from various organs.

Materials and methods

Materials

Biotinylated *Anguilla anguilla* agglutinin (AAA) as well as biotinylated and unlabelled *Ulex europaeus* agglutinin 1 (UEA-I) were purchased from E-Y Laboratories (San Mateo, California, USA). Monoclonal antibodies (mabs) 12-4LE [17], 19-0LE (cross-reacting between H type 2 and Le\text{\alpha}) [18, 19], 7-LE (anti-Le\text{\alpha}) [19] and 121-SLE (anti-sialosyl-Le\text{\alpha}) were generated in the laboratory of J. Bara. Their specificities were extensively studied previously [20]. Mab Bw494 detecting a terminal Gal\text{\beta}1-3HexNAc unit and cross-reacting between Le\text{\alpha} and T/\beta antigen [21] was a kind gift from Behringwerke (Marburg, Germany). Monoclonal antibody LeuM1 (anti-Le\text{\alpha}) was from Becton-Dickinson (Heidelberg, Germany). Fuc\text{\alpha}1-2Gal\text{\beta}1-0(CONH)\text{\alpha}HSA was a kind gift from Dr Kolar (Behringwerke, Marburg, Germany). The following oligosaccharides derived from human milk were purchased from Oxford Glycosystems (Abingdon, Oxon, United Kingdom):

1. Fuc\text{\alpha}1-2Gal\text{\beta}1-3GlcNAc\beta1-3Gal\text{\beta}1-4Glc (LNFP I) [H type 1],
2. Gal\text{\beta}1-3(Fuc\text{\alpha}1-4)GlcNAc\beta1-3Gal\text{\beta}1-4Glc (LNFP II) [Le\text{\alpha}],
3. Fuc\text{\alpha}1-2Gal\text{\beta}1-3(Fuc\text{\alpha}1-4)GlcNAc\beta1-3Gal\text{\beta}1-4Glc (LNDFH I) [Le\text{\alpha}],
4. Gal\text{\beta}1-4(Fuc\text{\alpha}1-3)GlcNAc\beta1-3Gal\text{\beta}1-4Glc (LNFP III) [Le\text{\beta}],
5. Fuc\text{\alpha}1-2Gal\text{\beta}1-4(Fuc\text{\alpha}1-3)Glc (LDFT),
6. Gal\text{\beta}1-3GlcNAc\beta1-3Gal\text{\beta}1-4Glc (LNT).

Gastric juice was collected and pooled according to the secretor and Lewis phenotype of the donors. Mucus glycoproteins were isolated following previously published procedures [22] by extraction with 90% phenol.

Preparation of neoglycolipids

Neoglycolipids of LNFP I, LNFP II, LNFP III, LNDFH I and LDFT were prepared according to Stoll et al. [23] by coupling to dipalmitoylglyceroerythritolamine via reductive amination.

Immunohistochemical analysis

Antigens diluted in 0.1 M carbonate buffer, pH 9.6 (glycoproteins) or in 40% aqueous methanol (neoglycolipids) were coated to 96-well polystyrene microtitre plates (Nunc, Wiesbaden, Germany) at 37 °C overnight. After blocking of unspecific binding sites with PBS (20 mM phosphate, 0.15 M NaCl, pH 7.2) containing 5% bovine serum albumin (BSA, Sigma, Munich, Germany) for 1 h at 37 °C, biotinylated lectins were incubated for 2 h at room temperature. All washing steps and lectin dilutions (to 10 \mu g.mL\text{\textsuperscript{-1}}) were performed with PBS/0.5% BSA. Streptavidin-alkaline-phosphatase conjugate, diluted 1:5000, was incubated for 30 min at room temperature. The reaction was developed with p-nitrophenylphosphate (1 mg.mL\text{\textsuperscript{-1}}) in 50 mM diethanolamine buffer pH 9.8 containing 0.5 mM MgCl\text{\textsubscript{2}} (30 min, 22 °C).

Competition assays with mabs 12-4LE, 19-0LE, 7-LE, 121-LE, Bw494 and LeuM1 and unlabelled UEA-I were carried out on gastric mucins pooled from Le\text{\alpha}(\text{\textsuperscript{\textcircled{\textalpha}}}) individuals (25 \mu g.mL\text{\textsuperscript{-1}}) with AAA and UEA-I in a dilution of 10 \mu g.mL\text{\textsuperscript{-1}}. Mabs were used as undiluted cell culture supernatants (12-4LE, 19-0LE, 7-LE, 121-LE), at 50 \mu g.mL\text{\textsuperscript{-1}} (Bw494) or 1:10 (by vol) (LeuM1), respectively. Non-biotinylated UEA-I was employed at 50 \mu g.mL\text{\textsuperscript{-1}}. AAA and mabs were co-incubated for 1 h at 37 °C.

Immunohistochemical labelling

Human carcinoma tissues were derived from the files of the Institute of Pathology at the University of Cologne. Normal pyloric tissues were obtained from kidney donors with proven brain death and provided from the Centre de Recherches Paris Saint-Antoine. These specimens were fixed with 95% ethanol and defined with regard to their secretor and Lewis status [20]. Human tissues were fixed in 5% neutral-phosphate buffered formalin and embedded in paraffin. Five \mu m thick sections were cut and deparaffinized following standard histological techniques. After blocking of endogenous peroxidase using 1% H\text{\textsubscript{2}}O\text{\textsubscript{2}} in methanol for 30 min, slides were incubated with biotinylated lectins (100 \mu g per ml TBS per 2.5% BSA) for 1 h. Specimens were incubated with streptavidin-peroxidase complex P396 (Dakopatts, Copenhaghen, Denmark, diluted 1:400 in TBS per 2.5% BSA) for 1 h. Between all steps, which were performed at room temperature, the slides were threefold washed in TBS. The reaction was developed by 200 \mu g.mL\text{\textsuperscript{-1}} (w/v) 3- amino-9-ethyl-carbazole (Sigma, Munich, Germany) in...