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Detection of glycosylated sites in embryonic rabbit kidney by lectin chemistry

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Abstract The reciprocal cell biological interaction between mesenchymal and epithelial tissue plays a critical role during nephrogenesis. It is unknown to date whether the tissues interact during nephron induction by pure diffusion of substances or whether cellular contacts via gap junctions or focal adhesion molecules are involved. In neonatal rabbit kidney the interface between both tissues shows unique features. It consists of a distinct space, which is filled with specific extracellular matrix consisting of glycosylated proteins such as fibronectin, laminin, collagen, and proteoglycans. In the present experiments we tested by histochemistry whether it is possible to detect additional glycosylated proteins using Soybean agglutinin (SBA), *Dolichos biflorus* agglutinin (DBA), *Ulex europaeus* I agglutinin (UEA I), and Peanut agglutinin (PNA) as molecular markers. All tested lectins showed distinct labeling patterns in embryonic renal tissue. Within the collecting duct ampulla, DBA and UEA I revealed intensive cellular reaction. In contrast, PNA and SBA reacted at the basal aspect of the collecting duct ampulla tip in addition to a cellular reaction. To identify the individual molecules labeled by the lectins, embryonic tissue was fractionated and separated by electrophoretic methods. For the first time, we were able to show by two-dimensional electrophoresis and subsequent western blot experiments that lectins bind to a series of individual protein spots, which have not been identified to date.

Keywords Kidney · Development · Lectins · Histochemistry · Two-dimensional electrophoresis

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Introduction

The tissue interface between the basal aspect of the collecting duct (CD) ampulla and the surrounding mesenchyme is most important for the molecular transduction process during nephron induction. During kidney growth the CD ampullae of the arborizing CD are surrounded by nephrogenic mesenchymal (Saxen 1987) and endothelial (Kloth et al. 1992) cells. As the organ increases in volume the CD ampullae maintain a constant distance to the overlaying organ capsule. Correct geometric positioning is important for the following dichotomous CD branchings that guide successive nephron induction. When the exact positioning of the branching point is impaired, it will cause malformation during further kidney development (Woolf and Winyard 1998). One would expect that the CD ampulla and the nephrogenic mesenchyme are found in close cellular vicinity. However, the light microscopic views of human (Woolf and Winyard 1998), rabbit (Strehl and Minuth 2001), rat (Plisov et al. 2001), and mouse (Saxen 1987) kidney reveal that there is no close tissue contact between the CD ampulla and the surrounding mesenchyme. The epithelial–mesenchymal interface appears as a remarkably broad space, where close cell contacts do not seem to occur.

Electron microscopy of the embryonic cortex in neonatal rabbit kidney shows that the epithelial–mesenchymal interface contains an individual extracellular matrix. It is most prominently developed in the CD ampullar tip region, diminishes in the neck–shaft region, and is completely absent in the more matured CD (Strehl et al. 1999). This specific matrix forms an individual compartment at the interface between the CD ampulla and the surrounding mesenchyme.

Known extracellular matrix components between the CD ampulla and the surrounding mesenchyme are fibronectin (Mounier et al. 1986), tenascin (Aufderheide et al. 1987), glycoproteins (Brandenberger et al. 2001; Bullock et al. 2001; Denda et al. 1998; Durbeej et al. 1996; Korhonen et al. 1990; Kreidberg et al. 1996), proteoglycans (Kispert et al. 1996; Pellegrini et al. 1998; Vainio et

al. 1989), and glycosaminoglycans (Pohl et al. 2000). Matrix metalloproteinases such as MMP 2 and MMP 9 are involved in the degradation and maintenance of the matrix (Legallicier et al. 2001).

A common molecular feature of extracellular matrix proteins is their glycosylation status. It is known that lectins bind to glycosylated molecules and recognize structures in the embryonic kidney (Holthöfer 1987, 1988; Laitinen et al 1987; Ojeda et al. 1993). A profound morphological, histochemical, and biochemical analysis of the epithelial–mesenchymal interface, however, has not been performed to date. In order to find glycosylation sites in this specific area, we analyzed in the present experiments the binding profiles of Soybean agglutinin (SBA), *Dolichos biflorus* agglutinin (DBA), *Ulex europaeus* I (UEA I), and Peanut agglutinin (PNA) in neonatal rabbit kidney by histochemical and biochemical methods. The data revealed that the four lectins bind to different regions of the investigated tissue area. For the first time it could be shown by two-dimensional (2-D) electrophoresis with subsequent western blot that lectins bind to several molecules, which are present in nephrogenic tissue. The experiments suggested that the lectin-positive molecules belong to a group of glycosaminoglycans.

Materials and methods

Tissue preparation

For histochemistry 1-day-old New Zealand rabbits were anesthetized with ether and killed by cervical dislocation. Both kidneys were removed immediately and frozen in liquid nitrogen.

Light microscopic techniques

Corticomedullary orientated cryosections (8 µm) of neonatal rabbit kidneys were performed with a cryomicrotome (Microm, Heidelberg, Germany). After fixation of the sections in ice-cold ethanol washing steps in phosphate-buffered saline (PBS) followed. The sections were then incubated in blocking solution (PBS + 1% bovine serum albumin + 10% horse serum) for 30 min. To visualize the glycosylation pattern fluorescein isothiocyanate-conjugated lectins (all obtained from Vector Laboratories, Burlingame, Calif., USA) were applied for 60 min in blocking solution 1:2,000. Soybean agglutinin preferentially binds to oligosaccharide structures with terminal α- or β-linked *N*-acetylgalactosamine and to a lesser extent to galactose residues. *Dolichos biflorus* agglutinin reveals carbohydrate specificity toward α-linked *N*-acetylgalactosamine. Peanut agglutinin binds preferentially to galactosyl (β-1,3) *N*-acetylgalactosamine and UEA I to α-linked fucose residues. Following several washes in PBS the specimens were embedded with Slow Fade Light Antifade kit (Molecular Probes, Eugene, Ore., USA) and analyzed using an Axiovert 35 microscope (Zeiss, Oberkochen, Germany).

Co-incubation experiments

Co-incubation experiments were performed to correlate the distribution of lectin-binding sites with immunological antigen detection in the CD ampulla by mab anti-P_{CD}Amp 1 (Strehl et al. 1997), mab anti-osteopontin (Rogers et al. 1997; obtained from the Developmental Studies Hybridoma Bank, Iowa, USA), and mab anti-cytokeratin 19 (kindly provided by Prof. Dr. R. Moll, Marburg, Germany). Fixation of tissue and blocking of unspecific binding sites were done according to the above-described protocol. Sections were then incubated with the antibodies (diluted 1:10) and after washing steps a donkey anti-mouse IgG Texas red-conjugated secondary antibody (Jackson Immunoresearch Laboratories, West Grove, Pa., USA; diluted 1:150) was applied together with the lectins. As controls labeling experiments with either lectin or antibody alone were performed to elucidate a possible interference on binding sites.

Isolation of the embryonic kidney zone

For biochemical analysis cortical explants from kidneys of newborn New Zealand rabbits were isolated according to methods described earlier (Minuth 1987). In brief, after removing the kidneys both poles were cut off. From the remaining piece of tissue the capsule was stripped off using forceps. The resulting explants consisted of the fibrous organ capsule with adherent CD ampulla, S-shaped bodies, and nephrogenic mesenchyme.

SDS-PAGE with subsequent western blot analysis

Embryonic cortical renal explants were homogenized in sample buffer containing 2% SDS, 10% glycerin, 125 mM TRIS-HCl, and 1 mM EDTA (all obtained from Sigma-Aldrich-Chemie, Deisenhofen, Germany), and centrifuged at 10,000 *g* for 10 min. The supernatants were used in the following experiments. The amount of protein was determined by a protein microassay (BioRad Laboratories, Hercules, Calif., USA). Twenty-gram protein samples were separated first by SDS-PAGE in 10% Laemmli minigels according to methods described earlier (Laemmli 1970), which were then electrophoretically transferred to P-Immobilon membranes (Millipore, Eschborn, Germany; Pluskal et al. 1986). In order to detect glycosylated proteins, the blots were first equilibrated for 1 h (PBS, pH 7.2, 0.05% Tween; Sigma, 10% horse serum; Boehringer, Mannheim, Germany) following an incubation with lectins (DBA, PNA, SBA, and UEA I) diluted 1:2,000 overnight at 4°C. After several washing steps the membranes were incubated with antibodies to the lectins (anti-DBA, anti-PNA, anti-SBA, anti-UEA I; Vector Laboratories) diluted 1:1,000 in PBS-Tween for 1 h. A horseradish peroxidase-conjugated donkey anti-goat immunoglobulin antiserum (1:1,000; Dianova, Hamburg, Germany) was applied for 45 min and served as detection marker for the antibody–lectin complex. Blot development was started by addition of 0.5 mg/ml diaminobenzidine, 0.02% H_20_2, and 0.03% cobalt chloride dissolved in citrate buffer (17 mM), pH 6.3. The reaction was stopped by washing the membrane in tap water. Immunoblots were documented with a Scan Jet 6200 C scanner (Hewlett Packard, Greely, Colo., USA). Determination of apparent molecular weight was performed in conjunction with a broad range molecular weight protein standard (BioRad Laboratories).

Two-dimensional electrophoresis with subsequent western blot analysis

To separate lectin binding proteins by 2-D electrophoresis embryonic cortical renal explants were homogenized in lysis buffer (pH 7.5) containing 8 M urea, 4% CHAPS, 40 mM TRIS-HCL, 2 mM DTT, and 0.5% carrier ampholytes. Solubilized protein samples, 100 µg, were loaded on a gel tube for isoelectric focusing. The gels were run for 14 h using increasing voltage (100–1,000 V) in a model 175 Tube Cell (BioRad Laboratories) according to described methods (O'Farrel 1975). After a 15-min equilibration in 2% SDS, 10% glycerin, 125 mM TRIS-HCL, and 1 mM EDTA, the focused gel tubes were laid on the surface of 3-mm-thick 10% Laemmli gels. Proteins were separated by SDS-PAGE at 100 V and 120 mA for 7 h (O'Farrel 1975). To detect lectin binding sites the gel plates were equilibrated in a borate acid buffer. Proteins were electrophoretically transferred to a P-Immobilon membrane (Pluskal et al. 1986). Finally the membranes were incubated with the lectins (1:2,000) and then with anti-lectin antibodies (1:1,000). A horseradish peroxidase-conjugated donkey

anti-goat antibody (1:1,000) was used for visualization of the antibody–lectin complex. To determine the molecular weights and isoelectric points of the 2-D separated proteins 2-D SDS-PAGE standards (BioRad Laboratories) were separated in parallel to the individual experiments.

Specificity of lectin binding

Specificity of lectin binding was tested on immunoblot membranes by incubating in PBS-Tween containing 1 M *N*-acetylgalactosamine for DBA and SBA, 1 M galactose for PNA, and 1 M L-fucose for UEA I. Lectin incubation without or with inhibiting carbohydrates was performed in parallel after SDS-PAGE and 2-D electrophoresis with following western blot. An identical procedure was performed to test the specificity of lectin binding on cryostat sections.

Number of experiments

For the present experimental series 22 kidneys of neonatal rabbits were examined by light microscopic analysis. Fifty-seven renal explants were isolated and investigated by electrophoretic techniques.

Results

Histochemistry

A cryosection through the cortex of neonatal rabbit kidney shows the tip of the CD ampulla surrounded by nephrogenic mesenchyme, while on the lateral side S-shaped bodies at early stages of nephron development are found (Fig. 1a). The cells of the CD ampulla can be identified by immunohistochemistry. They display a distinct reaction for cytokeratin 19 (Fig. 1b). The basal aspect of the CD ampulla is visualized with antibodies reacting with P_{CD} Amp1 (Strehl et al. 1999; Fig. 1c) and osteopontin (Denda et al. 1998; Fig. 1d). The label of the last two antigens is most prominent at the ampulla tip and is continuously diminished towards the neck and shaft region.

The ampulla tip is the site where nephron induction takes place by exchange of morphogenic signals between the basal aspect of the CD epithelium and the surrounding mesenchyme. The prominent expression of P_{CD} Amp1 (Fig. 1c) and osteopontin (Fig. 1d) at this site indicates a peculiar composition of the tissue. To test whether a specific glycosylation pattern is present in this region incubation with SBA, DBA, UEA I, and PNA was performed (Fig. 2).

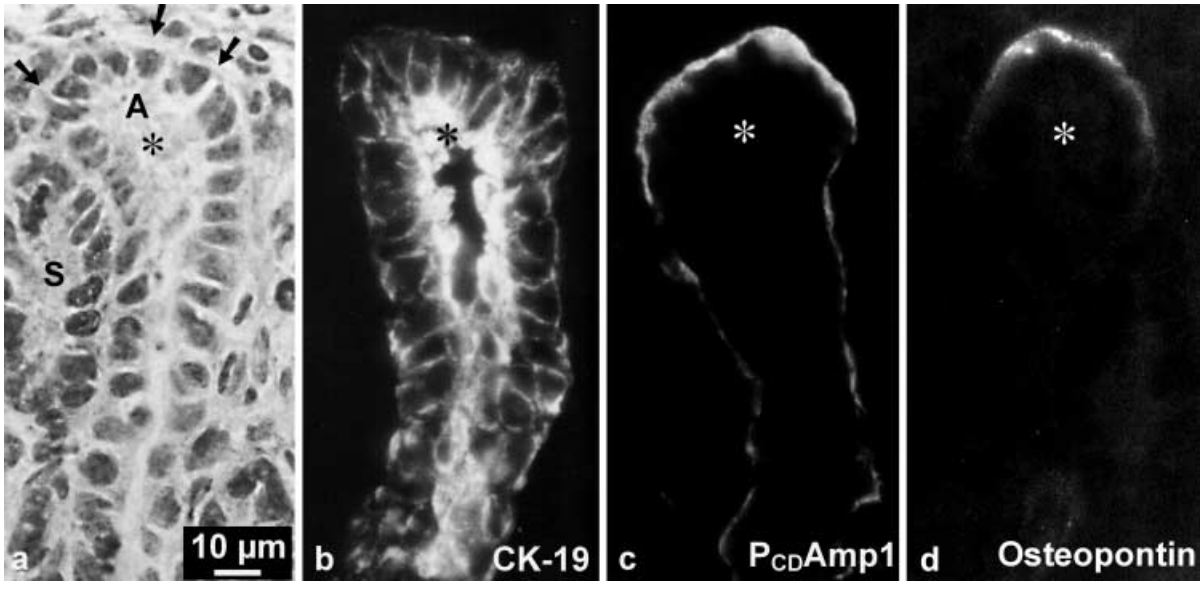


Fig. 1a–d Light microscopic identification of the interface between the nephron inducer and the surrounding mesenchyme. **a** Toluidine blue-stained longitudinal cryosection through the cortex of neonatal rabbit kidney. *Arrows* indicate the wide space between the basal aspect of collecting duct ampulla and surrounding mesenchyme. **b** Immunohistochemistry reveals that all ampulla cells are positive for cytokeratin 19 (*CK-19*). **c**, **d** P_{CD} Amp1 (**c**) and osteopontin (**d**) are expressed at the basal aspect of the ampulla tip. *A* Ampulla, *S* S-shaped body. *Asterisks* show the lumen of the ampulla

Tissue binding sites

All four probed lectins reacted within the CD ampulla of the embryonic zone in neonatal rabbit kidney. SBA (Fig. 2a), DBA (Fig. 2b), and UEA I (Fig. 2c) showed exclusive binding with the CD ampulla, while the metanephrogenic mesenchyme, S-shaped bodies, and developing vessels were negative. In contrast, PNA (Fig. 2d) labeled the CD ampulla and neighboring S-shaped bodies.

Cellular binding sites

Within the ampulla all of the lectins showed a heterogeneous labeling. Soybean agglutinin (Fig. 2a) and PNA (Fig. 2d) revealed binding at the ampulla tip, where no reaction was seen with DBA (Fig. 2b) and UEA I (Fig. 2c).

Table 1 Soybean agglutinin (*SBA*), *Dolichos biflorus* agglutinin (*DBA*), *Ulex europaeus* I agglutinin (*UEA I*), and peanut agglutinin (*PNA*) binding along the maturing collecting duct (CD) in the neonatal rabbit kidney. + Binding sites. (*B* Basal aspect of CD cell, *C* cytoplasmic cell side, *L* luminal cell side, *CCD* cortical collecting duct, *MCD* medullary collecting duct)

	SBA			DBA			UEA I			PNA		
	B	C	L	B	C	L	B	C	L	B	C	L
Tip	+									+		
Neck–shaft	+	+	+		+	+		+	+	+		+
CCD	+	+	+		+	+		+				+[a]
MCD	+	+			+							

[a] Binding for PNA only on matured B-type intercalated cells

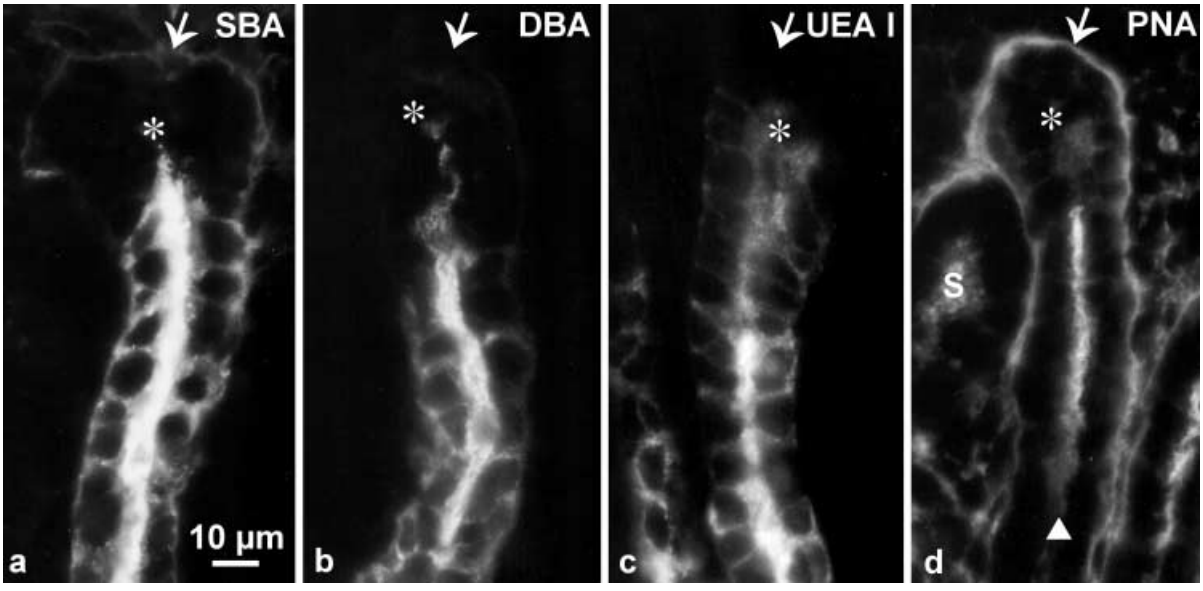


Fig. 2a–d Histochemistry of lectin binding in the embryonic cortex of neonatal rabbit kidney. **a** Soybean agglutinin (*SBA*) binds to the basal aspect of collecting duct ampulla tip (*arrow*). Ampulla neck–shaft region reveals luminal and cytoplasmic binding. **b**, **c** *Dolichos biflorus* agglutinin (*DBA*; **b**) and *Ulex europaeus agglutinin* I (*UEA I*; **c**) show luminal and cytoplasmic binding in the neck–shaft region. **d** Peanut agglutinin (*PNA*) reacts with the basal aspect of the ampulla (*arrow*) and the S-shaped body. *Triangle* indicates the loss of luminal PNA binding. *Asterisks* show the lumen of the ampulla. *Arrows* indicate the basal aspect of the ampulla

Labeling with SBA, DBA, UEA I, and PNA appeared most prominent at the luminal and lateral surface of cells within the ampulla neck region. Most interesting, at the transition between the ampulla neck and the matured CD, PNA binding disappeared abruptly (Fig. 2d *triangle*). The distribution of lectin binding in the different portions of maturing CD is summarized in Table 1.

Extracellular binding sites

Soybean agglutinin (Fig. 2a *arrow*) and PNA (Fig. 2d *arrow*) binding was associated with the basal aspect of the CD ampulla tip. In contrast, binding of DBA (Fig. 2b *arrow*) and UEA I (Fig. 2c *arrow*) was not detectable at this site.

In addition, at higher magnification label of SBA could be observed as a reaction with reticular structures at the interface between the ampulla and the nephrogenic mesenchyme. Thin fibers protrude form the basal aspect of the ampulla towards the organ capsule (Figs. 2a *arrow*, 3). PNA (Fig. 2d) also labels the basal aspect of the ampulla, but does not reveal a network of fibers as observed after SBA (Figs. 2a, 3) labeling.

Specificity of lectin binding

To evaluate specific or unspecific labeling of lectins, incubations were performed in the presence of competitive carbohydrates. Soybean agglutinin and DBA were incubated in a solution containing 1 M *N*-acetylgalactosamine, UEA I in a solution containing 1 M L-fucose, and PNA was incubated in a solution containing 1 M galactose. Binding of all four lectins on tissue samples could be completely eliminated by the addition of carbohydrates during lectin histochemistry (no figure), which indicates specificity of binding.

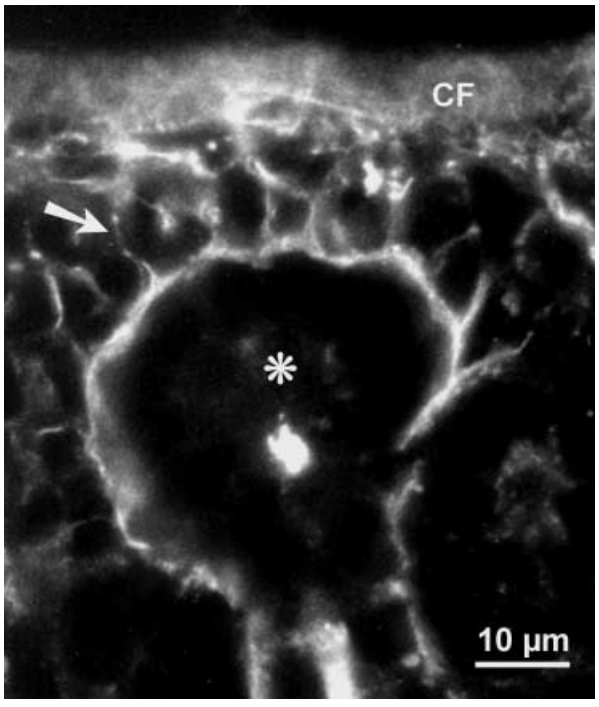


Fig. 3 Soybean agglutinin labeling of the collecting duct ampulla tip. Micrograph shows the SBA binding pattern in the region between the ampulla, surrounding mesenchyme, and organ capsule. Abundant SBA binding is present at the basal aspect of the ampulla. In addition, thin fibers are labeled, which originate from the ampulla and extend through the mesenchyme toward the organ capsule. *Asterisk* in the lumen of the ampulla tip. *CF* Capsule fibrosa. *Arrow* indicates a positive fiber

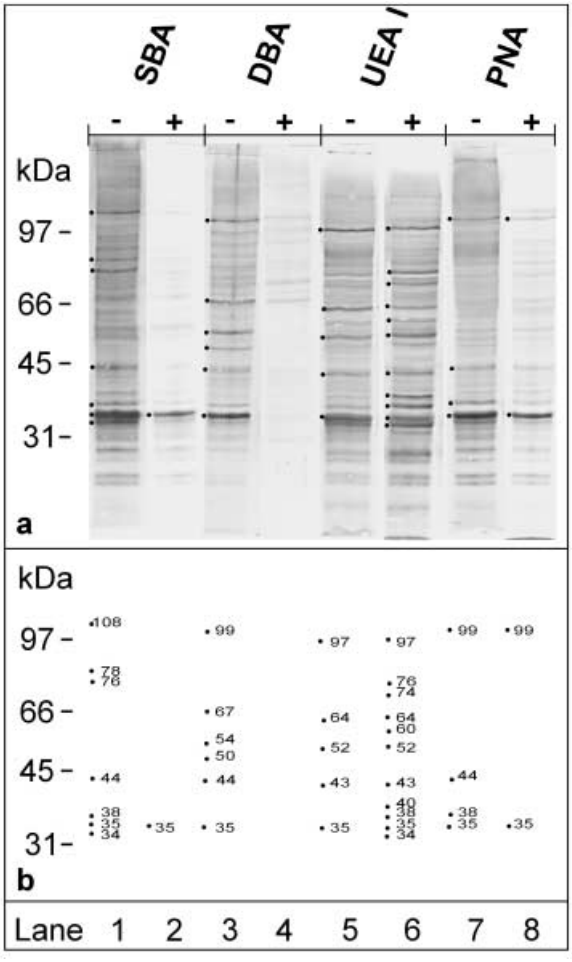


Fig. 4 **a** SDS-PAGE with lectin western blot of isolated embryonic kidney tissue. All probed lectins react with several protein bands (*lanes 1, 3, 5, 7*). *Minus symbol* indicates lectin incubation without inhibiting carbohydrate (*lanes 1, 3, 5, 7*) and *plus symbol* indicates lectin incubation with specific inhibiting carbohydrate (*lanes 2, 4, 6, 8*). **b** Determined molecular sizes of detected bands in kilodaltons

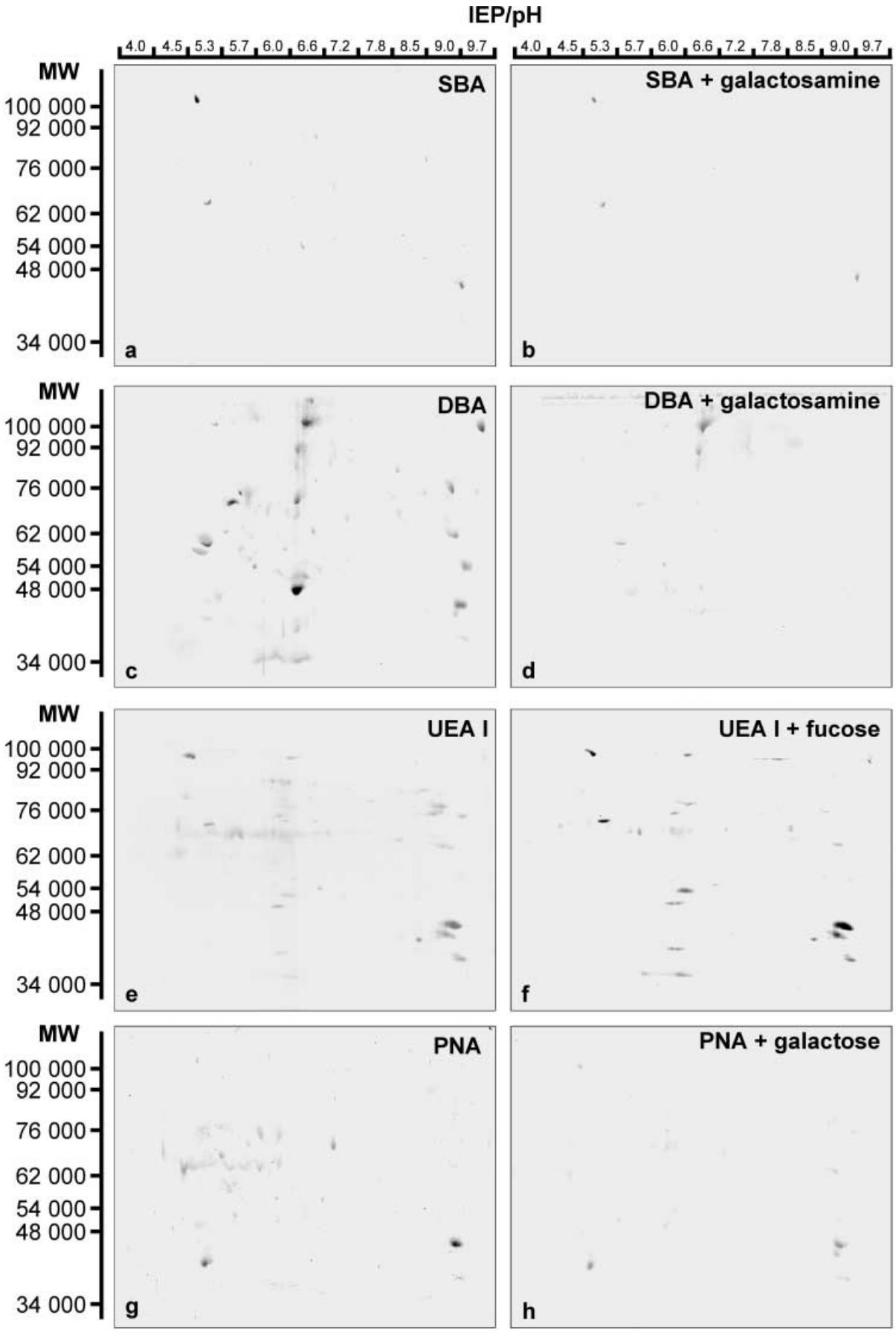
SDS-PAGE and subsequent lectin western blot

To elucidate the binding of lectins to proteins, the embryonic cortex of neonatal rabbit kidney was isolated, fractionated and separated by SDS-PAGE. Lectins were probed on blotted nitrocellulose (Fig. 4). All tested lectins reacted with more than one protein band. Soybean agglutinin revealed binding to 34, 35, 38, 44, 76, 78, and 108 kDa bands (Fig. 4a, b *lane 1*). As compared to SBA, DBA recognized a different pattern with bands related to 35, 44, 50, 54, 67, and 99 kDa proteins (Fig. 4a, b *lane 3*). In contrast, UEA I reacted with bands of 35, 43, 52, 64, and 97 kDa (Fig. 4a, b *lane 5*). Peanut agglutinin yielded bands of 35, 38, 44, and 99 kDa (Fig. 4a, b *lane 7*). Thus, the major protein band with 35 kDa was detected by all of the lectins tested (Fig. 4a, b *lanes 1, 3, 5, 7*).

To determine the specificity of binding, incubation of lectins in the presence of inhibiting carbohydrates was performed (Fig. 4a, b *lanes 2, 4, 6, 8*). Inhibition experiments with 1 M *N*-acetylgalactosamine for SBA (Fig. 4a, b *lane 2*) and DBA (Fig. 4a, b *lane 4*) and 1 M galactose for PNA (Fig. 4a, b *lane 8*) demonstrated a strong reduction but not complete elimination of lectin binding. SBA binding was completely eliminated in the presence of inhibition carbohydrate except remaining binding to a 35-kDa band (Fig. 4a, b *lane 2*). This result indicates specific binding to all labeled proteins (*lane 1*) except to the 35-kDa band. Complete elimination of all labeled bands was obtained for DBA in the presence of the inhibition carbohydrate (Fig. 4a, b *lane 4*). Unspecific binding was observed when UEA I was incubated in the presence of 1 M L-fucose (Fig. 4a, b *lane 6*). Peanut agglutinin labeling in the presence of inhibiting carbohydrate showed specific binding of proteins except for a 99- and a 35-kDa band (Fig. 4a, b *lane 8*). Thus, highest specificity for lectin binding investigated by one-dimensional electrophoresis and western blot was observed for DBA and to a lesser extent for SBA and PNA.

Two-dimensional electrophoresis and subsequent lectin western blot

Two-dimensional electrophoresis was performed in order to elaborate the molecular features of proteins which are


IEP/pH
4.0 4.5 5.3 5.7 6.0 6.6 7.2 7.8 8.5 9.0 9.7
MW
100 000
92 000
76 000
62 000
54 000
48 000
34 000
SBA
a
SBA + galactosamine
b
DBA
c
DBA + galactosamine
d
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PNA + galactose
h

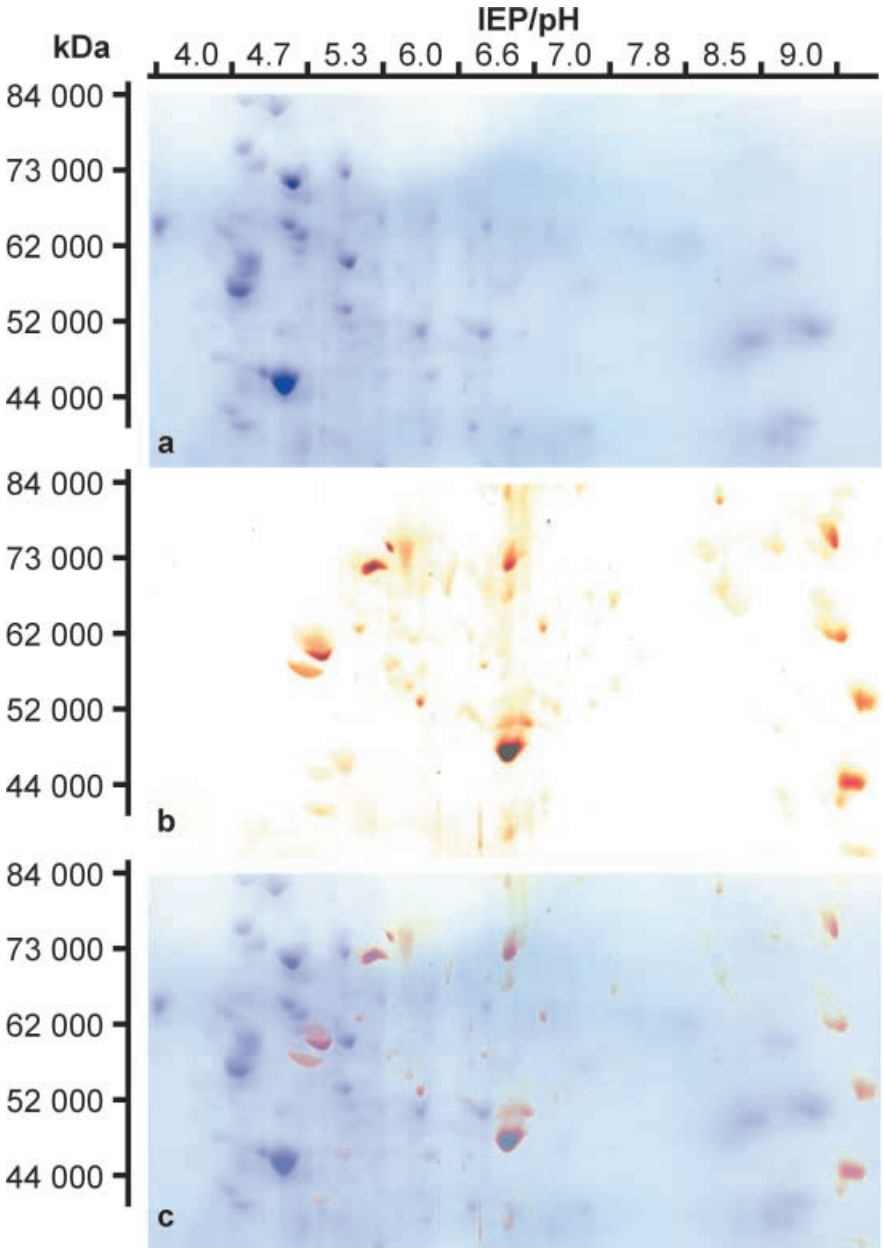

Fig. 6 **a** Two hundred micrograms protein of embryonic tissue was separated by 2-D electrophoresis. The gel was stained by Coomassie blue. **b** Detail of DBA western blot after 2-D electrophoresis of the same protein sample. **c** Images **a** and **b** were merged to identify lectin-positive spots in a Coomassie blue-stained gel. None of the lectin-labeled spots matched with the Coomassie blue-stained spots

bound by lectins (Fig. 5). In the first dimension, protein extracts were separated by their isoelectric point while separation in the second dimension occurred by their molecular size. Subsequently, the 2-D gels were blotted and probed with SBA (Fig. 5a), DBA (Fig. 5c), UEA I (Fig. 5e), and PNA (Fig. 5g). The blots revealed an individual and broad spectrum of labeled proteins. One common spot of 45 kDa and pI 9.5 was positive for all tested lectins. All the other detected spots yielded different molecular sizes and isoelectric points as illustrated in Table 2. Soybean agglutinin (Fig. 5a) reacted with 3 spots, DBA showed 12 positive spots (Fig. 5c), UEA I labeled 9 spots(Fig. 5e), and PNA (Fig. 5g) bound to 4 spots.

To determine the specificity of lectin binding on 2-D western blots, inhibiting carbohydrates were applied in addition to the lectins (Fig. 5b, d, f, h). Soybean agglutinin showed the same binding pattern in the presence of inhibiting galactosamine (Fig. 5b) as found without carbohydrate (Fig. 5a). Consequently, this results showed unspecific labeling of all SBA-positive spots. *Dolichos biflorus* agglutinin incubation together with galactosamine (Fig. 5d) demonstrated a significant change of binding as compared to DBA applied alone (Fig. 5c).

◀ **Fig. 5a–h** Two-dimensional electrophoresis and lectin western blot of renal embryonic tissue. Lectin incubation revealed that SBA (**a**), DBA (**c**), UEA I (**e**), and PNA (**g**) bind to different protein spots (see also Table 2). Only one common spot of 45 kDa and pI 9.5 is detected by all probed lectins. **b**, **d**, **f**, **h** The blot membrane was incubated with SBA together with the related inhibiting carbohydrate galactosamine (**b**), DBA incubation together with galactosamine (**d**), UEA I incubation together with fucose (**f**), and PNA incubation together with galactose (**h**). *IEP* Isoelectric point

Table 2 Apparent molecular weights (*kDa*) and isoelectric points (*pI*) of spots detected with SBA, DBA, UEA I, and PNA in two-dimensional electrophoresis and subsequent western blot experiments

SBA		DBA		UEA I		PNA	
kDa	pI	kDa	pI	kDa	pI	kDa	pI
45	9.5	45	9.5	37	9.6	40	5.3
63	5.5	48	6.7	41	9.1	45	9.5
102	5.2	54	9.7	42	9.3	64	5.0
		57	5.2	45	9.5	72	7.5
		60	5.3	50	6.9		
		62	9.4	74	5.6		
		72	5.7	75	9.7		
		72	6.7	78	9.4		
		76	9.4	99	5.5		
		92	6.8				
		100	9.9				
		102	7.0				

Complete inhibition of binding to 10 different spots could be documented. Only 2 spots (92 kDa, pI 6.8; 102 kDa, pI 7.0) remained unchanged during the inhibition experiments. Surprisingly, UEA I incubation with fucose (Fig. 5f) showed a unexplainable increase of binding intensity as compared to UEA I label obtained without fucose (Fig. 5e). Only two spots (75 kDa, pI 9.7; 78 kDa, pI 9.4) disappeared during the inhibition experiments. Peanut agglutinin incubation in the presence of galactose (Fig. 5h) leads to a reduced binding to only one spot (72 kDa, pI 7.5) as compared to PNA label obtained without galactose (Fig. 5g).

The most impressive specificity of lectin label was shown for DBA (Figs. 5c, d, 6b) as compared to the other probed lectins (Fig. 5). However, staining of the 2-D gel (Fig. 6a) revealed that the DBA-labeled spots (Fig. 6b) do not merge with Coomassie-positive spots (Fig. 6c). This results can not be explained yet but may be due to the low amount of sample protein or to the low content of amino acids within the molecules detected by the lectins.

Discussion

The CD ampulla induces the formation of all nephrons. During this process the cells within the ampulla tip interact with the surrounding nephrogenic mesenchyme. As a consequence some of the mesenchymal cells condense, polarize, and form the comma-shaped body as a first visible sign of nephron development. It is assumed that the nephron induction process requires close contact between the ampulla tip and the surrounding mesenchyme. However, light microscopy revealed a large gap between the epithelial and mesenchymal cells (Fig. 1a). As shown in earlier investigations by scanning and transmission electron microscopy the epithelial–mesenchymal interface is filled with a specific extracellular matrix consisting of a dense fiber network (Strehl et al. 1999). Proteins such as P_{CD} Amp1 (Strehl and Minuth 2001; Fig. 1c) and osteopontin (Denda et al. 1998; Fig. 1d) are found to be localized on this highly specific extracellular matrix surrounding the ampulla tip and facing the nephrogenic mesenchyme.

It has been shown that the extracellular matrix in kidney contains extracellular matrix proteins such as fibronectin (Mounier et al. 1986), tenascin (Aufderheide et al. 1987), and a high degree of glycosylation sites (Hay 1981). In order to investigate whether a specific glycosylation pattern is present at the epithelial–mesenchymal interface in embryonic kidney, we labeled cryosections (Fig. 2) and separated protein samples (Figs. 4, 5) with conjugated lectins. It was found that only SBA (Figs. 2a, 3) and PNA (Fig. 2d) showed a distinct label at the basal aspect of the CD ampulla, while DBA (Fig. 2b) and UEA I (Fig. 2c) revealed no reaction. In contrast, downward from the ampulla neck toward the shaft and the cortical CD, SBA (Fig. 2a), DBA (Fig. 2b), and UEA I (Fig. 2c) showed an intensive cytoplasmic and luminal cell label. Peanut agglutinin (Fig. 2d) demonstrated also an intensive luminal, but no cytoplasmic label. Taken together, histochemistry with diverse lectins displayed different cellular binding sites on the CD ampulla (Fig. 2). In the surrounding nephrogenic mesenchyme, however, cellular binding sites were absent.

Fibers between the basal aspect of the CD ampulla, the surrounding mesenchymal cells, and the organ capsule reacted with SBA (Figs. 2a, 3). Lectin histochemistry shows that the detected fibers occur at the interface, where nephron induction takes place. Whether these structures are extracellular matrix fibers or cellular protrusions has not been elaborated yet. We assume that the components of the extracellular matrix at this particular site could regulate the release of signaling molecules such as sonic hedgehog (Burrow 2000), BMP-7 (Vukicevic et al. 1996), Wnt-11 (Kispert et al. 1996), BF-2 (Hatini et al. 1996), Pax-2 (Torres et al. 1995), and WT1 (Kreidberg et al. 1993) secreted by both tissues during kidney development. On the other hand, the fibers could serve as a structural bridge connecting the CD ampulla and the nephrogenic mesenchyme.

In order to obtain information on molecules labeled by the different lectins within the embryonic zone of the kidney, we performed 2-D electrophoresis and western blot experiments with isolated embryonic tissue. These experiments showed numerous labeled protein bands (Fig. 4), which reflect the various tissue binding sites obtained by lectin histochemistry (Fig. 2). In contrast to light microscopic observations, co-incubation of the lectins with competitive carbohydrates during western blot experiments showed the highest degree of specific binding for DBA (Fig. 4 *lanes 3, 4*) and to a minor degree for SBA (Fig. 4 *lanes 1, 2*) and PNA (Fig. 4 *lanes 7, 8*). Since on cryosections the binding of lectins could be completely reduced by competitive carbohydrates, the result on western blots yielded a discrepancy between the morphological and biochemical situation. This may be caused by alterations of the molecules occurring during biochemical isolation, so that the original three-dimensional structure is impaired and unspecific binding sites appear.

The amino acids sequence of the detected spots is to date unknown. According to the molecular weight at least P_{CD} Amp1 (Strehl and Minuth 2001) and osteopontin (Denda et al. 1998) can be excluded. In the next experimental steps MALDI mass spectrometry analysis will reveal which kind of proteins are recognized by the different lectins shown for the first time in 2-D electrophoresis. It is most probable that the lectin-labeled molecules are not identical with laminin (Durbeej et al. 1996), collagens (Mounier et al. 1986), and other glycoproteins (Brandenberger et al. 2001; Bullock et al. 2001). The fact that the detected spots were not stained by Coomassie blue (Fig. 6) points out a low protein and a high carbohydrate proportion as it has been shown for proteoglycans (Kispert et al. 1996; Pellegrini et al. 1998; Vainio et al. 1989).

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