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The cytoskeletal protein villin as a parameter for early detection of tubular damage in the human kidney

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ABSTRACT

Villin is a cytoskeletal protein of brush borders in the kidney and gut. After renal tubular cell injury the brushborder fragments are shedded into the tubular lumen and excreted with urine indicating renal tubular damage (so called "renal antigen" shedding). In urine villin appears as intact molecule (95 000 dalton) and as fragment with 70 000, 45 000 and 22 000 dalton. The major villin fragment (70 000 dalton) was purified after ammonium sulphate precipitation from urine of human renal transplant recipients. Final purification of the villin 70 000 dalton fragment was achieved by gel filtration with TSK 3000 SWG preparative grade. Purification was varified by sodium dodecyl sulphate–polyacrylamide gel eletrophoresis and western blotting.

INTRODUCTION

Kidney damage often leads to renal failure with the consecutive hazard of kidney replacement therapy [1,2]. As kidney failure at early onset does not demonstrate early subjective recognizable warning signs, it is important to develop markers of renal tubular and glomerular function that are able to detect early on any dysfunction with a view to preventing renal damage. A new approach in this direction was the determination of "renal antigens" in urine. The idea was that any cell damage will be followed by a shedding of tubular fragments into urine, which can be detected by enzyme immunoassay [3,4].

Villin is a cytoskeletal protein of brush borders with a molecular weight of 95 000 dalton as the intact molecule [5]. It is linked to actin and stabilizes the actin filaments that anchor the individual microvilli [5–8]. This is its role under normal Ca^{2+} concentrations of $< 10^{-7}$ *M*. However, in conditions of high Ca^{2+} concentrations of $> 10^{-6}$ *M*, which are present during cellular damage such as hypoxia or direct toxic agents, villin acts as an F-actin severing protein [9]. This causes a release of brush-border bundles which are shed into the lumen of the tubule and excreted with urine [3]. The appearance of villin in the urine is therefore an indicator of renal tubular damage. In urine villin appears as the intact protein and also as fragments of 70 000, 45 000 and 22 000 dalton. The most abundant fragment is the 70 000-dalton fragment. Brush borders in the kidney are present in the proximal tubule only. Shedding of villin would therefore indicate damage to the proximal tubular cells.

In order to develop an assay to detect human villin in urine, we stratified our approach as follows: (1) development of a polyclonal antibody against villin, (2) development of a monoclonal antibody against villin and (3) purification of human villin 70 000-dalton fragment.

The production of antibodies has partially been reported elsewhere [3]. In this paper we focus on the purification of a 70 000-dalton villin fragment from urine of patients with routine techniques.

EXPERIMENTAL

Fast protein liquid chromatography (FPLC)

For gel filtration under low-pressure conditions (FPLC), an HPLC pump and controller (LKB–Pharmacia, Freiburg, Germany) was used. The detection of the effluent was performed with a monochromatic UV detector at 280 nm and recorded on strip-chart recorder or computer (Shimadzu CR3-A). The columns used were TSK 3000 SWG (Pharmacia LKB) and Superdex 200 16/60 (Pharmacia–LKB). The buffer used was 20 m*M* Tris HCl (pH with 8 *M* urea.

Flow conditions

The typical flow-rate was between 0.5 and 1.0 ml/min in all instances. The effluent was collected at 2–8-min intervals and further processed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE).

SDS-PAGE

SDS-PAGE was performed on 13% slab gels with Coomassie Brilliant Blue staining as routinely used in our laboratory or by automated SDS-PAGE (Phast system; Pharmacia–LKB) with automated silver staining (Pharmicia product information).

Samples

Urine from two children following renal cadaver transplantation was collected daily without the addition of any preservative and immediately stored in a refrigerator. Urine from one child with acute renal failure following haemolytic uraemic syndrome (HUS) was collected similarly. The procedure was approved by our local ethics committee.

RESULTS

To 500-ml aliquots of urine, ammonium sulphate was added to achieve a final concentration of 50%. After centrifugation the pellet was further processed. After dissolution in sterile distilled water the solution was freeze-dried and stored until processing.

Fig. 1 depicts SDS-PAGE with silver staining of the original urine. The gel filtration of the crude solution on a Superdex 200 60/16 preparative column is shown in Fig. 2. According to the SDS-PAGE and the gel filtration patterns, the greatest amount of protein is in the range 45 000–75 000 dalton. Although the higher-molecular-weight proteins are separated very effectively, the lower-molecular-weight proteins are not cut off. In addition, with this column a final separation of albumin with a

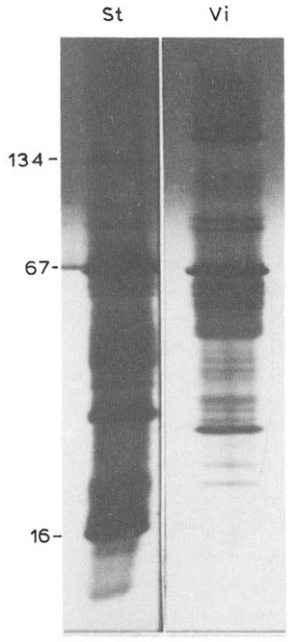

Fig. 1. SDS-PAGE with Phast system with automated silver staining. Discontinuous gel with 8–25% PA. St = standard (molecular weights in kilodalton); Vi = crude villin preparation.

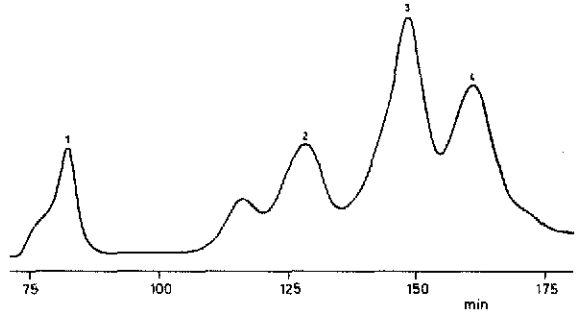

Fig. 2. Separation of crude villin preparation by gel filtration on Superdex 200 16/60 together with two marker proteins, aldolase (158 000 dalton) and ovalbumin (43 000 dalton) in the presence of 8 *M* urea. Flow-rate, 0.5 ml/min; absorbance, 0.64; attenuation, 8. Peaks: 1 = elution peaks; 2 = aldolase; 3 = villin (+albumin); 4 = ovalbumin.

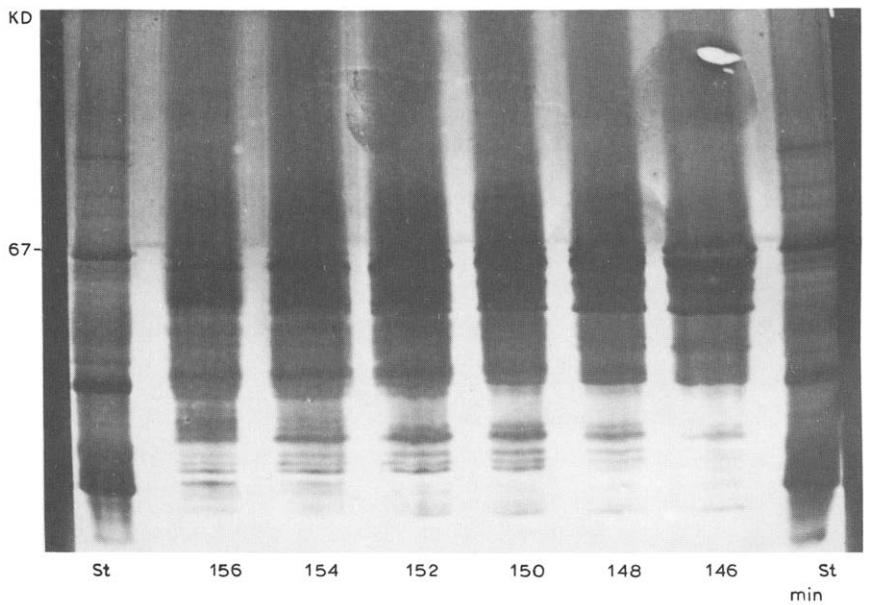

Fig. 3. SDS-PAGE with Phast system (see also Fig. 1) from effluent of the gel filtration separation shown in Fig. 2. The time corresponds to the time scale of the gel filtration tracing. St = standard proteins (molecular weights in kilodalton, kD).

molecular weight of 67 000 dalton and the villin fragment with a molecular weight of 70 000 dalton could not be achieved (Fig. 3).

A better resolution of the villin preparation was achieved with another gel filtration column, TSK 3000 SWG. As can be seen in Fig. 4., the main villin peak as demonstrated by Fig. 2 is separated into two clearly distinguishable peaks. The two peaks correspond to the different molecular weights of villin and albumin, as shown in the corresponding SDS-PAGE patterns in Fig. 5.

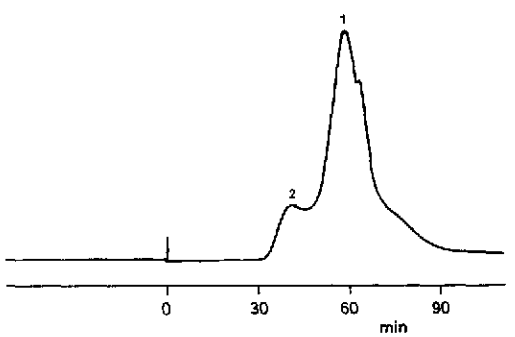

Fig. 4. Separation of a 70-kilodalton villin fragment by gel filtration on a TSK 3000 SWG collumn in the presence of 8 *M* urea. Flow-rate, 0.5 ml/min; absorbance, 0.1; injection volume, 500 μl. Peaks: 1 = albumin; 2 = villin.

DISCUSSION

The original proposal to purify villin derived from brush-border vesicles of chicken intestine was reported by Bretscher and Weber [10], but the technique is time consuming, difficult and yields protein of low purity. In addition, from fifteen chicken intestines a maximum of 5 mg of villin can be isolated. In the urine of patients with severe renal damage, *e.g.*, those who have undergone kidney transplantation, which in experimental terms is nothing but a recovery from acute renal failure with brush-border shedding, we observed villin concentrations up to 20 mg/l [3].

With the present approach it was possible to highly enrich villin substantially by gel filtration in the presence of 8 *M* urea. We used two different gel filtration media that were not equally suitable for the present problem. The advantage of Superdex 200 is the very clear separation between high- and low-molecular-weight proteins in the range 200 000–20 000 dalton. The separation of human

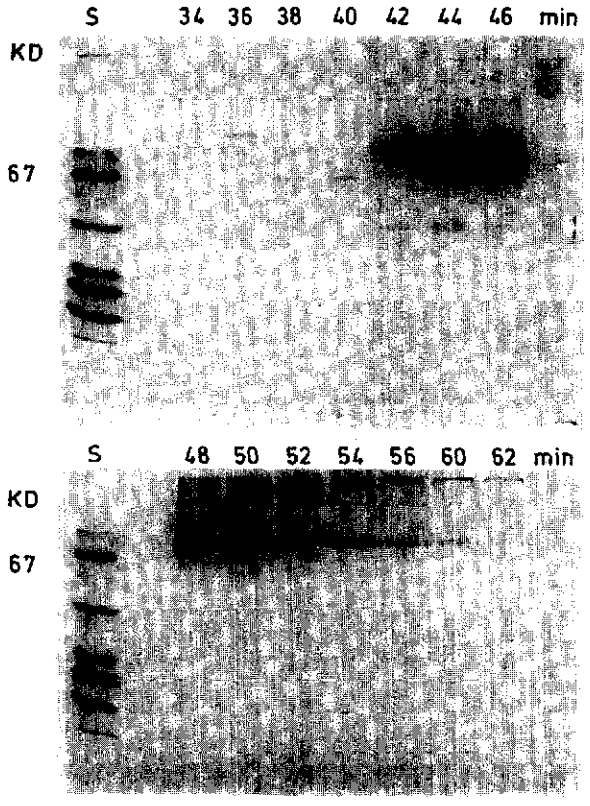

Fig. 5. SDS-PAGE with continuous slab gel with 13% PA of the gel filtration given in Fig. 4. The time corresponds to the time scale of the gel filtration tracing. S = standard proteins (molecular weights in kilodalton, KD).

serum albumin (67 000 dalton) and the villin fragment (70 000 dalton) was not possible on Superdex 200. In contrast, the TSK 3000 SWG column resolved these two proteins. This approach is simple, straightforward and inexpensive.

Owing to the detection of the gene locus and the exact sequence of villin, further developments such as tumour markers are coming into the field [11,12].

In conclusion, renal antigens, that is, proteins which are released by the kidney under conditions of damage, are new parameters of tubular cell injury. With classical biochemical methods and the development of improved separation media, the purification of proteins can be resolved by simple and fast techniques.

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