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Protein Z and protein Z-dependent protease inhibitor and renal tubules

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Dear Sirs,

Protein Z (PZ) is a vitamin K-dependent, plasma protein that serves as a cofactor for the inhibition of factor Xa by protein Z-dependent protease inhibitor (ZPI) in the presence of calcium ions and phospholipids. ZPI is a member of the serpin superfamily of proteinase inhibitors and not only inhibits factor Xa in a PZ-dependent fashion, but also inhibits factor XIa in the absence of cofactors. Both PZ and ZPI are thought to be produced predominantly by the liver (1).

To assess other potential sources of PZ besides the liver, tissue northern analysis was performed and showed substantial PZ mRNA expression (~1.4 kb) in the kidney (▶Fig. 1A). Subsequent immunohistochemistry studies using two monoclonal anti-PZ antibodies (Mab 2048, Mab 2306.BF12.2) (2) demonstrated PZ staining in the renal distal and collecting tubules based on tubular morphology and location in paraffin and frozen sections of the renal cortex (not shown) and the renal medulla (Fig. 1C). ZPI was also detected in kidney tubules by immunohistochemical analysis using two monoclonal antibodies (Mab 4242.2, Mab 4336.1E5) (3) in the same location as PZ (Fig. 1D). As Northern analysis has shown little if any ZPI expression in the kidney, compared to that in the liver (4), it is conceivable that the ZPI detected by immunohistochemistry was produced elsewhere and then bound to tubular PZ (2). Notably, PZ and ZPI immune-reactivity was not seen in glomeruli or other renal vascular structures. PZ and ZPI expression was also detected in the liver by immunohistochemistry, but not in lung, heart, spleen, or vasculature (not shown).

Madin-Darby canine kidney cells (MDCK, CCL34, ATCC, Manassas, VA, USA) are derived from canine distal tubule cells, have retained a polarised phenotype, and have been used extensively to examine the sorting of proteins and other molecules. Studies of MDCK cells stably expressing recombinant human PZ, ZPI and both PZ and ZPI cultured on Transwell filter units (0.4 μm, Fisher Scientific, Pittsburgh, PA, USA) (5) demonstrated that these proteins were secreted apically, i.e. toward the tubular lumen (not shown). Random urine testing of six laboratory personnel showed the presence of PZ (61.3 ± 9.3 ng/ml; 2.4 ± 0.4% of the plasma level), but no ZPI by monoclonal sandwich immunoassays (2,3). Western blotting using rabbit polyclonal antibodies of concentrated (YM 10, Millipore, Billerica, MA, USA) urine specimens demonstrated apparent full-length PZ (62,000 molecular weight [MW]), with little evidence of proteolytic degradation and a trace of ZPI reactivity at 72,000 and 50,000 MW.

These results suggest that at least some of the PZ expressed by tubular cells is secreted into the urine, whereas the bulk of ZPI is either not secreted into the urine or is degraded to forms

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of low molecular weight. Glomerular filtration appears less likely as a source of urinary PZ, since PZ and ZPI circulate as a relatively high-molecular-weight complex in plasma (2). Prothrombin, factor XI and prekallikrein are also produced by kidney tubules (6–10) and the F1 fragment of prothrombin (PTF1) and PZ (11,12) have been detected in kidney stones.

Northern analysis of mouse tissues demonstrates kidney expression of PZ (not shown) and mouse urine (n=5) contains PZ at 1.9 ± 0.6% the PZ level of mouse plasma. Therefore the mouse will likely provide an appropriate model to investigate the physiologic relevance of urinary PZ.

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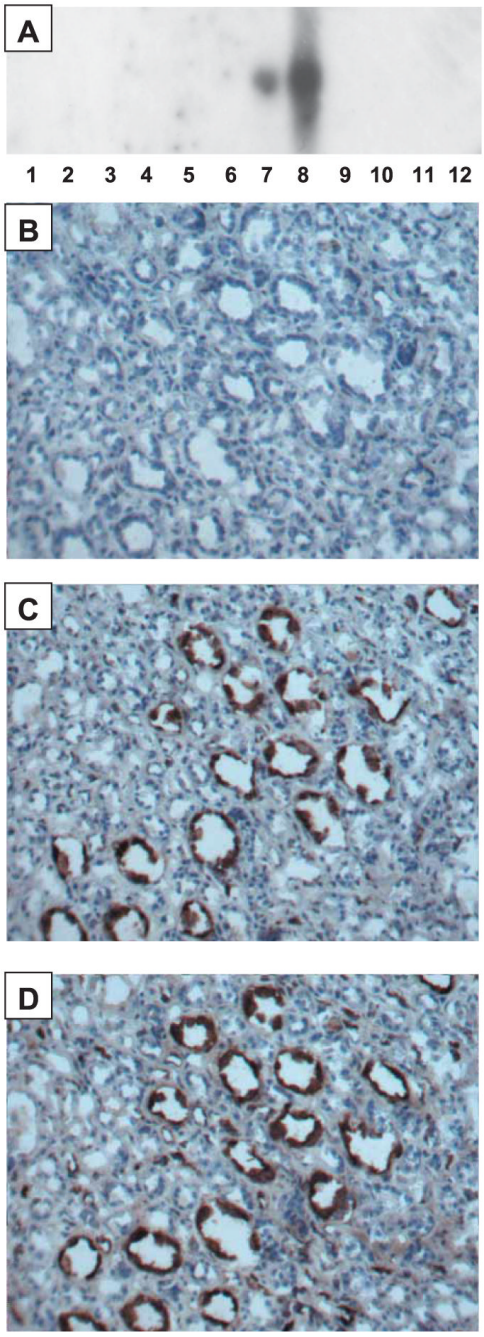

Figure 1. PZ and ZPI in human kidney tubules

A) Human tissue Northern blot (Clontech, Mountain View, CA, USA) probed with PZ cDNA. 1: Brain, 2: Heart; 3: Skeletal muscle; 4: Colon; 5: Thymus; 6: Spleen; 7: Kidney; 8: Liver; 9: Small intestine; 10: Placenta; 11: Lung; 12: Neutrophils. B) Frozen section (20×) stained with isotype control monoclonal IgG. C) Frozen section (20×) stained with Mab 2048 anti-PZ IgG. D) Frozen section (20×) stained with Mab 4336.1E5 anti-ZPI IgG. H_2O_2 (3%) inactivation of endogenous peroxidase, biotin-insensitive detection (ImmPRESS, Vector Laboratories, Burlingame, CA, USA) with diaminobenzidine (DAB) substrate (brown). B, C & D are adjacent sections. Human kidney sections were from Zyagen (San Diego, CA, USA).