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DEFICIENCY OF A MOUSE KIDNEY METALLOENDOPEPTIDASE ACTIVITY: IMMUNOLOGICAL DEMONSTRATION OF AN ALTERED GENE PRODUCT

Malcolm J. McKay[+], Cheryl L. Garganta, Robert J. Beynon* and Judith S. Bond[‡]

Department of Biochemistry, Virginia Commonwealth University, Richmond Virginia, 23298

*Department of Biochemistry, University of Liverpool, P.O. Box 147, Liverpool L69 3BX U.K.

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Summary: Meprin, an 85,000 molecular weight metalloendopeptidase is a major component of the kidney brush border membrane in mice. Some inbred mouse strains exhibit low levels of meprin activity. These strains were characterized by little, if any, protein in brush border preparations corresponding to the native enzyme. However, material exhibiting partial identity to meprin was identified by Ouchterlony immunodiffusion. Immunoblots of brush border proteins confirmed that this immunoreactive material was present but of higher molecular weight than the native enzyme. The implication of these data is that the structural gene for meprin is expressed, albeit incorrectly, in the low-meprin strains.

Meprin is a membrane-bound metalloendopeptidase that is present in the brush border membrane of mouse kidney (1,2). Some inbred mouse strains express activities of meprin that are less than 5% of normal (3). The gene (Mep-1) that regulates the activity of meprin has been localized to chromosome 17, telomeric to the D region of the histocompatiblity complex (4). The Mep-1^a genotype denotes the high meprin trait; Mep-1^b denotes low meprin animals.

Previous work has eliminated the possibility of a dissociable meprin inhibitor in Mep-1^b mice (3). Further, it is unlikely that subtle changes in meprin, such as a modification in pH optimum or substrate affinity could be responsible for such a dramatic change in specific activity (2). The activities of other renal brush border enzymes are unchanged in Mep-1^b animals (2), precluding a widespread alteration in the enzymatic complement of this membrane. Accordingly, we have compared the Mep-1^a mice with Mep-1^b mice to establish whether meprin is absent or alternatively, is present in an altered form.

[+]Present address: Abbott Laboratories, Abbott Park, IL 60064
[‡]To whom correspondence should be addressed.

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MATERIALS AND METHODS

Adult male mice (20-25 g) were obtained from Jackson Laboratory, Bar Harbor, ME (CBA/J, BALB/cJ), Harlan-Sprague-Dawley, Gilbertsville, PA (C3H/HeN) or Dominion Laboratories, Dublin, VA (ICR) and were maintained at 23°C on a 12 h dark/12 h light cycle with free access to standard laboratory mouse chow and water.

Microvilli were prepared as described (5) except that whole mouse kidney was used as starting material. The degree of enrichment of brush border enzymes was assessed routinely by measurement of alkaline phosphatase (2). Detergent solubilization of brush border proteins was achieved by incubating microvilli at a protein concentration of 2 mg/ml in the presence of 1% (v/v) Triton X-100 at 37°C for 1h.

Meprin was purified from frozen ICR mouse kidneys obtained from Rockland Farms, Inc. (Gilbertsville, PA). Purification of the enzyme commenced with 200 kidneys as previously described (1).

Antiserum was raised by injection of 250 µg meprin in Freund's complete adjuvant (Colorado Serum Co. Laboratories, Denver, CO) intradermally into the flanks of a male New Zealand rabbit (Charles River Laboratories, Wilmington, MA). Three additional injections of 250 µg of meprin in Freund's incomplete adjuvant were administered to the rabbit at 10 day intervals. Four days after the final injection, the rabbit was bled, blood was allowed to clot at 4°C overnight and the serum was collected and stored at -20°C. Pre-immune serum was obtained from the rabbit immediately prior to the first injection.

Immunodiffusion plates were prepared with 1.2% (w/v) electrophoresis grade agarose (Bethesda Research Laboratories, Inc., Gaithersburg, MD) in 10 mM Tris-HCl, pH 7.5, containing 0.01% (w/v) sodium azide and 1 M sodium chloride. The wells were cut 5 mm apart and between 3 and 4 mm deep.

For immunofluorescent labelling of tissue sections, kidneys were frozen at -20°C in OCT embedding compound (Scientific Products, McGaw Park, IL) and sections prepared on a cryostat at -10°C. After air drying, sections were fixed with 2% paraformaldehyde for 10 min and washed with phosphate buffered-saline (PBS) containing 0.15 M NaCl, 2 mM KCl, 1 mM $CaCl_2$ in 10 mM sodium phosphate, pH 7.2. To remove non-specific binding of the goat anti-rabbit IgG, the sections were incubated with normal goat serum (Cappel Laboratories, Westchester, PA, diluted 1:10) for 30 min at 23°C. Sections were then incubated at 37°C with pre-immune serum or antiserum which had been absorbed with rabbit liver powder (Cappel Laboratories) for 18 h at 4°C and subsequent centrifugation at 30,000 x g for 15 min; both the pre-immune serum and antiserum were diluted 1:80 before incubation with the kidney sections. The sections were washed three times with PBS (10 min each) and then incubated with fluorescein-conjugated goat anti-rabbit IgG (diluted 1:20; Cappel Laboratories). Unbound goat anti-rabbit IgG was removed from the sections by three washes (5 min each) of PBS. The sections were allowed to air dry before the coverslips were mounted with Aquamount (Scientific Products).

Sodium sulfate precipitation was used to prepare immunoglobulin fractions from pre-immune serum and antiserum. The serum proteins were sequentially precipitated and centrifuged (27,000 x g, 10 min) with 0.17 g and 0.3 g/ml sodium sulfate. The final sedimented precipitate was resuspended in 10 ml of 70 mM Tris-HCl, pH 8.0, at a final concentration of 3 mg/ml.

Mouse kidney brush borders were subjected to sodium dodecyl sulfate (SDS) polyacrylamide slab gel electrophoresis (6) and were stained for protein or were immediately electro-blotted onto a nitrocellulose membrane (Schleicher and Schuell, Inc., Keene, NM). Electro-blotting was performed in a 25 mM Tris-base transfer buffer with 150 mM glycine and 20% (v/v) methanol at 23°C with 200V for 1.5h The nitrocellulose membrane was then incubated with rabbit anti-meprin IgG (X1000 dilution) for 1h at 2°C and developed with the Bio-Rad Immun-Blot (goat anti-rabbit IgG horseradish peroxidase conjugate) assay kit (Bio-Rad Laboratories, Richmond, CA).

RESULTS AND DISCUSSION

Renal brush border membrane preparations from several mouse strains contained numerous protein bands, evident after SDS polyacrylamide gel electrophoresis, in the molecular weight range of 20,000 to 200,000. The protein profiles of all Mep-1^a strains examined thus far (including BALB/c, DBA/2, A, C57BL/10S, ICR, CD/1 and Swiss) were very similar and showed no consistent differences (data not shown). The profiles of the brush border membranes prepared from Mep-1^b mice were also similar to those from Mep-1^a mice, with a notable exception (Fig. 1). A major protein band (with an approximate molecular weight of 85,000) that was present in the profiles from Mep-1^a strains was missing or dramatically reduced in intensity in the profiles from all Mep-1^b strains tested. Purified preparations of meprin migrated as a single band to the same position as this 85,000 molecular weight band. The simplest interpretation of these observations is that the 85,000 molecular weight band in the brush border preparation is meprin and that this protein is absent in Mep-1^b strains.

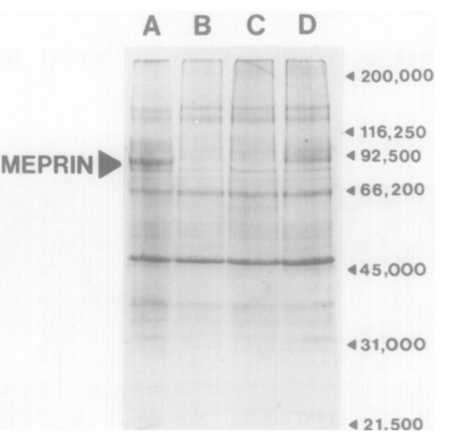

Fig. 1. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of mouse kidney brush border membranes. Samples (100 µg) of kidney brush border membranes from two Mep-1a mouse strains (ICR, lane A; BALB/c, lane D) and two Mep-1b strains (C3H, lane B; CBA, lane C) were subjected to SDS-polyacrylamide gel electrophoresis on 10% (w/v) acrylamide gels as described in the Materials and Methods section and stained with Coomassie Brilliant Blue.

To investigate this further, an anti-serum to purified meprin was raised in rabbits and used as a probe for the presence of meprin-related antigens in the brush border membranes of Mep-1^b mice. The rabbit anti-meprin IgG was tested on Ouchterlony plates and single precipitin lines were observed over a wide range of concentrations of meprin or anti-meprin IgG; no precipitin lines were detected with IgG from pre-immune serum. In addition, the anti-meprin IgG was able to precipitate purified meprin preparations from solution in the presence of staphylococcal clumping factor (results not shown).

The anti-meprin IgG was thus used for immunofluorescent localization of cross-reactive material in sections of kidney from Mep-1^a and Mep-1^b mice (Fig. 2). Sections prepared from a Mep-1^a mouse (BALB/c) showed strong fluorescence specifically at the luminal surface of the proximal tubule, confirming the brush border localization of the enzyme. Sections from a Mep-1^b mouse (C3H), showed neither localized fluorescence in the luminal region nor pronounced fluorescence within the cell.

Purified preparations of meprin and brush border preparations from Mep-1^a mice (ICR and BALB/c) all formed strong precipitin lines against anti-meprin IgG (Fig. 3A). The lines of identity indicated no evidence of antigenic variation in meprin between the two different strains. Pretreatment of brush borders with detergent produced stronger precipitin lines. Figure 3 (B-D) shows the results of the antigenic reactions observed with brush border preparations from Mep-1^b

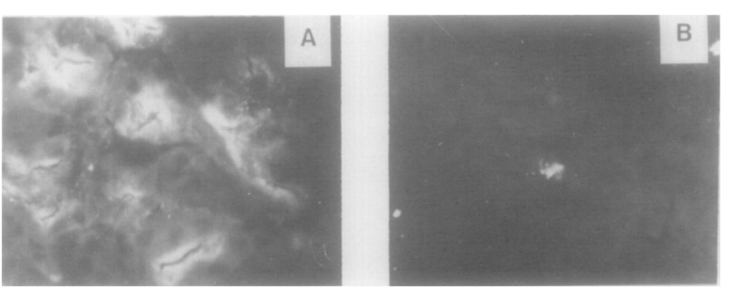

Fig. 2. Immunofluorescent staining of mouse kidney cross-sections. Mouse kidney sections were fixed in paraformaldehyde and subjected to immunofluorescent staining with anti-meprin IgG. Plate A shows a fluorescent-stained kidney section of a BALB/c (Mep-1a) mouse. Plate B shows a fluorescent-stained kidney section from a C3H (Mep-1b) mouse.

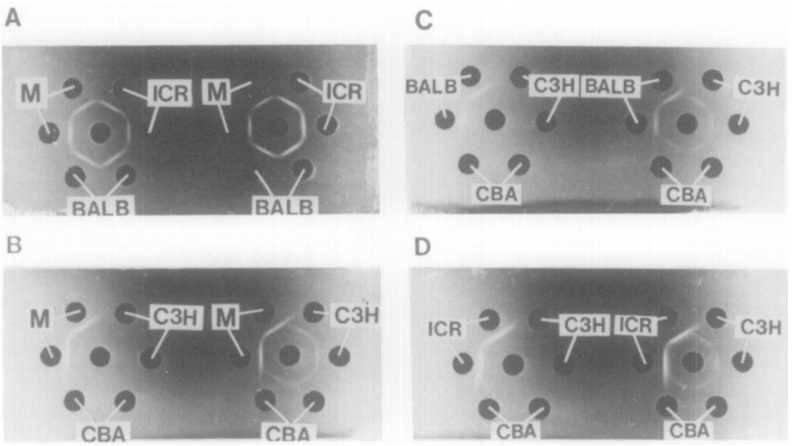

Fig. 3. Immunodiffusion patterns of meprin and mouse kidney brush border membranes on Ouchterlony plates. An anti-meprin IgG was placed in the center wells of each of plate. In each the four plates, preparations of meprin and kidney brush border membranes without pretreatment with Triton X-100 were placed in the left hand set of wells. Preparations of meprin and kidney brush border membranes pretreated with Triton X-100 were placed in the right hand wells. A: Samples of meprin and brush border preparations from ICR and BALB/c (Mep-1a) mice. B: Samples of meprin and brush border preparations from C3H and CBA (Mep-1b) mice. C: Samples of brush border preparations from a BALB/c (Mep-1a) mouse and from C3H and CBA (Mep-1b) mice. D: Samples of brush border preparations from an ICR (Mep-1a) mouse and from C3H and CBA (Mep-1b) mice.

mice (C3H/He and CBA), and Mep-1^a mice (ICR and BALB/c). No cross-reaction was evident with Mep-1^b brush border membranes unless they were pretreated with detergent. The detergent-solubilized C3H and CBA brush borders formed weak precipitin lines against the anti-meprin IgG. The Mep-1^b membranes showed partial identity to meprin and to the Mep-1^a brush border preparations. The union of precipitin lines between detergent-solubilized brush borders from C3H and CBA mice (Fig. 2B-D) consistently showed complete identity. These data indicate that brush border preparations from Mep-1^b mice contain some antigenic determinants that are recognized by the anti-meprin IgG. The fact that this reaction was only seen with brush borders that had been pretreated with detergent indicates that either the detergent facilitated the passage of the antigen(s) through the agarose gel or that unfolding of brush border protein(s) in the Mep-1^b preparations was necessary to expose epitopes.

To further elucidate the nature of the various antigenic determinants in the Mep-1^a and Mep-1^b mouse brush borders, SDS-polyacrylamide gel electro-

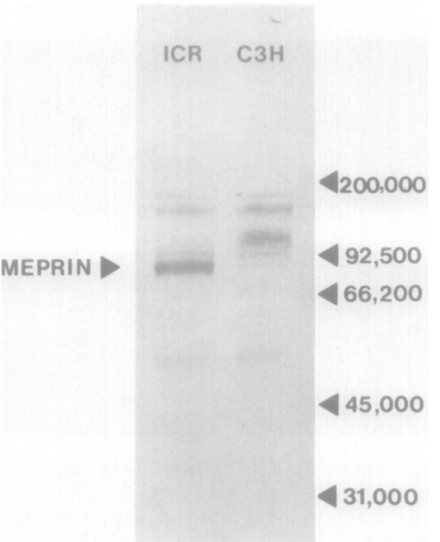

Fig. 4. Immublotting of mouse kidney brush border membranes. Samples (40 µg) of kidney brush border membranes from a Mep-1a mouse strain (ICR) and a Mep-1b mouse strain (C3H) were subjected to SDS-polyacrylamide gel electrophoresis on 10% (w/v) acrylamide gels and electroblotted onto nitrocellulose paper and immunoprobed with anti-meprin IgG as described in the Materials and Methods section.

phoresis and immunoblotting were employed. The 85,000 molecular weight meprin subunit previously identified in brush border preparations, stained with Coomassie Brilliant Blue, was readily detectable in the immunoblots from Mep-1^a mice. This band was virtually absent in the Mep-1^b brush border preparations (Fig. 4). The chromogenic immunoprobe also detected other protein bands that were common to Mep-1^a and Mep-1^b animals. The most distinct of these included two bands with molecular weights of 145,000 and 135,000. In the molecular weight region of 90,000-120,000 there was a complicated pattern of bands. In comparing Mep-1^a and Mep-1^b-derived tracks, at least one band with a molecular weight of 112,000 was unique to Mep-1^b strains, implying that some form of meprin is synthesized in animals deficient in enzyme activity. We estimate that meprin constitutes approximately 5% of the brush border membrane in Mep-1^a mice, as determined from densitometric analyses of electrophoretograms and on the basis of the specific activity of purified enzyme preparations (results not shown). If the 112,000 molecular weight protein in Mep-1^b mice is a meprin

precursor, it is present at much lower levels, conceivably because this or other altered forms of the meprin subunit are extensively degraded.

It was possible that meprin was synthesized in the Mep-1^b mouse kidney but not directed to the correct subcellular compartment. Thus, a number of subcellular fractions were prepared from the kidneys of Mep-1^a and Mep-1^b mice (ICR and C3H, respectively). Samples of the nuclear, lysosomal-mitochondrial, microsomal and cytoplasmic fractions were subjected to SDS-polyacrylamide gel electrophoresis, electroblotted and immunoprobed with the anti-meprin IgG. With the exception of the microsomal fraction (which contains brush border membranes), no bands were visualized by the chromogenic immunoprobe (results not shown). These observations indicate that the meprin subunit protein does not accumulate in another cellular compartment of the Mep-1^b kidney as a result of errors in its synthesis or post-translational processing.

The data demonstrate that the meprin structural gene is expressed in Mep-1^b animals. Whether the difference between Mep-1^a and Mep-1^b animals is due to a fault within the structural gene or is due to defective transcriptional or translational events is unknown.

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