Zonulin Is Structurally And Functionally Related To The Mucosal Mast Cells-Derived Mast Cell Protease II

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Background: Zonulin is a molecule involved in the regulation of intestinal tight junctions both in health and disease. While zonulin mechanism of action has been partially defined, its biochemical characteristics are still largely unknown.

Aim: To purify and structurally characterize the zonulin protein.

Methods: Rat small intestinal tissues were analyzed by a combination of gel filtration chromatography and zonulin sandwich ELISA. Rat intestine homogenates were loaded on a sephacryl column and fractions collected and assayed for their zonulin content. Each fraction was also resolved by SDS-PAGE, transferred, and immunoblotted with zonulin-immunoreactive, anti-Zot antibodies. Both wild type and WBB6/F1-W/W^{V} mice intestinal tissues were tested in the microsnapwell assay and transepithelial electrical resistance (TEER) and zonulin release from stimulated tissues determined.

Results: Of the 6 fractions (F1-F6) collected and tested with zonulin ELISA, F5 contained the highest zonulin concentration. Western analysis revealed two major bands that migrated with approximate apparent Mr of 24,000 and 23,000 in the zonulin-positive fraction, F5, while the zonulin-negative fractions, F1-4, and 6, each revealed only one immunoreactive band (~24 kDa). Therefore, the ~23 kDa band from F5 was excised from a Comassie blue-stained gel and subjected to Matrix Assisted Laser Desorption Ionization mass spectrometry. Search using the Profound search engine for protein matches revealed a high similarity of this protein (estimate Z score 1.58) with the rat mast cell protease (MCP)-II. To establish whether zonulin and MCP-II are the same moiety, microsnapwell experiments were conducted in WBB6/F1-W/W^{V} mice characterized by pleiotropic defects in mucosal mast cells and, therefore, lack MCP-II. Tissues mounted in the microsnapwell system and exposed at increasing time intervals (up to 3 h) to zonulin-releasing stimuli showed a TEER decrease (Δ TEER= -170±15.8 Omhs/cm^2 vs -43±11 in untreated tissues, $p<0.001$) and a parallel increase in zonulin release (10.0±0.8 ng/mg protein vs 0.2±0.7 in untreated tissues, $p<0001$) similar to that observed in stimulated wild type animals (-120±20 and 15.1±3.1, respectively).

Conclusions: zonulin is structurally and functionally similar, but not identical, to MCP-II and may represent one of the possible intestinal luminal PAR-2 activators involved in the pathophysiological regulation of intercellular tight junctions.