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October 2001

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Recent *AAPS PharmSci* Articles of Interest

Three-Dimensional Structure of Fibrolase, the Fibrinolytic Enzyme from Southern Copperhead Venom, Modeled from the X-Ray Structure of Adamalysin II and Atrolysin C

Michael B. Bolger, Steve Swenson, and Francis S. Markland, Jr.

The fibrinolytic enzyme from southern copperhead snake venom, fibrolase, contains 1 mole of zinc per mole of protein, belongs to the major family of metalloproteinases known as the metzincins, and has been shown to degrade fibrin clots *in vitro* and *in vivo.* The purpose of this study was to develop a 3-dimensional model of fibrolase to investigate the geometry of conserved and variable sequences between members of the snake venom metalloproteinases. When compared to atrolysin C (form D) or adamalysin II (metzincins with completely different substrate specificity), fibrolase has approximately 60% overall sequence identity and nearly 100% sequence similarity in the active site. We used the crystal structure of adamalysin II to build a 3-dimensional homology model of fibrolase. Three disulfide bonds were constructed (the highly conserved disulfide bond [118-198] was maintained from the adamalysin II structure and 2 new disulfide bonds were introduced between residues 158-182 and 160-165). We used Sculpt 2.5 and HyperChem 5.0 to "dock" a substrate fragment octapeptide (IITEKLVTS), and a water molecule into the active site cleft. We calculated the differential average homology profile for fibrolase compared to 8 hemorrhagic and 5 nonhemorrhagic metzincins. We then determined the sequence regions that might be responsible for their substrate specificity. Our 3-dimensional homology model shows that the variable sequences lie on the periphery of the identified active site region containing the His triangle; this indicates that substrate specificity may depend on surface residues that are not directly associated with the active site.

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Visualization of the Lipid Barrier and Measurement of Lipid Pathlength in Human Stratum Corneum

Priya S. Talreja, Nancy K. Kleene, William L. Pickens, Tsuo-Feng Wang, and Gerald B. Kasting

Detailed models of solute transport through the stratum corneum (SC) require an interpretation of apparent bulk diffusion coefficients in terms of microscopic transport properties. Modern microscopy techniques provide a tool for evaluating one key property¾lipid pathway tortuosity¾in more detail than previously possible. Microscopic lipid pathway measurements on alkali expanded human SC stained with the lipid-soluble dyes methylene blue, Nile red, and oil red O are described. Brightfield, differential interference contrast, fluorescence, and laser scanning confocal optics were employed to obtain 2-dimensional (2-D) and 3-dimensional (3-D) images. The 2-D techniques clearly outlined the corneocytes. Confocal microscopy using Nile red yielded a well-delineated 3-D structure of expanded SC. Quantitative assessment of the 2-D images from a small number of expanded SC samples led to an average value of 3.7 for the ratio of the shortest lipid-continuous pathway to the width of the membrane. This was corrected for the effect of alkaline expansion to arrive at an average value of 12.7 for the same ratio prior to swelling.

Spatial Expression Patterns of Peptide Transporters in the Human and Rat Gastrointestinal Tracts, Caco-2 *in vitro* Cell Culture Model, and Multiple Human Tissues

Dea Herrera-Ruiz, Qing Wang, Olafur S. Gudmundsson, Thomas J. Cook, Ronald L. Smith, Teresa N. Faria, and Gregory T. Knipp

This study sought to identify the spatial patterns of expression of peptide transporter 1 (PepT1), peptide transporter 3 (PTR3), peptide/histidine transporter 1 (PHT1), and the human peptide transporter 1 (HPT-1) mRNA in complementary DNA (cDNA) libraries of the human and rat gastrointestinal tracts (GIT), Caco-2 *in vitro* cell culture model, and in a human multiple tissue panel. Human PTR3 and PHT1 are putative peptide transporters recently discovered. Using sequence-specific primers designed to amplify regions of PepT1, PTR3, PHT1, and HPT-1, we were able to identify the expression of mRNA for each of these transporters in human cDNA panels (Clontech, Palo Alto, CA), the rat GIT, and in Caco-2 cDNA libraries by the polymerase chain reaction (PCR) and Southern Blot analysis. These studies suggest that in the human GIT, PepT1 appears to be localized predominantly in the duodenum, with decreasing expression in the jejunum and ileum. In contrast, PTR3 and HPT-1 were widely expressed in the human GIT, with predominant expression in the different regions of the colon. PHT1 appeared to be expressed in low levels throughout the human GI tract. Interestingly, the mRNAs for all 4 peptide transporters were expressed in Caco-2 cells throughout 30 days of culture. PepT1, PTR3, PHT1, and HPT-1 were also widely expressed in the rat GIT. Human tissue cDNA panel screening suggests that PTR3 and PHT1 are more uniformly expressed, whereas PepT1 and HPT-1 demonstrated site-specific expression. These results suggest that PepT1, PTR3, PHT1, and HPT-1 all may act to facilitate the diffusion of peptides and peptide-based pharmaceuticals in the GIT. PTR3, PHT1, and HPT-1 expressions in Caco-2 cell monolayers strongly suggest that their function needs to be further elucidated and their contribution to peptide transport not ignored. Taken together, these results demonstrate the potential for molecular biological characterization in localizing active transporter systems that can potentially be targeted for enhancing the absorption of peptide-based pharmaceuticals.

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Jeff Glassie, Esq., Partner, Shaw Pittman

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