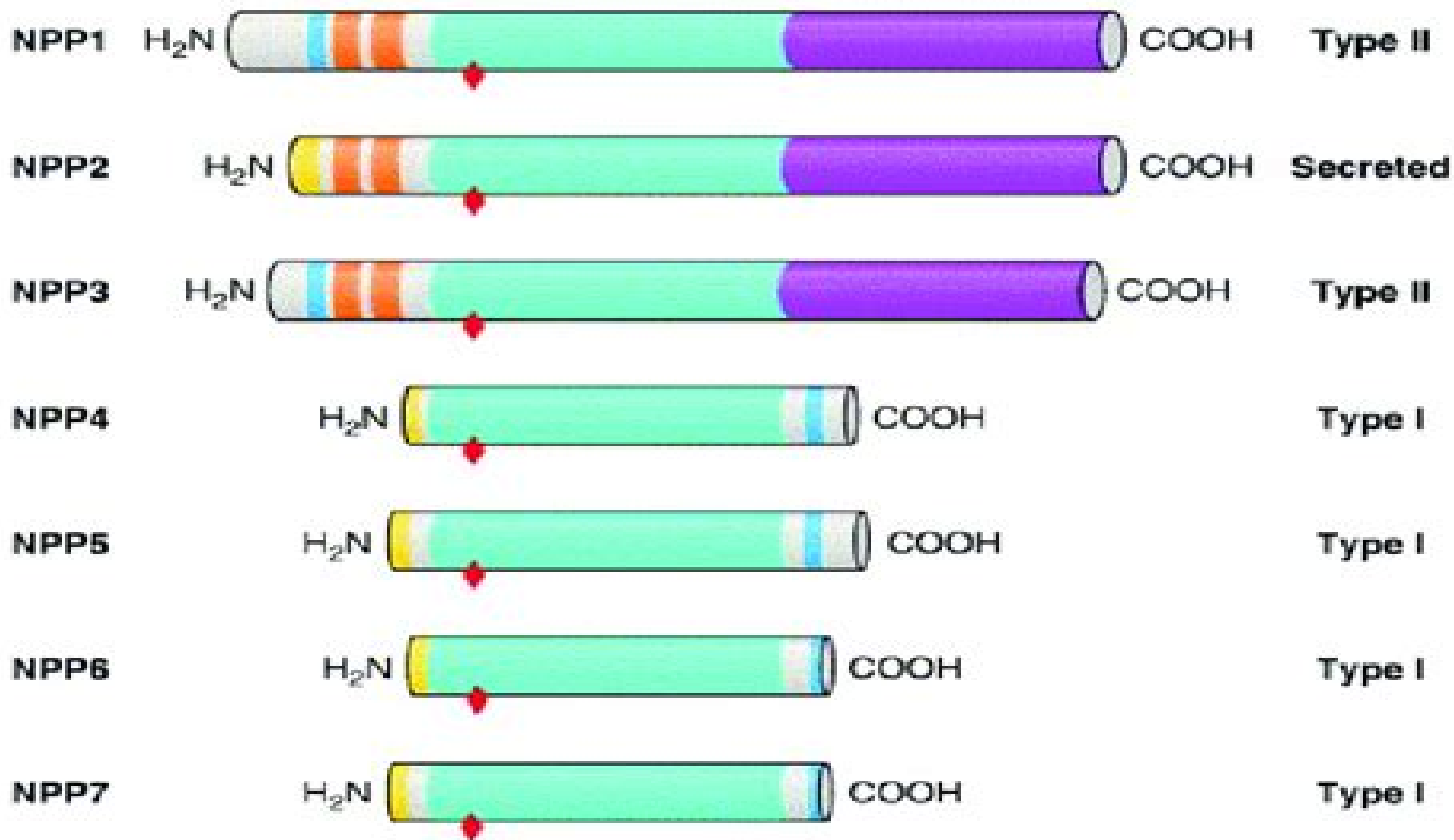


Isoenzyme

Orientation



Key: Transmembrane domain

Catalytic domain

Nuclease-like domain

Somatomedin-B-like domain

Catalytic-site residue

Signal-peptide sequence

Domain Structure Function Of Nucleotide Pyrophosphatasesphosphodiesterases Npps

Emmanuel Paye Bessay



Domain Structure Function Of Nucleotide Pyrophosphatases/phosphodiesterases Npps:

Domain Structure and Function of Nucleotide Pyrophosphatases/Phosphodiesterases Rik Gijsbers, 2003-09 Ecto nucleotidases Extracellular nucleotides and their metabolism Nucleotide Pyrophosphatase Phosphodiesterase NPPs Mammalian NPP isoforms NPPs are modular proteins Enzyme activities of NPPs Functional diversity of NPPs Aims and strategies Materials and methods Results and discussions A family of NPP proteins Characterization of the catalytic domain of NPPs Characterization of the non catalytic ectodomains of NPPs NPP1 and the pathogenesis of insulin resistance Conclusions and Perspectives **Issues in Biochemistry and Geochemistry: 2011 Edition**, 2012-01-09 Issues in Biochemistry and Geochemistry 2011 Edition is a ScholarlyEditions eBook that delivers timely authoritative and comprehensive information about Biochemistry and Geochemistry The editors have built Issues in Biochemistry and Geochemistry 2011 Edition on the vast information databases of ScholarlyNews You can expect the information about Biochemistry and Geochemistry in this eBook to be deeper than what you can access anywhere else as well as consistently reliable authoritative informed and relevant The content of Issues in Biochemistry and Geochemistry 2011 Edition has been produced by the world's leading scientists engineers analysts research institutions and companies All of the content is from peer reviewed sources and all of it is written assembled and edited by the editors at ScholarlyEditions and available exclusively from us You now have a source you can cite with authority confidence and credibility More information is available at <http://www.ScholarlyEditions.com> *Kelley's Textbook of Rheumatology* Gary S. Firestein, William N. Kelley, 2013 Helps you to better understand scientific underpinnings of rheumatic diseases so that you can better manage your patients

Purinergic Signalling in Neuron-Glia Interactions Derek J. Chadwick, Jamie A. Goode, 2006-05-01 ATP the intracellular energy source is also an extremely important cell cell signalling molecule for a wide variety of cells across evolutionarily diverse organisms The extracellular biochemistry of ATP and its derivatives is complex and the multiple membrane receptors that it activates are linked to many intracellular signalling systems Purinergic signalling affects a diverse range of cellular phenomena including ion channel function cytoskeletal dynamics gene expression secretion cell proliferation differentiation and cell death Recently this class of signalling molecules and receptors has been found to mediate communication between neurons and non neuronal cells glia in the central and peripheral nervous systems Glia are critical for normal brain function development and response to injury Neural impulse activity is detected by glia and purinergic signalling is emerging as a major means of integrating functional activity between neurons glia and vascular cells in the nervous system These interactions mediate effects of neural activity on the development of the nervous system and in association with injury neurodegeneration myelination and cancer Bringing together contributions from experts in diverse fields including glial biologists neurobiologists and specialists in purinergic receptor structure and pharmacology this book considers how extracellular ATP acts to integrate communication between different types of glia and between neurons and

glia Beginning with an overview of glia and purinergic signalling it contains detailed coverage of purine release receptors and reagents purinergic signalling in the neural control of glial development glial involvement in information processing and discussion of the interactions between neurons and microglia Dissertation Abstracts International ,2008 **Middleton's Allergy** N. Franklin Adkinson,2003 Presents information on the basic laboratory and clinical aspects of the immunology physiology pharmacology and clinical science features of allergy Includes chapters on molecular biology signal transduction mechanisms and the biology of cell types such as lymphocytes macrophages and platelets **Seikagaku** ,2004

Molecular Biology of the Cell ,2001 Structural and Functional Studies of Nucleotide Pyrophosphatase Angela Howard,2011 Nucleotide pyrophosphatase phosphodiesterase 6 NPP6 is a membrane anchored ectoenzyme initially expressed in the brain and kidney It is the sixth member of the NPP superfamily Currently the superfamily consists of seven members NPP1 NPP7 Each member was numbered according to the order in which it was associated with the family The NPP superfamily hydrolyzes a wide range of substrates such as lipids nucleotides and their derivatives NPP1 NPP2 and NPP3 hydrolyze nucleotides and derivatives NPP6 and NPP7 along with NPP2 hydrolyze phosphodiester bonds in lipids and derivatives Thus far NPP4 and NPP5 have yet to be characterized in terms of which substrates they act upon or the activity which they possess Many of the hydrolytic pathways of NPP enzymes lead to the formation of bioactive lipids that affect numerous pathological as well as physiological processes This combined with their extracellular action makes them suitable targets for therapeutic intervention NPP6 is expressed predominately in the kidneys where it plays a role in choline reabsorption Choline is needed to maintain healthy kidneys however over time choline degrades and the body does not replenish it Therefore structurally and functionally characterizing NPP6 is essential for potential kidney therapeutics Here we focused on testing and identifying more substrates for NPP6 Our results show that NPP6 is not a very promiscuous enzyme Overall NPP6 preferred hydrolyzing smaller substrates opposed to larger substrates Like another member of the NPP superfamily NPP7 NPP6 has lysophospholipase C activity We identified lysophosphatidic acid 16 0 LPC 16 0 and para nitrophenyl phosphocholine pNPPC as highly potent substrates for NPP6 Next we focused on characterizing the structure and function of NPP6 Given that very little is known about the structure and function of NPP6 insight into this area may assist in the design of chemical probes of NPP6 physiological roles All NPP superfamily members hydrolyze a wide range of substrates but exhibit unique substrate specificity profiles Currently little is known what defines the varying substrate specificity among the enzymes To explore substrate specifying determinants for NPP6 we used computational modeling as a rational tool to guide selection of substrate recognition residues to experimentally mutate We selected known substrates such as LPC 16 0 and pNPPC and docked each into the NPP6 homology model The experimental results validated the model suggesting the presence of a common binding pocket for the different substrates of NPP6 Furthermore the experimental studies provide validation of our model for hydrogen bond interactions between the substrate and specific amino acid

residues either during substrate recognition or during the catalytic process. The catalytic domain of the NPP superfamily has been associated with other sulfo and phospho coordinating metalloenzymes which include the alkaline phosphatase superfamily. Both the alkaline phosphatases and NPPs possess two divalent metal cations within their binding pocket and are inactivated by removal of the endogenous divalent metals. Until now the role of the endogenous divalent metals has been unknown. Here we show that the secondary structure is not affected by the presence or absence of the endogenous divalent metals although the catalytic activity is eliminated upon overnight incubation with the metal chelator EDTA. Attempts to restore the catalytic activity of NPP6 were made. Results showed that Ca^{2+} and Zn^{2+} restored almost complete function of the enzyme.

Characterization of the Structure and Function of Nucleotide Pyrophosphatase Irene Werengekha Wanjala, 2010. Nucleotide pyrophosphatase phosphodiesterase NPP 7 also known as alkaline sphingomyelinase Alk SMase is a membrane anchored ectoenzyme initially discovered in the intestinal tract in 1969. It is the most recently identified NPP isoform. The NPP family of enzymes currently consists of seven members NPP1-7 numbered according to the order in which they were first associated with the NPP family. NPP enzymes hydrolyze a wide range of substrates. NPP1 and 3 are nucleotide pyrophosphatases that cleave inorganic phosphate from nucleotides and their derivatives. In contrast NPP2, NPP6 and NPP7 are phosphodiesterases that hydrolyze phosphodiester bonds in lysolipid substrates and their derivatives. NPP4 and NPP5 are yet to be characterized in terms of substrate preference and biological function. Numerous lysolipid substrates have been shown to be hydrolyzed by NPP2 and NPP6 but only three main lysolipid substrates have been previously reported for NPP7. One of the goals of this work was to characterize the substrate preference of NPP7 through exhaustive kinetic means. Our results show that NPP7 like NPP2 and NPP6 hydrolyzes numerous choline containing substrates including glycerolipids lysophosphatidylcholine, platelet activating factor, lyso platelet activating factor and sphingolipids sphingomyelin and sphingosylphosphorylcholine. A synthetic small molecule para nitrophenyl phosphocholine was also identified as a substrate for NPP7. Most hydrolytic activities of NPP isoforms lead to formation of bioactive lipids that affect numerous physiological and pathological processes. This coupled with their extracellular activity makes them attractive targets for therapeutic intervention. A major bottleneck to their utilization is that very little is known about their structure and function. Although NPP enzymes hydrolyze a wide range of substrates and sometimes share a common substrate they exhibit different substrate preference profiles. The factors influencing these different preferences have not been investigated. Our second goal therefore was to employ computational modeling as a rational tool to guide experimental procedures in exploring NPP7 substrate specificity determinants. Our modeling results suggest a common binding pocket for the different substrate groups and the presence of essential non covalent interactions between the substrates and specific amino acid residues. Like alkaline phosphatases NPP isoforms require divalent metal cations for catalytic activity but it is not clearly known whether the divalent metals play both a functional and structural role within the enzyme. Our third goal was therefore to explore the role played by

divalent metal cations in NPP7 Our results indicate that the secondary structure of NPP7 is not altered in the absence or presence of divalent metal cations However its catalytic function is eliminated with prolonged exposure to sufficient amounts of metal chelators The current work significantly advances our understanding of the NPP7 in terms of preferred substrates the amino acid determinants that underlie these preferences and the role of divalent catalytic site metal ions in the structure and function of the enzyme

Cyclic Nucleotide Phosphodiesterases Joe Beavo, 1990-11-30 Second volume in a series addressing topical areas important to the understanding of the control of biological processes This volume focuses on the structure and molecular biology of isoenzymes and their role in signal transduction processes necessary for coordinating cellular functioning

Cyclic-Nucleotide Phosphodiesterases in the Central Nervous System Nicholas J. Brandon, Anthony R. West, 2014-03-07 This book reviews advances in understanding phosphodiesterases within the central nervous system and their therapeutic applications A range of expert authors from both academia and industry describe these then focus on the areas of greatest scientific and medical interest to provide more detailed coverage Therapeutic and drug discovery applications are covered for diseases including Alzheimer's Parkinson's schizophrenia erectile dysfunction and spinal cord injuries There is also a chapter on drug discovery tools such as in vitro assays and X ray structures for medicinal chemistry studies

Crystallographic Studies of Ectonucleotide Pyrophosphatase/phosphodiesterase 1 Jenifer Alexandra Brown, 2021 The STING STimulatory of INterferon Genes pathway is an innate immune pathway that senses the presence of double stranded DNA dsDNA in the cytosol dsDNA binds to cyclic GMP AMP synthase which catalyzes the production of 2'3' cyclic GMP AMP cGAMP a small molecule cGAMP then binds to the STING protein leading to the production of Type 1 interferons and an immune response Ectonucleotide pyrophosphatase phosphodiesterase 1 ENPP1 is an extracellular regulator of the STING pathway that hydrolyzes cGAMP to AMP and GMP and acts as an innate immune checkpoint molecule STF 1084 is an ENPP1 inhibitor we developed to further study ENPP1 activity in the cancer context I generated a 3.2 Å crystal structure of ENPP1 in complex to STF 1084 to understand its mechanism of inhibition and guide subsequent inhibitor designs Using this structural information we were able to guide development of more potent ENPP1 inhibitors To further understand how ENPP1 hydrolyzes cGAMP and explain why we can create substrate specific ENPP1 mutants I generated a 1.9 Å crystal structure of catalytically inactive bacterial Xac NPP in complex with linearized cGAMP These data revealed no substantial interactions between the GMP of cGAMP and NPP side chains contradicting proposals postulated by previous publications We generated an NPP H214A mutant that degrades ATP but not cGAMP Using these data in conjunction with structural data to understand cGAMP hydrolysis we hypothesized that His214 coordinates a zinc ion that is necessary for leaving group stabilization in cGAMP hydrolysis but not ATP hydrolysis To address this question we solved a 2.0 Å structure of NPP H214A in complex with a non hydrolyzable bisphosphorothioate cGAMP analog This structure revealed only one coordinated zinc ion in the active site supporting the hypothesis that His214 is necessary for coordination of a leaving group

stabilizing zinc ion To further study ENPP1 in various contexts I attempted to generate macromolecular tools for a number of biochemical applications Nanobodies are single chain macromolecules that recognize specific protein sequences and or conformations Nanobodies can be used as tools in a variety of applications including X ray crystallography Western blotting flow cytometry and enzymatic studies Using the Kruse yeast display nanobody library I isolated 9 unique nanobody sequences that had some affinity for ENPP1 and characterized their ENPP1 binding affinity and their utility as macromolecular tools This work enhances our understanding how ENPP1 functions as a hydrolase and provides new avenues for modulating ENPP1 activity

The Biology of NPP1 (Nucleotide Pyrophosphatase/Phosphodiesterase I) Ilia Banakh,2005 Structure-function Relations in the Calcium-dependent Protein Modulator of Cyclic Nucleotide Phosphodiesterase Michael P. Walsh,1978 *Characterization of Nucleotide Pyrophosphatase/phosphodiesterase 4, a Dinucleotide Hydrolase, and Its Promotion of Platelet Aggregation* William Chosen Chang,2012 Reciprocal Modulation of Phosphodiesterase-5 Structure and Function by Ligand Binding to Its Catalytic Site and Phosphorylation of Its Regulatory Domain Emmanuel Paye Bessay,2007 **Quaternary Structure of Human Phosphodiesterase-11 (PDE11) Isozymes and Interaction of Cyclic Nucleotides and Inhibitors with the Catalytic Site** James Louie Weeks (II.),2008

Structural Elements that Regulate Interactions Between the Extracellular and Transmembrane Domains of Human Nucleoside Triphosphate Diphosphohydrolase 3 Keith J. Gaddie,2009 The nucleoside triphosphate diphosphohydrolases NTPDases are a family of constitutively expressed endogenous nucleotidases some of which regulate purinergic signaling by divalent cation dependent hydrolysis of nucleotides acting as agonists at purinergic receptors Due to the scarcity of specific inhibitors and genetically modified animals the functions of most individual NTPDases are poorly understood and still under investigation However the NTPDases have been implicated in many biological and physiological processes including secretion cell adhesion pain perception cancer and malignant transformation adenosine recycling and platelet aggregation Membrane bound NTPDase3 expressed on the cell surface has a large extracellular domain a linker region and a transmembrane domain Evidence exists for interactions between the transmembrane domain and the active site lobes that govern the function of rat NTPDase1 However the specific mechanisms regulating this cross talk for NTPDases as well as how the N and C terminal transmembrane helices in NTPDase3 interact within and between monomers to mediate oligomerization and modulation of enzymatic activity is still unknown The roles of the conserved proline residues of human NTPDase3 located in the linker region that connects the N and C terminal transmembrane helices with the extracellular active site were examined by proline to alanine substitutions coupled with single cysteine substitutions strategically placed in the transmembrane domain to serve as cross linking sensors of helical interactions Mutation of several proline residues resulted in decreased nucleotidase activities and some uncoupled the effect of ATP binding on TMD movements The data suggest a role for proline residues 53 and 481 in the linker region of human NTPDase3 for coupling nucleotide binding and

hydrolysis at the enzyme active site to movements and or rearrangements of the transmembrane helices necessary for optimal nucleotide hydrolysis To investigate the structural functional roles of the conserved polar residues in the transmembrane helices of human NTPDase3 each was singly mutated to alanine All mutants were properly glycosylated and had specific activities similar to wild type except Q44A The Q44A mutation decreased specific activities by approximately 50% 70% and nearly eliminated Triton X 100 detergent inhibition The same conserved polar residues were mutated to cysteine singly and in pairs to allow a disulfide cross linking strategy to map potential inter and intra molecular hydrogen bond interactions The results support the centrality of Q44 for the strong inter molecular interactions driving the association of the N terminal domains of two NTPDase3 monomers in a dimer while S39 and T495 may contribute to helical interactions involved in forming higher order oligomers These results suggest a model for putative hydrogen bond interactions of the conserved polar residues in the transmembrane domain of native dimeric NTPDase3 that are important for protein expression activity and susceptibility to membrane perturbations This dissertation provides additional insights to the structural elements that regulate the enzymatic activity of the NTPDases With the recent elucidation of the crystal structure of the extracellular portion of rat NTPDase2 the major remaining structural questions regarding the cell membrane NTPDases are how the TM helices interact and how these interactions are coupled to modulation of enzyme activity and NTPDase function This dissertation addresses these questions for human NTPDase3 and suggests that similar structure function relationships are relevant to the family of NTPDases controlling purinergic signaling

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