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Biochemical characterization of the NTPDases family and thrombosis

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Abstracts. This paper provides insight into the key role of the Nucleoside Triphosphate Diphosphohydrolase (NTPDase) family in thrombosis and its therapeutic potential. Thrombosis is an important mechanism to prevent bleeding, but its abnormal formation can lead to serious health problems such as myocardial infarction and stroke. This paper employs the method of literature analysis, obtaining materials through the investigation of previous literature, thereby conducting a literature review on the biochemical characteristics of some members of the NTPDase family. This paper begins with a description of the basic mechanisms of thrombosis and the biochemical characterization of the NTPDase family, including the structure, function, and specific substrate specificity of its members. The NTPDase enzymes regulate platelet activation by hydrolyzing extracellular Adenosine Triphosphate (ATP) and Adenosine Diphosphate (ADP), thereby preventing excessive thrombosis. The article describes in detail the catalytic roles of the NTPDase family members, their relationship to platelets and thrombosis, and discusses their role in regulating platelet function and blood coagulation. Finally, the article identifies NTPDase family members as promising targets for the treatment of thrombosis and explores the possibility of utilizing these enzymes in the clinical prevention and treatment of thrombosis-related diseases, highlighting their therapeutic potential in the control of inflammatory vascular diseases.

Keywords: NTPDases, thrombosis, P2 receptors

1. Introduction

Thrombosis is an important part of the blood clotting system, which plays a key role in stopping bleeding. However, when blood clots form abnormally in blood vessels, they can lead to serious health problems such as myocardial infarction, stroke and pulmonary embolism. These conditions are usually triggered by damage to blood vessels, slow blood flow, or changes in blood components such as increased clotting factors. The clinical importance of thrombosis lies in its close association with a wide range of cardiovascular diseases, which are the leading causes of death worldwide. Understanding the mechanisms of thrombosis is therefore essential for the prevention and treatment of these diseases.

The discovery journey of the Nucleoside Triphosphate Diphosphohydrolase (NTPDase) family has been progressively advanced with the development of biochemical and molecular biology techniques. Initially, scientists observed the presence of activities in tissues capable of hydrolyzing nucleotides triphosphate. With advances in enzymology, researchers began to identify and characterize these

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activities in the mid-20th century, and in the 1980s, advances in molecular biology led to a more detailed classification and nomenclature of these enzymes, resulting in the term "NTPDase" In the 1990s, utilizing molecular cloning and expression profiling, scientists began to reveal the identity of the NTPDase family. In the 1990s, using molecular cloning and expression analysis techniques, scientists began to unravel the mysteries of the NTPDase family by revealing the genetic structures of its members and the structures of the encoded proteins.

There is an important link between thrombus formation and extracellular adenosine triphosphate (ATP) and adenosine diphosphate (ADP) levels. Extracellular ATP and ADP are important signaling molecules for platelet activation, and they promote platelet activation and aggregation by binding to P2 receptors on platelets, thereby promoting thrombosis. The NTPDase family enzymes, particularly NTPDase1, regulate platelet activation and prevent excessive thrombus formation by hydrolyzing extracellular ATP and ADP and reducing the concentration of these nucleotides formation. Therefore, the activity and expression level of NTPDase enzymes are important for the control of thrombosis. The main focus of this paper is to investigate the interrelationship between NTPDase enzymes and thrombosis.

This paper's review of the biochemical characteristics and the role in thrombosis formation of members of the NTPDase family provides a detailed knowledge framework for future researchers. This helps researchers better understand the functions and regulatory mechanisms of these enzymes, enabling more in-depth study of thrombosis mechanisms. This is significant for the prevention and treatment of cardiovascular diseases, as abnormal thrombosis formation is a primary cause of serious health issues like myocardial infarction and stroke. A thorough understanding of this process offers a theoretical foundation for developing new treatment strategies.

2. Biochemical characterization of the NTPDase family

2.1. Structure and function of family members

The four main classes of extracellular nucleotidases include extracellular nucleotide triphosphodiesterases (E-NTPDases), extracellular 5'-nucleotidases (eN), phosphodiesterases (E-NPPs), and alkaline phosphatases (APs) [1].

E-NTPDases are nucleotide-specific enzymes (all extracellular nucleotidases in the following articles refer to NTPDases) that are primarily responsible for the hydrolysis of nucleotide triphosphate (ATP) and nucleotide diphosphate (ADP) in organisms, ultimately resulting in the production of nucleotide monophosphates. There are several members of their family, each with its own unique structure and function. They have been previously categorized as E-type ATPases, ATPDases, extracellular ATPases, or extracellular apyrases [2]. The NTPDases family differentially hydrolyzes the terminal and βphosphate groups of nucleotides to rapidly generate the corresponding nucleosides diphosphate and/or monophosphate.

And they play a key role in extracellular signaling. They can activate a variety of P2 receptors in different forms and concentrations, which are classified as P2X and P2Y. P2X receptors are ion-channel type receptors, sensitive to ATP, which when activated lead to the influx of calcium and sodium ions into the cell, and play a signaling role, while P2Y receptors are G-protein-coupled receptors, which can be activated by a variety of nucleotides, and play a role by activating the second messenger system in the cell. Functions. These receptors play a role in a variety of physiological processes, such as inflammation, immune response, blood coagulation, and neurotransmission.

2.2. Specific substrate specificity and tissue distribution

Eight members of the NTPDase family have been identified, including NTPDase1 through NTPDase8. NTPDase5 and NTPDase6 are normally located inside the cell, but they can be secreted outside the cell upon heterologous expression. This implies that these enzymes may be involved in different physiological processes inside the cell, such as nucleotide metabolism and signal transduction, but in some cases, they may also affect the extracellular environment through a secretory mechanism.

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NTPDase4 and NTPDase7, on the other hand, are located exclusively inside the cell, and their catalytic sites are oriented towards the lumen of the cytoplasmic organ. Such a localization suggests that they may be primarily involved in nucleotide metabolic processes within the cell, such as functions within organs such as lysosomes or the endoplasmic reticulum. The remaining four NTPDases, NTPDase1, 2, 3, and 8, are typical cell-surface enzymes with extracellularly oriented catalytic sites. NTPDase1 is widely expressed on vascular endothelial cells and is capable of hydrolyzing ATP and ADP. It plays a key role in the regulation of blood coagulation and inflammatory responses by controlling the extracellular levels of ATP and ADP to regulate platelet aggregation and immune cell activation. NTPDase2 is commonly expressed in tissues such as neural tissues and liver, where it has a high hydrolytic specificity for ATP.NTPDase2 may play a role in the regulation of neurotransmitter release and neural signaling [2]. NTPDase3 is commonly expressed in a variety of tissues, including the digestive and endocrine systems. It has a moderate preference for hydrolysis of ATP and ADP and may be involved in digestive processes and hormone regulation. NTPDase8 is expressed in some tissues, such as kidney and vascular endothelial cells, where it also hydrolyzes ATP and ADP, but the specific physiological functions are not as well understood as those of NTPDase1.

2.3. Catalysis

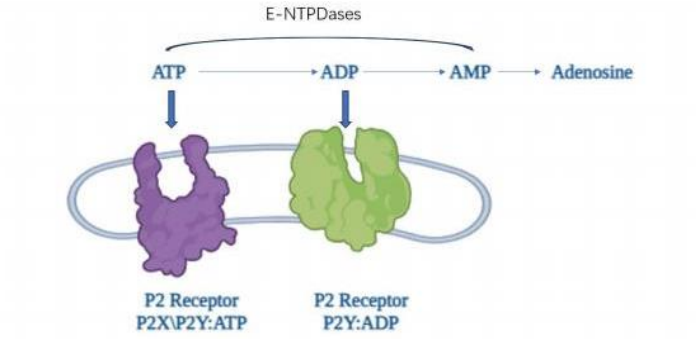

Figure 1. The main catalytic properties of the E-NTPDase family members and the degradation of extracellular nucleotides and activation of the P2 receptor at the cell surface site

NTPDase enzymes sequentially convert ATP to ADP and Pi and ADP to AMP and Pi. NTPDase1 is unique among these enzymes in that it directly dephosphorylates ATP to AMP without releasing a significant amount of ADP.ATP activates both the P2X receptor and a subtype of the P2Y receptor. Upon degradation, ADP may activate additional P2Y receptor subtypes [3]. Differences in cellular localization and functional properties of different isoforms of the NTPDase family lead to differences in catalytic function. This paper focuses on four cell surface-located NTPDases, namely NTPDase 1, 2, 3, and 8. They can be distinguished on the basis of substrate preference, use of divalent cations, and product formation. All cell surface-located NTPDases require Ca^{2+} or Mg^{2+} ions in the millimolar range for maximum activity, and they are usually inactive in the absence of these ions.

The rate of hydrolysis of nucleotide diphosphates varies considerably between isoforms. NTPDase1 hydrolyzes ATP and ADP approximately equally, whereas NTPDase3 and NTPDase8 prefer ATP to ADP as substrate. NTPDase2 has a particular preference for nucleotide triphosphates and has therefore been previously classified as an ecto-ATPase. Unlike NTPDase1 and NTPDase2, NTPDase3 and NTPDase8 prefer to be activated by Ca^{2+} rather than Mg^{+}. NTPDase3 and NTPDase8, unlike NTPDase1 and NTPDase2, prefer to be activated by Ca^{2+} rather than Mg^{2+}. Membrane-bound NTPDase1 hydrolyzes ATP almost directly to AMP, temporarily generating a small amount of free ADP in the process. This property effectively avoids activation of the P2Y receptor for nucleoside diphosphates [4]. In contrast, NTPDase2 releases ADP upon hydrolysis of ATP, after which ADP gradually accumulates and is slowly converted to AMP. This not only removes agonism for nucleoside triphosphate-sensitive P2Y receptors, but also produces agonists for nucleoside diphosphate-sensitive receptors (P2Y1 and P2Y12 receptors

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in platelets) [5]. At the same time, the action of NTPDase3 and NTPDase8 leads to the temporary coexistence of nucleotide diphosphates and triphosphates, presenting a pattern of formation of intermediates.

3. The relationship between NTPDase and platelets and thrombus

Platelet activation and integrin binding are known to occur in response to a variety of agonists, a process that is dependent on the release of extracellular nucleotides and can be modulated by specific antagonists targeting P2Y1, P2Y12, and P2X1 receptors [6]. In the physiological state, when platelets are activated, they expose phosphatidylserine, forming a catalytic surface that promotes coagulation and exposes tissue factors to the bloodstream through interactions with leukocytes and particles, which promotes clot formation.

One might naturally assume that the thrombus would grow as platelets accumulate and ADP is released leading to the activation of more platelets. However, this is not what actually happens. Bound platelets depolymerize and reenter the circulation, resulting in a decrease in thrombus size. Experimental data suggest that unstimulated purified platelets exhibit little or minimal CD39 activity, whereas particles that naturally circulate in plasma exhibit the biochemical activity of NTPDase. Thus, we suggest that NTPDase1 attached to particles may play a critical regulatory role in thrombosis. Specifically, monocyte-derived microparticles are able to bind to activated platelets in thrombi through the mediated action of platelet P-selectin and microparticle PSGL-1. During platelet thrombosis, particles gradually accumulate in growing aggregates carrying more NTPDases. implying that NTPDases are not an important component of the initial formation of thrombi, but begin to accumulate only after the platelet thrombus has developed and matured. Thus, the spatial and temporal expression of NTPDases accumulated in the microparticles may control thrombus size by regulating the hydrolysis of ADP, which in turn inactivates it, and ultimately leads to platelet depolymerization, which is responsible for the reduction in thrombus size we observed [7].

4. Therapeutic potential

Currently, therapeutic success with platelet microthrombotic drug interventions in certain patient populations with high rates of adverse clinical events is modest. Today's research suggests that the evolution of rational drug delivery systems utilizing extracellular nucleotidases (e.g., NTPDase1) may help to control the thrombotic component of inflammatory vascular disease and be able to reduce the inflammatory response. It may also help to cure heart disease, stroke and other cardiovascular diseases caused by thrombosis. Overall, the role of NTPDases in regulating platelet function and blood coagulation makes them promising targets for the treatment of thrombosis. Further studies may reveal how these enzymes can be effectively utilized to combat thrombosis-related diseases in the clinic.

5. Conclusion

This paper primarily investigates the biochemical characteristics of NTPDase family members and their role in thrombosis formation. The study reveals that NTPDases play a crucial role in regulating platelet activation and thrombosis, highlighting their potential as therapeutic targets for cardiovascular diseases. The paper could further explore the specific molecular mechanisms of how different NTPDase family members interact with platelets. Future research could focus on developing specific inhibitors or activators of NTPDases as potential therapeutic drugs, and exploring the role of NTPDases in other physiological processes and diseases could also be valuable.

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