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Characterization of Apyrase from the Salivary Glands of *Aedes aegypti* Based on Bioinformatics Database

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Abstract

The salivary glands of mosquitoes, which act as disease vectors, contain biological components that facilitate the blood-feeding process and transmit pathogens to the human body. Apyrase, a protein from the salivary glands of *Aedes aegypti*, is known to be an immunogenic protein that influences the host's immunity. It can prevent platelet aggregation by hydrolyzing ATP or ADP into AMP and thus facilitate the blood-feeding process. This mechanism is also exploited by pathogens to obtain entry into the host's body. Vaccination with the apyrase protein could control dengue virus transmission by enhancing the immune response to inhibit the pathogen's entry. To examine the potential of apyrase, it is critical to explore its nature and characteristics by using bioinformatics databases. This quantitative descriptive study involved apyrase protein from the salivary glands of *Ae. aegypti* obtained from several bioinformatics databases, namely UniProt, SWISSMODEL, ENZYME, Rhea, CFSSP, GlyCosmos, STRING, and QuickGo. The findings contribute to a more fine-cut understanding of apyrase as a potential vaccine to inhibit dengue transmission.

Keywords: apyrase, immunogenic, pathogen, transmission, vaccine

Introduction

Infectious diseases still prevail in multiple parts of the globe, especially in tropical regions (Pujara et al., 2019). Numerous studies have investigated the transmission of tropical diseases mediated by arthropod vectors into the human body to prevent pathogen transmission and infection (Senjarini, 2013). The pathogen transmits through several organs in the vector's body until it finally penetrates the host (Luplertlop, 2014). The vector's salivary glands aid in the blood-feeding process and this transmission of pathogens (Liu et al., 2023; Wathon et al., 2015a). For example, the salivary glands of the *Ae. aegypti* contains various biological components that can facilitate blood-feeding in the human body. This mechanism enables the entry of dengue virus into the human body, leading to infection in the host body.

The salivary glands contain substances that aid in transmitting pathogens to the host body, such as vasodilator and immunomodulatory components (Gomez et al., 2018). Vasodilators can widen blood vessels, making it easier for mosquitoes to suck the host's blood. The immunomodulator also possesses immunosuppressive properties to suppress the host's immune system, fostering pathogen transmission (Marin-Lopez et al., 2023). Multiple studies have delved into these immunomodulatory properties of mosquito salivary glands (Arora et al., 2021).

Previous research shows that sustained exposure to vector saliva can protect the host body through shifting the immune response which provides immunity to the host body (Montiel et al., 2020; Wathon et al., 2022). This signifies the potential to control virus transmission through vaccination of the host body with immunomodulatory proteins from the salivary glands of *Ae. aegypti*. The host body will form antibodies to fight the injected immunomodulatory proteins, preventing the transmission of dengue virus.

Immunomodulatory components in the salivary glands of *Ae. aegypti* have different structures and functions (Sun et al., 2020). These components contain protein molecules assisting the blood-feeding process in the human body (Gavor et al., 2022). Several of these proteins are immunogenic, affecting hemostasis and the host's immune response (Oktarianti et al. 2014; Wathon et al., 2020). The immunogenicity of these protein groups has been extensively investigated. One of the immunogenic proteins from the salivary glands *Ae. aegypti* is the apyrase protein (Oktarianti et al., 2015).

The apyrase protein is pivotal in hydrolyzing ATP or ADP into AMP (Oktarianti et al., 2022a). ATP

and ADP molecules are inducers that can bind to platelet cell membrane receptors to trigger aggregation between platelet cells (Oktarianti et al., 2022b). This ability to degrade ATP and ADP can prevent platelet aggregation, subsequently facilitating the blood-feeding on the host body (Fontaine et al., 2011). Apyrase activity can disrupt hemostasis and the host's immune response, thereby increasing the opportunity for pathogen transmission. Further studies are necessitated to understand the characteristics of apyrase from the salivary glands of *Ae. aegypti*.

The potential of apyrase can be explored by analyzing critical information in the bioinformatics database. This study examines the constituent amino acids and biological as well as physicochemical properties of apyrase protein. It also explores various post-translational modifications to the protein. This study is significant for vaccine development, as a measure to inhibit dengue transmission in dengue-endemic areas.

Materials and Methods

Research Site and Time

This research was conducted from February to May 2024 at the Biotechnology Sub-Laboratory of Biology Department, Faculty of Mathematics and Science in Jember University.

Equipment and Materials

This study operationalized both hardware and software. The hardware was an ASUS laptop with Intel® CoreTM i3-1035G1 processor specifications (8 CPUs), ~1.2GHz with 4GB RAM. Some of the bioinformatics database software employed were UniProt (https://www.uniprot.org), SWISS-MODEL (https://swissmodel.expasy.org), ENZYME (https://enzyme.expasy.org), Rhea (https://www.rhea-db.org), CFSSP (https://www.biogem.org/tool/chou-fasman/), GlyCosmos (https://glycosmos.org), STRING (https:/ /string-db.org), QuickGo (https://www.ebi.ac.uk/QuickGO).

The Retrieval of the Amino Acid Sequence of the Apyrase from *Ae. aegypti* Salivary Gland

As the primary data for analyses, the amino acid sequence of apyrase was retrieved from the UniProt database. The search focused on the pre-determined accession number corresponding to the apyrase protein from the salivary glands of the *Ae. aegypti* species. The amino acid sequences were downloaded in fasta format.

The 3D Structure of the Apyrase

The 3D structure of apyrase protein was obtained from the SWISS-MODEL database. The structure was visualized based on the apyrase amino acid sequence previously retrieved from UniProt. Subsequently, the identification and characterization of the protein's secondary structure were conducted using the CFSSP web tool, analyzing each segment of the apyrase protein.

The Characterization of the Apyrase Properties

The information concerning the properties and characteristics of apyrase were collected from the UniProt, ENZYME, Expasy, and Rhea databases. The characterization was conducted using the accession number and amino acid sequence of apyrase, focusing on its functional roles, catalytic activity, cofactors, and binding sites.

The Post-translational Modification of the Apyrase

Information on the post-translational modifications of apyrase was obtained from the UniProt and GlyCosmos databases by using the accession number and amino acid sequence of the target protein. The retrieved information pertained to signal peptide sequences and various glycosylation processes.

The Interaction of the Apyrase

Information regarding the interaction of apyrase with other proteins was obtained from the STRING database. The search for this data was accomplished by using the accession number and amino acid sequence of apyrase. The interactions between proteins were visualized using interaction line models, accompanied by descriptions of the biological activities of each protein projected to interact with apyrase.

Results

Profile of Apyrase Sequence from *Ae. aegypti*

This study utilized the amino acid sequence of apyrase from the salivary glands of *Ae. aegypti*, obtained from the UniProt database with accession number P50635. The gene encoding this protein was referred to as the apy gene. Apyrase from *Ae.*

aegypti mosquito (Yellow fever mosquito) (*Culex aegypti*) consists of 562 amino acids. The molecular weight of apyrase protein is 62,754 kDa. The protein code and the complete amino acid sequence of the apyrase protein are presented in Table 1.

Table 1. The amino acid sequence of apyrase from the salivary gland of *Ae. Aegypti*

>sp|P50635|APY_AEDAE Apyrase OS=Aedes aegypti OX=7159 GN=APY PE=1 SV=2
MAGRPGYSEVIFLYVVSVAVIARATDNMPVNKDVSKLFPLTLIHINDLHARFEETNMKSNVCTQKDQCIAGIARV
YQKIKDLLKEYESKNPIYLNAGDNFQGTLWYNLLRWNVTADFIKKLKPAAMTLGNHEFDHTPKGLAPYLAELN
KEGIPTIVANLVMNNDPDLKSSKIPKSIKLTVGKRKIGIIGVLYDKTHEIAQTGKVTLSNAVEAVRREAAALKKDN
IDIIVVLSHCSYEEDKKIAAEAGDDIDVIVGAHSHSFLYSPDSKQPHDPKDKVEGPYPTLVESKNKRKIPIVQAKSF
GKYVGRLTLYFDEEGEVKNWEGYPVFIDHKVQQDPQILKDLVPWRAKVEAIGSTVVGETMIELDRDSCRDQEC
TLGVLYADGFADQYTNDTFRPFAIIQAGNFRNPIKVGKITNGDIIEAAPFGSTADLIRLKGADIWDVAEHSFALDD
EGRTNCLQVSGLRIVIDISKPVRSRVKKIEVMDYTNPKSDKLKPLDKEAEYYIVVPSYLADGKDGFSAMKRATAR
RTGPLDSDVFKNYVEKIKKVDNLKLGRVIVCKGSKCT

The 3D Visualization of the Apyrase

The 3D structure of apyrase from *Ae. aegypti* was visualized through homology modeling using the SWISS-MODEL database. The protein's sequence data from *Ae. aegypti* was used to generate the 3D structure, identified by the STML ID code 7qga.1. This model was further refined using X-ray crystallography, achieving a resolution of 1.5 Å, which is indicative of high-quality structural data (Ramírez and Caballero, 2018). Generally, protein structures resolved to <2.5 Å are deemed highly accurate. The 3D structure (ID: 7qga.1) is illustrated in Figure 1, showcasing the detailed visualization achieved through these advanced modeling techniques.

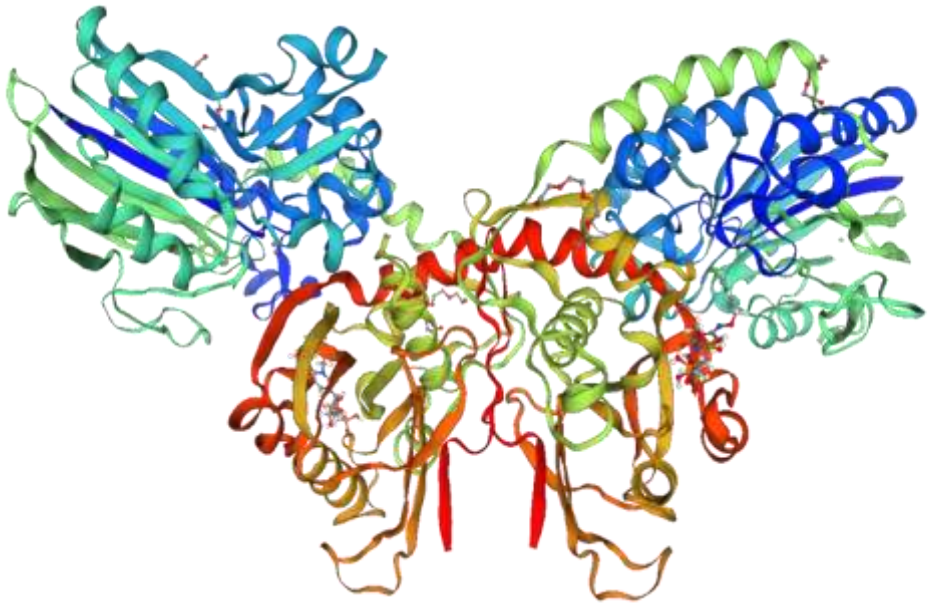

Figure 1. The 3D structure of apyrase (STML ID: 7qga.1) (SWISS-MODEL, 2024)

The 3D structure, based on its conformation, forms a homodimer consisting of two identical protein chains (chains A and B). A protein homodimer is a complex formed by two identical protein subunits that are non-covalently bound together. These subunits share a common amino acid sequence and structural configuration, interacting to create a functional dimer (Zhanhua et al., 2005). Apyrase serves as an enzyme-forming homodimer to become catalytically active. In a homodimer, both subunits possess identical primary, secondary, tertiary, and quaternary structures. The

homodimeric structure of enzymatic proteins is crucial for their biological activity. Dimerization can enhance the thermal and proteolytic stability of the protein, thus leveraging its resilience. Additionally, homodimerization can regulate the protein's activity through allosteric effects, where the binding of a substrate or ligand to one subunit influences the activity of the other subunit. This allows for functional diversity, as different conditions or modifications (e.g., phosphorylation) can influence dimer formation and eventually regulate the protein's function (Mou et al., 2015; Dang, 2022).

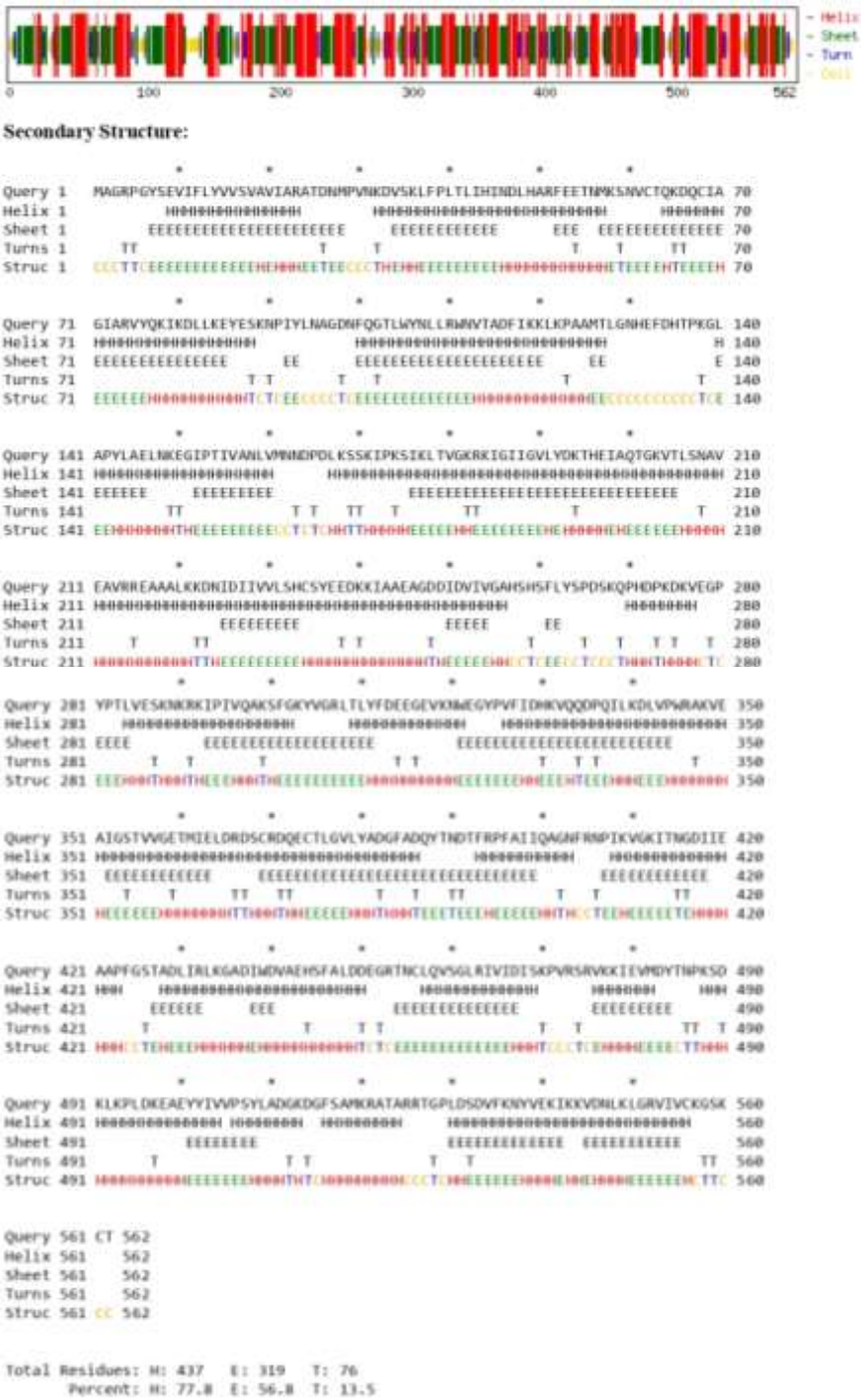

Figure 2. The identification of secondary structure of apyrase (CFSPP, 2024)

The observation of the secondary structure of the protein using the CFSSP webtool identified a secondary structure of the 562 amino acid sequences that made up the apyrase protein. This

structure was found in the form of a helix, sheet, turn, and coil. Based on the secondary structure, the helix structure was the most common (dominant) structure, followed by the sheet, turn, and coil structures. A protein helix structure, specifically referring to an alpha-helix, is one of the common secondary structures found in proteins (Swanson and Sivaramakrishnan, 2014). The alpha-helix provides a stable and flexible structure, contributing to the overall stability of the protein. Helices protein structure can be involved in various functions, including forming the structural framework of proteins, participating in protein-protein interaction, protein-RNA interaction, protein-DNA interactions, and facilitating the passage of ions or molecules across membranes (Woolfson, 2023). The alpha-helix is a fundamental element of protein architecture, contributing to the diverse functionalities, structural integrity and stability of proteins in various biological contexts.

In addition, understanding the interactions between apyrase and its ligands is crucial for biochemistry and pharmacology, including developing therapeutic agents that target nucleotide metabolism. In the 3D structure, several ligands have been successfully identified, as documented more detail in the Table 2.

Table 2. Several ligands in the protein's 3D structure (STML ID: 7qga.1)

Ligands Name	Ligands Name Detail	Number of Ligands	Type of Bonding
Zn	Ion Zinc	2	Non-Covalent
Ca	Ion Kalsium	2	Non-Covalent
BOI	[[(2~{R},3~{S},4~{R},5~{R})-5-[(4~{E})-4-[(4-chlorophenyl)methoxyimino]-3-methyl-2-oxidanylidene-pyrimidin-1-yl]-3,4-bis(oxidanyl)oxolan-2-yl]methoxy-oxidanyl-phosphoryl]methylphosphonic acid	2	Non-Covalent
PG4	Tetraethylene Glycol	4	Non-Functional Binders

Generally, apyrase can associate with and hydrolyze such natural substrates as ATP, ADP, UTP, GTP, and CTP. ATP is the primary substrate for apyrase, which hydrolyzes into ADP (adenosine diphosphate) and inorganic phosphate. ADP is another substrate for apyrase, which hydrolyzes into AMP (adenosine monophosphate) and inorganic phosphate. Similar to ATP, UTP can also be hydrolyzed by apyrase. Another triphosphate serves as a substrate for apyrase. Similarly, CTP can be hydrolyzed by apyrase. Some of the inhibitors to apyrase include suramin and reactive blue 2. Suramin is a known inhibitor of apyrase, which interferes with the enzyme's ability to hydrolyze ATP. Reactive Blue 2 is another inhibitor that can bind to apyrase and prevent its activity (Maloney et al., 2010). Some cofactors of apyrase are calcium and magnesium ions as well as adenosine. Calcium and magnesium (divalent cations) are crucial cofactors for apyrase activity, stabilizing the enzyme structure and participating in the catalytic process. Adenosine is the product of ATP and ADP hydrolysis by apyrase, which can influence inhibition mechanisms (Reno and Novak, 2005).

The Characterization of the Apyrase

Apyrase is crucial for the blood-feeding process of mosquitoes to humans as their hosts (Hughes, 2013). That is achieved by preventing platelet aggregation which depends on the presence of inducers, namely ATP and ADP molecules (Oktarianti et al., 2022). Apyrase can reduce probing time by facilitating the rate of finding the blood-feeding location (Hamasaki et al., 2009).

Apyrase has alternative names such as adenosine diphosphatase, ADPase, ADP-diphosphatase, and ATP-diphosphohydrolase (Expasy, 2024). The catalytic activity of apyrase is to catalyze the change in ribonucleoside 5'-triphosphate + 2 H2O <=> a ribonucleoside 5'-phosphate + 2H+ + 2 phosphate (Rhea, 2024). Apyrase can hydrolyze ATP molecules into ADP. Furthermore, ADP molecules can be hydrolyzed to AMP (Figure 3).

Most hydrolase enzymes belong to the ecto-ATPase group whose biological activity occurs on the cell surface and hydrolyzes extracellular nucleotides (Goding et al., 2003). The catalytic ability of apyrase is different from that of ATPase because ATPase only hydrolyzes ATP molecules. In contrast, apyrase can hydrolyze ATP and ADP molecules (Madry et al., 2018). The majority of cofactors of apyrase are divalent metal cations (UniProt, 2024). These are metal cations with valence electrons of 2 charges, such as Ca2+ and so on.

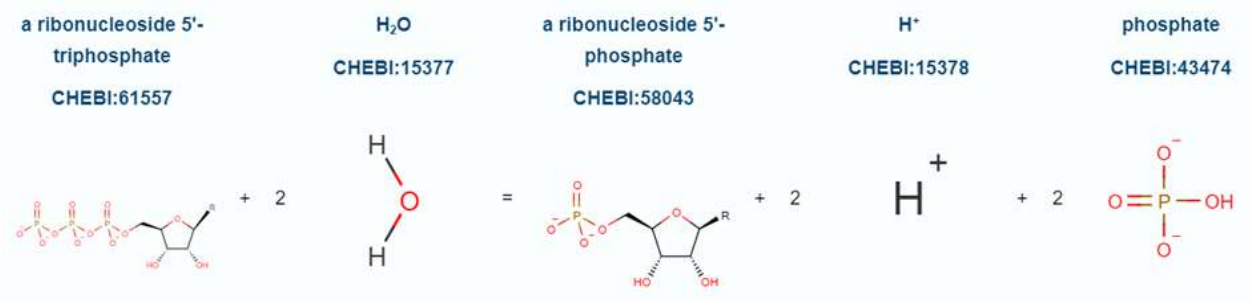

Figure 3. Chemical reactions catalyzed by apyrase (UniProt, 2024)

Apyrase exhibits multiple binding sites, indicating its interactions with various molecular entities and chemical compounds. These entities include metal ions, cofactors, substrates, enzyme products, allosteric regulators, physiological inhibitors/activators, receptor/sensor ligands, transporters/channels, transcription regulator effectors, and chromophores. Additionally, there are individual amino acid sites within the protein that can interact with these chemical entities. Functional proteins, like apyrase, possess these binding sites and interaction sites, which are crucial for their biological activity. For instance, cofactors are essential for the enzymatic activity of many proteins (Bachosz et al., 2023). Table 3 provides a detailed list of the binding sites and interaction sites identified in apyrase. Furthermore, Figure 4 illustrates the specific positions of these binding sites and interaction sites on the amino acid sequence of apyrase.

Table 3. The binding sites and sites on apyrase (UniProt, 2024)

Description	ID Position(s)	Amino Acid of Apyrase	Binding to Chemical Entities
Binding site	47	D	a divalent metal cation 1
Binding site	49	H	a divalent metal cation 1
Binding site	98	D	a divalent metal cation 1
Binding site	98	D	a divalent metal cation 2
Binding site	130	N	a divalent metal cation 2
Site	131	H	transition state stabilizer
Site	134	D	transition state stabilizer
Binding site	233	H	a divalent metal cation 2
Binding site	257	H	a divalent metal cation 2
Binding site	424	F	substrate
Binding site	508-514	YLADGKD	substrate

Figure 4. The position of binding sites and sites of amino acid constituting apyrase from the salivary gland of *Ae. aegypti* (GlyCosmos, 2024).

Post-translational Modification of the Apyrase

Apyrase features a signal peptide sequence spanning amino acid positions 1 to 24, with the specific sequence being MAGRPGYSEVIFLYVVSVAVIARA. This signal peptide, located at the N-terminal end of the protein, directs the newly synthesized protein to the endoplasmic reticulum for subsequent post-translational modification and processing (Hiss and Schneider, 2009). The presence of a signal peptide indicates that this protein is a secretory protein (Owji et al., 2018), released extracellularly. Signal peptides are associated with secretory proteins. Furthermore, signal peptides are known to influence the immunogenicity of proteins. This suggests that the signal peptide sequence of apyrase may contribute to its immunogenicity, making it a potential candidate for future vaccines (Cheng et al., 2022; Vatakis and McMillan, 2011; Kovjazin and Carmon, 2022). Figure 5 illustrates the position of the signal peptide sequence relative to the overall amino acid sequence, underlining its significance in the protein's structure and function.

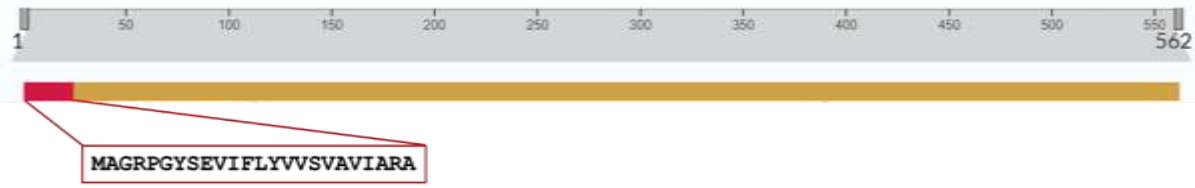

Figure 5. The position of peptide signal (red) in relation to the overall sequence of apyrase (UniProt, 2024).

Apyrase consists of a main sequence of amino acids spanning positions 25 to 562, totalling 537 amino acids. It is composed of two subunits, labeled subunit A and sub-unit B (Oktarianti et al., 2022), indicating its classification as a complex protein. Complex protein structures, characterized by multiple subunits, are often considered potential vaccine due to their immunogenic properties (Cid and Bolivar, 2021). The information regarding the main of amino acid sequence from apyrase is presented in Table 4.

Table 4. The main sequence of the apyrase amino acid

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>sp|P50635|25-562
TDNMPVNKDVSKLFPLTLIHINDLHARFEETNMKSNVCTQKDQCIAGIARVYQKIKDLLKEYESKNPIYLNAG
DNFQGTLWYNLLRWNVTADFIKKLKPAAMTLGNHEFDHTPKGLAPYLAELNKEGIPTIVANLVMNNDPDLKSS
KIPKSIKLTVGKRKIGIIGVLYDKTHEIAQTGKVTLSNAVEAVRREAAALKKDNIDIIVVLSHCSYEEDKKIA
AEAGDDIDVIVGAHSHSFLYSPDSKQPHDPKDKVEGPYPTLVESKNKRKIPIVQAKSFGKYVGRLTLYFDEEG
EVKNWEGYPVFIDHKVQQDPQILKDLVPWRAKVEAIGSTVVGETMIELDRDSCRDQECTLGVLYADGFADQYT
NDTFRPFAIIQAGNFRNPIKVGKITNGDIIEAAPFGSTADLIRLKGADIWDVAEHSFALDDEGRTNCLQVSGL
RIVIDISKPVRSRVKKIEVMDYTNPKSDKLKPLDKEAEYYIVVPSYLADGKDGFSAMKRATARRTGPLDSDVF
KNYVEKIKKVDNLKLGRVIVCKGSKCT
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Apyrase also undergoes a post-translational modification in the form of glycosylation. Glycosylation occurs when a glycan (carbohydrate) molecule attaches to an amino acid from a protein (Eichler, 2019). The majority of proteins developed for therapeutics and vaccines undergo a glycosylation process (Zhang et al., 2016; Adamo et al., 2013). The stability of a therapeutic protein may depend on changes in temperature, pH, and storage time (Wathon et al., 2015b). Glycosylation of a protein plays an important role in protein folding, interactions, signal transduction, mobility, and stability (Roth et al., 2012). Glycan molecules can also serve as specific receptors for proteins that act as enzymes (Gimeno et al., 2019). Glycan molecules can increase the half-life of a protein, especially therapeutic proteins (Fares and Azzam, 2019).

Based on its structure, glycan molecules consist of N-Linked and O-Linked (Yang et al., 2017). N-Linked glycosylation occurs when a glycan molecule is attached to the amino acid asparagine. Meanwhile, O-Linked glycosylation occurs when the glycan molecule is bound to the amino acid serine and/or threonine (Krautter and Iqbal, 2021). Glycosylation in apyrase occurs at the amino acid asparagine (N) at sequences 112 and 390 with the N-Linked type (GlcNAc). The position of glycosylation (glycan molecules) on the apyrase of *Ae. aegypti* is shown more detail in the Figure 6.

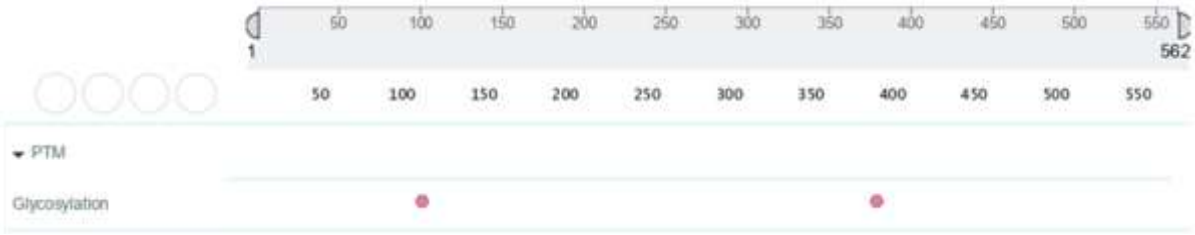

Figure 6. The position of glycan molecule attached to amino acid of apyrase (GlyCosmos, 2024)

The Visualization of Apyrase Interaction

The STRING database provides information on protein-protein interactions that is accessible through computational approaches (Szklarczyk et al., 2019). The data output in STRING shows that there are 10 types of proteins that interact with the apyrase. These 10 proteins are reported in Table 5.

The protein interactions visualized using the STRING database reveal the relationship between the apyrase protein and 10 interacting proteins (Figure 7). This network illustrates the direct and indirect interactions between proteins, with the strength of interactions indicated by the number of connecting lines. The apyrase is directly linked to 7 predicted functional partners, including proteins AAEL004457, AAEL005672, AAEL009191, AAEL006485, AAEL012172, AAEL002269, and AAEL015410. Some of these proteins may serve as mediators for other proteins that interact indirectly with the apyrase protein, highlighting the complexity of protein-protein interactions in the network.

Additional proteins that interact indirectly with the apyrase protein include AAEL015233, AAEL008661, and AAEL009273. The interaction between the apyrase and AAEL015233 is mediated by predicted functional partners, including AAEL012172, AAEL002269, and AAEL015410. Likewise, the interaction between the apyrase and AAEL008661 is facilitated by predicted functional partners AAEL002269, AAEL015410, and AAEL009273. Finally, the interaction between the apyrase and AAEL009273 is mediated by predicted functional partners AAEL002269, AAEL015410, and AAEL009191. These findings indicate that while some proteins may not directly interact with the apyrase protein, several predicted functional partners act as strong mediators, establishing a connection between these proteins.

Proteins AAEL002269 and AAEL015410 are predicted functional partners of apyrase, showing the highest number of interactions with other associated proteins. AAEL002269, in particular, exhibits a wide array of protein interaction relationships compared to other proteins. This protein is believed to play a significant role in mediating interactions among proteins involved in the expression and biological activity of the apyrase protein from *Ae. aegypti* salivary glands. The intricate pattern of protein interactions suggests a complex network that can modulate the immune response of an organism. This implies that apyrase is crucial in modulating host-immunity.

Table 5. List of Predicted Functional Partners of Apyrase Protein from *Ae. aegypti* Salivary Glands (STRING, 2024; QuickGo; 2024)

Accession Number (Protein Number)	**Protein Name**	**Biological Function**
Q1HRI9 (AAEL004457)	Mitochondrial cytochrome c	Electron carrier protein. The oxidized form of the cytochrome c heme group can accept an electron from the heme group of the cytochrome c1 subunit of cytochrome reductase. Cytochrome c then transfers this electron to the cytochrome oxidase complex, the final protein carrier in the mitochondrial electron-transport chain
Q16EF1 (AAEL015233)	Adenosine deaminase	Catalysis of the removal of an amino group from a substrate, producing a substituted or nonsubstituted ammonia
Q16WK9 (AAEL009191)	AAEL009191-PA	Catalysis of the removal of an amino group from a substrate, producing a substituted or nonsubstituted ammonia
Q175Y5 (AAEL006485)	Inosine-uridine preferring nucleoside hydrolase	Catalysis of the hydrolysis of any N-glycosyl bond
Q16WB3 (AAEL009273)	Inosine-5'-monophosphate dehydrogenase	Catalyzes the conversion of inosine 5'-phosphate (IMP) to xanthosine 5'-phosphate (XMP), the first committed and rate-limiting step in the de novo synthesis of guanine nucleotides, and therefore plays an important role in the regulation of cell growth
Q179D4 (AAEL005672)	Adenosine deaminase	Catalysis of the reaction: 2'-deoxyadenosine + H2O = deoxyinosine + NH3
Q17IS2 (AAEL002269)	Purine nucleoside phosphorylase	catalyze the phosphorolytic breakdown of the N-glycosidic bond in the beta- (deoxy)ribonucleoside molecules, with the formation of the corresponding free purine bases and pentose-1-phosphate
Q1DGZ2 (AAEL015410)	AAEL015410-PA	Catalysis of the reaction: AMP + H2O = IMP + NH3
Q16MW6 (AAEL012172)	S-methyl-5'-thioadenosine phosphorylase	Catalyzes the reversible phosphorylation of S-methyl-5'-thioadenosine (MTA) to adenine and 5-methylthioribose-1-phosphate. Involved in the breakdown of MTA, a major by-product of polyamine biosynthesis. Responsible for the first step in the methionine salvage pathway after MTA has been generated from S-adenosylmethionine. Has broad substrate specificity with 6-aminopurine nucleosides as preferred substrates
Q16Y55 (AAEL008661)	AAEL008661-PA	Protein involved in the biochemical reactions with fatty acids

The characterization of apyrase with the aid of bioinformatics databases has revealed its complexity, as indicated by its structural conformation and composition of homodimer subunits. This complexity corresponds to a desirable trait for vaccine candidates. Unlike many proteins, apyrase structures lack an alpha helix, crucial for maintaining protein stability. Apyrase possesses binding sites for ligands/substrates, cofactors, and inhibitors, which determines its biological activity. The presence of a signal peptide at the N-terminal end corroborates that apyrase is a secretory protein. Signal peptides contribute to the immunogenicity of proteins. Additionally, the glycosylation of apyrase can elevate its activity, stability, and immunogenicity. Apyrase exhibits sophisticated protein interactions, underscoring its potential as a vaccine due to its stable and immunogenic characteristics. Such a property plays a key role in modulating an organism's immunity.

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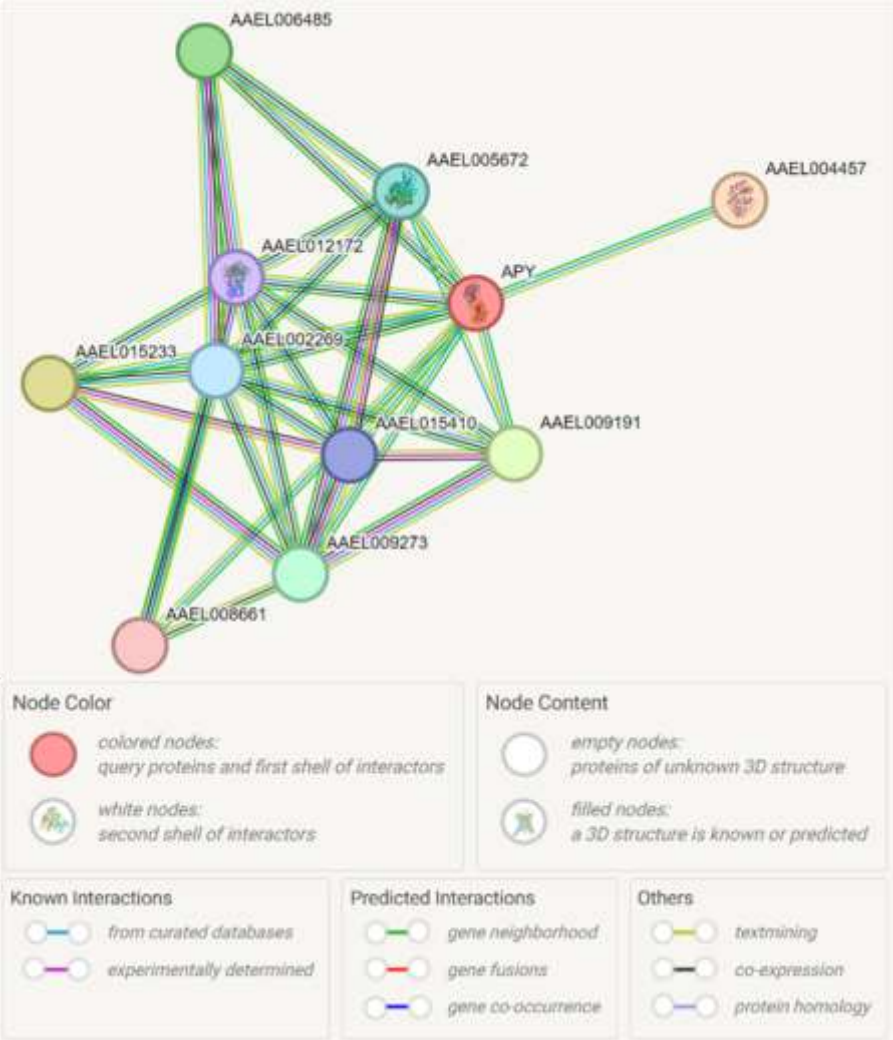

Figure 7. The visualization of the interaction between apyrase and associated proteins (STRING, 2024).

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Proteins AAEL002269 and AAEL015410 are predicted functional partners of apyrase, showing the highest number of interactions with other associated proteins. AAEL002269, in particular, exhibits a wide array of protein interaction relationships compared to other proteins. This protein is believed to play a significant role in mediating interactions among proteins involved in the expression and biological activity of the apyrase protein from *Ae. aegypti* salivary glands. The intricate pattern of protein interactions suggests a complex network that can modulate the immune

response of an organism. This implies that apyrase is crucial in modulating host-immunity.

The characterization of apyrase with the aid of bioinformatics databases has revealed its complexity, as indicated by its structural conformation and composition of homodimer subunits. This complexity corresponds to a desirable trait for vaccine candidates. Unlike many proteins, apyrase structures lack an alpha helix, crucial for maintaining protein stability. Apyrase possesses binding sites for ligands/substrates, cofactors, and inhibitors, which determines its biological activity. The presence of a signal peptide at the N-terminal end corroborates that apyrase is a secretory protein. Signal peptides contribute to the immunogenicity of proteins. Additionally, the glycosylation of apyrase can elevate its activity, stability, and immunogenicity. Apyrase exhibits sophisticated protein interactions, underscoring its potential as a vaccine due to its stable and immunogenic characteristics. Such a property plays a key role in modulating an organism's immunity.

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