A Study of Structural Basis of Collagen-GPVI Interaction

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Abstract

While the immune-type GPVI receptor activates platelets, the way this receptor developed into a platelet under rapid blood flow conditions is not understood. Moreover, the preservation of collagen reactions in non-mammalian animals not expressing GPVI is not apparent. According to this research, the proline-rich GPVI domain enhances GPVI signalling kinetics and is required for maximal adherence to flow collagen. The proline-rich domain accelerates signalling by binding and directly activating the Src family Lyn kinase. Collagen stimulates chicken thrombocytes, but, in strong contrast to platelet activity, they do not form 3-dimensional aggregates under circumstances of arterial flow. Despite being selective, thrombocytes exhibit a decreased integrin density of 2b3 on the cell's surface, with most platelet-specific genes expressed. These results reveal that the GPVI enhances immune signalling kinetics by means of a novel molecular mechanism and demonstrate that weather-cell responses to collagen under flow are partly maintained. In conjunction with stimulus-specific tyrosine phosphorylation of the GPVI/FcR-chain complex and/or CLEC-2, Src and Syk tyrosine kinases are responsible for aggregation of human and mouse platelet. Receptor-deficient platelets show a key role in mediating aggregation for GPVI and/or CLEC-2.

Keywords: *Collagen-GPVI Interaction, 3-dimensional aggregates, blood flow, GPVI enhances.*

1. INTRODUCTION

There are several bimolecular structures in human body. They are also known as macronutrients. The macronutrients are three: protein, lipids and carbs. Calories or energy come from macronutrients. In order to maintain life, the body needs great quantities of macronutrients, thus the word 'macro.' The distinct and unique functions of all bio molecules. Fat compounds present in the human body, proteins, carbohydrates, nucleic acids and. The significance of every bio molecule in the body is its own.

Other than carbohydrates, lipids or fats and nucleic acids, such as DNA and RNA, proteins are one of the four different macromolecules. Macromolecules are huge molecules that carry out tasks inside living beings. In line with its function, the molecular structure of the protein molecule is different. For the normal development of the organism, proteins are necessary. Protein is an important macronutrient for muscle mass development. The nutrients such as proteins are primarily present in animal goods and also in the plant product to some degree. It also occurs in other sources, including legumes and nuts.

Around 15% of the person's body weight is composed of protein. In the healing of injured tissues and cells, proteins play an important function. The human body utilises proteins to repair and construct tissues, function as enzymes, assist the immune system, and act as hormones. Every major function needs a slightly different protein shape. All proteins have the same fundamental components despite their structural variations.

2. REVIEW OF LITERATURE

Isuru Induruwa et al., (2016) examined platelets as important to the development of pathological or physiological nostases of thrombus. Current anti-platelet medicines limit the aggregation of platelets but risk systematic side-effects such as bleeding in patients. New therapeutical methods may need that the thrombogenic collagen receptors on the surface of the platelet be targeted at early platelet adhesion or activation via inhibitory effects on certain

glycoproteins. This review highlights the key role played by GPVI in ischemical stroke and the current methods to limit their activity. In this review, we address the typical hamostatic method and the function of GPVI at plate rupture locations. We talk about how the unique GPVI structure enables collagen to be interactive and generates downstream signals leading to thrombus development.

T. Harma and others (2016) One of the most extensively distributed immune receptors, leukocyte associated immunoglobulin-1 (LAIR-1), attenuates immunological active cell activation in certain locations of collagen. The LAIR-1 collagen-binding domain is similar to the collagen receptor of the platelet activation Glycoprotein VI (GPVI). Since the overlapping collagen binding specificities are likewise shown in both LAIR-1 and GPVI, the common foundation for identification of collagen would emerge. Therefore, to avoid undesired cross reactions during therapeutic intervention, it is essential to acquire knowledge into the molecular interaction of both receptors with their ligand. We have determined LAIR-1's Kristal structure and have mapped it using the titration and mutagenesis of nuclear magnetic resonance (NMR). The essential residues to collagens interaction are R59, E61 and W109. Our data. Strictly conserved in the GPVI and LAIR-1, these residues are not only found in the GPVI collagen binding site previously suggested. Our results show that collagen recognition mechanisms similar to LAIR-1 and GPVI are unexpected. This basic understanding will help to explore special interventions in the immunity and haemostasis of collagen-induced signals.

J H.H. et al (2016) The OSCAR is an immunoglobulin receptor (ig) like that of the collagen which is osteoclastically up-regulated and manifested in a variety of myeloid cells. Oscar has a high sequence and structural similarity with other collagen réceptors in this familia, including glycoprotein VI, Ig-like receptor 1 with a leukocyte related, and B4 with a single collagen sequence as part of the leukocyte receptor complex family. Here we show OSCAR crystal structures in their free and complex form with a three-helic peptide-like collagen (CLP). The structures show the CLP peptide binding exclusively with a total of 661 Å2 solvent-accessible collagen surface, to one of two Ig-style domains, the membrane-proximal (domain 2) of OSCAR. The unique architecture of the OSCAR protein, which has an obtuse angle and a rotation of domain 2 with respect to the membrane-distal domain, facilitates this form of attachment.

KONO et al., (2010), GPVI is the main platelet activation receptor. Loss or inhibition of GPVI produces relatively little bleeding times in animal models but inhibits the development of arterial thrombus. GPVI is thus seen as a powerful target molecule for the treatment of thrombotic illnesses. The AT1 receiver losartan (DuP-753) and EXP3179 have recently been described as an inhibitor of platelet adhesion and aggregation via GPVI. However, the association between losartan and GPVI binding on the molecular level remains unclear.

Taylor, Lewis et al (2014) Inadequate aggregation of platelets presents a cardiovascular risk that is mainly controlled by thienopyridines and aspirin. These medicines have the danger of increased bleeding and 'resistance,' which underlie a push for novel antiplatelet medications. Although they are effective, In order to discover these medications, one approach is the identification of an appropriate pharmacological target and then the identification of tiny modulators. The collagen platelet receptor, GPVI, increases the development of thrombus, is a promising and untapped objective.

In Mishra, and others (2014) Acute heart disease is one of the world's most prominent causes of death and disease. Although recent advances in current anti-platelet treatment substantially decreased the burden of cardiovascular mortality, their clinical advantages are often frequently restricted by bleeding risk due to their worldwide effect on primary haemostasis. Recent advances in platelet biologie explain the interaction between the platelet and collagen at the key position in the arterial thrombosis sequence of events. The development of new therapeutic strategies for platelet-collagen receptors glycoprotein VI (GPVI) is therefore highly interested, because it is promised not only a selective blockage or deficiency of the platelet collagen receptor, but also the exclusive GPVI expression of plates and megakaryocytes, to prevent the off-target harmfulness. Recent progress in platelet function knowledge during arterial thrombosis and the structural and functional characteristics of GPVI platelet are summarised in the current review in order to recognise it as a new antiharvest target.

Molica Filippo et al (2017) All the three main kinds of blood cells are generated in the bone marrow. These include platelets, erythrocytes and leucocytes. While red blood cells are the biggest, white blood cells are tiny pieces and account for a modest portion of blood volume. While red blood cells are smaller. However, by stopping bleeding, platelets have a vital role. Platelets attach, activate and form the platelet plug blocking blood sugar in exposed extracellular matrix in case of artery wall damage. However, the same process may result in acute thrombosis,

producing severe ischemical events, such as myocardic infarction or stroke, when platelets are increased as in atherosclerotic plaque break-up.

3. AMINO ACIDS & PROTEINS

The monomer units of proteins are amino acids. Amino acids are compounds that have an amine group (NH2), a carboxylic acid group (R-C=O-OH), and a side chain (namely R). Carbon, hydrogen, oxygen and nitrogen are the main components of amino acid. The protein chain consists of amino acids which are combined with a peptide bond, which has disulphide connections and are also referred to as polypeptide channels because of the peptide bonding proteins. In biochemistry, the words typically relate to alpha-amino acids, they are very significant. Proteins are biochemical substances comprised of one or more polypeptides that are normally physiologically useful folded into a globular or fibrous shape.

A polypeptide consists of one linear polymer chain of amino acids that links the carboxylic and the next amino acid residues via peptide connections. The amino acid sequence of a protein is determined by the gene sequence encoded in the genetic code. A triple genetic code is applied to each amino acid. The normal amino acids are 20 and the genetic coding for each amino acid is distinct. There cannot be the same genetic code for two amino acids. In general 20 conventional amino acids are specified by the genetic code; however selenocysteine and arcaea-pyrrolysine may be included in some species under particular genetic code. Proteins are produced according to the kind of function to which a protein is performed via a sequence process of core dogma. DNA replication occurs when a DNA replica is produced to the cells that constitute the desired proteins for the production of any protein. This transcript is followed by the transcription of DNA to mRNA. The mRNA molecule decodes the DNA information it is transcribed from.

Shortly after or even during a synthesis, protein residues are frequently chemically changed by post-translation changes that affect the physical and chemical characteristics, folding properties, stability, activity and ultimately protein function. Proteins may be linked to non-peptide groups, which may be termed cofactors or prosthesis groups. Proteins may also cooperate to accomplish a certain function and frequently form stable complexes. The capacity of polypeptides to fold into a globular form, or structure, is one of the most distinctive characteristics. Proteins are quite different in terms of how they fit into a defining structure. Some protéins fold into a very stiff structure and are thus regarded to be one structure with minor variations. Other proteins are rearranged extensively between conformations. This shift in conformity is frequently related to a signalling event. The protein structure therefore acts as a medium through which the function of a protein or the activity of an enzyme may be regulated. Not all proteins that need a folding mechanism to work, as some functions are unfolded.

Protein consists of amino acid macromolecules. Long strands of amino acids are proteins. The protein components are amino acids. That is, the amino acids constitute a protein molecule, similar to the links in a chain. In order to produce particular compounds, protein chains are twisted and folded together in a precise manner then. An amino acids chain is a protein molecule, then twisted to the helical form, plyated into a sheet and twisted back into an intricate globular form. This may infer that a protein molecule consists of the primary, secondary, tertiary and quaternary structures.

1. Sources of Protein

We stated before that protein is a tissue healing factor, and that is why protein in your diet is so essential. However, what are the finest protein sources? And are there several protein types? Let's look at these issues. You might next take a peek in your fridge and determine whether your diet is high in protein or low protein.

2. Types of Proteins

In all living organisms, protein may be found. The kind and quantity of protein in meals may vary but in some way necessarily exists. The protein level in meats, cheeses and nuts is more pronounced than many plant-based alternatives. We will need to study its nutrition label in order to ascertain the protein content of a meal. In the human body, there are many kinds of proteins that perform their own function. There is another biomolecule linked to some protein molecules and they are thus called. One such example is glycoprotein.

3. Conjugated protein

A conjugated protein is a protein that works in contact via covalent or weak connections with other chemical groups. Many proteins are termed simple proteins as they include just amino acids and no additional chemical groups. However, some proteins produce, in addition to amino acids, and they are referred to as conjugated proteins in hydrolysis and other chemical components. In a conjugated protein, the nonamino portion is typically termed its prothesis group. The vitamins constitute most prothesis categories. Based on the chemical composition of its prothetic group, combined proteins are categorised. Examples of conjugated proteins include

4. Lipoproteins

A lipoprotein is a biological component which includes water-bound proteins as well as lipids. Many lipoproteins are enzymes, conveyors, structural proteins, antigenes, adhesins and poisons. Examples include the high (HDL) and low (LDL) density lipoproteins which allow lipids to be transported into the blood stream, the mitochondrane, chloroplastic and bacterial lipoprotein transmembrane proteins.

5. Phosphoproteins

Phosphoproteins are proteins chemically linked to a phosphoric acid substance (see phosphorylation for more). The organic chemical category including Fc, Ulc, Calcineurin, C and urocortin receptors.

6. Metalloprotein

A protein which includes a Metalloprotein ion cofactor. The activities of metalloproteins in cells include enzymes, proteins for transportation, storage and the proteins for signal transduction. In fact, approximately 1/4 of all proteins need metals to perform their activities. The metal ion is typically co-ordinated with the polypeptide and/or macrocyclic ligands of a nitrogen, oxygen or sulphur atoms of amino acids. Metal ion presence enables metalloenzymes, such as redox processes, to execute tasks not readily by a small range of functional groups present in amino acids.

7. Glycoproteins

Glycoproteins are proteins that are covalently linked to side chains of polypeptide, with oligosaccharide chains (glycans). In cotranslational or posttranslational modifications, the carbohydrate is linked to the protein. Glycosylation is recognised for this process. Extracellular portions are frequently glycosylated in proteins that have extracellular regions. Glycoprotein frequently plays an essential part in cell-cell interactions as an integral membrane protein. Glycoproteins are also present in cytosol, although their roles and routes are less well-understood in this section. The biggest and most extensive category of conjugated proteins is glycoproteins. Their spectrum ranges from glycoproteins in glycocalyx-containing cell surface membranes to major leukocyte antibodies.

Since the rough ER and Golgi proteins pass through, they have the addition of carbohydrate to make them glycoproteins. While glycosylation takes occur in the raw ER, it is more often done in the Golgi device. Glycosylation enhances the polarity of the released protein that adds to its solubility and stability. For example, for this reason, the growth hormone is a protein with a certain amount of glucose added.

But some secreted proteins are highly glycosylated to absorb and retain a lot of water. Mucus is an example of highly glycosylar proteins that generate the physical characteristics of the material via glycosylation

4. COLLAGEN

Collagen is a secreted protein that we often encounter in the laboratory. It is the body's most plentiful protein. For instance, a tendon is almost all collagen. It is plentiful, and binds the whole body's cells together in the skin, the bone, and indeed, in extracellular spaces. The cell that produces the collagen is typically a fibroblast.

During the synthesis the three chains are individually synthesised, followed by the removal of signal sequences. Then the three polypeptide chains, remaining in the endoplasmatic reticulum, wrap in the quaternary design around each other. However, this rigid, rod-like structure only forms the middle part of each polypeptide chain. There is a significantly distinct amino acid content on the two ends of every polypeptide chain. So a long bar-like structure in

the medium, with a tangled area at either end, comprises the first quaternary structure. This structure has a relative solubility, termed the procollagen. This is essential because it would be deadly to the cell if the molecules were to be aggregated within the cell.

Collagen is a very versatile protein family consisting of more than 28 members who are highly complex through the use of collagen type combinations, multiple initiatory locations for transcription, alternative splicing models, translation changes and a wide range of expression patterns. Collagen is a very diverse proteins family. Despite this variability, collagen research in the last 60 years have shown that collagen is frequently found as huge fibrillary structures consisting of various right-hand three-helic structures, produced by the combination of 3 left-handed type-II helixes with a one-residue stagger). The G-X-Y repeating triple amino acid sequences, of which G-P-O is the most commonly seen triplet, with O being the 4-hydroxyproline, are critical to the creation of these triple helical structures. In such structures, the obligatory glycine of the triplets is buried within the core of the helix and is inaccessible to solvent, while residues of X andY may be accessed with solvents, with collagen fibrils providing essential stability via interchain hydrogen connection. The variety of imino and amino acids within these repeated sequences of X and Y lead to variations in collagen helical parameters that range between a tight left hand 72 and 103 helical symmetry, thus offering a flexible and more accessible motive for the detection of collagen-binding proteins

Collagen is one of the vessel wall's main components. If it damages the ship wall, platelets stick to the exposed collagen surface and are activated, resulting to the development of the thrombus. Many proteins on the platelet surface have been reported to be potential colagen receptors, however only two of them exhibit their respective collagen receptor characteristics under normal conditions: glycoprotein (GP)1 VI, and integrate α2β1(GPIa/IIa) respectively. (GPIa/IIa).

Platelets with either α2β1 or GPVI integrin deficiency exhibit a lack of collagen reactivity and integrin α2β1 antibodies such as FAB 6F1 and P1E6 and a collagen-induced platelet aggregation with the FAB fragment of anti-GPVI blocked antibody. Snake venom convulxin (Cvx) and collagen-related peptides (CRP), which imitate the triple collagen helix, may individually activate the plates by selectively binding to GPVI. There were many studies showing the connection of collagen, CRP and Cvx to GPVI led to platelet activation via tyrosine phosphorylation of the α-chain FC receptor, Syk, phospholipase C, and many other proteins, indicating that GPVI is a major receptor for platelet activation generated by collagen.

5. WHAT ARE GLYCOPROTEINS?

In the cell membrane or surrounding it, proteins are seen floating. They move and interact with the surroundings of the cell. Glyco is a scientific prefix which meaning 'sugar.' Single proteins with a sugar linked to them are glycoproteins.

On the exterior of the plasma membrane, the sugar faces out, glycoproteins are always present. This is a picture of the glycoprotein-marked plasma membrane.

Glycoproteins are proteins containing sugar residues which are covalently linked. The hydrophilic and polar properties of sugars may alter the protein to a significant degree of chemical characteristics. Sugars are frequently needed to work effectively in a glycoprotein and reach the cell or organism's final goal. Glycoproteins are often present as membrane proteins or as an extracelular matrix on the surface of cells in which they operate. In cell-cell interactions and mechanisms for infections of bacteria and viruses these cell surface glycoproteins play a crucial role.

In two areas of the cell, sugar is attachable to a protein, the N-linked reticulum, and the Golgi apparatus, which generates O-linked sugars. The N-linked glycoproteins are sugar-linked and O-linked glycoproteins are oxygenated to the sugar. The glycoproteins are sugar. The various N- and O-linked sugar structure gives them various functionalities.

The structure and mechanism of the glycoproteins are three types: N-linked glycoprotein, O-linked glycoprotein and nonenzymatic glycosylated glycoprotein.

In two membrane-bound cell organelles, the raw endoplasmic reticulum, and the Golgi apparatus, n-lined glycoproteins are produced and modified. On the surface of the raw endoplasmic reticulum, the glycoprotein protein component is composed of a consecutive amino acid addition to create a linear polymer termed polypeptide. For polypeptide synthesis, 20 different amino acids may be utilised. The particular order of the amino acids in the polypeptide is crucial and is referred to as the sequence of amino acids. The production of N-linked glycoproteins depends on one of twenty amino acids utilised in polypeptide synthesis: asparagine (C 4 H 8 N 2 O 3).

Glycoproteins N-linked: In glycoprotein from the mammalian cells, the main carbohydrates attachment is N-glycoside connection. A consensus sequence of amino acids, Asn-X-Ser/Thr (N-X-S/ T,) where X is any amino acid except proline is located on the attachment of carbohydrate to n-linking glycoproteins. Approximately 65% of all proteins include at least one incidents of N-X-S/T consensus, analysed by the human protein sequences.

The many enzymes of the superfamily glycosyltransferase are used for all sugar attachments of the N-linked glycoproteins. N-linked glycoproteins contain asparagine residues in a polypeptide connected to the R side chain. Carbonates are found always in the amino acid sequences, followed by some more amino acid (-Asn-Xaa-Ser/Thr) followed by a serine or threonine residue. One of the sugars in a polypeptide is not linked to carbohydrate. Rather than that, the protein is translated into the raw, endoplasmic reticula; a big prefabricated carbonate comprising 14 or more sugar residues is linked to asparagine. The carbon dioxide of the glycoprotein will then be changed by enzymes to eliminate some sugars and bind others when the glycoprotein is freshly produced from the raw endoplasmic reticulum to the Golgi device and to other cell places. Various N-related glycoproteins will ultimately become part of or secreted from the cell membrane.

Glycoproteins O-linked: O-linked glycoproteins are synthesised directly onto the polypeptide via the step-by-step addition of nuclear activated sugars. Atomic suger is combined with UDP, GDP (as with mannose) or CMP (for instance, NANA). As with N-linked glycoproteins, the many enzymes of the glycosyl transferase superfamily carry out all the sugar compounds of O-linked glycoproteins. The addition of sugar residues in Golgi apparatus to the hydroxy side chain of the serine and threonine residue is typically generated by the addition of O-linked glycoproteins. O-linked glycoproteins are synthesised by addition of a single sugar residue at a time, as opposed to N-linked glycoproteins. Many o-linked glycoproteins are released into the extracellular matrix around the cell.

6. CONCLUSION

The last few years have seen major advances in understanding how platelets interact with collagen and the events that initiate primary hemostasis and arterial thrombosis. The cloning of GPVI, the generation of mice deficient in collagen receptors and their associated signaling molecules, and the availability of new antibodies and collagen-derived peptides allowed detailed in vitro and in vivo studies on the role of the individual candidate receptors in the complex process of platelet tethering, activation, adhesion, aggregation, and procoagulant activity. These studies have changed the long-standing concept of platelet-collagen interactions, the so-called 2-site, 2-step model, which proposed 21 as the major collagen receptor in hemostasis and thrombosis. This hypothesis had been based on the assumption that the integrin is constitutively in a high-affinity conformation and is essential for the initial firm arrest of platelets on collagen. However, it is now recognized that 21, like the other integrin's, is in a low-affinity state on resting platelets and requires inside-out signals to efficiently bind to collagen.

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