Understanding Bacterial Endotoxin

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Abstract:

Bacterial endotoxins, also known as lipopolysaccharides (LPS), are essential components of the outer membrane of Gram-negative bacteria. This abstract explores the importance of the biochemical structure of endotoxins, their toxicity, conventional methods of endotoxin detection, and the intriguing interactions between endotoxins and proteins.The biochemical structure of endotoxins is characterized by their complex, amphiphilic nature. This amphiphilic character, with lipid A being the hydrophobic component and the polysaccharide chain being hydrophilic, is crucial for their biological activity. The specific arrangement of lipid A, core oligosaccharide, and O-antigen plays a significant role in their pathogenicity and immunogenicity, making it vital to understand these structural aspects.Endotoxins are notorious for their potent toxicity, and even trace amounts can induce severe inflammatory responses in humans. This toxicity is attributed to the ability of endotoxins to activate the immune system through Toll-like receptor 4 (TLR4) signaling, resulting in the release of proinflammatory cytokines. Therefore, the detection of endotoxins in pharmaceuticals and medical devices is of paramount importance to ensure product safety.Conventional methods of endotoxin detection have evolved over the years and are predominantly based on the Limulus amebocyte lysate (LAL) assay. This method relies on the clotting of the LAL in the presence of endotoxins and is highly sensitive and widely used. However, alternative methods such as recombinant Factor C-based assays and mass spectrometry are emerging as viable options, offering improved specificity and reliability.Endotoxin-protein interactions constitute a fascinating aspect of endotoxin research. These interactions play a crucial role in modulating the immune response, influencing host-pathogen interactions, and have potential applications in biotechnology and medicine. The characteristics of endotoxin-protein interactions, including the role of proteins like LPS-binding proteins (LBP), CD14, and MD-2, are central to understanding the mechanisms underlying endotoxin recognition and signaling.

Keywords: Polymerase chain reaction, Rabbit pyrogen test, Limulus amebocyte lysate test

Introduction:

O-antigen, core polysaccharide, and lipid A make up the majority of an endotoxin. The hydrophilic and outermost region of the endotoxin is where the O-antigen is found. It is composed of 1 to 40 repetitive units that are peculiar to each species and give bacteria their serological specificity.The inner core, which connects to lipid A, and the outer core, which connects to O-antigen, are the two components of the core polysaccharide[1]. The outer core is more diversified than those in the inner core, which primarily comprises residues of Kdo and L-glycerol-D-manno-heptose (HEP).[2] A tool-like receptor (TLR4) on immune cells secretes pro-inflammatory cytokines as a result of the highly conserved lipid A.[3] The outer membrane of Gram-negative bacteria is made up of endotoxins. A wide range of biological activities are elicited by isolated endotoxins when given to laboratory animals. These biological activities are also present during Gram-negative septic shock.Lipopolysaccharides (LPS) are endotoxins. The lipid A, the core oligosaccharide, and the O-specific polysaccharide are three covalently connected sections that make up LPS in Enterobacteriaceae and many other Gram-negative bacteria. Gramnegative bacteria have a wide range of O-polysaccharide structure and composition, which affects the parent bacterial strain's serological specificity. The structure and composition of the core oligosaccharide are less varied because many different bacterial species share the same core structure. . The least structurally variable component of the LPS molecule is lipid A, which shares a common structure and content with most Gram-negative bacteria.[4-6] All three components of the LPS molecule are immunogenic, causing the production of antibodies that bind to particular epitopes in the affected area. LPS only has lipid A as its biological component because the polysaccharide lacks any harmful properties. 2 The broad range of biological functions that were discovered to be expressed by isolated free lipid A or purified LPS are summarized in Table I. As can be seen from the table, endotoxin's actions are not necessarily deleterious; in fact, some of them, like the ability to induce tumor necrosis and act as an adjuvant, might be advantageous to the host. The

biological effects of LPS are not caused directly by the LPS molecule, but rather by endogenous mediators that are created after endotoxin interacts with LPS-sensitive cells. Cells called macrophages mediate the harmful effects of LPS[7-9]. and one of the main mediators of endotoxin's lethal action is turnout necrosis factor alpha (TNF).[10-12] Lipopolysaccharide-binding protein (LBP), bactericidal/permeability-increasing protein (BPI), an amyloid P component, a cationic protein, enzymes used in the biological endotoxin assay (anti-LPS), a lysozyme, and a lactoferrin are some of the biomolecules that have reportedly been shown to interact with endotoxins.[13] Hypothetically, either affinity interaction, hydrophobic interaction, or ionic binding can affect how endotoxins interact with biomolecules. Following the interaction of biomolecules with endotoxins, both molecules typically aggregate or disaggregate. It is believed that the endotoxin aggregate's physicochemical change causes its toxic effects to manifest in vivo.[14] These interactions may also mask endotoxins, making it challenging to remove them in later processes. The'masking of endotoxin' effect may also alter the Limulus coagulation cascade in the LAL-based endotoxin assay, leading to false-positive results.[15] Endotoxin contents in some biopharmaceutical products are frequently underestimated when LAL are used during the quantitative analysis, according to Chen and Vinther, because of the endotoxin masking effect.[16]

Biochemical Structure of Endotoxin:

Endotoxin refers to an inherent fraction found in the outer membrane of all gram-negative bacteria, and its more accurate biochemical name is lipopolysaccharide (LPS). Endotoxins are thought to be a non-concrete category of biomolecules that are released when bacterial cells die and result in toxic effects like fever, septic shock, multiorgan failure, and even death. Lipid A, the active component of the LPS biochemical structure, is actually controlled by these immune responses. With a hydrophilic, negatively charged bisphosphorylated diglucosamine backbone and a hydrophobic domain made up of six (Escherichia coli) or seven (Salmonella) acyl chains connected by amide and ester linkages, lipid A serves as the membrane anchor for LPS molecules. Lipid A's distinct structural features give LPS molecules their biological properties, including their specificity and affinity for related proteins.The core oligosaccharide and O-specific polysaccharide chain make up the other two components of LPS molecules. An inner subdomain, which is directly connected to Lipid A, and an outer subdomain, which offers an attachment site for the O-specific polysaccharide chain, make up the core oligosaccharide of LPS. Compared to inner cores, the covalent structures of outer cores are more variable. The role of the core oligosaccharide is unknown other than to provide linkage.The O-specific polysaccharide chain is connected to the outer core's terminal and is out in the bacterial cells' environment. The O-specific polysaccharide chain functions as a type of immunogenic substance that may assist bacteria in evading the immune system. The O-specific polysaccharide chain has a very diverse range of structural characteristics when compared to core oligosaccharide and lipid A. Salmonella's O-specific polysaccharide chain has more than 1000 unique immunochemical variants, whereas some LPS molecule structures lack O-specific polysaccharide chains entirely.[17-20]

Toxicity of Endotoxin:

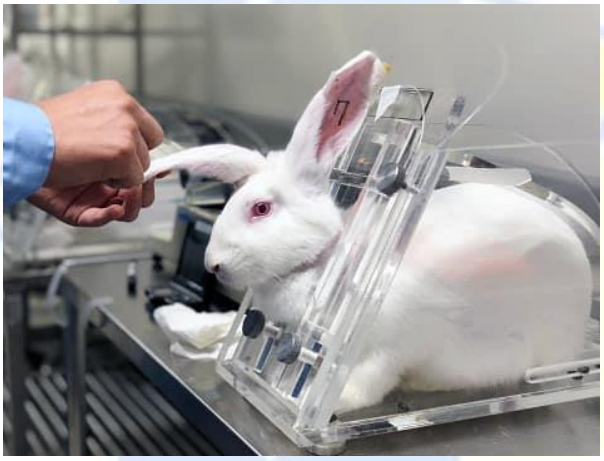
The death of gram-negative bacteria cells releases LPS molecules with a high degree of chemical stability into the environment. Due to damaged intestinal mucosa, LPS molecules can enter the bloodstream and cause endotoxemia, which can result in symptoms such as altered cellular and organ structure and function, increased body temperature, altered hemodynamics, and septic shock. Tumor necrosis factor, interleukin-6, platelet activating factor, and other inflammatory cytokines are overexpressed by the activation of the innate immune system when LPS molecules enter the human body through the liver. This results in systemic inflammatory response syndrome, which has been linked to severe acute respiratory syndromes, cancers, large-area burns, and acute peritonitis. Endotoxin-induced shock's high mortality rate continues to be a significant clinical issue, particularly in patients who are immunosuppressed and in poor health.Additionally, when an organism is sterilized, endotoxin is not removed; rather, the release of LPS occurs when cells die. If gram-negative organisms were present before sterilization, the endotoxin of the organism still exists even though the products may have been sterilized. In the fields of biological products, medical devices, parenteral drugs, food and water security, etc., testing for this LPS in the finished products is a crucial component of ensuring the safety of the sterilized products. The development of new detection techniques is discussed in this article along with an introduction to LPS detection methods. Because of their inherent qualities of quick response, simple operation, low cost, high sensitivity, and high specificity, which satisfy the development requirements of endotoxin detection, the biosensors using LPS affinity components as sensing elements are summarized. Future integrated and miniature endotoxin detection devices with high sensitivity and outstanding stability will be commercially available thanks to advancements in microfabrication and nanomaterials.[21-27]

Endotoxin detection:

Conventional method of endotoxin detection:

Rabbit pyrogen test:

Hort and Penfold made the initial discovery of the rabbit pyrogen test (RPT) in 1912, and Florence B. Seibert later presented it.[28] Its detection method is based on administering pharmaceutical parenteral drugs to rabbits and monitoring the creatures for a rise in body temperature or a fever.[29] Endotoxin concentrations as low as 0.5 EU/ml can be detected using this method.[30] Since rabbits exhibit a sensitive and focused immune response similar to that of humans, they were chosen as the test model.[31] The in vivo technique, still used on the majority of blood products, especially in Japan, is crucial in preventing endotoxin contamination. The application of this method has been constrained by the use of animal models, particularly when a large number of samples need to be analyzed. Additionally, Ochiai et al reported that when compared to other traditional methods of endotoxin detection, the RPT had a limited sensitivity and accuracy.[32] RPT, the oldest and most basic endotoxin detection method, involves injecting the questioned biological sample into live rabbits and watching for a fever to appear. This approach is based on the idea that when endotoxins are present, fever patterns in rabbits and people are similar. It was decided that a fever was defined as a temperature increase of 0.5°C over the course of 180 minutes following injection. Even though the method appears simple, the limit of detection (LOD) is 0.5 EU/ml, which was thought to be accurate when this method was created in 1912. This method has received praise for its accuracy; since it is an in vivo method, it is simple to accept the test's findings because the researchers can see the test subject displaying infection symptoms. But observing the test subject experience the side effects of endotoxins offers a strong case for their presence in the sample. This approach is frequently criticized. In general, the scientific community is moving away from using live subjects in experiments, especially when using animals. Although this test was once regarded as the best in the field and is still used in some areas of Japan, it is now criticized for the large number of samples required and its near-obsolete sensitivity and accuracy in comparison to other techniques.[33-37]

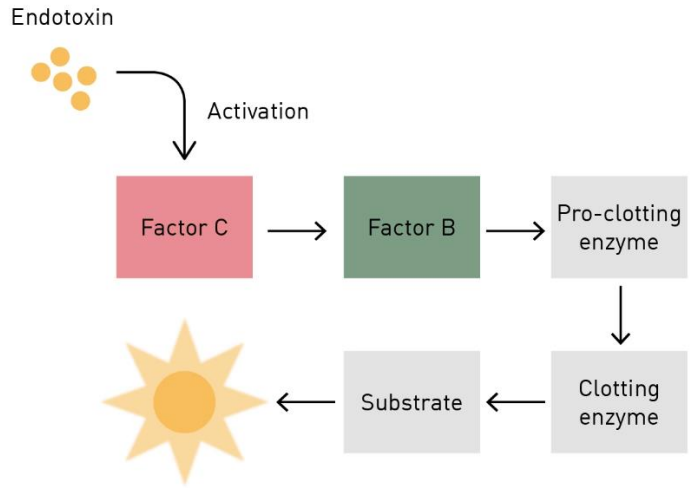


Img: Rabbit pyrogen test

Limulus amebocyte lysate test:

In the 1960s, Levin and Bang made the discovery of the Limulus Amebocyte Lysate (LAL) test. It is most commonly referred to as the indirect animal test and uses a blood extract from horseshoe crabs (Limulus polyphemus). The gel-clot, turbidimetric, and chromogenic techniques can be divided into three basic categories for this method (Figure 2). The gel-clot method is less precise and sensitive than turbidimetric and chromogenic LAL tests.[38] The horseshoe crab blood extract used in the LAL test, which is exposed to endotoxins, causes clots to form. According to[29] the LAL test is three to 300 times more sensitive than the RPT technique. Compared to the RPT test, which can only detect 0.5 EU/ml, it can detect as little as 0.03 EU/ml.[39] Because it is based on the endotoxin-induced coagulation response that is triggered by the binding of endotoxin to factor C in LAL, the LAL test is also known as the Bacterial Endotoxin Test (BET).[40] Despite being widely recognized as a valid technique for determining pyrogenicity, the LAL test is only applicable to the part of Gram-negative bacteria's cell wall. As a result, it is not applicable to samples with a lot of protein.[41] The LAL test is unable to accurately quantify in vivo endotoxin activities in the case of endotoxin contamination in veterinary vaccines.[42] The LAL test cannot be used on samples that contain free metal ions because they can change the measurement's sensitivity, which lowers the test's accuracy. As a result, efforts to develop ion-based endotoxin removal methods have been hampered. In order to reduce the measurement variability brought on by the presence of metal ions in the samples being examined, the LAL method was modified in 201.[43] In a nutshell, the

modification involved reconstructing the sample so that all metal ions in each sample had an analogous composition. . To further reduce the inhibition/enhancement effect of metal ions on the LAL activity without compromising endotoxin sensitivity, a minimum sample dilution (MSD) of 1000-fold was introduced. To lessen the interaction between the endotoxin and the metal ions, 5 mM EDTA was added to each sample.Aside from the problems with LAL specificity and sensitivity, the overfishing of horseshoe crabs for their blood poses a threat to their population.[41] Even though the creatures are put back in the water after the blood is collected, it seems that 20% of the horseshoe crabs perish.[45] Factor C, the key element of the endotoxin-mediated cascade, has been converted into recombinant LAL. Recombinant factor C,[46] functions as an endotoxin biosensor because it can identify the presence of endotoxin following thorough analysis of enzymatic activity. Recombinant factor C was added as a potential replacement for the LAL test in the 2014 revision of the Guidelines for Using the Test for Bacterial Endotoxin by the European Directorate for the Quality of Medicines and Healthcare (EDQM).[47]

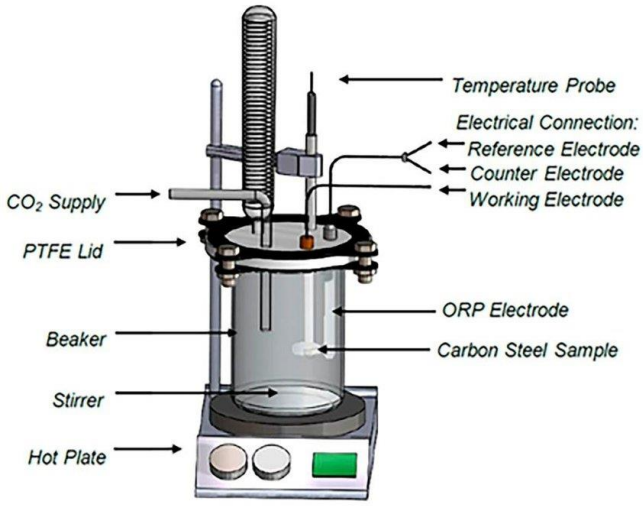


Img: Limulus amebocyte lysate test

Electrochemical technique:

A concept known as electrochemical impedance spectroscopy (EIS) is the foundation for the majority of electrochemical biosensors. Electrodes must be inserted into the desired solution to be tested for EIS, and a sinusoidal alternating current signal—typically ranging from 2 to 10 mV—must be delivered through the solution. The frequency of these sinusoidal waves can be changed to produce an impedance spectrum.[48] To lower electric resistance, the electrodes have a metal coating. When endotoxins come into contact with the electrode-protein complex, they bind to the proteins because the proteins are highly selective to endotoxin components. Endotoxin neutralizing proteins are the name given to these proteins.[49] Endotoxins increase the electrode's resistance when they bind to the ENPs on the electrodes. This was the case in an experiment conducted by[50] who built an electrode out of gold and a complex of myeloid differentiation 2 (MD2) and human recombinant toll-like receptor 4 (rhTLR4) proteins. They subjected these electrodes to a variety of endotoxin-containing solutions, and for each concentration, they generated impedance spectra. At higher endotoxin concentrations, the maximum current across all potential differences was lower.[51]The study also revealed that this specific biosensor had high endotoxin specificity to avoid producing erroneous positive results. The sensor's LOD was 0.0002 EU/ml, which was less than the 0.03 EU/ml standard LAL test limit. Once endotoxins are bound to TLR4-MD2 complexes, this system's single-use electrode becomes a significant drawback. Endotoxins could be detected using metal complexes immobilized on a gold electrode at concentrations as low as 0.001 EU/ml.[52] An array of nanochannels that have been modified with polymyxin B (PMB) and have been used to make porous silicon membranes (pSim)-based electrochemical biosensors have a high affinity for endotoxins. The LOD of 18 EU/ml was displayed. These sensors demonstrated the ability to detect endotoxins from different bacterial strains, including E. coli and S. typhimurium, without the use of labels.[53] Studies have also described highly sensitive electrochemical biosensors based on gold electrodes modified with peptides that were used to detect endotoxins with a LOD of 0.04 EU/ml.[54] In comparison to biologically based techniques, this method was quicker, more accurate, and, in most cases, more cost-effective.[55] Amperometric and potentiometric methods are two additional electrochemical techniques. The most typical electrochemical sensor for the detection of endotoxins has been described as an amplimetric transducer.[56] They operate according to the same EIS tenet, which states that the concentration of the studied sample has a linear relationship with the measured

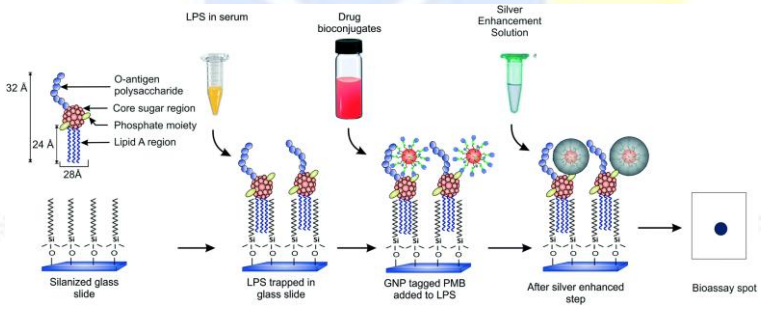
current. For quick and affordable testing, this method can use disposable, premade test strips.[57] Potentiometric methods are noteworthy because they were the first biosensor to be able to detect endotoxins in real-time, despite having LODs that are relatively high, 1–5 EU/ml.[48,58] In comparison to biological methods, the processes used to create the electrodes, measure them, and use them are more labor- and complexity-intensive.[59] They need more advanced personnel and tools to be operated than RPT or LAL tests.[60]



Img: Electrochemical technique

Optical techniques:

A liquid crystal (LC)-based optical sensor for highly sensitive endotoxin detection is one such example. Endotoxin-specific single-stranded DNA aptamers, which are endotoxin-selective biosensor probes, are used to create LC-based optical biosensors. The linear endotoxin detection range of the LC-based aptamer optical biosensors is from 0.05 to 1,000 EU/ml, with a LOD of 5.5 EU/ml. The biosensors' recovery is maximized because they barely interact with the biomolecules. These optical methods can be broadly categorized into three groups: luminescence, surface plasmon resonance (SPR), and electrochemiluminescence, all of which rely on visual changes.[61] When it comes to the direct or indirect detection of pathogenic bacteria and their toxins, optical transducers are particularly appealing. Due to the availability of various variants with various spectrochemical properties, it gained popularity right away. When the target analyte binds to receptors immobilized on the transducer surface, tiny changes in spectophotometric parameters like refractive index or thickness can be detected by these sensors.[62]

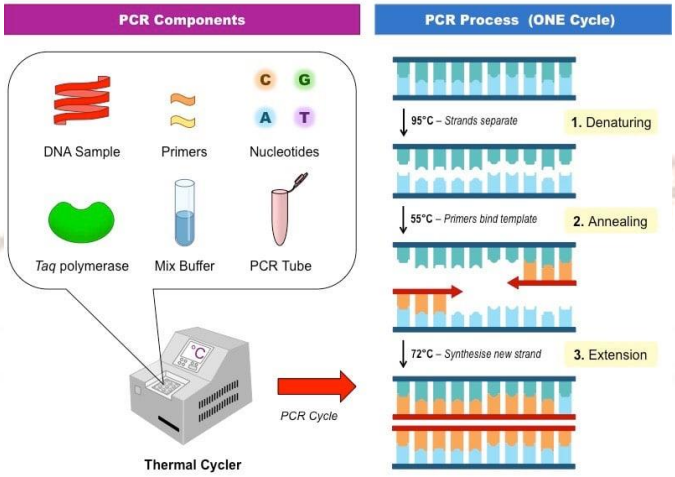


Img: optical techniques

Polymerase chain reaction:

PCR is a widely used, common method that is essential for accurately amplifying DNA from one or a few copies to millions of copies. A variety of PCR variants, including Real-time PCR, Reverse Transcriptase PCR (RT-PCR), Nested PCR, and Multiplex PCR, have been developed to identify pathogenic bacterial endotoxin. Additionally, it has the benefit of being able to be connected to other methods such as Surface Acoustic Wave Sensor (SAW), Fluorescence in Situ Hybridization (FISH), Most Probable Number (MPN-PCR), Lightcycler real-time PCR (LC-PCR), PCR-Enzyme linked immunosorbent assay (PCR-ELISA), and sandwich

hybridization assay. Compared to other available conventional techniques, PCR is significantly quicker and takes up less time. Additionally, PCR is fully automated, doesn't call for pre-enrichment procedures, can be coupled with other methods, and yields accurate results. Multiplex PCR, out of all the PCR variations that are currently available, has the distinct advantage of simultaneously detecting multiple organisms by using various primers to amplify DNA regions. The main drawback of the majority of PCR variants is their inability to differentiate between living, viable cells and dead, non-viable cells. RT-PCR and EMA-LAMP can, however, be used to fill this gap. Despite all the benefits, there are still some drawbacks, such as the labor and time requirements and the need for post-amplification processing steps like gel electrophoresis. Quantitative PCR analysis and endotoxin concentration successfully established a correlation.[63-65]



Img: Polymerase chain reaction

Endotoxin–protein interaction:
Analysis of endotoxin–protein interactions:

Electron microscopy:
The structure and chemistry of biomolecules have been determined using scanning electron microscopy (SEM) and transmission electron microscopy (TEM) at resolutions ranging from micron to subnanometer.[66] used SEM to examine the surface morphology of bacterial cells following incubation with peptides and eosinophil cationic proteins (ECP).[67] They looked at the degree of cell surface damage and hypothesized that endotoxin and ECP interaction was to blame for the damage. Additionally, they examined the samples' agglutination level using TEM, which was consistent with the SEM analysis.The disaggregation of endotoxins following incubation with apolipophorin III (apoLp-III) was successfully observed under TEM in[68] The ability of lipopolysaccharide transport protein (Lpt) to disassemble endotoxin aggregates even at low concentrations has also been demonstrated using TEM.[69]

Dynamic light scattering (DLS) analysis:
The size distribution profile of nanoparticles in suspension has been determined using DLS, also referred to as Photon Correlation Spectroscopy[70,71] DLS results in decreasing mean endotoxin diameter after incubation with apoLp-III in the study of endotoxin-apoLp-III interaction, further demonstrating the function of apoLp-III in endotoxin disaggregation.[68]Polymyxin B has been discovered to promote endotoxin aggregation in contrast to apoLp-III.[72] The polymyxin B-endotoxin interaction was confirmed by the DLS analysis to be concentration-dependent. Despite the high peptide concentrations, polymyxin B was unable to enlarge endotoxin aggregates at a concentration of 0.3 mg/ml. However, at a concentration of 3 mg/ml of endotoxin, higher levels of polymyxin B may cause the endotoxin aggregates to change into a larger form. The aggregative interaction of mobile divalent metal cations with endotoxins and plasmid DNA has been studied using DLS analysis. This analysis was done to determine the hydrodynamic size of endotoxin aggregates when certain divalent cations were present. It was determined that Zn2+ was better able than plasmid DNA to selectively bind endotoxins, removing endotoxins from plasmid DNA by 490 percent. Endotoxins and green fluorescent protein (GFPuv) have been found to interact.[73] performed DLS analyses on endotoxin-GFPuv and pure GFPuv. Based on the significant correlation between GFPuv aggregate size and endotoxin concentration, they came to the conclusion that endotoxins caused GFPuv to aggregate.

Fluorescence resonance energy transfer (FRET):

When used with optical microscopy, FRET relies on the energy transfer between two molecules separated by a few nanometers. It is currently popular in biomedical and drug discovery research.[74] A donor molecule absorbs light, followed by an acceptor molecule receiving energy emission-free transfer via a dipole-dipole coupling and the acceptor molecule emitting light.[75] The ability of peptides (Pep 19-2.5, Pep 19-2.5KO, and Pep 19-8) to intercalate into phospholipid liposomes or endotoxin aggregates from Salmonella minnesota strain R60 has been studied using FRET.[76] It was discovered that all three peptides intercalated into endotoxin aggregates almost at the same amplitude. The peptides' ability to intercalate into typical phosphatidylcholine and phosphatidylserine liposomal membranes was also revealed by the FRET analysis. FRET was used by Brauser[75] to investigate how endotoxins and the antibiotic enrofloxacin interact. In that experiment, the donor was the compound 7-nitrobenzo-2-oxa-1,3-diazole (NBD), and the acceptor, 9-(2-carboxyphenyl)-3,6-bis(diethylamino)-xanthyliumchlorid (Rhodamine), was a phosphatidylethanolamine. Before performing the FRET analysis, the endotoxin vesicles were prepared and incubated with enrofloxacin. The outcomes demonstrated that after the incubation with enrofloxacin, the signals from the donor and acceptor molecules did not change, and thus Brauser[75] concluded that the endotoxin vesicles did not interact with anything.[77] created a high-throughput screening method to find new substances that prevented endotoxin from adhering to CD14. The time-resolved intermolecular FRET (TR-FRET)-based cell-free screening system was used to find new inhibitors of the interaction between endotoxin and CD14 in a library of secondary metabolites from microorganisms.

Docking program:

A computational technique called docking "docks" small molecules into the structures of large target molecules and "scores" how complementary those molecules might be to binding sites.[78] In the study of endotoxin behavior, docking programs are primarily used to: (1) produce an accurate structural model; and (2) produce an accurate prediction of activity. The study of the relationship between endotoxin and antimicrobial peptides (AMPs) used a docking program. Kushibiki and others[80] used a docking program to understand the interactions between tachyplesin I (TP I), a horseshoe crab-produced antimicrobial peptide, and endotoxin. Based on the calculated structural model, they strongly suggested that the cationic residues of TP I interacted with the phosphate groups and saccharides of endotoxin while the hydrophobic residues of TP I interacted with the acyl chains of endotoxin. These discoveries provided a thorough structural understanding of how TP I and endotoxin bind.The same strategy was applied by.[67] to research the relationship between eosinophil cationic protein (ECP) and endotoxin. They came to the conclusion that Arg71, which formed hydrogen bonds on the binding sites of the tripeptide arginylglycylaspartic acid (RGD), was the cause of the binding selectivity of the b-glucan complex with LGBP. Additionally, they proposed that four amino acid residues in LGBP, Arg34, Lys68, Val135, and Ala146, were essential in the binding mechanism because they interacted with one another via hydrogen bonds in the protein's active site.[80]

Characteristics of endotoxin–protein interactions:

In general, under fixed physicochemical conditions (such as pH, temperature, and viscosity), endotoxins behave differently toward various protein types. The hydrophobic interaction is primarily responsible for the interaction between endotoxin and lysozyme, a crucial part of the innate immune system.[81] Through a noncompetitive inhibition, the interaction reduces the enzymatic activity of the lysozyme and changes the biological activity of the endotoxin. Additionally, endotoxins have the potential to aggregate heavily, which would inhibit lysozyme activity.[82] A negative result will be obtained for the filtrate when a lysozymeendotoxin mixture is subjected to ultrafiltration and the LAL test, indicating the absence of monomeric LPS molecules.[83] Endotoxins' inactivity is frequently linked to monomeric endotoxins. Endotoxin and endotoxin-neutralizing protein (ENP) interact in a dose-dependent manner.The LAL assay and the production of TNFa in human mononuclear cells (MNC) demonstrate that a nearly complete neutralization can be achieved at an ENP/Endotoxin molar ratio of 20:1. Endotoxin's three-dimensional structure, specifically the transition of the lipid A structure from a cubic to a multilamellar phase, can be altered by ENP to transform it from an active into an inactive form. The endotoxin aggregate structure affects the stability of the endotoxin-ENP binding, though the direction of the dependency is still unclear. Eosinophil cationic protein (ECP), a human-secreted protein frequently used as a readout for the assessment of active inflammatory diseases, is another type of protein that exhibits a high affinity for endotoxin. By calculating the minimal agglutination concentration (MAC) or the minimal peptide concentration that could induce bacterial agglutination, Pulido et al.[67] investigated the endotoxin-agglutinating activity of ECP. Five different strains of E. coli (D21, D21e7, D21e19, D21f1, D21f2)

were used, and the agglutination was tested using ECP and peptides. The D21f2 strain with the shortest endotoxin did not agglutinate despite being incubated with 5 mM ECP for 12 hours, according to the findings. Similar outcomes for the incubation with peptides were also found, indicating a low affinity for endotoxins. It was also noted that, as seen under a transmission electron microscope (TEM), the degree of endotoxin agglutination decreased with decreasing length of endotoxin strain. Apolipoproteins can interact with endotoxins, adding to the body's defenses against septic shock.[68] Apolipophorin III (apoLp-III) from the moth species Galleria mellonell can be used as a model to study the interaction between apolipoproteins and endotoxins and to comprehend its function in septic shock defense. Endotoxin and apoLp-III interact primarily through hydrophobic interactions, which are influenced by the LPS phase transition temperature (30–37 C).[83] Fast protein liquid chromatography (FPLC) [68] to characterize the interaction between endotoxin and apoLp-III. By size exclusion, FPLC was used to isolate the complexes, which were then analyzed for size and apoLp-III/LPS contents. The findings demonstrated that LPS disaggregation had taken place as the large apoLp_III/LPS complexes were broken down into smaller aggregates. The outcomes were further supported by transmission electron microscopy, where it was evident that the LPS molecules had changed from their typical long-rod shape to that of tiny spheres. The apoLp-III-endotoxin interaction's binding mechanism. In a nutshell, endotoxin disaggregation begins when apoLp-III first enters the interior of the endotoxin micelles, gaining access to the hydrophobic Lipid A region. This direct interaction between the hydrophobic protein region and the lipid A region, enabled by an apparent change in the protein conformation, results in the formation of a stable apoLp-III/endotoxin complex. Previous research has demonstrated that a few endotoxin-interacting proteins also function as mediators of endotoxin-induced cell activation. For instance, it has been demonstrated that the innate immune system protein lipopolysaccharide-binding protein (LBP) mediates the transfer of endotoxins to CD14 on macrophages or monocytes.[84-86] The activation of macrophages and monocytes can result from the LBPmediated interaction between endotoxins and CD14.[87] The transfer of endotoxins to MD-2/TLR4 is then catalyzed by the endotoxin-CD14 complex, which results in cell inflammation.[88] In animal models, CD14 is also said to be a strong moderator of inflammation intensity.[89] By preventing LBP from transmitting endotoxins to CD14, bactericidal/permeability-increasing protein (BPI), in contrast to LBP, has been found to control inflammation.[90] The interactions between LBP and endotoxins and BPI and endotoxins are probably governed by a competitive electrostatic interaction, with BPI displaying the greatest affinity for the endotoxin.[88] When LBP and BPI interact with endotoxins, they are most effective on surfaces with a dense concentration of endotoxin molecules.[90] According to Drago-Serrano et al. [91], the primary factor that caused lactoferrin (Lf), a multifunctional protein of the innate immune system, to act as a permeabilizing agent was its interaction with enterobacterial endotoxin. Through electrostatic interactions, bonds between Lf and endotoxin were formed, which neutralized the activity of the endotoxin and stopped the inflammatory response.[92]

Conclusion:

Understanding the intricate biochemical structure of endotoxins, which comprises lipid A, core oligosaccharide, and O-antigen, is essential for appreciating their role in pathogenicity and immunogenicity. The amphiphilic nature of endotoxins is a fundamental aspect that governs their biological activities and interactions with other molecules.Endotoxins are infamous for their potent toxicity, capable of triggering severe inflammatory responses even at trace levels. The activation of the immune system through Toll-like receptor 4 (TLR4) signaling leads to the release of proinflammatory cytokines, making their detection and removal paramount in healthcare and pharmaceutical industries.The conventional method of endotoxin detection, primarily based on the Limulus amebocyte lysate (LAL) assay, has been a cornerstone in ensuring product safety. The sensitivity and reliability of this assay have made it a widely adopted technique. However, alternative methods, such as recombinant Factor C-based assays and mass spectrometry, are gaining prominence due to their improved specificity and reliability, contributing to the continued evolution of endotoxin detection techniques.the study of bacterial endotoxins and their detection methods goes beyond ensuring product safety; it delves into the very foundations of microbial biology, immunology, and biochemistry. Recognizing the importance of endotoxin structure, toxicity, detection methods, and the intricate interactions between endotoxins and proteins not only advances our understanding of the microbial world but also paves the way for innovative applications in medicine, biotechnology, and beyond. Continuous research and development in this field are essential for maintaining and improving the safety and quality of healthcare products and expanding the frontiers of scientific knowledge.

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