Inorganic polyphosphate is a linear polymer of orthophosphate units linked by high-energy phosphoanhydride bonds (Figure 1). Polyphosphate is widespread throughout biology, with polymer lengths that can vary from just a few phosphates to many hundreds or even thousands of phosphates long, depending on organism and cell type.1–3 It is intensely anionic because each phosphate unit carries a negative charge at physiological pH. In many organisms, polyphosphate is stored in intracellular organelles in a highly condensed state together with metal ions. Although these acidic, polyphosphate-rich, and calcium-rich compartments have different names in different organisms, a general term of acidocalcisomes has been proposed.4

See Insight Into James H. Morrissey on page 1305

In 2004, the laboratory of Roberto Docampo reported that polyphosphate is a major constituent of the dense granules of human platelets, and further noted that platelet dense granules share several features in common with acidocalcisomes in unicellular eukaryotes—namely, both are acidic, electron-dense, and contain abundant stores of both polyphosphate and divalent metal ions.5 As expected for a dense granule payload, polyphosphate is secreted on platelet activation. Dr Docampo alerted us to his discovery before publication, prompting us to work with him to investigate whether polyphosphate secreted from platelets might contribute to blood clotting. In 2006, we published the first report that polyphosphate is indeed a potent modulator of the blood clotting system, both accelerating the clotting cascade and slowing fibrinolysis.6 Our initial report has since been followed by a series of studies from our laboratory and others exploring the unique contributions of polyphosphate to hemostasis, thrombosis, and inflammation. In this article, we review our current understanding of the prohemostatic and prothrombotic roles of polyphosphate and discuss the possibility of targeting polyphosphate to develop novel antithrombotic drugs.

Background: Biological Roles of Polyphosphate

Metabolism of polyphosphate and its biological roles have been studied most extensively in microorganisms. In bacteria, polyphosphate is synthesized enzymatically from ATP7 and is typically heterodisperse, ranging in size from a few phosphates to hundreds or even thousands of phosphates long. Polyphosphate can be enzymatically degraded by specific endopolyphosphatases (which cleave internal phosphoanhydride bonds in polyphosphate) and exopolyphosphatases (which release terminal phosphates from polyphosphate), as well as some nonspecific phosphatases, such as alkaline phosphatase. As noted above, most microorganisms store polyphosphate in intracellular organelles, although Neisseria bacteria express long-chain polyphosphate on their capsule.8,9 Proposed roles for polyphosphate in microbes include serving as a storage molecule for phosphate and a backup energy source for ATP, sequestering metal ions to reduce metal toxicity, regulating gene transcription and enzymatic activity, biofilm development, quorum sensing, virulence, stress responses, growth and development.8,9 Although functions of polyphosphate in multicellular organisms have been studied less extensively, roles for this
polymer are now being identified. A variety of mammalian cell types are reported to contain polyphosphate, including platelets, mast cells, myeloma cells, erythrocytes, and astrocytes. Extracts from mammalian heart, liver, lung, and kidneys contain polyphosphate of varying size ranges, with brain reported to have relatively long-chain polyphosphate (>800 phosphates in length). In contrast, polyphosphate secreted by activated platelets and mast cells has a rather narrow size distribution of ~60 to 100 phosphates long. Subcellular compartments in mammalian cells described to contain polyphosphate include lysosomes, platelet dense granules, serotonin-containing secretory granules of mast cells, mitochondria, nuclei, and nuclei. Polyphosphate in organelles is often in close association with metal ions, such as calcium, magnesium, and zinc, and with organic cations, such as polyamines and basic amino acids. Polyphosphate decays with a half-life of ~1.5 to 2 hours in human blood or plasma in vitro. Mammalian alkaline phosphatase is a potent exopolyphosphatase, although in most cases, the enzymes responsible for degrading polyphosphate in mammals have not been extensively studied. Crude extracts that from a variety of mammalian tissues can degrade polyphosphate, and brain has 10- to 30-fold more degradation capability than other tissues.

Functions ascribed to polyphosphate in mammalian systems include roles in angiogenesis, apoptosis, cell proliferation, energy metabolism, tumor metastasis, osteoblast activity, and bone mineralization. In the rest of this article, we focus on the understanding of roles of polyphosphate in hemostasis, thrombosis, and inflammation.

Polyphosphate Triggers Clotting and Accelerates Thrombin Generation
Polyphosphate acts at multiple steps in blood coagulation in a manner that is dependent on polymer length. Figure 2 gives an overview of the plasma clotting cascade, depicting the 5 points at which polyphosphate acts. The contributions of polyphosphate are procoagulant, shortening the time to achieve a thrombin threshold. It is likely that polyphosphate released from platelets is physiologically relevant because platelets lacking dense granules are less able to support thrombin generation, whereas this activity can be restored by adding exogenous polyphosphate.

Initiation of the Contact Pathway
The contact pathway is triggered when plasma comes into contact with a variety of artificial surfaces, such as glass, clay, and diatomaceous earth. For years, investigators have sought to identify the true (patho)physiological activators of this pathway. We recently showed that polyphosphate is a potent activator of the contact pathway (Figure 2, point 1), and therefore may represent at least one of the long-sought, biologically relevant activators of this pathway of blood clotting. Polyphosphate binds kallikrein with high affinity, and polyphosphate-mediated contact activation likely occurs via a template mechanism. Interestingly, zymogen factor XII has enzymatic activity when in complex with tissue factor pathway inhibitor (TFPI); (4) greatly accelerating the activation of FXI by thrombin (and also promoting FXI autoactivation—not shown here); and (5) enhancing the structure of fibrin fibrils, rendering them more resistant to fibrinolysis. These actions of polyphosphate are dependent on polyphosphate polymer length, as described in detail in the text.

Figure 1. Inorganic polyphosphate is a linear, anionic polymer of orthophosphates held together by the same type of high-energy phosphoanhydride bonds found in ATP. Polyphosphate secreted by activated platelets and mast cells is ~60 to 100 phosphate units long, whereas microbial polyphosphate ranges in size from a few phosphates to many hundreds or even thousands of phosphates long.

Figure 2. Overview of the plasma clotting cascade, showing the principle points at which polyphosphate acts. The clotting cascade can be triggered via either the contact pathway or the tissue factor pathway. The contact pathway is initiated by a combination of factor (F) XII autoactivation and the reciprocal activation of FXII by kallikrein and of prekallikrein by FXIIa. The tissue factor pathway is initiated by the cell-surface complex of tissue factor (TF) and FVIIa. Polyphosphate acts by (1) triggering the contact pathway; (2) accelerating FV activation; (3) abrogating the anticoagulant activity of tissue factor pathway inhibitor (TFPI); (4) greatly accelerating the activation of FXI by thrombin (and also promoting FXI autoactivation—not shown here); and (5) enhancing the structure of fibrin fibrils, rendering them more resistant to fibrinolysis.
consistent with the idea that platelets are good at accelerating clotting, but are poor at triggering clotting.

Activation of Factor V
Factor V (FV) circulates in plasma as a single-chain protein (procofactor) with a molecular weight of 330 kDa. Limited proteolysis removes the B domain and generates mature FVa, the essential protein cofactor for factor Xa (FXa). The complex of these 2 proteins on a suitable membrane is termed the prothrombinase complex, cleaving prothrombin to release thrombin. Polyphosphate enhances the rate of FV activation by FXa, thrombin, and FXIa (Figure 2, point 2). Short polyphosphate polymers (≤60 phosphates long) have minimal ability to enhance generation of FVa, but platelet-sized polymers have robust ability to promote FV activation.

Abrogation of Tissue Factor Pathway Inhibitor
Tissue factor pathway inhibitor (TFPI) is a Kunitz-type protease inhibitor found on endothelial cells, in plasma, and in platelets. TFPI targets the active sites of FXa and factor XIIa in the tissue factor–factor VIIa complex. In vitro experiments indicate that both exogenous polyphosphate and polyphosphate in platelet releasates profoundly abrogate the inhibitory function of TFPI (Figure 2, point 3).

One mechanism by which polyphosphate seems to abrogate TFPI inhibition is by accelerating the rate of FVa generation. This is because, once FXa assembles into the prothrombinase complex, it becomes highly resistant to inhibition by TFPI, especially in the presence of its substrate, thrombin.

Conditions exist in which FVa is incompletely cleaved and thus retains portions of the B domain. This happens when FXa activates FV, and also when FV is secreted by activated platelets. Interestingly, TFPI can inhibit the prothrombinase complex when FXa binds to these partially activated versions of FVa, and furthermore, polyphosphate can abrogate the anticoagulant function of TFPI toward these incompletely activated versions of prothrombinase.

FXI Activation by Thrombin
Coagulation FXI circulates in plasma as a homodimer with a molecular weight of 160 kDa. Limited proteolysis by FXIIa or thrombin generates active FXIa. FXIa then activates factor IX to propagate the clotting cascade.

Although FXIIa can activate FXI, it has long been known that this reaction is not relevant to hemostasis in vivo because FXII deficiency is not associated with bleeding. Nevertheless, severe FXI deficiency in humans is associated with clinical bleeding diatheses. The source of FXIa in vivo rather seems to be back-activation of FXI by thrombin. Early studies described this reaction as being slow in the absence of an artificial anionic surface to act as a template. We recently reported that platelet polyphosphate enhances the rate of thrombin-catalyzed FXIa generation by ~3000-fold (Figure 2, point 4). In addition, polyphosphate potently accelerates both FXI autoactivation and FV activation by FXIa. Therefore, it is possible that platelet polyphosphate is the missing cofactor that explains how FXI contributes to hemostasis.

Polyphosphate Enhances Fibrin Clot Structure and Inhibits Fibrinolysis
Thrombin, the last protease in the clotting cascade, removes 2 small, inhibitory peptides from fibrinogen, converting it to fibrin. These fibrin monomers then spontaneously polymerize into fibrin polymers. The structure of the fibrin clot influences both its mechanical properties and susceptibility to fibrinolysis. Alterations in the structure of fibrin fibrils can, therefore, influence hemostasis and thrombosis. In 2008, we showed that polyphosphate enhances fibrin clot structure (Figure 2, point 5).

Changing the rate of thrombin generation influences clot structure, so one mechanism by which polyphosphate can affect clot properties is through accelerating the thrombin burst. In addition, the presence of polyphosphate during clotting of plasma also directly modifies the structure of the resulting fibrin clot. Polyphosphate seems to become incorporated directly into fibrin clots, resulting in thicker fibrin fibrils with greater elastic strength and resistance to fibrinolysis. Inorganic pyrophosphate, which is also present in platelet dense granules, abrogates the ability of polyphosphate to enhance fibrin clot structure, while having no effect on fibrin clots formed in the absence of polyphosphate.

Fibrin clots are ultimately removed in vivo via digestion by plasmin, which is generated when plasminogen is activated by either tissue-type or urokinase-type plasminogen activator. For optimal rates of plasmin generation, these proteins bind to fibrin via C-terminal lysine residues. A circulating carboxypeptidase (term thrombin-activatable fibrinolysis inhibitor; also known as procarboxypeptidase B2, R, or U) removes these C-terminal lysines from fibrin, thereby decreasing binding of fibrinolytic proteins and inhibiting fibrinolysis. We showed that polyphosphate, by shortening the lag time to the thrombin burst, results in earlier activation of thrombin-activatable fibrinolysis inhibitor, giving this carboxypeptidase more time to modify fibrin and thus render the clot resistant to fibrinolysis. Furthermore, Mutch et al showed that the presence of polyphosphate attenuates the binding of both plasminogen and tissue-type plasminogen activator to partially degraded fibrin (possibly by masking access to C-terminal lysines), which additionally attenuates fibrinolysis.

Polyphosphate in Hemostasis
The potential contributions of polyphosphate to hemostasis are just beginning to be elucidated. Patients with Hermansky–Pudlak syndrome and other platelet dense granule defects experience bleeding tendencies, but it is unknown whether the reduction of platelet polyphosphate in these patients contributes to their bleeding diatheses. In a mouse model, knocking out the gene for inositol hexakisphosphate kinase 1 led to a substantial reduction in accumulation of polyphosphate in platelet dense granules. As with humans with similarly reduced polyphosphate levels in their
Polyphosphate in Thrombosis

Recent studies of mechanisms underlying thrombosis have led to a new appreciation that thrombosis is not always triggered by the same activators of blood clotting that function in normal hemostasis. Such prothrombotic activators include proteins in the contact pathway of coagulation (also known as the plasma kallikrein–kinin system); products released from inflammatory cells and necrotic cells such as polyphosphate, extracellular histones, and nucleic acids; and other inflammatory mediators such as the complement cascade.

Triggering of the plasma clotting system via the contact pathway, while irrelevant for hemostasis, may contribute to thrombosis. Studies of patients with atherosclerosis or myocardial infarction have demonstrated associations with elevated plasma levels of FXII, FXI, or prekallikrein. Furthermore, patients with severe FXI deficiency have reduced risk of ischemic stroke and deep vein thrombosis. In animal models, FXII deficiency is protective against arterial and venous thrombosis, whereas anti-FXII antibodies inhibit thrombus formation in primate models. Real-time fluorescent imaging of clot formation in vitro and in vivo indicates that excessive contact factor activation and release of dense granule contents during platelet activation contribute to thrombosis and vessel occlusion.

The identity of the true (patho)physiological activator(s) of the contact pathway in vivo has yet to be definitively determined, but extracellular nucleic acids, misfolded proteins, and polyphosphate have been proposed. The ability of polyphosphate to activate the contact pathway in vitro is profoundly dependent on polymer length, with optimal activity requiring long polyphosphate polymers (of the size that accumulates in infectious microorganisms).

The procoagulant and proinflammatory activities of polyphosphate may contribute to thrombosis. Polyphosphate substantially enhances the procoagulant activity of extracellular histones, which activate platelets via toll-like receptors. Polyphosphate also activates nuclear factor-kB and fibroblast growth factors to induce differentiation of mesenchymal cells. Activation of the contact pathway leads to generation of kallikrein and release of the vasoactive peptide, bradykinin from high molecular weight kininogen. When bradykinin binds to its receptors on the endothelial cell, it causes release of nitric oxide and endothelium-derived hyperpolarizing factor, resulting in vasodilation.

Activation of the contact pathway can also activate the complement cascade: FXIIa initiates the classical pathway and kallikrein directly activates complement components C3 and C5. Interestingly, polyphosphate destabilizes C5b,6 in the terminal pathway of complement, reducing the lytic capacity of the membrane attack complex. This latter finding indicates that polyphosphate can also be anti-inflammatory.

Research using animal models has suggested that polyphosphate may contribute to thrombosis in vivo. When administered intravenously, polyphosphate leads to lethal pulmonary embolism in mice, the occurrence of which is dependent on FXII. A separate study demonstrated that mice were resistant to thrombosis if their FXI was mutated to have decreased ability to interact with polyphosphate.

Polyphosphate-Based Therapeutics

Although many hemostatic agents have been developed to treat bleeding in surgery, trauma, and patients with congenital clotting disorders, substantial room for improvement still exists. The intriguing possibility that the procoagulant nature of polyphosphate could be harnessed to treat bleeding warrants further investigation. In fact, recent studies have shown that addition of polyphosphate enhances the hemostatic action of chitosan, that polyphosphate/silica nanoparticles have increased hemostatic function compared with silica particles or polyphosphate alone, and that addition of polyphosphate to fibrin sealants might increase their effectiveness. Adding soluble polyphosphate to plasma reverses the anticoagulant activity of heparins and of direct inhibitors of thrombin or FXa. Polyphosphate also shortens the prolonged clotting times of plasma from patients with hemophilia A or B or patients taking vitamin K antagonists.

The contributions of polyphosphate to thrombosis suggest that polyphosphate could be a novel target for antithrombotic therapy. Proof-of-principle studies have recently demonstrated that certain cationic polymers, dendrimers, and other compounds can neutralize polyphosphate procoagulant activity in vitro and attenuate both venous and arterial thrombosis in mice, with minimal bleeding side effects. The compounds used in these initial studies have significant toxicity, however. To address this, we recently reported the use of highly biocompatible, nontoxic, dendrimer-like scaffolds that are also highly effective polyphosphate inhibitors. These compounds were as effective as heparin in protecting mice against arterial thrombosis, while having far less bleeding side effects compared with heparin.

Conclusions

Polyphosphate is a newly recognized regulator of blood clotting that is secreted by activated platelets and mast cells, and which is present in infectious microorganisms. It acts in a strongly procoagulant manner that modulates blood clotting at 4 principal steps (Figure 2) although the precise contributions of polyphosphate to the plasma clotting system depend on the polyphosphate polymer length. The contributions of polyphosphate to hemostasis and thrombosis suggest that this molecule may be a useful therapeutic target in coagulation disorders. It is, therefore, possible that some form of polyphosphate might be useful in the future as a parenteral or topical hemostatic agent, provided its proinflammatory activity can be adequately
controlled. Polyphosphate inhibitors may also have utility in preventing or treating thrombosis. Many of the detailed molecular mechanisms by which polyphosphate contributes to hemostasis, thrombosis, and inflammation remain to be determined.

Sources of Funding

This study was supported by grant R01 HL047104 from the National Heart, Lung and Blood Institute of the National Institutes of Health.

Disclosures

The authors are coinventors on patents and pending patent applications on medical uses of polyphosphate and of inhibitors of polyphosphate.

References


Polyphosphate is secreted by activated human platelets and mast cells and is also present in many infectious microorganisms. In 2006, our laboratory first showed that polyphosphate is a potent modulator of the blood clotting system. Further work from our group and others has now revealed that polyphosphate is strongly procoagulant and likely plays roles in normal hemostasis, thrombotic diseases, inflammation, and host responses to pathogens. Polyphosphate is a potential drug target for the development of antithrombotic, anti-inflammatory treatments that may have fewer bleeding side effects compared with conventional anticoagulants. It seems likely that there are still many contributions of polyphosphate to human biology that remain to be elucidated.

**Significance**

Polyphosphate is secreted by activated human platelets and mast cells and is also present in many infectious microorganisms. In 2006, our laboratory first showed that polyphosphate is a potent modulator of the blood clotting system. Further work from our group and others has now revealed that polyphosphate is strongly procoagulant and likely plays roles in normal hemostasis, thrombotic diseases, inflammation, and host responses to pathogens. Polyphosphate is a potential drug target for the development of antithrombotic, anti-inflammatory treatments that may have fewer bleeding side effects compared with conventional anticoagulants. It seems likely that there are still many contributions of polyphosphate to human biology that remain to be elucidated.
ATVB Named Lecture Reviews—Insight Into Author

ATVB Named Lecture Reviews—Sol Sherry Distinguished Lecture

Insight Into the Author: James H. Morrissey, PhD, University of Illinois at Urbana-Champaign

Why did you choose the profession of scientific investigation?
I've always wanted to be a scientist, ever since I was a little child. Science fascinates me, and I couldn't imagine doing anything different.

Who have been your role model(s) in your scientific and professional life?
I've had lots of good and bad examples of how to behave as a scientist, and I've learned a great deal from all of them!

What have been important influences on your professional life?
My wife, Genevieve, has given me the best guidance and grounding in my life. (We were high school sweethearts, so we've been together all our adult lives.) She's helped me focus on my work while also becoming a more complete human being.

What are your scientific inspirations?
I became fascinated with biology when I was in high school. Biological systems are both incredibly complicated but also remarkably self-regulating, and it seemed to me that we understood very little of the mechanisms that made biological systems work. This made biology mysterious and exciting. I like focusing on hard problems, and I try to work on areas that aren't the obvious next steps to take.

How have mentors contributed to your professional development?
Running a research lab is essentially running a small business, and you really have to spend a lot of time and energy supervising others. Scientists get no training for this, however! My best mentors have provided great examples of how to treat other people with kindness, dignity, and respect, while also being effective managers. Fletcher Taylor and Bill Thurman (formerly of the Oklahoma Medical Research Foundation) stand out in my memory.

If you knew then what you know now, would you do anything different?
I haven't had a lot of regrets in my life. But I do wish I had learned to speak a foreign language well when I was young.

What wisdom do you impart on new investigators?
If you do get a position as a head of a laboratory, be sure to keep working in the lab for several years. At first, you will have the best hands of anyone in your lab, and the people you hire will need a lot of attention and help. Avoid the pressures that take you away from the lab bench for the first 5 years at least.

If you were not a scientist, which profession would you pick?
Computers, definitely.

Which direction do you envisage your science taking?
In recent years, I've found myself collaborating more and more intensely with others, especially with scientists who don't work in my field but whose expertise is crucial to tackling a problem we are addressing. This has been highly rewarding to all concerned, and I see myself engaging in more extensive collaborations of this nature in the future.

What are your nonscientific activities?
Reading (especially, nineteenth-century literature), traveling, and riding my tandem bike with my wife.

What sports do you follow?
I have a passion for minor-league baseball. I try to attend a game whenever I visit a town that has minor league ball.

What are your favorite books, movies, music (pick one or all)?
One of my favorite books is also one of my favorite movies: To Kill a Mockingbird.

What are you favorite foods and are they heart healthy?
Well, that would be bacon. It isn't heart healthy, so I only eat it on special occasions.
Stephanie A. Smith and James H. Morrissey

Arterioscler Thromb Vasc Biol. 2015;35:1298-1305; originally published online April 23, 2015; doi: 10.1161/ATVBAHA.115.301927
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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