

# Scientific and Standardization Committee Communications: Inventory Of Exogenous Factors From Animal Sources that Induce Platelet Aggregation

On behalf of the Registry of Exogenous Hemostatic Factors of the Scientific and Standardization  
Committee of the International Society on Thrombosis and Haemostasis

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Platelets clump together by two distinctly different processes; agglutination and aggregation. Agglutination is a passive process, independent of the metabolic status of the platelets. It is a physical phenomenon of binding of a protein or glycoprotein ligand, which has two or more binding domains, to cell surface components. This is in contrast to platelet aggregation, an active, transmembrane process that requires metabolically active platelets. It involves activation, shape change, secretion and adhesion. Both the processes, agglutination and aggregation, can be monitored by following the change in light transmission in turbidometric aggregometers. Formaldehyde-fixed platelets can be used to distinguish between these two processes; only agglutination can be induced in these platelets, but not aggregation. PGE<sub>1</sub> inhibits aggregation, but not agglutination. Metabolic inhibitors, such as antimycin A and 2-deoxy-D-glucose, are also used to distinguish aggregation and agglutination. Some exogenous factors isolated from animal sources induce platelet agglutination, whereas others induce aggregation. In this inventory, when possible, we will distinguish between these effects. This updated inventory includes, in addition to several new inducers of platelet aggregation from snake venoms, those that were isolated from other animal sources. Some of these factors exhibit phospholipase A<sub>2</sub> (PLA<sub>2</sub>) and proteinase activities, whereas others do not exhibit any enzymatic activity. We will use the same classification described in a previous inventory by Smith and Brinkhous (1). Accordingly, aggregation inducers are divided into three main groups. Group I is comprised of factors that directly act on platelets to induce platelet aggregation. Group II consists of factors that require a plasma component for their activity. Group III is made up of heterogeneous and partially characterized factors for which the mechanisms of action are largely unknown.

## I. Exogenous Factors with Direct Action on Platelets

### A. Enzymatic Components:

*Serine Proteinases* Several serine proteinases, particularly thrombin-like enzymes (for an inventory of thrombin-like enzymes see reference 2), interfere in coagulation and platelet aggregation. The thrombin-like enzymes preferentially release either fibrinopeptide A or B from fibrinogen unlike thrombin, which releases both fibrinopeptides. Some of these thrombin-like enzymes induce platelet aggregation by direct action on platelets. However, they are less potent compared

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to thrombin (3). There is no significant correlation between the procoagulant activities of these enzymes and their ability to induce platelet aggregation. The platelet aggregation induced by thrombin-like enzymes is different from that induced by thrombin and is mainly due to ADP released from platelets (3). Interestingly, not all thrombin-like enzymes induce platelet aggregation (2). Several new members of serine proteases that induce platelet aggregation have been isolated and characterized since the publication of the earlier inventory (1). They are namely bothrombin (4), cerastobin (5), cerastocytin (6), cerastotin (7), Fraction IVa (8), afaâcytin (9), MSP1 and MSP2 (10) and PA-BJ (11) (Table 1). Some of these proteinases require the presence of fibrinogen for their activity (4). Some proteinases, such as cerastotin, induce both aggregation and agglutination of platelets (7). Afaâcytin also activates factor X to factor Xa (9)

*Phospholipase A<sub>2</sub> Enzymes* Phospholipase A<sub>2</sub> (PLA<sub>2</sub>) is another group of enzymes that induce platelet aggregation directly (Table 2). In general these agents act by cleaving platelet membrane phospholipids resulting in the release of arachidonic acid leading to formation of thromboxane A<sub>2</sub> (20). The aggregation is independent of release of ADP from platelets and can be inhibited by indomethacin and aspirin (inhibitors of cyclooxygenase and hence thromboxane A<sub>2</sub> formation). The PLA<sub>2</sub> from *Agkistrodon contortrix contortrix* venom, in contrast, initiates platelet aggregation by an unknown mechanism, which is independent of arachidonate pathway and is not mediated by ADP, serotonin or thrombin (21). There are some PLA<sub>2</sub> enzymes from the venoms of cobra, bee and Russell's viper venom (22-26) that induce biphasic effects. At lower concentrations they initiate a reversible aggregation, whereas at higher concentrations they inhibit platelet aggregation. These biphasic effects are due to the formation of arachidonate and lysophospholipids and can be negated by the addition of serum albumin, which binds and neutralizes the products of enzymatic hydrolysis (23-25). A PLA<sub>2</sub> from *Agkistrodon acutus* venom, however, initiates aggregation in washed platelet suspension and inhibits platelet aggregation in platelet rich plasma (27). Interestingly, human platelet secretory PLA<sub>2</sub> enzyme fails to release arachidonate and induce aggregation of rabbit platelets (28). The physiological relevance of the venom PLA<sub>2</sub> enzymes, which interfere in platelet aggregation only in the absence of serum albumin, is not clear. For details, see (29, 30).

## Non-Enzymatic Components

*Lectins* Among the group of non-enzymatic components with direct effect on platelet aggregation are Ca<sup>2+</sup>-dependent lectins (C-type lectins; Table 3). These snake venom lectins bind to platelets most likely through their carbohydrate recognition domains and induce platelet secretion and aggregation (32-35). Lactose, a specific inhibitor of hemoagglutination mediated by these lectins, is also a potent inhibitor of platelet aggregation. The aggregatory response is also inhibited by prostaglandins I<sub>2</sub> and E<sub>1</sub>, and RGD containing peptides.

*Components inducing aggregation via acetyl glyceryl ether phosphorylcholine (AGEPC) pathway* Collagen interacts with two different receptors glycoprotein Ia/IIa (GP Ia/IIa) and glycoprotein VI (GP VI) and induces aggregation through AGEPC pathway. Similarly some agonists (Table 4) isolated from snake venoms specifically bind to either GP Ia/IIa or GP VI and induce platelet aggregation. Convulxin is one member of this group that has been well studied (36-46). It is a C-

type lectin related protein. Platelet aggregation mediated by convulxin involves a phosphoinositide-specific phospholipase C as well as other mechanisms. Convulxin induces signal transduction in part like collagen and binds to GPVI (46). In contrast, aggrexin and rhodocytin, C-type lectin related proteins, binds to platelet via GPIa/IIa and elicit platelet aggregation through the activation of endogenous phospholipase C, leading to hydrolysis of phosphoinositide and subsequent mobilization of intracellular  $Ca^{2+}$  (47-50). Trimucylin, a potent platelet aggregation inducer isolated from *Trimeresurus mucrosquamatus* (51-53) also activates collagen receptors on platelet membrane and causes aggregation and release mainly through phospholipase C-phosphoinositide pathway. Interestingly, there is a run of Gly-Pro-Xaa repeats in the amino terminal end of the protein (53). The complete structure of this protein is not yet known. Whether this protein is similar to another aggregoserpentin isolated from the same snake venom (54) is also unknown. Two other platelet aggregation inducers, platelet aggregoserpentin and triwaglerin isolated from *T. gramineus* and *T. wagleri*, respectively, also activate platelets by similar mechanisms (55, 56). Platelet aggregoserpentin activates platelets by an endogenous phospholipase A<sub>2</sub> or C resulting in the formation of platelet activating factor (55). Triwaglerin appears to activate platelets independent of formation of thromboxane A<sub>2</sub> and platelet activating factor or release of ADP, suggesting the involvement of phospholipase C mediated formation of diacyl glycerol and inositol triphosphate (56). However, currently no structural information of these proteins is available. Equinatoxin, a cytolyisin isolated from sea anemone *Actinia equina*, also induces platelet aggregation in washed rabbit platelet suspension (57, 58). Phospholipase C-mediated phosphoinositide breakdown appears to play a role in initiation of platelet aggregation.

*Glycoprotein Ib agonists* This category of non-enzymatic platelet inducers bind to platelet glycoprotein Ib (GP Ib) and leads to platelet aggregation (Table 5). They also induce platelet agglutination. Some of the members of this group are mamushigin (59) and alboaggregin A and B (60-62). Structurally, these proteins belong to C-type lectin related protein family. Mamushigin and alboaggregin B has two chains, and , whereas alboaggregin A contains four chains, A1, A2, A3 and A4. Both alboaggregins induce platelet agglutination of human fixed platelets with similar potency (62). However, alboaggregin A is more potent in inducing platelet secretion and aggregation (62). These proteins are structurally similar to other GP Ib-binding proteins, such as echicetin (63), agkicetin (64), flavocetins (65), tokaracetin (66) and GPIb-BP (67). However, this latter group of proteins inhibits platelet adhesion and aggregation.

## II. Exogenous Factors Requiring Plasma Factors

Several factors that induce platelet aggregation or agglutination require the presence of plasma factor for their activity (Table 6). Botrocetin (also called venom coagglutinin) (68-79) interacts with von Willebrand factor (vWF) forming a platelet-active complex, which binds to GPIb resulting in platelet agglutination. Bitiscetin, a basic protein, in contrast to acidic botrocetin, also binds to vWF and induces platelet agglutination (80). Both botrocetin and bitiscetin are C-type lectin related proteins. Cerastotin induces platelet aggregation of washed platelets in the presence of fibrinogen (7). It also induces agglutination of formalin-treated platelets in the presence of fibrinogen and vWF. Some proteinases, which activate prothrombin, induce platelet aggregation via thrombin formation. These factors, such as ecarin (81, 82) and *Enhydrina schistosa* platelet aggregating factor (83), require the presence of prothrombin.

### III. Partially Characterized Exogenous Factors

Several exogenous factors have not been completely characterized in terms of both their structure and mode of action. All these factors have been put together in this group (Table 7). Two low molecular weight protein polysaccharide complexes have been isolated and purified from *Trimeresurus okinavensis* and *T. purpureomaculata* venoms (84, 85). These complexes induce ADP release and platelet aggregation in platelet rich plasma. A large acidic mucopolysaccharide from *Stichopus japonicus* Selenka (sea cucumber) also induces platelet aggregation through ADP release and cyclooxygenase pathway (86, 87). This protein requires fibrinogen.

#### Acknowledgements

This inventory is derived from the work of the Registry of Exogenous Hemostatic Factors of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis. The members of this registry are Carmen Luisa Arocha-Pinango, Patrizia Gempeler-Messina, Agnes Henschen, Francis S. Markland, Neville A. Marsh, Takashi Morita, Jan Rosing and R. Manjunatha Kini.

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Table 1. Serine proteinases with direct action of platelets.

<b>Name</b>	<b>Species</b>	<b>Physical Properties</b>	<b>Mode of action</b>	<b>References</b>
Acutin	<i>Agkistrodon acutus</i>	33,500 Da thrombin-like enzyme	Via platelet ADP release	2
Afaâcytin	<i>Cerastes cerastes</i>	2 subunits = (43,000 Da) and with constituted of two disulfide-linked chains ( =35,500, ' =10,200 Da), pI= 6.25,	Independent of prostaglandin/ thromboxane A <sub>2</sub> synthesis pathway. Mechanism probably similar to that by ADP and serotonin release	9
Bothrombin	<i>Bothrops jararaca</i>	33,000 Da, 232 aa, glycoprotein	Bothrombin interaction with GPIb is directly or indirectly linked to activation of GPIIb/IIIa; requires the presence of exogenous fibrinogen.	4
Batroxobin	<i>Bothrops atrox</i>	42,000 Da, single chain glycoprotein	Via ADP release	2
Factor VIII Activating Enzyme	<i>Bothrops jararacusso</i>	28,000 Da, single chain polypeptide	Via ADP release	79
Cerastobin	<i>Cerastes vipera</i>	Thrombin-like enzyme	Hydrolyses platelet's cytoskeleton such as actin, actin-binding protein and P235	5
Cerastocytin	<i>Cerastes cerastes</i>	38,000 Da, single polypeptide, pI>9, thrombin like enzyme	Via ADP release	6
Crotalocytin	<i>Crotalus h. horridus</i>	55,000 Da, single chain polypeptide	Via prostaglandin intermediates and thromboxane formation; ADP may also be required	80, 81
Fraction IVa	<i>Cerastes cerastes</i>	62,000 Da, dimer, pI>9.6 Serine protease with thrombin-like enzyme	Mechanism similar to that of thrombin	8
MSP1 MSP2	<i>Bothrops moojeni</i>	2 glycoprotein species 34,000 and 32,500 Da Glycoprotein, 38,000 Da	Via platelet ADP release	10
PA-BJ	<i>Bothrops jararaca</i>	30 000 Da, 232 aa, single glycoprotein chain, one N- and one O- linked carbohydrate moiety	Similar to that of thrombin	11
Thrombocytin	<i>Bothrops atrox marajoensis</i>	36 000 Da, single chain glycoprotein; 5.6 % carbohydrate	Via ADP release	2,82-85
Venzyme	<i>Agkistrodon contortrix contortrix</i>	68 000 Da, thrombin-like enzyme		86

Table 2. Phospholipase A<sub>2</sub> enzymes with direct action of platelets.

<b><u>Protein</u></b>	<b><u>Physical Properties</u></b>	<b><u>Mode of action</u></b>	<b><u>References</u></b>
<i>Agkistrodon acutus</i> Acidic PLA <sub>2</sub>	16,400 Da, pI 4.9	Arachidonic acid release from platelet membrane phospholipids and the thromboxane A <sub>2</sub> formation	19
<i>Agkistrodon contortrix contortrix</i> PLA <sub>2</sub>	14,000 Da, single chain polypeptide	Independent of arachidonic acid	13
<i>Apis mellifera</i> PLA <sub>2</sub>	Glycoprotein, 134 aa, single chain	Arachidonic acid release and thromboxane A <sub>2</sub> formation	15
<i>Crotalus durissus terrificus</i> Crotoxin	Two chain; acidic nonenzymatic subunit and basic PLA <sub>2</sub>	Independent of the formation of arachidonic acid metabolites	87
<i>Crotalus t. terrificus</i> PLA <sub>2</sub>	-----	Arachidonic acid release from platelet membrane phospholipids and the thromboxane A <sub>2</sub> and prostaglandin formation	14
<i>Naja naja atra</i> PLA <sub>2</sub>	Single chain, 119 aa	Arachidonic acid release from platelet membrane phospholipids and the thromboxane A <sub>2</sub> and prostaglandin formation	15, 16
<i>Naja mossambica mossambica</i> PLA <sub>2</sub>	Single chain, 118 aa	Arachidonic acid release and thromboxane A <sub>2</sub> formation	20
<i>Vipera russelli</i> PLA <sub>2</sub>	Single chain	Arachidonate liberation from platelet membrane	17, 18
<i>Trimeresurus mucrosquamatus</i> PLA <sub>2</sub>	Single chain	Arachidonic acid release and thromboxane A <sub>2</sub> formation	15

Table 3. Lectins with direct action of platelets.

<u>Name</u>	<u>Species</u>	<u>Physical Properties</u>	<u>Mode of actions</u>	<u>References</u>
Bushmaster lectins (BML)	<i>Lachesis muta</i>	28,000 Da, dimer	Lectin binding to the platelet induces platelet secretion with the activation of GP IIb/IIIa complexes. Ca <sup>2+</sup> dependent. Interactions most likely through carbohydrate recognition domains.	23, 24
Jameson's-mamba lectin	<i>Dendroaspis jamesonii</i>	26,000 Da, monomer		25, 26
Thrombolectin	<i>Bothrops atrox</i>	28,000 Da, dimer		24, 25
Western cottonmouth lectin	<i>Ancistrodon piscivorous leukostoma</i>	28,000 Da, dimer		24, 26
Western diamondback rattlesnake lectin	<i>Crotalus atrox</i>	28,000 Da, dimer		24, 26
Southern copperhead lectin	<i>Agkistrodon contortrix contortrix</i>	28,000 Da, dimer		Aggreagation is induced only in the presence of lectin antisera

Table 4. Components inducing aggregation via acetyl glyceryl ether phosphorylcholine (AGEPC) pathway

<b>Name</b>	<b>Species</b>	<b>Physical Properties</b>	<b>Acceptor/Receptor</b>	<b>References</b>
Aggregoserpentin	<i>Calloselasma rhodostoma</i>	28,200 Da, 231 aa, glycoprotein	Not Known	38
Aggretin	<i>Calloselasma rhodostoma</i>	29,000 Da, heterodimer =18,000 and =15,000 Da)	Most likely GPIa/IIa agonist	39
Convulxin	<i>Crotalus durissus terrificus</i> <i>Crotalus durissus cascavella</i>	300,000 Da, multisubunit acidic glycoprotein complex ( =13,500, =12,500 Da), 5% carbohydrate	GPVI	27-37
Equinatoxin	<i>Actinia equina</i>	20,000 Da, pI 12.5	Not known	48, 49
Platelet Aggregoserpentin	<i>Trimeresurus gramineus</i>	43,400 Da, 307 aa, single chain glycoprotein	Not known	46
Rhodocytin	<i>Calloselasma rhodostoma</i>	30 000 Da, disulfide-linked heterodimer (18,000, 15,000 Da)	GPIa/IIa agonist	40, 41
Trimucytin	<i>Trimeresurus mucrosquamatus</i>	68,000 Da, 340 aa, single chain, 50 % carbohydrate, pI 5.4	GPIa/IIa agonist	42-44
Triwaglerin	<i>Trimeresurus walgeri</i>	68,000 Da	Not known	47

Table 5. Exogenous glycoprotein Ib agonists

<b>Name</b>	<b>Species</b>	<b>Physical Properties</b>	<b>Mode of action</b>	<b>References</b>
Alboaggregin-A	<i>Trimeresurus albolabris</i>	52,000 Da , 4 subunits (16,000 - 18,000 Da)	Binds to GPIb directly and induces direct platelet aggregation	51-53
Alboaggregin-B		26,000 Da, 2 subunits (17,000 and 1, 000 Da)		
Alboaggregin-C		121,000 Da, 2 subunits (19,000 and 17,000 Da)		
Mamushigin	<i>Agkistrodon halys blomhoffii</i>	2, 000 Da, disulfide-linked heterodimer (17,000, 136 aa, 15,000 Da, 123 aa)	Binds to GPIb and induces direct platelet aggregation	50

Table 6. Exogenous factors requiring plasma cofactor

<b>Name</b>	<b>Species</b>	<b>Physical Properties</b>	<b>Mode of action</b>	<b>References</b>
Bitiscetin	<i>Bitis arietans</i>	25,000 Da, disulfide-linked heterodimer ( = 16,000, = 13,000 Da), pI 9.1	Bitiscetin binds to vWF forming a complex which binds to GPIb resulting in platelet agglutination	71
Botrocetin/Venom coagglutinin	<i>Bothrops jararaca</i> <i>Bothrops alternatus</i> <i>Bothropd medusa</i> <i>Bothrops neuwiedii</i>	26,500 Da, disulfide-linked heterodimer	Binds to vWF forming a complex which binds to GPIb resulting in platelet aggregation or agglutination	59-70
Cerastotin	<i>Cerastes cerastes</i>	40,000 Da, single chain glycoprotein, pI 6.6	Requires the presence of fibrinogen and vWF for platelet agglutination. Binding of cerastotin on GPIb could result in the secretion of vWF.	7
Ecarin	<i>Echis carinatus</i>	Metalloproteinase associated with C-type lectin related protein	Indirectly by the conversion of prothrombin to thrombin	72, 73
Venom Platelet-Aggregating Factor	<i>Enhydrina schistosa</i>		Requires the presence of prothrombin and Ca <sup>2+</sup>	74
Venom Platelet-Aggregating Factor	<i>Loxosceles reclusa</i>		Requires plasma component, aggregation via ADP and serotonin release	88

**Table 7.** Exogenous factors which induce platelet aggregation through unknown mechanisms

<b>Name</b>	<b>Species</b>	<b>Physical Properties</b>	<b>Mode of action</b>	<b>References</b>
Acidic mucopolysaccharide	<i>Stichopos japonicus</i> Selenka	30,000-50,000 Da, heparin-like substance containing galactose, glucuronic acid, fucose and sulfate	Dependent upon the release of ADP and cyclo-oxygenase pathway; requires the presence of fibrinogen	77, 78
Venom Platelet-Aggregating Factor	<i>Trimeresurus okinavensis</i>	4,000 Da, Protein polysaccharide complex	Causes ADP release and aggregation in PRP	75
Venom Platelet-Aggregating Factor	<i>Trimeresurus purpureomaculata</i>	Protein polysaccharide complex	Causes ADP release and aggregation in PRP	76
Venom Platelet-Aggregating Factor	<i>Atheris squamiger</i>			89
Venom Platelet-Aggregating Factor	<i>Bitis arietans</i>		Via ADP release?	90-92
Venom Platelet-Aggregating Factor	<i>Bitis nasicornis</i>			93
Venom Platelet-Aggregating Factor	<i>Crotalus m. molossus</i>		Direct action via a membrane effect?	94
Venom Platelet-Aggregating Factor	<i>Notechis s.scutatus</i> <i>Notechis ater flinder</i> <i>Notechis ater niger</i> <i>Notechis ater occidentalis</i> <i>Notechis ater serventyi</i> <i>Notechis ater humphreysi</i> <i>Australaps superba</i> <i>Pseudonaja affinis</i> <i>Pseudonaja nuchalis</i> <i>Pseudonaja textilis</i> <i>Pseudechis australis</i> <i>Pseudechis papuanus</i> <i>Pseudechis porphyriacus</i> <i>Hoplocephalus stephensi</i> <i>Tropidechis carinatus</i> <i>Acanthophis antarcticus</i> <i>Oxyuranus scutellatus</i> <i>Oxyuranus microlepidotus</i> <i>Cryptophis nigrescens</i>		Direct action on fresh platelets; irreversible aggregation with degranulation	95
Green Pit Viper Venom Platelet-Aggregating Factor	<i>Trimeresurus erythrurus</i> <i>Trimeresurus popeorum</i>			96
Venom Platelet-Aggregating Factor	<i>Rhabdophis subminiatus</i>			97
Venom Platelet-Aggregating Factor	<i>Trimeresurus flavoviridis</i>			95