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Cellular and Molecular Life Sciences

# Review

# Serglycin – Structure and biology

**S. O. Kolset[a,*] and H. Tveit[b]**

[a] Department of Nutrition, Institute of Basic Medical Sciences, Faculty of Medicine, University of Oslo, Box 1046 Blindern, 0316 Oslo (Norway), Fax: +47-22851398, e-mail: s.o.kolset@medisin.uio.no
[b] Institute of Molecular Bioscience, Faculty of Mathematics and Natural Sciences, University of Oslo, Box 1041 Blindern, 0316 Oslo (Norway)

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**Abstract.** Serglycin is a proteoglycan found in hematopoietic cells and endothelial cells. It has important functions related to formation of several types of storage granules. In connective tissue mast cells the covalently attached glycosaminoglycan is heparin, whereas mucosal mast cells and activated macrophages contain oversulfated chondroitin sulfate (type E). In mast cells, serglycin interact with histamine, chymase, tryptase and carboxypeptidase, in neutrophils with elastase, in cytotoxic T cells with granzyme B, in endothelial cells with tissue-type plasminogen activator and in macrophages with tumor necrosis factor-α. Serglycin is important for the retention of key inflammatory mediators inside storage granules and secretory vesicles. Serglycin can further modulate the activities of partner molecules in different ways after secretion from activated immune cells, through protection, transport, activation and interactions with substrates or target cells. Serglycin is a proteoglycan with important roles in inflammatory reactions.

**Keywords.** Serglycin, proteases, cytokines, hematopoietic cells, storage granules, secretion, heparin, chondroitin sulfate E.

## Introduction

Proteoglycans (PGs) constitute an important part of the glycoconjugates of mammalian cells and tissues. PGs are also found in more simple systems, such as *Drosophila*, *Caenorhabditis elegans* and even in *Hymia*. These highly negatively charged molecules have been the subject of a series of reviews [1–4]. Several reviews have focused on PGs in the extracellular matrix (ECM) [5] or on the cell surface [6]. More recently, reviews have addressed PGs in relation to interactions with growth factors, proteases or in developmental or pathological conditions [2,7]. However, limited attention has so far been granted PGs located intracellularly, which also reflects on the limited number of reviews that have addressed this topic [8, 9]. There are several interesting issues that are likely to be more extensively studied in the future, such as the cellular uptake and intracellular degradation of PGs located either on the cell surface or in the ECM, the presence and function of PGs in the nucleus, and more in depth studies on the regulatory aspects of the biosynthesis of the carbohydrate part of the PGs, the glycosaminoglycans (GAGs). The role of PGs in the formation of intracellular storage and secretory vesicles has been addressed in recent studies (see below) and is likely to provide us with new information on the functions of intracellular PGs.

The cell-associated PGs have traditionally been classified in two groups, belonging either to the syndecan or the glypican family. These cell-surface

* Corresponding author.

PGs differ in their mode of interaction with the cell surface, the former having transmembrane protein domains in the core protein, the latter being associated with the cell surface through glycosyl phosphatidyl inositol (GPI) linkages [10]. The most important PG identified so far in intracellular locations is serglycin. This PG could strictly be referred to as an intracellular PG and thereby distinguish it from the syndecans and the glypicans, or it could be included in the class of cellular PGs, as opposed to matrix-associated PGs. However, serglycin has been documented to be a secretory product and can therefore easily be incorporated into the ECM or associate with surfaces of target cells. Several aspects of the biology of serglycin have not been studied in any great detail, and a pragmatic view would be to regard it as an intracellular PG, awaiting further studies on the functions of secreted serglycin.

In this review we focus on the structure of serglycin, with emphasis on the serglycin gene and the GAG chains attached. Finally, we discuss the biological functions of serglycin. The main aim of this review is to present and discuss issues we regard as important for future research on this PG, and to refer to publications relevant for such a discussion.

## Serglycin gene

The first partial sequenced PG cDNA (named pPG1) was isolated from the rat yolk sac carcinoma cell line L2 in 1985 [11], and this cDNA was further used to identify the whole rat PG cDNA from rat yolk sac L2 cells [12]. The core protein of an intracellular chondroitin sulfate PG in both rat and mouse mucosal-like mast cells was later shown to be identical to the core protein for heparin PG core in rat and mouse serosal mast cells. Both the chondroitin sulfate and the heparin PG cores were also shown to be the same as the PG core isolated from rat yolk sac [13]. In 1986 it was shown that even if the rat PG from yolk sac and mast cell lineages had the same coding sequence, they differed in their mRNA sizes [12]. The same year the human platelet PG was isolated and partially characterized [14] and the N-terminal amino acid sequence was later shown to be highly homologous to the rat yolk sac PG [15]. The complete amino acid sequence of the human platelet PG was determined in 1988 [16], the same year in which the cDNA from rat yolk sac was used to determine the amino acid sequence of the PG from human promyelocytic leukemia HL-60 cells [17]. The PGs from non-transformed mouse bone marrow-derived mast cells (BMMC), human HL-60 cells and rat yolk sac L2 cells have been compared. Except for the repeating serine-glycine region, the N-terminal sequence of all the three species were conserved, while the C-terminal part of the mouse PG was more closely homologous to the rat PG than to the human PG [18], as shown in Fig. 1. After removal of the signal sequence, the translated mouse PG peptide core was determined to be 16.7 kDa and 14.3 kDa. Further processing of the core protein in mouse secretory granules has been reported [19] and N-terminal sequencing of serglycin isolated from monocytic cell lines has shown processing of the N-terminal part [20, 21].

In further studies the mouse gene of the secretory granule PG was cloned and characterized from a mouse liver genomic library, and the gene was expressed in rat-1 fibroblast cells [22]. The mouse gene consisted of three exons, the first exon (residues 1–25) contained 41 bp of 5′-untranslated nucleotides as well as the 25 amino acid signal peptide of the core protein. A large intron (~8 kb) follows before the second exon (residues 26–73) with its 48 amino acids, which is the N-terminal part of the core protein after removal of the signal peptide in the ER. The second intron is ~4 kb, and the last exon (residues 74–152) encodes a 79-amino acid sequence that includes the GAG attachment region. The last exon also contained a 424-bp 3′-untranslated sequence, while others reported the 3′-untranslated sequence to be 441 bp [23]. Serglycin was the first PG core protein to be cloned and sequenced, and it was given its name in 1989 [24]. The mouse serglycin gene does not have the classical TATA box ~30 bp upstream of the transcription initiation site, but a number of *cis*-acting elements in the 5′-untranslated region (UTR) have been detected [22]. The ~180-bp difference in size of the mRNA coding for the same PG in rat yolk sac (~1.3 kb) and rat mast cell lineages (~1.1 kb) had previously been demonstrated [25]. However, a more extensive investigation of the 5′-UTR and analysis of the upstream promoter regions of the serglycin gene showed two cap sites separated by approximately 180 bp [23]. These two different promoters could explain the two different sizes of mRNA. The upper presumptive rat yolk sac-like serglycin promoter (~1.3-kb mRNA) of the mouse gene had a TATA box with a 30-bp upstream *octa* binding site, together with upstream regulatory regions such as one cAMP response element-binding (*CREB*) site, one *AP2* site, one *AP1* site and one *E1I* site. The mast cell serglycin promoter (~1.1-kb mRNA) had no TATA-like box, and no upstream consensus sites, only partial sites for DNA binding regulatory proteins. Cell-specific expression of different regulatory factors [26] together with two possible promoter regions in the serglycin gene, can explain the cell-specific serglycin expression.

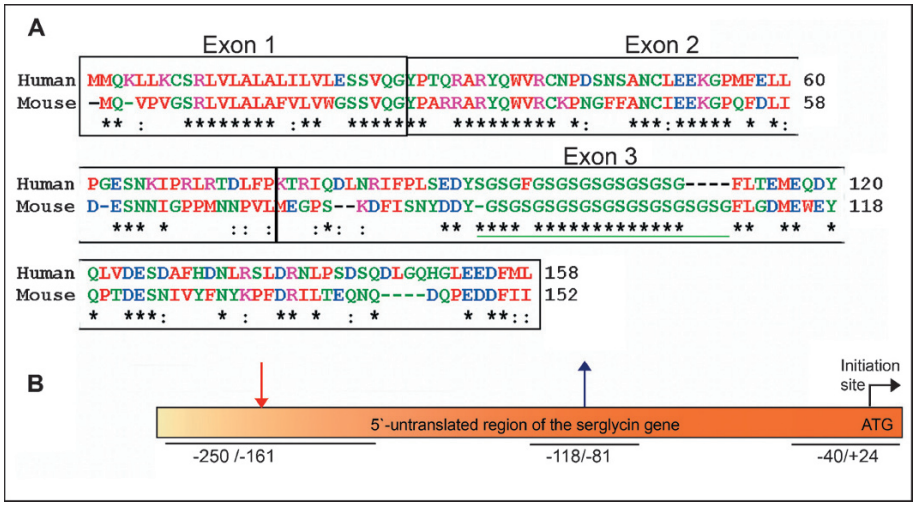


**Figure 1.** Serglycin. The human and mouse serglycin proteins are aligned. (*A*) The amino acid numbers are shown on the right side of the alignment, and the three exons are shown as three boxes. The GAG-attachment sites are underlined, and * means that the amino acids are identical, while : means that the amino acid substitution is conserved. (*B*) The 5′-untranslated region of the serglycin gene is presented. A negative *cis*-acting element is shown by a red arrow, while a positive *cis*-acting element is shown by a blue arrow.

Both positive (residues −118 to −81 and −40 to −20) and negative (residues −250 to −190) *cis*-acting elements in the 5′-UTR (Fig. 1) have been demonstrated in the mouse serglycin gene [26]. Based on similar mobilities in a gel mobility shift assay, both the serglycin expressing RBL-1 cells and rat-1 fibroblasts, which do not express serglycin, contained a common unknown *trans*-acting factor that binds to the suppressor element (residues −250 to −190) as well as the proximal promoter region (residues −40 to −20). In addition, the two different cell types contained distinct and different unknown *trans*-acting factors binding to the positive and negative *cis*-acting elements [26]. The negative regulatory element was dominantly active in the cells that do not express the serglycin (rat-1 fibroblasts), while the positive regulatory element was dominantly active in the serglycin expression cells (RBL-1 cells). After computer search for known conserved *cis*-acting elements, only the conserved *ets* domain within the enhancer region was found. This sequence is predicted to recognize DNA-binding proteins, like Erg, Elk, Ets-1, Ets-2, E74 and PU.1. The latter has been shown to be expressed in macrophages and B cells [27].

The partial human serglycin gene [28] was shown to consist of the same three exons as the mouse gene; exon 1 with the signal sequence (amino acids 1–27), exon 2 with amino acids 28–76, and the last exon with amino acids 77–158. The 5′-UTR of the human (residues −1 to −119) and the mouse (residues −1 to −123) gene were reported to be 96% identical, and both genes were without the classical TATA box [28]. The complete human serglycin gene (16.7-kb) was published 2 years later [29], and 7% of the serglycin gene comprised the three exons, while 93% of the gene contained the two introns. The serglycin gene had 19 *Alu* elements in both orientations in the two introns, and two *Alu* elements in the 5′-UTR. The mouse and human 5′-UTRs was shown to be 96% identical, and the *Ets* (−80) and *CRE* (−70) are the most critical regulatory elements for human serglycin gene expression [30]. The *CRE* site was determined to be responsible for the serglycin expression after both the PMA and dbcAMP treatment in HEL, CHRF 288-11 and HL-60 cells.

The expression of the serglycin gene is regulated by *cis*- and *trans*-acting elements in the 5′-UTR, but the availability of these elements to the transcription machinery is also essential. The chromatin modifications and structure play an important role in the regulation of serglycin expression. Firstly, the methylation pattern of the human serglycin gene has been shown to be of great importance. The human serglycin gene in HL-60 cells was less methylated than the highly methylated serglycin gene in the human T-lymphoblast Molt 4 cells that lack serglycin mRNA, demonstrating that the methylation pattern was related to the level of serglycin expression [29]. Secondly, DNase-I-hypersensitive sites (DHSS) have been shown to influence the serglycin gene expression. DHSS represent regions in the chromatin structure that are less compact, and such regions increase the possibility of gene transcription. The amount of DHSS in the human serglycin gene is tissue and signal dependent, and the high amount of DHSS in HL-60, compared to the HEL and CHRF 288-11 cells, correlates with the serglycin expression of the cells [30, 31]. It is interesting to note that an mRNA isoform in neutrophils has been detected that codes for a serglycin core protein with complete deletion of exon 2, while HL-60 only had a minor component of this mRNA isoform. Differential expression of serglycin has been demonstrated as promyelocytes mature to segmented neutrophils [32], and this

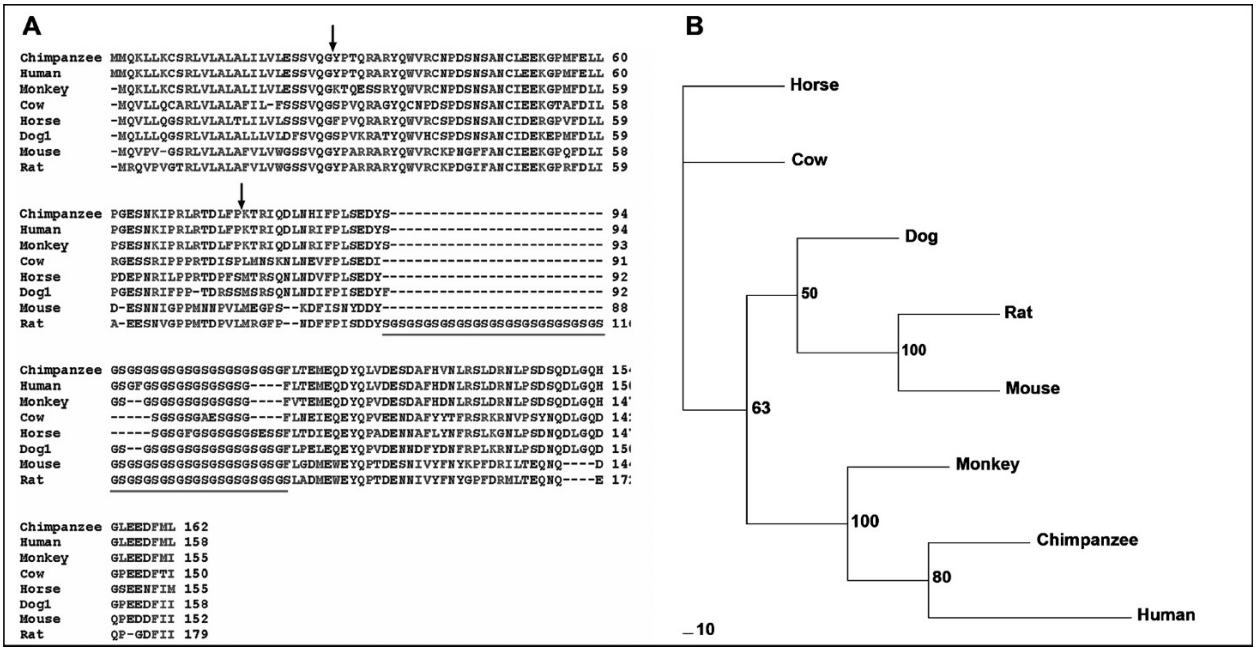


**Figure 2.** Serglycin from several species. Serglycin has only been cloned and characterized from human, mouse and rat cells. After recent genome and mRNA sequencing, several predicted serglycins are found in nucleotide databases. The predicted serglycin sequence references from the UniProt Source Sequences are: chimpanzee (UPI000036E75A), human (UPI000013188C), monkey (UPI0000D9C36D), cow (UPI00004F9640), horse (UPI000155D87E), dog (UPI00004BFC86), mouse (UPI0000001ABC) and rat (UPI000013188D). In (*A*), the serglycin sequences are aligned, and the arrows are where the exons are linked together. In (*B*), a neighbor joining (NJ) tree is produced using the Phylip package with 100 replicates.

stage-specific difference in serglycin expression could possibly be explained by the different isoforms detected in HL-60 compared to the neutrophils.

The serglycin gene has only been cloned and characterized from three species, and the amino acids of the serglycin from human (GeneID 5552), mouse (GeneID 19073) and rat (GeneID 56782) are presented in Fig. 2. Several genomes and mRNA libraries have been sequenced the last few years, and predicted serglycin core proteins from several species are available in the UniProt Source Sequences. Both cloned and predicted serglycins are aligned and presented in Fig. 2, and an evolutionary comparison of the different serglycin core proteins is also presented.

## Glycosaminoglycans

Serglycin is a PG mainly found in hematopoietic and endothelial cells [9]. Some reports suggest the presence of serglycin in other cell types, such as pancreatic acinar cells [33]. Also, the possible presence of serglycin in smooth muscle cells has been the subject of further studies [34, 35]. In the different types of blood cells the main GAG chains are chondroitin 4-sulfate (CS-4), chondroitin 6-sulfate (CS-6), chondroitin 4,6 disulfate (CS-E), chondroitin sulfate B (CS-B) and heparin [8] (as shown in Table 1; more detailed information on disaccharide structures can be found in [9]). The most highly sulfated of these GAGs is heparin, followed by CS-E and CS-B and finally CS-4 and CS-6. In relation to biological functions the sulfation pattern is an important issue, as some of the cell types with typical storage granules, such as mast cells and basophils, contain the highly sulfated GAG chains. This does not, however, seem to be a general rule as NK cells and cytolytic T lymphocytes contain GAG chains of the CS-4 type. In addition, monocytes express almost exclusively CS-4, but after differentiation to macrophages a mixture of CS-4 and CS-E is released from these cells [36]. An increase in the sulfation of CS is also seen in monocytes and macrophages after stimulation of the cells, suggesting that a shift in the sulfation pattern is linked to inflammatory conditions [37].

Of particular interest is the heterogeneity observed in mast cells [38]. Both connective tissue type mast cells and mucosal mast cells participate in similar, but not identical, immunological reactions. The different GAG profiles of these two cells types [39, 40] are an example of the importance of cell type for the expression of particular GAG structures. As these cells both contain serglycin, it is unlikely that the protein core itself has a major role in regulating whether CS or heparin is to be polymerized onto the

**Table 1.** Major GAGs found in serglycin.

| Type of GAG chain[a] | Cell type[b] |
|---|---|
| Chondroitin 4- sulfate (glucuronic acid-*N*-acetylgalactosamine 4-sulfate) | Platelets, monocytes, lymphocytes |
| Chondroitin 6-sulfate (glucuronic acid-*N*-acetylgalactosamine 6-sulfate) | Guinea pig platelets |
| Chondroitin sulfate E (glucuronic acid-*N*-acetylgalactosamine 4,6-sulfate) | Macrophages, mucosal and lung mast cells |
| Chondroitin sulfate B (iduronic acid ± 2-sulfate-*N*-acetylgalactosamine 4-sulfate) | Rat basophils |
| Heparin/heparan sulfate glucuronic acid or Iduronic acid ± 2-*O*-sulfate-glucosamine ± *N*-sulfate and/or 3 and 6-*O* sulfate | Mast cells |

[a] GAG chains with disaccharide units in parenthesis (±, with or without).
[b] See text for references. When not stated, the cells are of human origin.

protein core. The differences are more likely due to the other determinants in the biosynthetic process, such as the concentrations and activities of the biosynthetic enzymes and the concentrations of the different UDP sugars required for forming the different types of GAG chains [41].

An interesting point in relation to discussions on functions of the GAG moiety of PGs is why there is a difference in substitution of GAG chains in these mast cell types. Heparin and its relative heparan sulfate have been thoroughly studied, both with regard to its clinical use as an anticoagulant [42], and in relation to its ability to interact strongly with a series of ligands, such as lipoprotein lipase and several fibroblast growth factors [43–45]. The anticoagulant effect of heparin is well established [42], but is the result of exogenously administered heparin. The biological ligands for heparin in mast cells are mainly chymases, tryptases, carboxyypeptidases, histamine and other components [46, 47]. In addition, anticoagulant heparan sulfate has been found in the microvasculature in mast cell-deficient mice [48]. Another striking and challenging finding is the demonstration of antithrombin binding heparin isolated from clams that have no blood circulation system, further supporting the notion that heparin in mast cells is not involved in the regulation of blood coagulation.

The highly sulfated CS-E has in recent studies been shown to be an important ligand for several growth factors in the brain [49–51] and to interact with some of these factors with affinities similar to that of heparin [50]. It is therefore of interest to define in more detail if there are differences in the granule formation, ligand interactions, release of mediators, *etc.*, from connective tissue type mast cells and mucosal mast cells in wild-type and serglycin-knockout mice. Early studies on cells in an infection model in rats showed that the mucosal mast cell contained less histamine and enzymes than the connective tissue type [39, 52]. The question of biological functions of the CS-E GAG type is also relevant to the discussion of the functions of PGs in monocytes and macrophages. When monocytes differentiate into macrophages there is a shift in biosynthesis from CS-4 to a mixture of CS-4 and CS-E [36, 53]. The amount of CS-E expressed by macrophages can be further increased if they are activated after differentiation [37]. The PG serglycin is not stored in monocytes and macrophages, but is constitutively secreted. However, the secretion is increased after inflammatory stimuli. This may be due to increased secretion of ligands binding to serglycin or increased need of serglycin at inflammatory sites. Further studies on CS-E in mucosal mast cells and macrophages should potentially provide more information on functions of CS-E in immune cells. Some reports have shown that oversulfated CS found for example in mast cells and basophils contain significant amounts of CS-B [8, 19]. The biological functions of these GAG units have not been defined in detail. It is, however, interesting to note that some of the cell types expressing serglycin carry highly sulfated GAG chains of either heparin, CS-E or CS-B type. Human mast cells have been shown to contain both heparin and CS-E [54] and human mucosal mast cells mostly CS-E [55]. Furthermore, in mouse mast cells [56] serglycin has been shown to have both CS-E and heparin attached to the same protein core.

The polymerization of GAG chains onto the serglycin core has also been studied using β-D-xylosides. These compounds, with different aglycone groups, such as benzyl, nitrophenyl or hexyl, have been proven to be efficient tools in the studies of GAG biosynthesis in general [57]. Exogenously added xylosides will compete with xylosylated core proteins of PGs and result in the formation of free GAG chains on the xyloside compound. This experimental approach is most efficient in abrogation CS biosynthesis, although some compounds have been shown to initiate the formation

of HS chains. In mast cells it has been shown that xylosides abrogate heparin PG biosynthesis and stimulate the formation of free CS-E chains [58]. Also, in macrophages, which express serglycin with CS-E chains, exposure to xylosides resulted in the formation of free CS-E chains [59]. These results indicate that the serglycin core protein and/or structures therein or factors interacting with the core are important determinants for which type of GAG to polymerize onto the core protein. Furthermore, a cell type devoted to the biosynthesis of CS-E will not discriminate between a xylosylated serglycin core protein and xylose with an aglycone group, suggesting that the core protein in such a cell type is not a major regulator of either the initiation or the polymerization and modifications of the GAG.

## Biology

Studies on the biological functions of serglycin are now possible to much larger extent due to the important work of Pejler and co-workers [60], which has resulted in the generation of serglycin-knockout mice. These mice developed normally and were fertile. However, the generation of secretory granules in mast cells was compromised concomitant with a drastic reduction of several important proteases and histamine, without a detectable effect on the mRNA levels for the proteases. This study clearly demonstrated that serglycin is in some way involved in the process of granulopoiesis, *i.e.*, the generation of storage granules in mast cells. Further work on serglycin-knockout mice revealed that the secretory granules of cytotoxic T lymphocytes (CTL) also were compromised [61]. In this work it was shown that mRNA expression for the important granule components granzyme A and B was not affected. Interestingly, only the storage of granzyme B was affected in CTL of the serglycin-knockout mice. A finding along a similar line was recently published [62] where it was established that elastase was absent from azurophil granules of mature neutrophils, whereas other granule components were not affected. Neutrophils are important cells in antibacterial defense and when wild-type and serglycin-knockout mice were injected with the Gram-negative bacteria *Klebsiella pneumoniae* the virulence was increased in the latter mice. This result suggests that the immune defense can be affected by the lack of serglycin.

### *Serglycin and storage granules*

The role of serglycin in granulopoiesis was addressed in a study in neutrophils using an antibody raised against human serglycin [63]. Serglycin could be seen in Golgi area of immature cells and in some granules, whereas only minor amounts of serglycin could be seen in more mature cells. This finding is in support of a role for serglycin in the initial processes in granulopoiesis. Serglycin could not be detected in dense mature granules. This may suggest that serglycin is not present in granules of mature granulocytes. However, it might be that the epitope has been processed, as has been reported previously [28]. A heparin carrying PG was early on shown to be a protease-resistant PG [64, 65], which was later shown for the isolated PG from mouse mast cells [19]. N-terminal sequencing of serglycin purified from two different monocyte-like cell lines has also shown processing of the core protein [20, 21]. Another interesting possibility that has not been thoroughly studied is that serglycin expression could be changed during differentiation, due to alternative splicing [31]. If, however, serglycin disappears during maturation of some types of granules, as seen in granulocytes, it might be that its function is to be an initiator or early matrix scaffold for the organization of other granule components.

From studies on serglycin-knockout animals it has been shown that the process of granule formation is affected in mast cells, CTL and neutrophils when serglycin is not present. However, it is important to note that only one type of granules, the azurophil granules, is affected in neutrophils of serglycin knockout mice [62]. A general role for serglycin in granulopoiesis in hematopoietic cells does, therefore not seem to be the case. From these studies, it seems as if serglycin is found in only some granule types within a typical storage granule type of cell. In mast cells where serglycin is found in all granules there are some observations that there is heterogeneity within the granule itself, suggesting that different serglycin binding proteins such as chymase may be in electron-dense parts and tryptase may be in more translucent parts [66], possibly in different types of complexes [67]. The lack of serglycin in bone-derived mast cells resulted in the appearance of granules without electron-dense cores [68]. These results suggests that there can be a sub-compartmentalization within distinct granules, possibly to accommodate and efficiently organize the large number of serglycin partners.

It has been postulated that major determining factors in granule formation are timing of synthesis of major components, aggregation, possibly assisted by PGs, followed by retention inside granules [69–71]. It is also important to consider the importance of "cross-talk" between different granule components for a complete and proper organization of storage granules, as it has been shown that lack of one protease may affect the storage of other proteases [72, 73]. Furthermore, some proteases, like tryptases, can form

tetramers in complex with serglycin, which further adds to the heterogeneity within the mast cell granules [74]. Such tetramer formation has been shown to be stabilized by heparin [75]. Covalent linkage between serglycin and MMP-9 has also been demonstrated [76]. The importance of one granule component for the proper organizations of mast cells granules was also clearly demonstrated in mast cells of mice lacking histamine. This affected the content of proteases and PGs in granules of mast cells [77].

The possible functions of serglycin in granulopoiesis may turn out to be more complex than previously appreciated. Some points that need to be taken into consideration and addressed in detail in future work are the differences in functions between different cell types expressing serglycin, the structure and function of granule partners, the type and amount of GAG chains in the granules and also, the possible role of the serglycin core protein in sorting and retention of granules components. Studies in endothelial cells have shown that the release of, for example, IL-8 and GRO-α, residing in different types of granules, are stimulated by different mechanisms, showing that storage granules are not a uniform entity and that different granules components can be released by different signaling systems [78]. In neutrophils, which harbor at least four different types of granules with different types of cargos, serglycin has only been shown to be present in azurophilic granules [63]. Furthermore, different mast cell proteases may be in different types of complexes with serglycin [67]. Also, some granule proteases have been shown to be dependent on serglycin for storage, whereas others are not [79], most probably due to the positioning of basic charges, their density and the overall net charge of the proteases, as has recently been presented in an extensive review on mast cell proteases [80]. The reader is referred to this review for a more in-depth presentation of the interactions between serglycin and proteases, a topic that is also discussed to some extent in another recent review [81].

### *Serglycin and retention of proteases*

Serglycin has also been shown to be involved in retention of proteases in storage granules in mast cells [68]. This process may turn out to depend on the structure and properties of the proteases or other components interacting with serglycin [62, 79, 82]. Some granules components may, therefore, depend on the presence of serglycin, such as chymases, granzyme B and elastase, whereas other components, such as granzyme A and cathepsin G, are not affected by the lack of serglycin. It is also interesting to note that by comparing the three-dimensional structures of cathepsin G, proteinase 3 and elastase, using molecular modeling, a unique GAG-binding region was demonstrated only in elastase [62]. Similar analyses were performed to compare GAG-binding sites in granzyme A and B [61]. Both enzymes contained basic regions with the potential of interacting with GAGs but they were differently located in the two enzymes. It will be important to learn more about the structural requirements for interactions of partner molecules with serglycin. Hopefully this will also increase our understanding of the role of serglycin in granulopoiesis, and processes occurring downstream in immune reactions. We also need to obtain a more detailed understanding of the factors regulating synthesis of granule proteins. Many of these factors are linked to the development of myeloid leukemias.

### *Serglycin and release of partner molecules*

Early work on serglycin showed that complexes of serglycin and proteases were released from activated mast cells [83]. The release of proteases in complex with serglycin has later been shown by several investigators [80]. A major part of studies on serglycin and partner molecules has focused on proteases, mostly due to the importance of these molecules in mast cell biology. However, recent studies have shown that other important granule components in mast cells are histamine, cytokines, chemokines and prostaglandins [77, 84]. In other serglycin expressing cells several important ligands have been shown to be present. Azurophilic granules of neutrophils contain a series of components including defensins, myeloperoxidase and lysozyme, several of which are important in the antimicrobial defense [71]. Secretory granules of endothelial cells have been shown to contain both serglycin and tissue type plasminogen activator [85]. In macrophages the secretion of tumor necrosis factor-α has been shown to depend on serglycin [86] and in monocytes secretion of MMP-9 is linked to secretion of serglycin [76]. Secretion of chemokines complexed to PGs have been demonstrated in HIV-infected T cells [87]. From this it can be concluded that serglycin is involved in the regulation of, and in many cases, secretion of an impressive repertoire of molecules important in inflammatory reactions.

### *Serglycin and secretory vesicles*

Serglycin in monocytes/macrophages are not stored in granules as in mast cells. Histochemical and X-ray microanalysis has demonstrated that mast cells contained sulfur-rich (using microanalyses combined with electron microscopy) and heparan sulfate-rich granules, whereas monocytes did not [88, 89]. Serglycin is a secretory product from macrophages [20], and the secretion is increased following inflammatory stimulation [37]. Furthermore, cytokines and antibacterial

proteins secreted from activated macrophages have been shown to bind to serglycin [90], suggesting that secretion of serglycin and partner molecules, such as proteases [91], is linked to activation of monocytes and macrophages. It has been shown that abrogation of serglycin secretion in human monocytes also affected the secretion of several proteins. In addition, the lack of cell-associated serglycin resulted in the accumulation of empty vesicles, probably accumulated secretory vesicles lacking this PG [92]. This suggest that the lack of serglycin in monocytes affects secretory vesicle formation with interesting similarities to the effects observed on storage granules in serglycin-deficient mast cells [46, 47]. The process of granulopoiesis is fascinating and not only restricted to hematopoietic and endothelial cells, but also an issue for studies on neurendocrine cells and pancreatic cells [93]. If PGs are important for granulopoiesis in non-hematopoietic cells, it will be interesting to define which types are involved and if there are general mechanisms for PGs involved in granulopoiesis.

***Serglycin and regulation of partner molecules***

After release from mast cells, proteases can still be in complex with PGs [83] and during anaphylaxis mast cell protease 6 was found in the ECM, whereas mast cell protease 7 could be detected in the circulation [94]. The different fates of serglycin-associated proteins will depend on several factors including the strength of interactions and extent of processing after release. In recent studies it has been shown that the processing of carboxypeptidase A is compromised in mast cells of serglycin-knockout mice [68]. The proform of this enzyme is actually released through constitutively secreted vesicles in these cells. It is possible that the lack of other granule enzymes activating carboxypeptidase A, such as cathepsin E in storage granules of these animals, leads to this missorting. This finding highlights the interplay between different granule components. The lack of one component, dependent on serglycin, may have secondary effects on other granule components that may or may not depend on this PG for biological activity.

Serglycin released from cytolytic T cells have been proposed to participate in granzyme delivery to target cells [95]. In addition, secreted serglycin has been shown to interact with CD44 after release, suggesting that serglycin may be important in cell-cell interactions in immune reactions [96]. Studies of the activities of serglycin following secretion from storage granule type of cells, like mast cells and CTLs, and from constitutively secreting cells, like macrophages and endothelial cells, is a fascinating new aspect of PG biology and will probably provide insight into new biological functions.

After secretion from either hematopoietic cells or endothelial cells serglycin may have several interesting functions. Clusters of basic amino acids on the surface of proteases are important for the interactions with serglycin in granules with pH around 5.5 [97]. After release of granule content, which leads to an increase in pH, serglycin complexes can be dissociated, leading to the release of serglycin partner molecules. This clearly shows that both storage within and delivery from granules are important functions of serglycin. However, serglycin may have several additional functions following secretion. There are a few studies that have addressed this question focusing on serglycin. However, more studies have addressed functions linked to the GAG component of serglycin, with major emphasis on heparin. Heparin has shown to protect enzymes from inactivation by protease inhibitors that do not bind heparin [98, 99], to mediate encounter between protease and substrate [100] and to be involved in activation and processing of proteases [68, 101, 102]. It is possible that protection of serglycin partner molecules is also linked to transport, such that, for example, proteases will only be released to perform its function when the complex has reached its final destination.

The matrix metalloproteinase (MMP) system is important in inflammation, wound repair and other fundamental processes. The activation and regulation of these enzymes are subject to tight regulation [103], *e.g.*, through activation by membrane type MMPs and tissue inhibitors of MMPs (TIMPs). However, it has recently been shown by Pejler and co-workers [104] that mast cell proteases that are dependent on serglycin can participate in the activation of proMMP-2. The importance of different activation mechanisms for the different proMMPs will probably depend on tissue and the cell types involved. Interactions with serglycin have also been highlighted in other studies. A covalent linkage between serglycin from monocyte-like THP-1 cells has also been demonstrated [76], suggesting that PGs, like serglycin, and the MMP system can be more closely related than previously appreciated.

Human endothelial cells express mRNA for serglycin [34, 85], and, using an antibody raised against serglycin, it was shown that serglycin was located in granules of endothelial cells [85]. In the latter study co-localization of serglycin and tissue type plasminogen activator was demonstrated by immunohistochemistry. In these primary human endothelial cells serglycin was constitutively secreted [85], and PG secretion was increased after activation [34]. In this respect synthesis and secretion of serglycin in human endothelial cells resemble the turnover in human monocytes and macrophages [20].

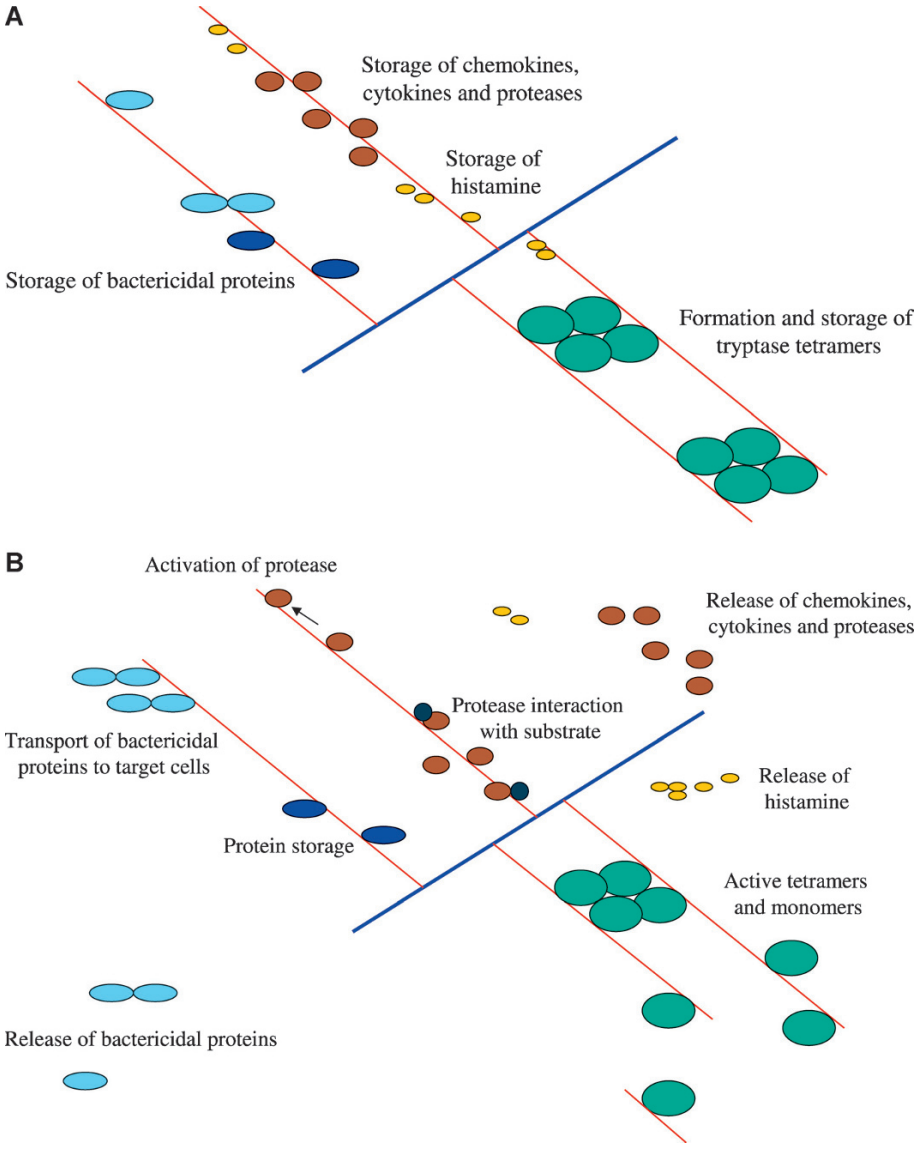


**Figure 3.** Schematic outline of biological functions of serglycin. (*A*) Inside cells serglycin is important for granule formation, although this is not general for all granule types in hematopoietic cells. The granules may either be of the storage type, where granule content is retained until the cells receive appropriate signals to secrete, as presented for mast cells, or the granules may be of the more constitutive secretory type, as in macrophages. Examples of important functions are storage and retention, protections, tetramer formation for some proteases, and keeping partner molecules in inactive form. (*B*) After release from the cells, serglycin can engage in different types of activities, either close to the cell of origin, in the extracellular environment regulating activities or transport of partner molecules, or in encounters with target cells or cells that inactivate or clear out serglycin. Human serglycin can potentially carry eight GAG chains, but is here shown with four. Core protein is in blue and GAG chains in red.

### *Elimination of serglycin*

The fate of serglycin after release from mast cells, CTL and macrophages has not been studied in any detail, but animal studies have shown that serglycin injected into the blood stream of rats are taken up by liver sinusoidal cells and degraded, probably through the hyaluronan receptor [21, 105]. The efficient uptake of serglycin may reflect a general phenomena with regard to PG turnover and explain the low concentrations of PGs found in plasma [106]. It is also interesting to note that the endocytic uptake through the hyaluronan receptor on liver endothelial cells is much more efficient than through CD44 [21]. It has been shown that serglycin can associate with cell surfaces through interactions with CD44 [96]. Interestingly, in one of the first reports on serglycin [107], before it was actually identified, a cell surface localization was demonstrated in rat yolk sac cells. Recently, a cell surface localization of serglycin was also demonstrated in human multiple myeloma [108]. Serglycin is also released from these cells and secretion has been suggested to be linked to inhibition of bone mineralization. The role of serglycin in hematopoietic malignancies is a topic that needs to be investigated in further detail.

As serglycin is released in most cases in complexes with other ligands [83, 109], the size of these molecular aggregates may prevent extensive diffusion outside inflamed tissues. Studies using dynamic laser scattering revealed that the serglycin has a hydrodynamic radius of approximately 140 nm with the potential of binding 30–60 granzyme B monomers [109]. The size of serglycin is close to that of some virus particles. The formation of such complexes may be important to

obtain high local concentrations of many of the biologically important serglycin partner molecules. Macrophages are also present in inflammatory tissues, as are mast cells. Removal of mast cell granules may be achieved through uptake and degradation by macrophages [110].

A schematic outline of biological functions of serglycin is given in Figure 3. Our understanding of serglycin functions has increased to a very large extent during the last years. A large part of this is due to generation of serglycin-knockout mice [60]. In the years to come we will learn more about the biological functions of serglycin from this interesting model system, also in relation to different disease models. A future challenge will be to expand studies done in mice to human studies. As of today we know that hematopoietic cells, endothelial cells and smooth muscle cells express serglycin. Future studies will show whether other cell types also express this PG. We need to learn more about the regulation and functions of serglycin in human pathological conditions such as bacterial infections, allergic reactions, chronic inflammatory conditions, coagulation disorders and atherosclerosis. In addition, we need to know more about the importance of having different types of GAG chains attached to the serglycin core protein in different serglycin-expressing cells. Furthermore, the regulation of the serglycin gene needs to be studied in more detail. It will be interesting to learn more about the signals generated during inflammatory reactions and how these influence serglycin expression in different serglycin-expressing cells.

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