A Serine Peptidase Inhibitor (Serpin) from *Gloeobacter violaceus*

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Abstract

Here we describe the inhibitory activity of serpin (Serine peptidase inhibitors) from *G. violaceus* that we named vioserpin.

Introduction

The serpins (Serine peptidase inhibitors) belong to the I4 family of protease inhibitors and constitute a large family of proteins found in animals, plants and different microorganisms.[1] Serpins regulate a wide range of physiological processes including blood coagulation, complement activation, inflammation, extracellular matrix remodeling, and tumor suppression[2], and their main characteristic is the inhibition of serine proteases.[3,4] Serpins are found in different bacterial strains[5], but their functional importance for these microorganisms is not clear. Here we describe the biochemical characterization of a serpin from *Gloeobacter violaceus* and its capacity to inhibit trypsin interacting in the presence of glycosaminoglycans.

Results and Discussion

The vioserpin showed specificity to inhibit trypsin by the formation of the covalent complex serpin-peptidase, demonstrated by SDS–PAGE analysis (Figure 1).

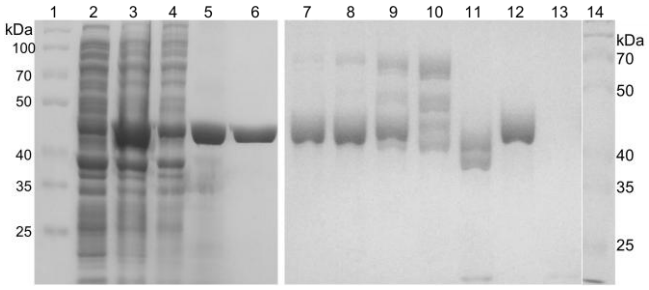

Figure 1. 10% SDS-PAGE Gel stained with coomassie blue. **1**, Molecular weight standard; **2**, Control not induced; **3**, *E. coli* BL21(DE3) pET28Gv transformed at 20 h of induction; **4**, induction pellet; **5**, supernatant of induction; **6**, Protein eluted with 200 mM imidazole. The complex formation: lanes **7** to **11**, incubations vioserpin:trypsin ratio of 8:1; 4:1; 2:1; 1:1 and 1:2; lane **12**, vioserpin control; lane **13**, bovine trypsin; lane **14**, Molecular weight standard.

The Stoichiometry of Inhibition (SI) for all inhibitory reactions was near 1 (Figure 2) and reactions of vioserpin with trypsin demonstrated rapid inhibition with rate constant 3,5 x 10^5 $M^{-1}.s^{-1}$, similar to that observed for reactions with glycosaminoglycans heparin, dermatan sulfate and chondroitin sulfate. It was observed that *k*' value for the test in the presence of heparin had the highest value of second-order rate constant, almost 5 times the value of second-order constant, (15,0 x 10^5 $M^{-1}.s^{-1}$), while dermatan and chondroitin sulfate did not influenced the values of *k*'.

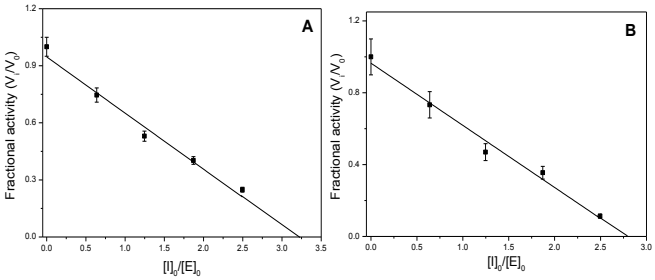

Figure 2. SI for the vioserpin-trypsin interaction was determined by extrapolation of the $[I]_0/[E]_0$ ratio ($[I]_0$ - initial concentration of inhibitor; $[E]_0$ - Initial enzyme concentration) with incubation of vioserpin-trypsin at different concentrations in absence (**A**) and in presence (**B**) of Heparin (1,0 µM).

Conclusions

In conclusion, our study defined the specificity of a serpin found in *G. violaceus* bacteria to inhibit trypsin-like enzymes, and it presented an inhibitory potency similar to others serpins with specificity for trypsin.[6,7] We were also able to show that the inhibitory activity of vioserpin can be influenced by heparin.

Acknowledgments

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