

Commentary

Trehalose: is it a potential inhibitor of antithrombin polymerization?

 Irene Martínez-Martínez^{1,2}

¹Servicio de Hematología y Oncología Médica, Hospital Universitario Morales Meseguer, Centro Regional de Hemodonación, Universidad de Murcia, IMIB-Arrixaca, Murcia, Spain;

²Grupo de Investigación CB15/00055, Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Instituto de Salud Carlos III (ISCIII), Madrid, Spain

Correspondence: Irene Martínez-Martínez (immlgi@um.es)



SERine Protease INhibitorS (Serpins) are a superfamily of proteins that are characterized by having a similar three-dimensional structure. The native conformation is not most thermodynamically stable, so polymerization is the main consequence when its stability is altered as a result of certain mutations. The polymerization of serpins has been a research topic for many years. Different mechanisms have been proposed and in the same way different compounds or strategies have been studied to prevent polymerization. A recent paper published in Bioscience Reports by Naseem et al. [*Biosci. Rep.* (2019) **5**, 39] studies the role of trehalose in the prevention of the polymerization of antithrombin, which belongs to the serpin superfamily. The main consequence of the antithrombin polymerization is the increased thrombotic risk, since antithrombin is the main inhibitor of the coagulation cascade. The authors demonstrate that trehalose is able to prevent the *in vitro* polymerization of antithrombin, under conditions in which it usually tends to polymerize, and demonstrate it by using different techniques. However, the binding site of trehalose in antithrombin should be defined by site-directed mutagenesis. On the other hand, it is not clear if all serpins polymerize *in vivo* through the same mechanism and it is also not clear if the same serpin can even polymerize through different mechanisms. Therefore, there are still doubts about the potential of trehalose or its derivatives to prevent *in vivo* antithrombin polymerization and, therefore, reduce thrombotic risk, as well as whether trehalose would be able to reduce polymerization in other serpins.

SERine Protease INhibitorS (Serpins) are a superfamily of structurally similar proteins but with diverse functions. Most serpins have the ability to inhibit serine proteases, although some inhibit caspases and cysteine proteases [1]. There are other serpins that do not exert any inhibitory function but act as molecular chaperones, in the transport of hormones or in the suppression of tumors [1–3]. Those serpins that have an inhibitory function perform a suicide inhibitory mechanism [4]. This mechanism is possible thanks to the fact that they present a great structural flexibility and that the native conformation is not the most thermodynamically stable [5]. But this structural flexibility has disadvantages, since certain mutations can alter the structural stability of the serpins causing the formation of inactive polymers [6]. In some serpins, polymerization not only reduces the amount of functional protein, but the intracellular accumulation of these polymers can result in cell death and organ failure [7]. The polymerization of serpins has been studied for many years but there is still no consensus on the polymerization mechanism [8–10]. However, defining the polymerization mechanism would help to rationally design molecules that are capable of reducing polymerization.

Antithrombin belongs to the serpin superfamily. It is the main inhibitor of the coagulation cascade and its main targets are FXa and thrombin. Therefore, antithrombin deficiency increases the risk of thrombosis significantly [11]. The congenital deficiency of antithrombin can be classified as type I or type II [12]. The intracellular polymerization of antithrombin results in type I deficiency increasing the thrombotic risk, although in some cases it is possible to detect disulfide-linked dimers in the plasma [13]. Unlike

Received: 10 April 2019

Revised: 08 May 2019

Accepted: 09 May 2019

Accepted Manuscript Online:
30 May 2019

Version of Record published:
18 June 2019

the mutations that cause polymerization of $\alpha 1$ -antitrypsin and that result in liver cirrhosis [14,15], the polymerization of antithrombin only results in increased risk of thrombosis. This is believed to be because antithrombin is synthesized at a concentration ten-times lower than $\alpha 1$ -antitrypsin. Antithrombin has been used as a model for the study of the mechanism of serpin polymerization [8]. Antithrombin is a serpin with certain characteristics that differentiate it from the rest, such as the presence of three disulfide bridges, a structural domain that binds heparin, and three or four N-glycans, which are essential for its correct folding and functionality [16]. Curiously, although most of gross deletions or insertions result in type I deficiency, our group described a case in which the insertion of eight amino acids in the helix F of antithrombin resulted in the secretion of a non-functional protein but was not able to polymerize [17].

The study of molecules capable of reducing or preventing the polymerization of serpins has been carried out with different serpins [18–24], in order to then extrapolate the results to other serpins. In the study by Naseem et al. [25], the effect of trehalose on the polymerization of serpins has been carried out with antithrombin. Although the authors present an *in-silico* study on the potential interaction of trehalose with other serpins and reference their effect in the prevention of polymerization with neuroserpin [22], the effect on the polymerization of other serpins should be explored. The compounds investigated by the authors are generally well-known osmolytes, which operate by altering the structure of water around proteins. This fact combined with the very (~ 1 M) high concentrations strongly support a non-specific effect for their action. This is acknowledged in the final paragraph of the Naseem et al. discussion [25], but they also propose that the interaction must occur in a hydrophobic area of the antithrombin. This must be demonstrated through site-directed mutagenesis, and thus define the residues involved and the type of interaction. Naseem et al.'s [25] paper showed using intrinsic fluorescence that trehalose stabilizes against the formation of the intermediate in the presence of GdnHCl. But they then choose a concentration of GdnHCl where they have shown that the intermediate is not populated (2 M) in an experiment using BisANS and infer that the low BisANS signal is due to shielding of a hydrophobic patch (rather than simply not being populated). They should have in fact chosen two GdnHCl concentrations at which the intermediate was equivalently populated in both the presence and absence of trehalose. Similarly, the possibility of spectroscopic effects on bisANS fluorescence (such as quenching) in the presence of high concentrations of sugars should be considered. Antithrombin has a dynamically regulated reactive center loop, whose conformation can affect kinetics of interaction/inhibition with different proteases. Therefore the changes induced by a heavily modified solvent could affect reactive center loop conformation rather than the process of RCL insertion as contended by Naseem et al. [25]. But the most important thing is to define the effect of trehalose or its derivatives *in vivo*. The authors demonstrate that trehalose reduces the polymerization of antithrombin in those conditions in which it usually polymerizes *in vitro*, such as at temperatures above 60°C, and they monitor this effect because antithrombin maintains its inhibitory function. *In vivo*, it would be necessary to establish a murine model of antithrombin deficiency caused by a mutation that induces polymerization, such as P80S [13], and see the effect of the administration of trehalose on the plasma activity of FXa and thrombin and on partial thromboplastin time. Although the authors state that the lowest concentration of trehalose that exerts an effect in the prevention of polymerization is 1 M, and higher concentrations have been used for the treatment of the oculopharyngeal muscular dystrophy therapy [26], it would be necessary to evaluate the absence of side effects at the concentrations required to observe the increase in inhibitory activity of antithrombin *in vivo*.

In summary, the paper of Naseem et al. [25] describes the potential of trehalose or molecules based on this scaffold to prevent the polymerization of antithrombin. Although more efforts should be made to obtain an efficient drug for use in patients, this is a step forward toward the ultimate goal.

Competing Interests

The author declares that there are no competing interests associated with the manuscript.

Funding

This work was supported by the Instituto de Salud Carlos III [grant numbers PI17/00050 and FEDER, CP13/000126 and FEDER]; and the Miguel Servet Contract from Instituto de Salud Carlos III [grant number CP18/00019].

Abbreviations

BisANS, 4,4'-Dianilino-1,1'-Binaphthyl-5,5'-Disulfonic acid, dipotassium salt; GdnHCl, Guanidinium chloride; RCL, reactive centre loop; Serpins, SERine Protease INhibitorS.

References

- Law, R.H., Zhang, Q., McGowan, S., Buckle, A.M., Silverman, G.A., Wong, W. et al. (2006) An overview of the serpin superfamily. *Genome Biol.* **7**, 216, <https://doi.org/10.1186/gb-2006-7-5-216>
- Silverman, G.A., Bird, P.I., Carrell, R.W., Church, F.C., Coughlin, P.B., Gettins, P.G. et al. (2001) The serpins are an expanding superfamily of structurally similar but functionally diverse proteins. Evolution, mechanism of inhibition, novel functions, and a revised nomenclature. *J. Biol. Chem.* **276**, 33293–33296, <https://doi.org/10.1074/jbc.R100016200>
- Gatto, M., Iaccarino, L., Ghirardello, A., Bassi, N., Pontisso, P., Punzi, L. et al. (2013) Serpins, immunity and autoimmunity: old molecules, new functions. *Clin. Rev. Allergy Immunol.* **45**, 267–280, <https://doi.org/10.1007/s12016-013-8353-3>
- Huntington, J.A. (2011) Serpin structure, function and dysfunction. *J. Thromb. Haemost.* **9**, 26–34, <https://doi.org/10.1111/j.1538-7836.2011.04360.x>
- Carrell, R.W. and Huntington, J.A. (2003) How serpins change their fold for better and for worse. *Biochem. Soc. Symp.* **70**, 163–178, <https://doi.org/10.1042/bss0700163>
- Carrell, R.W. and Lomas, D.A. (1997) Conformational disease. *Lancet* **350**, 134–138, [https://doi.org/10.1016/S0140-6736\(97\)02073-4](https://doi.org/10.1016/S0140-6736(97)02073-4)
- Stein, P.E. and Carrell, R.W. (1995) What do dysfunctional serpins tell us about molecular mobility and disease. *Nat. Struct. Biol.* **2**, 96–113, <https://doi.org/10.1038/nsb0295-96>
- Yamasaki, M., Li, W., Johnson, D.J. and Huntington, J.A. (2008) Crystal structure of a stable dimer reveals the molecular basis of serpin polymerization. *Nature* **455**, 1255–1258, <https://doi.org/10.1038/nature07394>
- Yamasaki, M., Sendall, T.J., Pearce, M.C., Whisstock, J.C. and Huntington, J.A. (2011) Molecular basis of α_1 -antitrypsin deficiency revealed by the structure of a domain-swapped trimer. *EMBO Rep.* **12**, 1011–1017, <https://doi.org/10.1038/embor.2011.171>
- Miranda, E., Pérez, J., Ekeowa, U.I., Hadzic, N., Kalsheker, N., Gooptu, B. et al. (2010) A novel monoclonal antibody to characterize pathogenic polymers in liver disease associated with alpha1-antitrypsin deficiency. *Hepatology* **52**, 1078–1088, <https://doi.org/10.1002/hep.23760>
- Martinelli, I., De Stefano, V. and Mannucci, P.M. (2014) Inherited risk factors for venous thromboembolism. *Nat. Rev. Cardiol.* **11**, 140–156, <https://doi.org/10.1038/nrcardio.2013.211>
- Corral, J., de la Morena-Barrio, M.E. and Vicente, V. (2018) The genetics of antithrombin. *Thromb. Res.* **169**, 23–29, <https://doi.org/10.1016/j.thromres.2018.07.008>
- Corral, J., Huntington, J.A., González-Conejero, R., Mushunje, A., Navarro, M., Marco, P. et al. (2004) Mutations in the shutter region of antithrombin result in formation of disulfide-linked dimers and severe venous thrombosis. *J. Thromb. Haemost.* **2**, 931–939, <https://doi.org/10.1111/j.1538-7836.2004.00749.x>
- Segeritz, C.P., Rashid, S.T., de Brito, M.C., Serra, M.P., Ordóñez, A., Morell, C.M. et al. (2018) hiPSC hepatocyte model demonstrates the role of unfolded protein response and inflammatory networks in α_1 -antitrypsin deficiency. *J. Hepatol.* **69**, 851–860, <https://doi.org/10.1016/j.jhep.2018.05.028>
- Giacopuzzi, E., Laffranchi, M., Berardelli, R., Ravasio, V., Ferrarotti, I., Gooptu, B. et al. (2018) Real-world clinical applicability of pathogenicity predictors assessed on SERPINA1 mutations in alpha-1-antitrypsin deficiency. *Hum. Mutat.* **39**, 1203–1213, <https://doi.org/10.1002/humu.23562>
- Aguila, S., Navarro-Fernández, J., Bohdan, N., Gutiérrez-Gallego, R., de la Morena-Barrio, M.E., Vicente, V. et al. (2014) Role of the C-sheet in the maturation of N-glycans on antithrombin: functional relevance of pleiotropic mutations. *J. Thromb. Haemost.* **12**, 1131–1140, <https://doi.org/10.1111/jth.12606>
- Martínez-Martínez, I., Johnson, D.J., Yamasaki, M., Navarro-Fernández, J., Ordóñez, A., Vicente, V. et al. (2012) Type II antithrombin deficiency caused by a large in-frame insertion: structural, functional and pathological relevance. *J. Thromb. Haemost.* **10**, 1859–1866, <https://doi.org/10.1111/j.1538-7836.2012.04839.x>
- Devlin, G.L., Parfrey, H., Tew, D.J., Lomas, D.A. and Bottomley, S.P. (2001) Prevention of polymerization of M and Z alpha1-Antitrypsin (alpha1-AT) with trimethylamine N-oxide. Implications for the treatment of alpha1-at deficiency. *Am. J. Respir. Cell Mol. Biol.* **24**, 727–732, <https://doi.org/10.1165/ajrcmb.24.6.4407>
- Mahadeva, R., Dafforn, T.R., Carrell, R.W. and Lomas, D.A. (2002) 6-mer peptide selectively anneals to a pathogenic serpin conformation and blocks polymerization. Implications for the prevention of Z alpha(1)-antitrypsin-related cirrhosis. *J. Biol. Chem.* **277**, 6771–6774, <https://doi.org/10.1074/jbc.C100722200>
- Zhou, A., Stein, P.E., Huntington, J.A. and Carrell, R.W. (2003) Serpin polymerization is prevented by a hydrogen bond network that is centered on his-334 and stabilized by glycerol. *J. Biol. Chem.* **278**, 15116–15122, <https://doi.org/10.1074/jbc.M211663200>
- Zhou, A., Stein, P.E., Huntington, J.A., Sivasothy, P., Lomas, D.A. and Carrell, R.W. (2004) How small peptides block and reverse serpin polymerization. *J. Mol. Biol.* **342**, 931–941, <https://doi.org/10.1016/j.jmb.2004.07.078>
- Sharp, L.K., Mallya, M., Kinghorn, K.J., Wang, Z., Crowther, D.C., Huntington, J.A. et al. (2006) Sugar and alcohol molecules provide a therapeutic strategy for the serpinopathies that cause dementia and cirrhosis. *FEBS J.* **273**, 2540–2552, <https://doi.org/10.1111/j.1742-4658.2006.05262.x>
- Zhou, A., Stein, P.E., Huntington, J.A., Sivasothy, P., Lomas, D.A. and Carrell, R.W. (2004) How small peptides block and reverse serpin polymerization. *J. Mol. Biol.* **342**, 931–941, <https://doi.org/10.1016/j.jmb.2004.07.078>
- Tanaka, M., Machida, Y., Niu, S., Ikeda, T., Jana, N.R., Doi, H. et al. (2004) Trehalose alleviates polyglutamine-mediated pathology in a mouse model of Huntington disease. *Nat. Med.* **10**, 148–154, <https://doi.org/10.1038/nm985>
- Naseem, A., Khan, M.S., Ali, H., Ahmad, I. and Jairajpuri, M.A. (2019) Deciphering the role of trehalose in hindering antithrombin polymerization. *Biosci. Rep.* **5**, 39
- Davies, J.E., Sarkar, S. and Rubinstein, D.C. (2006) Trehalose reduces aggregate formation and delays pathology in a transgenic mouse model of oculopharyngeal muscular dystrophy. *Hum. Mol. Genet.* **15**, 23–31, <https://doi.org/10.1093/hmg/ddi422>