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Note

Thrombin inhibition by antithrombin in the presence of oversulfated dermatan sulfates

Raoui M. Maaroufi,[a,*] Marcel Jozefowicz,[b] Jacqueline Tapon-Bretaudière[c] and Anne-Marie Fischer[c]

[a]*Institut Supérieur de Biotechnologie de Monastir, Monastir 5000, Tunisia*
[b]*Therapol, Saint-Denis 93200, France*
[c]*Laboratoire d'Hématologie, Hôpital Européen Georges Pompidou, 75908 Paris Cedex 15, France*

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Abstract—DSS_1 and DSS_2 are two oversulfated dermatan sulfate derivatives with sulfur contents of 7.8% and 11.5%, respectively. DSS_1 and DSS_2 both enhanced the rate at which antithrombin (AT) inactivates thrombin according to a concentration dependent manner. The analysis of the experimental data, using our previously described kinetic model [*Biomaterials* **1997**, *18*, 203] (i) suggested that both DSS_1 and DSS_2 catalyzed the thrombin–AT reaction according to a mechanism in which the oversulfated derivative quickly formed with AT a complex, which was more reactive towards thrombin than the free inhibitor and (ii) allowed us to determine the dissociation constants of the polysaccharide–inhibitor complexes, which were $(1.15 \pm 0.74) \times 10^{-7}$ and $(7.17 \pm 0.65) \times 10^{-9}$ M, and the catalyzed reaction rate constants, which were $(2.29 \pm 0.15) \times 10^8$ and $(8.71 \pm 0.08) \times 10^8$ M^{-1} min^{-1}, for DSS_1 and DSS_2, respectively. These data suggested that the oversulfation confers an affinity for AT to dermatan sulfate and that the higher the sulfur content the higher the affinity for AT. They also suggested that the reactivities of the polysaccharide–AT complexes formed towards the protease increased with the sulfur content.

Keywords: Heparin; Dermatan sulfate; Oversulfation; Antithrombin; Thrombin; Anticoagulant mechanism

Antithrombin (AT), a plasma derived single chain glycoprotein, is a serine-protease inhibitor (serpin), sharing about 30% homology with other serpins such as heparin cofactor II (HC II). AT exerts its inhibitory action by forming an inactive, extremely stable, equimolar complex between AT and the proteases such as thrombin. The interaction of AT with these serine-proteases is considerably enhanced by heparin,[1] a highly sulfated glycosaminoglycan with a widespread clinical use as an anticoagulant drug.[2,3] However, because bleeding[4] and heparin-induced thrombocytopenia[5] represent major side effects of heparin, it is likely that alternative drugs are being sought.[3,6]

Dermatan sulfate, a heparin-like sulfated galactosaminoglycan, exerts its anticoagulant effect through potentiating HC II inhibitory activity towards thrombin[7] but has no significant effect on thrombin inhibition by AT.[8] Chemically oversulfated dermatan sulfates enhance the rate of the thrombin–HC II reaction more than both the native dermatan sulfate[8–10] and heparin[11,12] whereas they have been reported to have no effect on the rate at which AT inactivates thrombin.[10,13] We found however that the oversulfation confers to dermatan sulfate a new acquired ability to enhance the rate of the thrombin–AT reaction.[8]

We present in this study kinetic data specifying the mechanism of the catalysis of the thrombin–AT reaction by two oversulfated dermatan sulfates.

Abbreviations: AT, antithrombin; HC II, heparin cofactor II; DS, dermatan sulfate.

* Corresponding author. Tel.: +216 97 406 124; fax: +216 73 465 404; e-mail: rmmaaroufi@yahoo.fr

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The inhibitor (AT) and the enzyme (E) were both set at equimolar initial concentrations ($C_{AT} = C_E = 10^{-8}$ M). The residual thrombin $[E_T]_t$ was measured for various incubation times t and for each initial polysaccharide concentration (C_{PS}) ranging from 10^{-10} to 5.10^{-5} M. The reciprocal of the residual enzyme was then plotted versus the incubation time for each C_{PS} (Fig. 1a and b). The curves $1/[E_T]_t = f(t)$ were linear and indicated that the total reaction was second order at any C_{PS}, when either DSS_1 or DSS_2 was used. We postulated thereafter that the data obtained here fit a kinetic model assuming a total reaction wherein the total protease (free (E) and/or polysaccharide-bound (PSE)) was inactivated by the total inhibitor (free (AT) and/or polysaccharide-bound (PSAT)). The slopes of these $1/[E_T]_t = f(t)$ curves were the experimental values of the total reaction rate constants k_{app}, which were subsequently plotted versus DSS_1 and DSS_2 concentrations, respectively (Fig. 2).

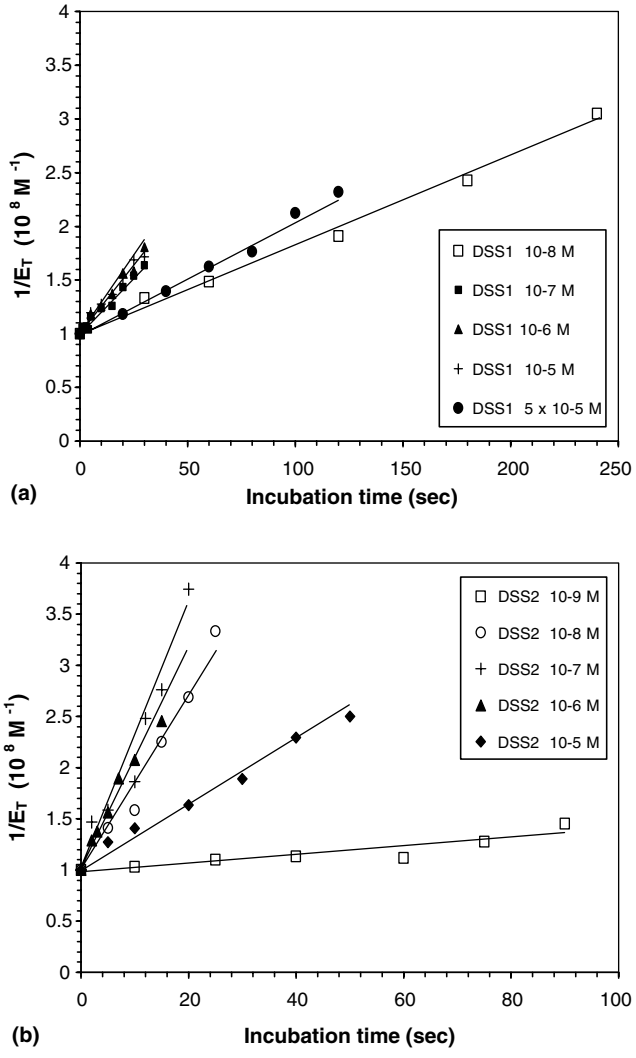

Figure 1. Thrombin inactivation by antithrombin in the presence of various DSS_1 (a) and DSS_2 (b) concentrations. The reciprocal of the residual enzyme was plotted versus the incubation time.

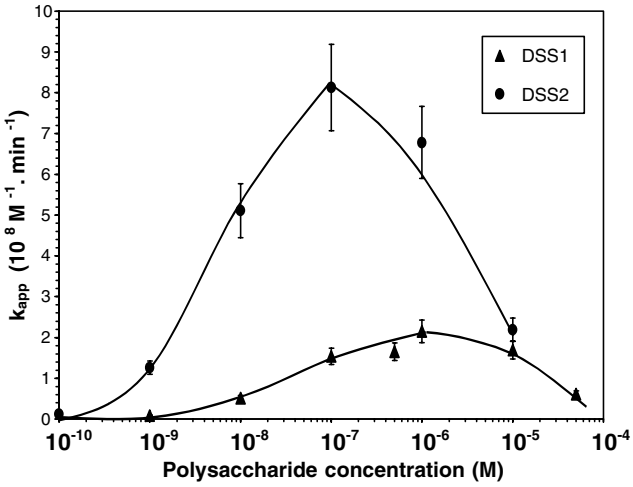

Figure 2. Thrombin antithrombin reaction rate as a function of DSS_1 (▲) and DSS_2 (●) concentrations. The total reaction rate constant (k_{app}) was plotted versus the polysaccharide concentration.

When DSS_1 was used, the graph obtained showed a significant increase in k_{app} as C_{PS} was raised up to 10^{-6} M, reaching the maximal value of $(2.15 \pm 0.24) \times 10^8$ M^{-1} min^{-1}; at higher concentrations, the reaction rate diminished significantly. In turn, when DSS_2 was used, at concentrations up to 10^{-7} M, the increase in k_{app}, up to $(8.15 \pm 0.24) \times 10^8$ M^{-1} min^{-1}, was also followed by a significant decrease.

At optimal concentrations, the acceleration of the reaction in the presence of DSS_1 or DSS_2 was 108-fold and 408-fold, respectively, by comparison with the non-catalyzed reaction (2×10^6 M^{-1} min^{-1}). Heparin was previously found to accelerate the thrombin–AT reaction 450-fold, under the same experimental conditions.[11]

We observed previously, at concentrations up to 10^{-6} M that neither DSS_1 nor DSS_2 prolonged the fibrinogen clotting time. Therefore, we assumed that neither DSS_1 nor DSS_2 could bind to thrombin, in this concentration range, and considered that fibrinogen is a heavy macromolecular substrate, which should be displaced from thrombin upon polysaccharide binding to the enzyme.[12] Taking this into account, we assumed that the formation of a polysaccharide–AT complex, which was more reactive than the free inhibitor towards thrombin was involved in the mechanism of the reaction catalysis, as already established when the inhibitor was HC II.[12] Thereafter, the higher C_{PS} the more polysaccharide–AT (PSAT) complex formed, until saturation of the inhibitor. This was illustrated by the sharp increase in k_{app} observed in the increasing part of the bell-shaped curve $k_{app} = f(C_{PS})$ for both DSS_1 and DSS_2 (Fig. 2).

The polysaccharide affinity for AT, K_{PSAT}, and the bimolecular rate constant, k, of the free thrombin