



COMMENT ON ÅGREN ET AL.

Increased Incorporation of Antiplasmin Into the Fibrin Network in Patients With Type 1 Diabetes. *Diabetes Care* 2014;37:2007–2014

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Antiplasmin (α_2 -antiplasmin or plasmin inhibitor) is the main physiological inhibitor of plasmin and efficiently attenuates fibrin clot lysis after being cross-linked to fibrin by the action of activated factor XIII (FXIIIa). Ågren et al. (1) recently demonstrated that the fibrin network of plasma clots from patients with type 1 diabetes contains more antiplasmin than the fibrin network of plasma clots from control subjects. This is an interesting finding, as increased clot resistance to fibrinolysis might contribute to cardiovascular disease frequently associated with diabetes.

The authors used a new, still unpublished method for the determination of the incorporation of antiplasmin into the fibrin network. A fibrin clot was formed after addition of calcium and thrombin to citrated plasma. The clot was washed by saline and solubilized with urea, and fibrin-bound antiplasmin was subsequently measured using an ELISA for antiplasmin. However, this method and the results raised several questions.

First, it is stated that the clots were completely resolved in 6 mol/L urea during incubation at 37°C for 120 min. We would request clarification on this issue, as from our understanding as what is reported in the literature, cross-linked fibrin clots (containing cross-linked antiplasmin) do not dissolve in urea (2). It is anticipated that only noncovalently fibrin-bound antiplasmin is extracted

by urea. Although noncovalently bound antiplasmin might theoretically be present in plasma clots (3), in a recent study we did not find evidence for the presence of this form of antiplasmin in plasma clots prepared and extracted in a comparable manner as in the study by Ågren et al. (4).

Second, resolution of cross-linked fibrin clots in urea requires the simultaneous presence of a reducing agent, such as β -mercaptoethanol or dithiothreitol, which cleaves the disulfide bridges in fibrin. It might be that the authors did use a reducing agent without mentioning the presence of such a compound. In that case, the reported antiplasmin antigen values might represent antiplasmin cross-linked to fibrin by FXIIIa. However, the amounts of fibrin-bound antiplasmin antigen (1.65 and 1.35 mg/L in patients and control subjects, respectively, corresponding to 2.1 and 1.6% of the total amounts of antiplasmin in plasma) were much lower than what other investigators, including Sakata and Aoki (5), found previously under comparable conditions (about 30%).

In conclusion, the observed difference in incorporation of antiplasmin into the fibrin clots of diabetic patients compared with control subjects might be interesting, but methodological issues should be solved before the findings make sense. These issues may include 1) the unknown efficiency of

the washing procedure of the fibrin clot to exclude that 1.6–2.1% antiplasmin just represents free antiplasmin not washed away, 2) the molecular identification of antiplasmin antigen (free antiplasmin vs. antiplasmin cross-linked to fibrin) by SDS-polyacrylamide gel electrophoresis followed by Western blotting, and 3) a validation of the ELISA for antiplasmin under the experimental conditions (effects of urea, reducing agent if present, or fibrin bound to antiplasmin). This information should explain the apparent contradictions with the literature.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

References

1. Ågren A, Jörneskog G, Elgue G, Henriksson P, Wallen H, Wiman B. Increased incorporation of antiplasmin into the fibrin network in patients with type 1 diabetes. *Diabetes Care* 2014;37:2007–2014
2. Lorand L. Factor XIII and the clotting of fibrinogen: from basic research to medicine. *J Thromb Haemost* 2005;3:1337–1348
3. Tsurupa G, Yakovlev S, McKee P, Medved L. Noncovalent interaction of α_2 -antiplasmin with fibrin(ogen): localization of α_2 -antiplasmin-binding sites. *Biochemistry* 2010;49:7643–7651
4. Talens S, Leebeek FW, Demmers JA, Rijken DC. Identification of fibrin clot-bound plasma proteins. *PLoS One* 2012;7:e41966
5. Sakata Y, Aoki N. Cross-linking of alpha 2-plasmin inhibitor to fibrin by fibrin-stabilizing factor. *J Clin Invest* 1980;65:290–297

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