Oxidative Modification of Fibrinogen Inhibits Its Transformation into Fibrin under the Effect of Thrombin

O. A. Azizova, A. P. Piryazev, A. V. Aseychev, and A. G. Shvachko

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Changes in the capacity of fibrinogen subjected to oxidative modification to transform into fibrin under the effect of thrombin and to form a fibrin clot were studied. The effects of oxidized fibrinogen preparations on the clot formation by citrate-treated donor plasma were evaluated by the thrombin time test. Oxidation impaired the capacity of isolated fibrinogen to form a fibrin clot under the effect of thrombin. Addition of oxidized fibrinogen solutions to donor plasma led to prolongation of the plasma clotting time. Maximum addition (33% volume) of oxidized fibrinogen led to a 10-26% prolongation of clotting time in comparison with addition of the same volume of the same solution without fibrinogen.

Key Words: *fibrinogen; thrombin; oxidative stress*

Oxidative stress plays the key role in the development of cardiovascular diseases. It was shown that the effect of oxidative stress is mediated through modified proteins and lipids [9], causing expression of various genes [5]. It was shown that oxidized lipoproteins play the key role in the development of atherosclerosis, in the plaque formation and its subsequent progress and rupture [1]. Fibrinogen (FB) is an independent risk factor for atherosclerosis and its complications [4]. In addition, FB is more sensitive to oxidative modification that other main plasma proteins (albumin, immunoglobulins, and transferrin) [7].

Oxidative modification of FB can modulate the clotting processes. It was shown that FB exposure to γ-rays or ultraviolet reduced its capacity to transform into fibrin [3,8]. A similar effect was observed after FB oxidation by peroxinitrite [6]. However in these studies FB was modified by irradiation under conditions much differing from its *in vivo* oxidation in oxidative stress.

We studied the effect of oxidative modification of FB on its transformation into fibrin and fibrin formation under the effect of thrombin in two systems: 1) isolated oxidized FB; and 2) blood plasma with oxidized FB.

MATERIALS AND METHODS

Fibrinogen was oxidized as described previously [2]. Lyophilized human FB (3 mg/ml; Sigma) was dissolved in phosphate buffer isotonic NaCl (pH 7.4). After addition of $FeSO_4$ and H_2O_2 (Fenton's reaction) the mixture was incubated during 1 h at 37°C, after which desferal (deferoxamine mesilate; Sigma) was added in the final concentration of 2 mM and 24-h single dialysis against the same buffer saline was carried out at ambient temperature.

Five FB preparations, differing by the concentrations of $FeSO_4$ and H_2O_2 (Fenton's reaction), were used. Fibrinogen preparation No. 1 was not

Laboratory of Biophysical Bases of Pathology, Institute of Physico-Chemical Medicine, Federal Agency for Public Health and Social Development, Moscow, Russia. ***Address for correspondence:*** o.azizova@mail.ru. O. A. Azizova, aseychev@mail.ru. A. V. Aseychev

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treated by these reagents, but was incubated in a thermostat under the same conditions and was dialyzed (spontaneous oxidation control). Other preparations were oxidized by $FeSO_4$ and H_2O_2 in the following proportions: 50 μM/50 μM for FB No. 2, 100 μM/10 μM for FB No. 3, 100 μM/250 μM for FB No. 4, and 500 μM/50 μM, respectively, for FB No. 5 (the final concentrations are shown).

Plasma or isolated FB clotting time were recorded on a Minilab-701 two-channel optical mechanical coagulometer (Yunimed), intended for studies of hemostasis by the plasma clotting method. Thrombin time was evaluated by Thrombin-test kit (Renam Firm). Analysis was carried out according to the instruction.

The effect of oxidized FB on citrate-treated donor plasma thrombin time was studied on plasma specimens with 4.8, 16.7, and 33.3% volume of oxidized FB (1 ml plasma+0.05, 0.2, or 0.5 ml FB, respectively). In order to take into consideration the plasma dilution in mixture with FB solution and the impact of low molecular-weight admixtures, plasma

TABLE 1. Effect of FB Oxidation on Thrombin-Catalyzed FB Transformation into Fibrin under Isolated Conditions without Plasma

Series	FB No. 1	FB No. 2	FB No. 3	FB No. 4	FB No. 5
I	10.00	>500	>800	>1000	>1000
	10.60	>500		>1000	>1000
	10.90				
	10.50				
	10.60				
	10.90				
	10.80				
X±*m*	10.61±0.12	N. e.	N. e.	N. e.	N. e.
II	19.00	>750	N. d.	>500	>500
	18.30	>750	N. d.	>500	>500
X±*m*	18.65±0.35	N. e.	N. e.	N. e.	N. e.
III	12.00	14.60	>300	>300	>300
	12.60	15.30			
	12.60	14.70			
	12.80				
X±*m*	12.5±0.18	14.87±0.22	N. e.	N. e.	N. e.
IV	24.10	80.40	>300	36.20	46.90
	>500	>250	>300	>450	164.00
	5.30			56.20	47.30
	23.10			68.90	8.10
X±*m*	N. e.	N. e.	N. e.	N. e.	66.6±33.8
V	82.60	42.00	27.10	32.10	29.60
	41.30	130.00	27.10	45.10	>200
	108.10	36.20	>500	>300	57.60
	159.00	148.80	>200	>300	>200
X±*m*	97.75±24.62	89.25±29.23	N. e.	N. e.	N. e.
VI	28.70	>100 c	>100 c	>100 c	>100 c
	30.80	>100 c	>100 c	>100 c	>100 c
	33.50				
	28.90				
X±*m*	30.48±1.12	N. e.	N. e.	N. e.	N. e.

Note. Results of 6 independent series of FB oxidation are presented. FB No. 1: spontaneous oxidation control; FB No. 2: 50 μM $FeSO_4$/50 μM H_2O_2; FB No. 3: 100 μM $FeSO_4$/10 μM H_2O_2; FB No. 4: 100 μM $FeSO_4$/250 μM H_2O_2; FB No. 5: 500 μM $FeSO_4$/50 μM H_2O_2 (the final concentrations are shown). N. e.: not evaluated. N. d.: no data.

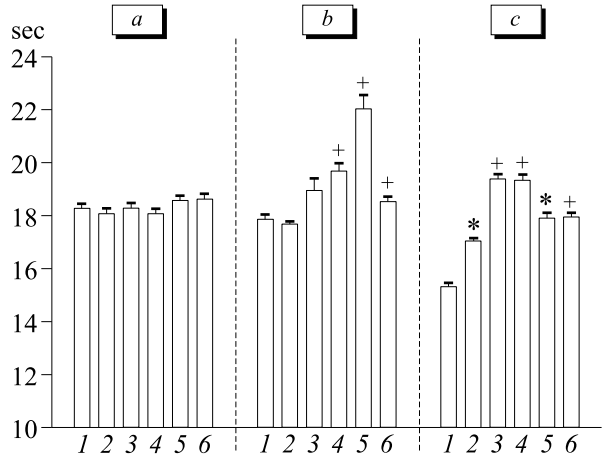

Fig. 1. Changes in donor plasma thrombin time after addition of oxidized FB preparations. Volume of added FB: *a*) 4.8%; *b*) 16.7%; *c*) 33.3%. *1*) control (addition of the same volume of dialysis solution); *2*) FB No. 1 (non-oxidized FB, subjected to dialysis: spontaneous oxidation control); *3*) FB No. 2 (50 μM $FeSO_4$/50 μM H_2O_2); *4*) FB No. 3 (100 μM $FeSO_4$/10 μM H_2O_2); *5*) FB No. 4 (100 μM $FeSO_4$/250 μM H_2O_2); *6*) FB No. 5 (500 μM $FeSO_4$/50 μM H_2O_2); the final concentrations are shown. $p<0.05$ *vs.* *control, +control and FB No. 1.

samples with similar volumes of the dialysis solution were used as the control. Thrombin time for each plasma sample incubated with FB was estimated by the results of 4-6 measurements. The significance of differences was evaluated using Student's *t* test with the probability of $p<0.05$.

RESULTS

The effect of oxidative modification of FB on its transformation into fibrin under the effect of thrombin was evaluated by changes in thrombin time (time during which FB transformed into fibrin) (Fig. 1). Addition of oxidized FB preparations led to prolongation of plasma clotting time. This effect started manifesting in the presence of FB intermediate concentration of 16.7% and increased with increase of the concentration to 33%. Plasma thrombin time was prolonged by 1-4 sec in comparison with the control values. Experiments demonstrated the emergence of anti-clotting activity during FB oxidation. Similar characteristics of oxidized FB were detected previously, when FB solutions were exposed to ultraviolet radiation [3]. However those studies did not show the changes in isolated oxidized FB characteristics in the thrombin time test.

We evaluated the effect of oxidation modification on thrombin-catalyzed transformation of FB proper without plasma. Six independent series of FB oxidation were tested (Table 1). Fibrinogen preparation No. 1 (FB spontaneous oxidation control) formed a clot within 10-30 sec (close to thrombin time for whole plasma) in 4 of 6 cases. With the rest FB preparations the clot formed only after a long time (about 100 sec), or in just part of specimens, or did not form during the time of recording (up to 100 sec) in the majority of other tests. Hence, oxidation impairs the FB capacity to transform into fibrin and form a fibrin clot. Presumably, two events take place: irreversible destruction of part of FB, leading to loss of capacity to form proper fibrin under the effect of thrombin, and manifestation of a sort of antithrombin activity by modified FB (observed after addition of oxidized FB to donor plasma).

The capacity of exogenous oxidized FB to prolong the clotting time (thrombin time) after its addition to native donor plasma was demonstrated previously [3] for FB exposed to ultraviolet radiation. The manifestation of this antithrombin activity after FB oxidation modified the proportion between blood clotting and anticlotting systems. This phenomenon can play the regulatory role in physiological adaptation to hypergeneration of active oxygen forms, while under conditions of oxidative stress, leading to failure of adaptation mechanisms, it can participate in the development of pathological processes. Oxidized FB can be involved in the development of the DIC syndrome and microthromboembolism.

Hence, oxidative modification of FB leads to reduction of its capacity to transform into fibrin. This violation is observed in solutions of oxidized FB and after its addition to the plasma.

REFERENCES

1. O. A. Azizova, E. V. Roitman, I. V. Dementyeva, *et al.*, *Byull. Eksp. Biol. Med.*, **129**, No. 6, 637-639 (2000).
2. E. M. Bekman, O. A. Baranova, E. V. Gubareva, *et al.*, *Ibid.*, **142**, No. 9, 268-272 (2006).
3. E. V. Roitman, O. A. Azizova, Yu. A. Morozov, and A. V. Aseychev, *Ibid.*, **138**, No. 9, 277-279 (2004).
4. M. Jink, C. H. Hennekens, and P. M. Ridker, *J. Am. Coll. Cardiol.*, **33**, 1347-1352 (1994).
5. H. Liu, R. Colavitti, I. I. Rovira, and T. Finkel, *Circ. Res.*, **97**, No. 10, 967-974 (2005).
6. G. Lupidi, M. Angeletti, A. M. Eleuteri, *et al.*, *FEBS Lett.*, **462**, No. 3, 236-240 (1999).
7. E. Shacter, J. A. Williams, M. Lim, and R. L. Levine, *Free Radic. Biol. Med.*, **17**, No. 5, 429-437 (1994).
8. E. Shacter, J. A. Williams, and R. L. Levine, *Ibid.*, **18**, No. 4, 815-821 (1995).
9. J. L. Witztum and D. Steinberg, *J. Clin. Invest.*, **88**, No. 6, 1785-1792 (1991).