# Effect of UV-Modified Fibrinogen on Platelet Aggregation in Platelet-Rich Plasma

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Oxidized UV-modified fibrinogen activates platelets in platelet-rich plasma. Kinetic turbidimetry showed that addition of oxidized fibrinogen to platelet-rich plasma led to platelet aggregation. Reversible aggregation is recorded starting from the 30th second and then constantly grows with the same rate. Nonoxidized fibrinogen produced no such effect. The relationship between aggregation intensity and rate and the degree of fibrinogen oxidation was described by a bell-shaped curve with a peak corresponding to 24% fibrinogen oxidation. The amplitude of aggregation increased with increasing the concentration of irradiated fibrinogen from 0.1 to 1.0 mg/ml and then plateaued. The rate of aggregation little depended on fibrinogen concentration.

**Key Words:** *free-radical oxidation; fibrinogen; platelets; aggregation; UV irradiation*

Plasma fibrinogen plays a key role in the maintenance of blood homeostasis. The development and exacerbation of cardiovascular diseases is often associated with increased plasma concentration of fibrinogen [4,5,10].

It is noteworthy that the development of atherosclerosis and cardiovascular diseases is closely related to activation of lipid peroxidation (LPO) [1]. LPO processes are most intensive during exacerbations of these diseases, for example in myocardial infarction or brain stroke [12]. Proteins are among the main targets of oxidative modification [6]. Fibrinogen is known as one of the most readily oxidizible plasma proteins. *In vitro* fibrinogen is 20-fold more oxidizable than serum albumin [8]. It can be hypothesized that the development of cardiovascular diseases is accompanied by fibrinogen oxidation and oxidized fibrinogen (OF) is an important factor stimulating the formation of thrombi.

The process of thrombus formation includes a cascade of reactions involving plasma proteins and platelets, which play an important role in blood clotting. Fibrinogen plays an important role in platelet activation, and, as it is easily oxidized, it can appear in the blood during the development of cardiovascular diseases closely associated with LPO. The question is whether fibrinogen, modified by oxidation, modulates platelet aggregation. We tried to answer this question in our study.

## MATERIALS AND METHODS

Human plasma fibrinogen (Sigma) containing about 65% protein (microbiuret method) was used. Fibrinogen was dissolved in a buffer containing (in mM): 5 D-glucose, 10 HEPES, 1 $NaH_2PO_4$, 1 $MgCl_2 \times 6H_2O$, 5 KCl, and 140 NaCl.

For oxidative modification, fibrinogen solution was exposed to UV radiation under a mercury lamp. Fibrinogen oxidation was evaluated by measuring tryptophan fluorescence at 280 and 340 nm excitation and emission wavelengths on a Perkin Elmer spectrofluorimeter.

Blood collected from 15 donors was stabilized with 3.8% sodium citrate (pH 6.0, 1:6 volume ratio).

Platelet-rich plasma (PRP) was prepared routinely [2]. Platelet aggregation was studied by kinetic turbidimetry on an automated analyzer of platelet

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aggregation (Biola). PRP was mixed with fibrinogen solution of a known concentration, and the aggregation curves were recorded. The mean size of platelet aggregates was taken as a quantitative parameter of aggregation. Platelet aggregation was evaluated by the peak of aggregation curve (degree of aggregation) and the rate of aggregation determined as the maximum slope of the aggregation curve. The relative values of aggregation rate and degree on different days were compared. The relative values of these parameters were estimated as the ratio of experimental values at each irradiation dose or OF concentration to the maximum amplitude or aggregation rate during the same experiment.

The data are presented as the mean±standard error for 5-6 experiment series.

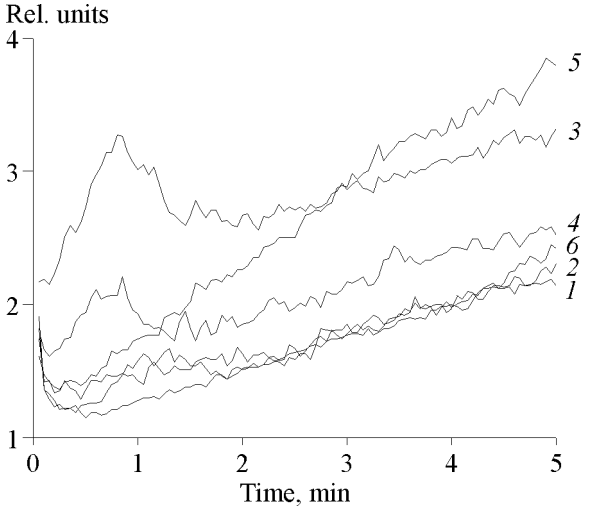


**Fig. 1.** Platelet aggregation induced by native fibrinogen (*1*) and fibrinogen oxidized by 12.7% (*2*), 24.0% (*3*), 38.2% (*4*), 46.3% (*5*), and 63.3% (*6*) in a representative experiment. Ordinate: mean size of aggregates.

## RESULTS

In preliminary experiments we investigated changes in the tryptophan fluorescence of fibrinogen after UV exposure; the decrease in this fluorescence was taken asa measure of protein oxidation. OF with oxidation degree of 12.7 to 63.3% was used in further experiments.

For evaluation of the effect of OF on platelet aggregation, UV-modified fibrinogen was mixed (1:1) with donor PRP and platelet aggregation curve was immediately recorded.

Addition OF to PRP induced reversible aggregation: the rate of aggregation slowly increased starting from the 30th sec and peaked 1 min after the beginning of recording (Fig. 1).

The relationship between the degree of fibrinogen oxidation and intensity of reversible aggregation was described by a bell-shaped curve; the maximum aggregation was observed when the intensity of tryptophan fluorescence decreased by 24%. Further oxidation of fibrinogen did not promote aggregation (Fig. 2). In some cases no aggregation was observed after oxidation of more than 50% tryptophan residues. Figure 1 presents kinetic curves of platelet aggregation induced by fibrinogen (2 mg/ml) of different degree of oxidation.

Then we evaluated the relationship between OF concentration and the degree of platelet aggregation. The amplitude of aggregation increased with increasing OF concentration from 0.1 to 1.0 mg/ml and then plateaued at a concentration of about 1.0 mg/ml (Fig. 3, *a*). The aggregation rate little depended on OF con-

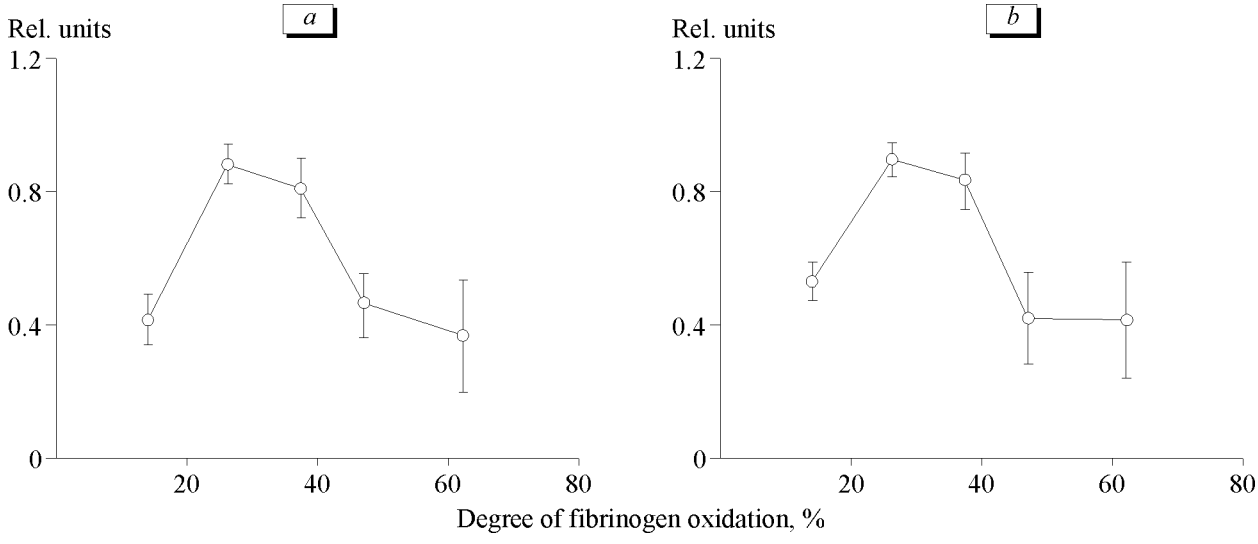


**Fig. 2.** Platelet aggregation in platelet-rich plasma induced by fibrinogen with different degree of oxidation (2 mg/ml). Here and in Fig. 3: ordinates: rate (*a*) and degree (*b*) of platelet aggregation.

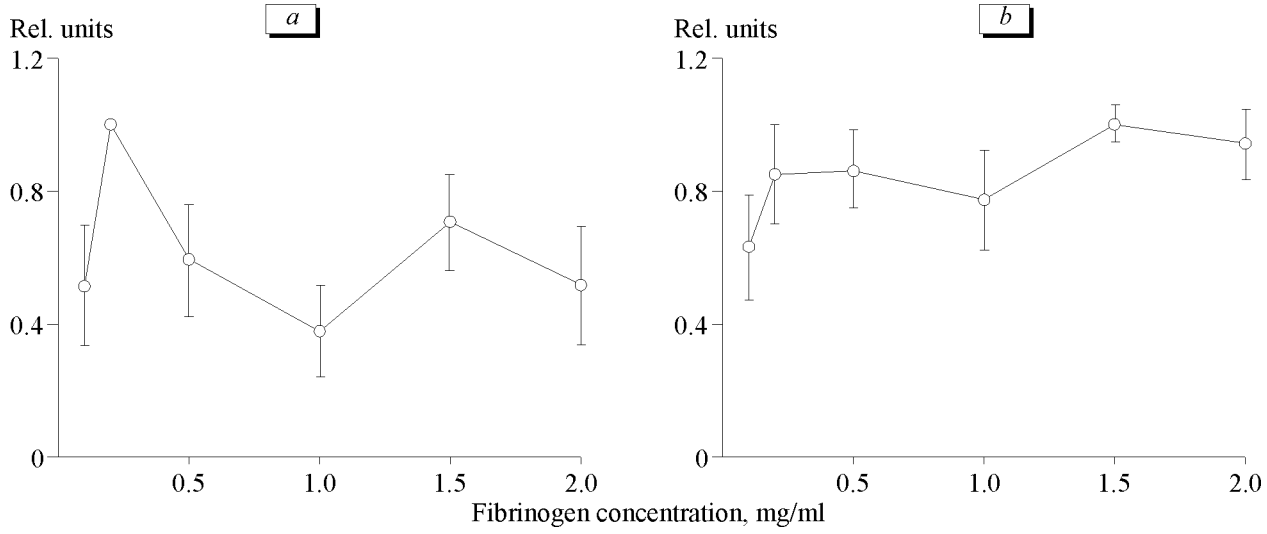


**Fig. 3.** Platelet aggregation in platelet-rich plasma induced by different concentrations of oxidized fibrinogen (single UV exposure, oxidation of 24% tryptophan residues).

centration (Fig. 3, *b*), while the amplitude decreased at OF concentration above 2.5 mg/ml.

Native fibrinogen in some experiments induced no platelet aggregation, but sometimes addition of normal fibrinogen led to a dose-dependent platelet aggregation. However, the aggregation rate was lower than in the presence of OF. Hence, platelets in PRP were activated by UV-oxidized fibrinogen.

It was previously shown that fibrinogen oxidation inhibits ADP- and thrombin-induced aggregation and thrombus formation [3,7-9]. These authors used various methods of fibrinogen oxidation: photooxidation, X-ray exposure, metal ions of alternating valence. Experiments with whole blood showed that photooxidation of fibrinogen in the presence of a prooxidant (methylene blue) in a high dose (50 μM) blocks fibrinogen binding to fibrinogen receptor (GP IIb/IIIa) on platelets and inhibits ADP-induced platelet aggregation [3,7]. In our experiments moderately oxidized fibrinogen directly activated platelet aggregation in the absence of other aggregation inductors, while highly oxidized fibrinogen had no effect on platelet aggregation. Presumably, inhibition caused by platelet aggregation inductors was due to higher degree of fibrinogen oxidation. Some reports describe thrombus formation stimulated by OF [11] similar to the phenomenon we observed.

Our findings indicate that OF can induce platelet activation and aggregation. The relationship between the intensity of aggregation and irradiation dose is described by a bell-shaped curve. The maximum aggregation was observed at moderate irradiation doses, while high doses were less effective (Figs. 1 and 2).

Presumably, free-radical oxidation of fibrinogen contributes to impairment of vascular homeostasis and stimulates thrombus formation. This hypothesis is based on the fact that the development of cardiovascular diseases is associated with enhanced thrombus formation and free-radical oxidation. These facts together with easy oxidation of fibrinogen and its key role in vascular homeostasis suggest that OF is present in the blood and affects thrombus formation processes.

These results indicate that OF can modulate blood clotting and play an important role in the development of cardiovascular diseases and atherosclerosis.

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