REVISTA ESPAÑOLA DE FISIOLOGIA
R. esp. Fisiol., 20, n.° 3, págs. 101-107, 1964

Department of Biochemistry
School of Medicine
University of Navarra
Pamplona

Fibrinolytic activity of the Hageman factor

by

J. Aznar*, E. Santiago and J. M. Macarulla

(Recibido para publicar el 6 de julio de 1964)

The Hageman factor (HF) is a hemocoagulative factor whose action on blood coagulation covers many aspects. It acts essentially in the development of the intrinsic activator of prothrombin (4-7, 15, 21-25, 27, 29, 30-33). It also seems to play a not clearly defined role in the intrinsic system; it acts on the platelet viscous metamorphosis by favoring its start (12, 28); it also has an effect on haemostasis, through its action on capillary walls (17, 26, 32); it releases a substance which causes pain on the smooth muscles (14). Finally, the aspect which will be dealt with in this article, is that it has a marked fibrinolytic activity.

NIEWIAROWSKI and PROU-WARTELLE (19) and SOULIER and PROU-WARTELLE (31) have shown that «native plasma» increases its fibrinolytic activity when it comes in contact with silica surfaces. Later on NIEWIAROWSKI *et al.* (20) demonstrated lytic activity on eluate of contact factor, described as having an arginine-estearase character. MARGOLIS (16) also confirms that a «native plasma» in contact with silica surfaces develops proteolytic activity and they showed that this effect is due to the HF (17). At the same time Iatridis *et al.* (8) showed lytic activity on a plasma fraction «Hageman like» and confirmed that the effect of the surface on the native plasma can play a role on the activation of plasminogen. He attributed the effect to the HF (9-10). He finally showed that this type of action belongs to the fibrinolytic system and believe that it functions by activating the plasmatic pro-activator (11).

RATNOFF (25-27) however, holds a different opinion, doubting about the fibrinolytic character of HF.

Because of these different points view it has seemed to us timely to review the problem and to try to explain clearly the role that HF plays within the fibrinolytic system.

We have previously confirmed the lytic activity of a «native plasma» after being activated with celite (3). Later we have studied (2) this point in more detail and at the same time we investigated the lytic activity of the two factors which make up the contact-factor. In the present article we describe the role of HF

* J. A. thanks the Patronato de Protección Escolar for a postgraduates fellowship.

within the fibrinolytic system. We define the HF as an activator of both plasminogen and pro-activator; it is therefore an indirect fibrinolytic agent.

Materials and methods

1. Streptokinase («Varidase» Lederle). 2. Celite («Dicalite» Speedex Great Lakes Carbon Corporation). 3. Silicone («Siliclad» Clay-Adams). 4. Thrombin («Topostasin» Roche). 5. Fibrinogen (Bovine Fibrinogen. Fraction I from bovine plasma. Armour Pharmaceutical Company). 6. «Native plasma» (2). 7. Eluate of Hageman factor (2). 8. Euglobulin precipitate (11). 9. *Estimation of fibrinolysis.* Fibrinolytic activity was determined by the fibrin-plate method of ASTRUP and MÜLLERTZ (1). The activities were recorded as the product (in mm^2) of two perpendicular diameters of the lysed zones. 10. Protein content of eluate of HF and euglobulin precipitate by the method of LOWRY *et al* (13).

Results

In the study of the fibrinolytic activity of the HF we have carried out the following experiments:

1. *Study of the fibrinolytic activity of HF eluate on a hot fibrin plate.*

TABLE I

Lysis activity of the HF eluate on hot fibrin plates.

Exper.	Diameter of lysis area mm.
1-43	0
44	5
45	4
46	5
47	5
48	5
49	4
50	0

We incubated several drops of HF eluate on fibrin plates, which had been previously heated at 85° C. for half an hour. The readings were done after 24 hours of incubation at 37° C. The results show (table I) that scarce lytic activity was developed; from 50 determinations only 7 exhibited lytic activity. This seems to indicate that the HF is an indirect activator of fibrinolysis. Similar determinations using streptokinaso were negative in all the tests.

2. *Study of the fibrinolytic activity of the HF eluate on a cold fibrin plate.*

Following the same technique we studied the fibrinolytic activity of the eluate problem but using a cold fibrin plate instead. All of the 100 determinations made showed evident lytic activity (table II) contrasting sharply with the results obtained with a hot plate.

3. *Study of the fibrinolytic activity of different solutions of streptokinase on a cold fibrin plate.*

The results were all negative. After 24 hours of incubation of different streptokinase solutions on a cold fibrin plate, no lytic activity could be observed.

4. *Fibrinolytic activity of a mixture of HF and streptokinase.*

Different amounts of streptokinase were incubated with 0.5 ml. of HF eluate at 37° C. for 10 minutes. At the end of this period in order to confirm its fibrinolytic activity we added a drop of the incubation mixture which contains approximately 40 μg of HF, and the quantities of streptokinase indicated on the table. The lysis areas obtained (table III) were approximately the same in all the trials. This seems at firts to indicate that the streptokinase has no fibrinolytic activity when bovine fibrinogen is used to prepare the plate. The lytic activity observed depends only on the quantity of HF present.

TABLE II

Lysis activity of the HF eluate on cold fibrin plates

Largest and smallest diameter of lysis area in mm	
13-13	7- 7
12-12	7- 7
9- 9	7- 7
10-10	10- 6
11-11	7- 7
11-11	7- 7
13-13	8- 8
12-13	11-13
10-10	8- 9
13-10	11-12
15-12	11-11
12-15	11-11
13-13	11-11
13-13	11-11
13-13	12-12
11-11	12-12
13-12	11-10
14- 9	11-11
13-11	10-10
10-10	10- 8
10- 9	9- 9
9-11	8- 8
11-11	8- 8
10-10	10- 8
12-12	14- 7
12-10	10-10
10-10	7- 9
10-10	8- 9
10-10	9- 9
15-15	9- 9
16-14	8- 8
14-14	10-10
17-17	11-10
15-16	11-11
15-15	10- 7
13-16	9- 7
13-13	7- 7
13-15	9- 9
15-15	8- 8
12-13	10- 8
13-13	10-10
14- 9	11-13
8- 8	9- 9
10-15	8- 9
16-12	8- 7
13-13	9- 9
10- 8	9- 9
11-11	9- 9
12-12	9- 9

5. *Study of the lytic activity of a sample of euglobulin precipitate.*

Samples of euglobulin concentrates obtained from human plasma display light fibrinolytic activity on a cold fibrin plate (table IV).

6. *Study of the fibrinolytic activity of a mixture of HF and a euglobulinic precipitate.*

A euglobulin concentrate obtained from human plasma and containing an approximate protein concentration of 4 to 6 mg. per ml. was incubated with the HF eluate. After 10 minutes of incubation at 37° C. several samples of the mixture were taken and their lytic activity tested on a cold fibrin plate. Each drop contained approximately 100 μg of euglobulin and 10 μg of HF.

The results obtained indicate an activation by the HF of the euglobulin precipitate since the lysis areas observed were larger than the values obtained by adding the effects of both reactants when used separately (table IV).

7. *Study of the activity of a mixture of euglobulin precipitate and streptokinase.*

Following the same technique as in 6, different quantites of euglobulin precipitate and streptokinase were incubated. Each drop placed on the fibrin plate contained approximately 100 μg of plasmatic euglobulins and an amount of streptokinase as indicated on table IV.

It can be seen that the streptokinase clearly increases the fibrinolytic activity of the euglobulin precipitate as it corresponds to its role of activator. In like manner it can be verified that the activity obtained is not a summation of effects but a direct consequence of the activation.

Discussion

To carry out this study the fibrin plate technique of ASTRUP and MÜLLERTZ (1)

TABLE III

Lysis activity of mixtures of HF eluate and variable amounts of streptokinase

Problem.	Largest and smallest diameter of lysis area in mm
Eluate of Hageman factor + 5 u.u. of Streptokinase	20-20 21-20 22-22 19-20 18-19 23-23 23-23 21-22 19-18 17-17 18-19 15-14 20-21
Eluate of Hageman factor + 20 u.u. of Streptokinase	19-19 20-20 20-14 21-23 23-23 22-22 19-18 27-16 21-21 23-21 21-21 21-21 19-20
Eluate of Hageman factor + 100 u.u. of Streptokinase	21-21 18-18 22-22 23-23 21-21 15-15 20-20 20-20 20-20 21-21 22-22 20-20 20-20

was used. As pointed out previously, the results vary with the type of fibrinogen used, since depending on the species from which it was obtained it can be contaminated with different substances. Our experiments have been performed using bovine fibrinogen which is, as it is known, contaminated with plasminogen (18) and therefore the results obtained should be judged bearing this in mind.

In the study of the fibrinolytic activity with the fibrin plate two types of plates can be used :

1. Hot plates which only contain fibrin.

2. Cold plates made basically o fibrin and plasminogen.

Two possibilities can occur in the study of a substance with fibrinolytic activity : that it be a direct or indirect fibrinolytic agent. The direct fibrinolytic agents are those having the capacity to cause lysis of fibrin directly. To the indirect type belong those which base their activity on the activation of substances which can subsequently change into plasmin. As a consequence of this they cannot act if there are no changeable substances present. There are exclusively only two such substances : plasminogen and proactivator.

The following results were obtained when we studied a direct fibrinolytic agent with a fibrin plate made with bovine plasminogen :

1. Hot plate : positive. 2. Cold plate : positive.

If an indirect fibrinolytic agent is used there can be two possibilities :

1. Hot plate : negative. Cold plate : negative. 2. Hot plate : negative. Cold plate : positive.

In the first case it will be an activator of the plasmatic proactivator which has no substrate on the plate to act on and thus cannot start the lysis. In the second case if it produces the lysis it must be because the plasminogen of the plate change to plasmin and so it must be a

plasminogen activator. However in this second case we cannot exclude the weak activating effect of the proactivator because it is always present in very small quantities.

In the light of the previous considerations we are going to study the fibrinolytic activity of the HF. In the first place we will try to specify if the HF is a direct or indirect fibrinolytic factor. We incubated an eluate problem on a hot fibrin plate, lacking plasminogen, the results obtained were always negative; this indicates clearly that the HF is not a direct fibrinolytic agent since it is unable to cause the lysis of a coagulum composed only of fibrin.

Repeating the same steps but using a cold plate we obtained a clear lytic effect which indicates the direct character of the fibrinolytic activity o the HF.

In the same manner similar experiments were done with streptokinase instead of the eluate problem. The lytic effects obtained were negligible. We can see one difference between the HF and streptokinase which makes us think that their activities are not completely identical. Thus the HF does not have exclusively the function of an activator of the plasmatic proactivator as in the case of the streptokinase.

With the purpose of verifying the previous aspects we did some crossed experiments between the HF and streptokinase, using in all of them identical quantities of HF and variable amounts of streptokinase. The areas of lysis obtained were practically the same in all the cases showing that it was independent of the amount of streptokinase but related exclusively to the quantity of HF added (table III).

As a result of all the facts and remembering that the coagulum formed in the fibrin plate contains an abundant quantity of plasminogen and very little or no proactivator we think that the effect obtained is due to an activation of the plasminogen.

From experiments done previously (2) we suspected that the HF eluate could also be an activator of the plasmatic proactivator. To this end a few crossed experiments were made using a sample of euglobulinic precipitate and HF eluate. When the plasma euglobulin precipitate was incubated alone on the fibrin plate it showed negligible fibrinolytic activity and which without a doubt is related to the very small amount of plasminogen

TABLE IV

Lysis activity of different streptokinase dilutions, HF factor, euglobulin precipitate and mixtures de those factors

Problem.	Average diameter in mm	± σ
Streptokinase 5 u.u.	0	0
Streptokinase 10 u.u.	0	0
Streptokinase 20 u.u.	0	0
Eluate of Hageman factor	10,2	1,1
Euglobulin precipitate	5,6	0,7
Euglo. prec. + 5 u.u. strep.	24,7	1,4
Euglo. prec. + 20 u.u. strep.	27,0	2,1
Euglo. prec. + 100 u.u. strep.	29,6	2,5
Euglo. prec. + Eluate HF	20,7	1,2

present. After incubating it for 10 minutes at 37° C. with a certain amount of HF eluate the lytic activity of the mixture increased sharply. We do not think that it could have been caused by a summation of the effects of the two reactants but it is rather an activation of the precipitate by the HF eluate. Since the euglobulin precipitate was obtained from human plasma which is rich in the plasmatic proactivator we think that the HF has a capacity to activate the fibrinolytic factor.

Identical experiments were made using streptokinase instead of HF eluate. The results which were all similar seem to indicate that in this respect the HF and the streptokinase have identical functions.

Since the eluate of contact factor contains only HF and PTA (3) and has no plasminogen nor plasmin (2) we think that all the described results could be attributed to the HF. Besides we have already shown how PTA, within the contact factor, lacks fibrinolytic activity.

Thus we can affirm that HF exhibits a marked indirect fibrinolytic activity. Therefore, we have established that HF produces both, a direct activation of plasminogen and an indirect activation through the previous activation of the plasmatic proactivator.

Summary

The fibrinolytic activity of the HF has been studied. This property of HF has been confirmed. It acts as an activator of both plasminogen and proactivator.

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