Biochimica et Biophysica Acta, 327 (1973) 207–212

BBA 67064

THE RELATION OF C1sa, A SUBUNIT OF THE ACTIVATED FIRST COMPONENT OF COMPLEMENT, TO OTHER PLASMA ENZYMES

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(Received June 1st, 1973)

SUMMARY

The relation of purified C1sa to other plasma enzymes was investigated. C1sa does not activate purified plasminogen or plasma kallikreinogen. No conversion of proenzyme C1s to C1sa was observed on incubation of purified C1s alone or with purified C1sa, but addition of C1r (one of the three subunits of C1) caused rapid conversion of C1s to C1sa. Moreover, it was found that purified plasmin and plasma kallikrein converted C1s to C1sa very slowly.

NTRODUCTION

It is well known that the first component of complement C1 (C1sa) initiates the directed sequence of reactions involving the remaining eight components of complement. However, there is little evidence for the interaction of C1sa with other plasma enzymes, such as plasmin or kallikrein. Lepow *et al.*[1] reported that addition of partially purified streptokinase to human serum inactivated the complement, and further work suggested that plasmin activated C1. Recently, Ratnoff and Naff[2] and Naff and Ratnoff[3] reported the conversion of proenzyme C1s to C1sa by plasmin, trypsin or C1r, respectively. On the other hand, Donaldson[4] suggested that kallikrein may directly or indirectly activate C1, but this has not been proved.

A highly purified preparation is required to examine these relationships in detail. This paper reports the relation of highly purified C1sa to other plasma enzymes.

MATERIALS AND METHODS

Materials

N^{α}-Acetyl-L-tyrosine ethyl ester (ATEE), N^{α}-tosyl-L-arginine methyl ester (TAME) and N^{α}-acetyl-L-arginine methyl ester (AAME) were purchased from the Foundation for Promotion of Protein Research, Institute for Protein Research,

Abbreviations: ATEE, N^{α}-acetyl-L-tyrosine ethyl ester; TAME, N^{α}-tosyl-L-arginine methyl ester; AAME, N^{α}-acetyl-L-arginine methyl ester.

Osaka University, Osaka. Trypsin was purchased from Sigma Chemical Co. "Varidase" was purchased from American Cyanamide Co., Pearl River, N.Y., and was used as streptokinase (human plasminogen activator). Purified C1sa was prepared as described previously[5]. This preparation was homogeneous on ultracentrifugation and disc-gel electrophoresis, and 1 mg of C1sa hydrolyzed 820.5 μmoles of ATEE in 0.1 M phosphate buffer, pH 7.4, at 37 °C, in 15 min. Purified C1s and partially purified C1r which are subunits of the first component, were prepared as described by Okamura *et al.*[6]. Purified C1s was homogeneous on ultracentrifugation and disc-gel electrophoresis. One mg of C1r hydrolyzed 45.0 μmoles of AAME in 0.1 M phosphate buffer, pH 7.4, at 37 °C, in 15 min. Purified human plasminogen was prepared and converted to plasmin by addition of streptokinase as described by Muramatu *et al.*[7]. This preparation was also homogeneous on ultracentrifugation and disc-gel electrophoresis, and the preparation of human plasmin obtained from 1 mg of plasminogen hydrolyzed 170.0 μmoles of TAME in 0.1 M Tris–HCl buffer, pH 8.5, at 37 °C, in 15 min. A partially purified preparation of kallikreinogen from human plasma was obtained as follows: The euglobulin fraction was treated with 0.03 M EDTA, at pH 4.8, as described previously[7]. The mixture was stirred overnight and insoluble protein was removed by centrifugation at 4000 rev./min for 15 min. The supernatant was dialyzed against 0.1 M Tris–HCl buffer, pH 8.5, overnight and then used as the preparation of plasma kallikreinogen. Purified human plasma kallikrein activated with acetone was prepared as described by Tsutsumi[8]. This preparation was homogeneous on ultracentrifugation and disc-gel electrophoresis, and 1 mg of the plasma kallikrein hydrolyzed 210.0 μmoles of TAME in 0.1 M Tris–HCl buffer, pH 8.5, at 37 °C in 15 min.

Assay of enzymes and protein

The esterolytic activity of C1sa was determined by incubating it with ATEE as substrate in 0.1 M phosphate buffer, pH 7.4, at 37 °C in 15 min. The esterolytic activities of plasmin and kallikrein were determined from the amounts of hydrolysis of TAME in Tris–HCl buffer, pH 8.5, at 37 °C in 15 min. The ester remaining was determined by Hesterin's method as modified by Roberts[9]. Protein concentration was determined by the method of Lowry *et al.*[10] using bovine serum albumin (Armour Pharmaceutical Co.) as standard.

RESULTS

Effects of C1sa on the activation of C1s, plasminogen and kallikreinogen

Volumes of 0.2 ml of C1s (372 μg/ml) were incubated for 0, 15, 30, 45 and 60 min at 37 °C with 0.2 ml of several dilutions (1–1/4) of C1sa (67.0 μg/ml) and then ATEE hydrolytic activity was assayed. ATEE hydrolytic activity of C1s alone was very low (0.5–0.7 μmole ATEE). However, when activated with 0.5 μg of trypsin for 5 min at 37 °C as described previously[6], it hydrolyzed 24.6 μmoles of ATEE at pH 7.4 and 37 °C in 15 min. Undiluted C1sa used (13.4 μg) hydrolyzed 10.8 μmoles of ATEE at pH 7.4 and 37 °C in 15 min. Under these conditions, no conversion of C1s to C1sa was observed on incubation of purified C1s alone or with purified C1sa. A typical experiment is shown in Table I.

Plasminogen was also not converted to plasmin by addition of purified C1sa.

TABLE I

EFFECT OF C1sa UPON C1s

ATEE hydrolytic activities were assayed in 0.1 M phosphate buffer, pH 7.4, and 37 °C in 15 min at a substrate concentration 15 mM. C1s contained 372 μg of protein per ml. C1sa contained 67.0 μg of protein per ml.

Incubation time at 37 °C (min)	*ATEE hydrolytic activity (μmoles)*				
	C1s, 0.2 ml + C1sa (in 0.2 ml)				
	*Undiluted**	*1/2*	*1/4*	*0*	*Undiluted + trypsin (0.5 μg)***
0	11.3	5.9	3.2	0.7	24.6
15	11.5	6.0	3.3	0.6	
30	10.0	5.7	3.0	0.6	
45	9.9	5.7	3.1	0.6	
60	9.8	5.1	2.7	0.6	

* Undiluted C1sa (13.4 μg) hydrolyzed 10.8 μmoles of ATEE at pH 7.4 and 37 °C in 15 min.
** Incubation was carried out at pH 7.4 and 37 °C for 5 min.

A typical experiment is shown in Table II. Volumes of 0.2 ml of plasminogen (800 μg/ml) and 0.2 ml of several dilutions (1–1/2) of C1sa (67.0 μg/ml), which had been dialyzed against 0.1 M Tris–HCl buffer, pH 8.5, were incubated for various times at 37 °C.

TABLE II

EFFECT OF C1sa UPON PLASMINOGEN

TAME-hydrolytic activities were assayed in 0.1 M Tris–HCl buffer, pH 8.5 and 37 °C in 15 min, at a substrate concentration 10 mM. Plasminogen contained 800 μg of protein per ml. C1sa contained 67.0 μg of protein per ml.

Incubation time at 37 °C (min)	*TAME-hydrolytic activity (μmoles)*			
	Plasminogen, 0.2 ml + C1sa (in 0.2 ml)			
	*Undiluted**	*1/2*	*0*	*Undiluted + streptokinase** (1000 units)*
0	8.5	4.4	0	25.0
15	8.3	4.4	0	
30	8.6	4.5	0	
45	8.5	4.3	0	
60	8.7	4.2	0	

* Undiluted C1sa (13.4 μg) hydrolyzed 8.5 μmoles of TAME at pH 8.5 and 37 °C in 15 min.
** Incubation was carried out at pH 8.5 and 37 °C for 5 min.

Undiluted C1sa used (13.4 μg) hydrolyzed 8.5 μmoles of TAME at pH 8.5 and 37 °C in 15 min. In these experiments, plasminogen had almost no detectable TAME hydrolytic activity. However, when activated with 1000 units of streptokinase, it hydrolyzed 25.0 μmoles of TAME at pH 8.5 and 37 °C in 15 min.

The effect of C1sa on kallikreinogen was examined (Table III). Volumes of 0.2 ml of kallikreinogen (34.0 mg/ml) were incubated for various times with 0.2 ml

TABLE III

EFFECT OF Clsa UPON KALLIKREINOGEN

TAME-hydrolytic activities were assayed in 0.1 M Tris–HCl buffer, pH 8.5 and 37 °C in 15 min, at a substrate concentration 10 mM. Kallikreinogen contained 34.0 mg of protein per ml. Clsa contained 67.0 μg of protein per ml.

Incubation time at 37 °C (min)	*TAME-hydrolytic activity (μmoles)*			
	Kallikreinogen, 0.2 ml + Clsa (in 0.2 ml)			
	*Undiluted**	*1/2*	*0*	*Undiluted + acetone treatment***
0	8.7	4.2	0.1	23.8
15	8.4	4.4	0.2	
30	8.6	4.4	0.2	
45	8.6	4.5	0.2	
60	8.6	4.4	0.2	

* Undiluted Clsa (13.4 μg) hydrolyzed 8.5 μmoles of TAME at pH 8.5 and 37 °C in 15 min.
** Treatment with 20% of acetone at 25 °C for 3 h, by the method of Webster and Pierce[11].

of several dilutions (1–1/2) of Clsa (67.0 μg/ml), which had been dialyzed against 0.1 M Tris–HCl buffer, pH 8.5. Undiluted Clsa used (13.4 μg) hydrolyzed 8.5 μmoles of TAME at pH 8.5 and 37 °C in 15 min. No conversion of kallikreinogen to kallikrein was observed on addition of purified Clsa under these conditions. On the other hand, kallikreinogen was rapidly converted to kallikrein on treatment with 20% acetone by the method of Webster and Pierce[11]. Activated kallikrein (6.80 mg) hydrolyzed 23.8 μmoles of TAME at pH 8.5 and 37 °C in 15 min.

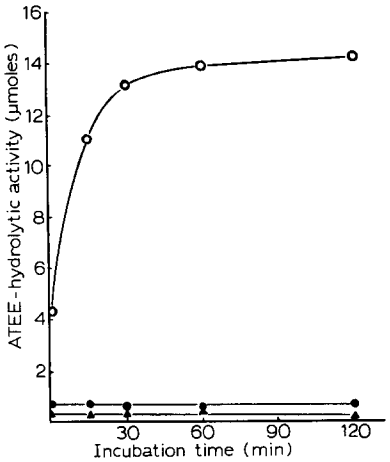
Effect of Clr, plasmin and kallikrein on the activation of Cls

Activation of Cls on incubation with Clr, plasmin or kallikrein was examined in 0.1 M phosphate buffer, pH 7.4.

Volumes of 0.2 ml of Cls (210 μg/ml) were incubated with 0.2 ml of Clr (2.78 mg/ml) for 0, 15, 30, 60 and 120 min at 37 °C. Clr used (556 μg) has almost no detectable ATEE hydrolytic activity, but hydrolyzed 25.0 μmoles of AAME at pH 7.4 and 37 °C in 15 min. As shown in Fig. 1, conversion of Cls to Clsa was rapid and maximum activity (13.4 μmoles ATEE) was obtained within 30 min under these conditions. On the other hand, when Cls was activated with 0.5 μg of trypsin at 37 °C for 5 min as described previously[6], it also hydrolyzed 13.8 μmoles of ATEE at pH 7.4 and 37 °C in 15 min.

A typical experiment on the effect of plasmin on Cls is shown in Fig. 2. Conversion was very slow and maximum activity (7.3 μmoles ATEE) was observed after incubation of 60 min, at 37 °C. Volumes of 0.2 ml of Cls (210 μg/ml) and 0.1 ml of plasmin (863 μg/ml), which had been dialyzed against 0.1 M phosphate buffer, pH 7.4, were incubated for various times at 37 °C and then ATEE hydrolytic activity was assayed. Plasmin used (86.3 μg) had almost no detectable ATEE hydrolytic activity. It hydrolyzed 11.5 μmoles of TAME at pH 7.4 and 37 °C in 15 min. In other experiments, 0.2, 0.3 and 0.4 ml of plasmin were used under the same conditions. More rapid activation was observed with these higher concentrations of plasmin.

Fig. 3 shows the effect of plasma kallikrein on Cls. Volumes of 0.2 ml of Cls



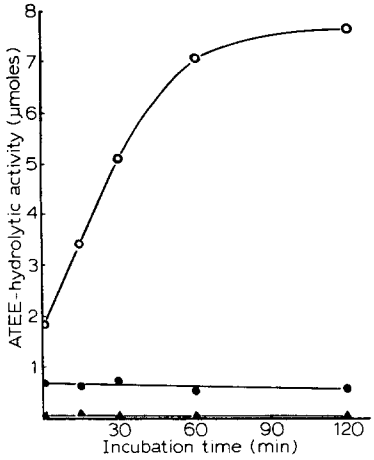


Fig. 1. Effect of Clr upon Cls. 0.2 ml of Cls (210 μg/ml) was incubated with 0.2 ml of Clr (2.78 mg/ml) in 0.1 M phosphate buffer, pH 7.4, at 37 °C, and at intervals, ATEE hydrolytic activity was assayed by incubation at pH 7.4 and 37 °C for 15 min. The Clr used (555 μg) hydrolyzed 25.0 μmoles of AAME, in 15 min. ○—○, Cls + Clr; ●—●, Cls only; ▲—▲, Clr only.

Fig. 2. Effect of plasmin upon Cls. 0.2 ml of Cls (210 μg/ml) was incubated with 0.1 ml of plasmin (863 μg/ml) in 0.1 M phosphate buffer, pH 7.4, at 37 °C, and at intervals, ATEE hydrolytic activity was assayed by incubation at pH 7.4 and 37 °C for 15 min. The plasmin used (86.3 μg) hydrolyzed 11.5 μmoles of TAME, in 15 min. ○—○, Cls + plasmin; ●—●, Cls only; ▲—▲, plasmin only.

(210 μg/ml) were incubated with 0.1 ml of kallikrein (732 μg/ml), which had been dialyzed against 0.1 M phosphate buffer, pH 7.4, under the same conditions as described above. Kallikrein used (73.2 μg) hydrolyzed 12.0 μmoles of TAME at pH 7.4 and 37 °C in 15 min. Under these conditions, kallikrein also converted Cis to Cisa very slowly. Maximum activity (5.2 μmoles ATEE) was observed after incubation

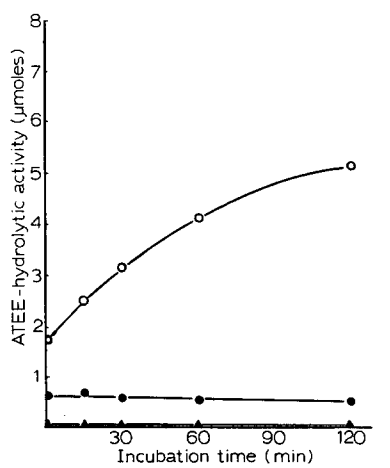


Fig. 3. Effect of plasma kallikrein upon Cls. 0.2 ml of Cls (210 μg/ml) was incubated with 0.1 ml of plasma kallikrein (732 μg/ml) in 0.1 M phosphate buffer, pH 7.4, at 37 °C, and at intervals, ATEE hydrolytic activity was assayed by incubation at pH 7.4 and 37 °C for 15 min. The kallikrein used (73.2 μg) hydrolyzed 12.0 μmoles of TAME, in 15 min. ○—○, Cls + kallikrein; ●—●, Cls only; ▲—▲, kallikrein only.

for 120 min, at 37 °C. Activation was found to depend on the concentration of kallikrein.

DISCUSSION

C1sa has been purified by several investigators[12–15]. Nagaki and Stroud[15] purified active C1s (C1sa), and suggested that formation of active C1s from its proesterase might be autocatalytic or due to traces of plasma proteolytic enzymes. However, this problem could not be decided because their enzyme was not homogeneous. Recently, in our laboratory, C1sa was highly purified from the euglobulin fraction of human plasma by successive column chromatographies on DEAE-cellulose, hydroxylapatite and TEAE-cellulose as described elsewhere[5]. This preparation was homogeneous on ultracentrifugation and disc-gel electrophoresis. To determine if C1sa affected C1s directly, highly purified C1sa was incubated with C1s at 37 °C for 60 min in 0.1 M phosphate buffer. As shown in Table I, no conversion was observed on incubation of purified C1s alone or with C1sa. This result is direct evidence that purified C1sa does not activate C1s and also proves that the purified C1sa did not contain C1r. Moreover, C1sa was found not to activate purified plasminogen or plasma kallikreinogen directly (Tables II and III). In contrast, C1r (and trypsin) caused rapid conversion of C1s to C1sa, and maximum activity was observed after incubation of 30 min at 37 °C (Fig. 1).

The effects of plasmin and plasma kallikrein upon C1s were also examined. Several investigators[1–3,16] reported that plasmin converts C1s to C1sa directly, but this has not been proved with a purified system. There are no previous reports of the activation of C1s by plasma kallikrein. As shown in Figs 2 and 3, highly purified plasmin and plasma kallikrein converted C1s to C1sa. However, these activities were very weak, and maximum activity was observed after incubation for 60 to 120 min at 37 °C.

The biological significance of these slow conversions of C1s to C1sa by plasmin and plasma kallikrein are unknown. Studies are in progress on this problem and on the activation mechanism.

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