Short Paper

A fibrinolytic enzyme from the green alga *Codium latum* activates plasminogen

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KEY WORDS: *Codium latum*, fibrinolytic enzyme, green algae, plasminogen, protease.

Marine algae contain various macromolecules that influence blood coagulation and fibrinolysis. Although it is well-known that algal polysaccharides show anticoagulant activity,1 there are few reports on the fibrinolytic enzymes from marine algae.2,3 We have found fibrinolytic activity in the extracts of marine green algae of the genus *Codium* and isolated fibrinolytic enzymes; 4–6 however, it remains unknown whether these fibrinolytic enzymes activate plasminogen to plasmin.

Plasmin has a central role in fibrinolysis. Plasminogen activators, tissue-type plasminogen activator (t-PA) and urokinase, which all convert plasminogen to plasmin, are also important in fibrinolysis. In the present study, we investigated the activity of algal fibrinolytic enzymes to activate plasminogen.

Fibrinolytic enzymes used in this experiment, CIP, CLP, and CDP, were isolated from *Codium intricatum*,4 *Codium latum*,5 and *Codium divaricatum*,6 respectively. *Codium intricatum* protease included two isoforms, CIP-I and -II. Fibrinolytic activity in the presence or absence of plasminogen was determined using a modified fibrin plate method.3,7 Ten milliliters of 0.4% human fibrinogen with or without plasminogen (Sigma Chemical Co., St Louis, MO, USA) in 0.1 M phosphate buffer, pH 7.4, was poured into a 10-cm Petri dish and then clotted by the addition of 0.2 mL of human thrombin (100 NIH U/mL; Sigma Chemical Co.) in the same buffer. The clot was allowed to stand for 1 h at room temperature. Then, purified protease solution (20 μL) was placed carefully on the plate. The plate was incubated for 18 h at 37°C and the diameter of the lytic circle formed was measured. Fibrinolytic activity was expressed in terms of the lytic area. Fibrinogen hydrolysis in the presence or absence of plasminogen was checked by sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS-PAGE). Fibrinogen in 50 mM Tris-HCl buffer, pH 7.8, containing 0.1 M NaCl at a final concentration of 10 mg/mL was incubated with algal enzyme (final concentration 10 μg/mL) in the presence or absence of Lys-plasminogen (final concentration 5.5 mM; Sigma Chemical Co.) at 37°C. At 0 min, 10 min, 30 min, 60 min, 90 min, and 120 min, a portion of the reaction solution was sampled, and analysed by SDS-PAGE according to the method of Schägger and von Jagow.8 The activation of human Lys-plasminogen by algal fibrinolytic enzymes was also investigated as follows. Algal fibrinolytic enzyme (100 μg/mL or 200 μg/mL) in 50 mM Tris-HCl buffer, pH 7.8, containing 0.01% Tween 80 was incubated with or without human Lys-plasminogen (final concentration 2.2 μM; Sigma Chemical Co.) at 37°C. At 1 h and 2 h, the amidolytic activity of the reaction solution was determined as follows. Twenty microliters of the reaction solution was added to 100 μL of the same buffer, to which was added 30 μL of S-2251 (3 mM; Chromogenix AB, Mölndal, Sweden). The change in absorbance at 405 nm was monitored with a microplate reader (Model 450; Bio-Rad, Richmond, CA, USA). The amidolytic activity of Lys-plasminogen in the absence of algal enzyme was determined in a similar manner.

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Received 1 February 2001. Accepted 21 May 2001.
that CLP activated plasminogen to plasmin. Codium divaricatum protease activated plasminogen weakly, and CIP was inactive. The fibrinogenolytic activity of CLP in the presence or absence of plasminogen is shown in Fig. 1, and a distinct difference in the fibrinogen degradation patterns was observed. All chains of fibrinogen were hydrolyzed completely within 90 min with CLP in the presence of plasminogen, whereas the Bβ and γ chains could still be detected after 120 min of incubation in the absence of plasminogen. The CDP hydrolyzed fibrinogen slightly faster in the presence of plasminogen than in its absence, whereas CIP showed no difference (data not shown). These results are consistent with the results of the fibrin plate assay. The amidolytic activity of CLP was higher in the presence of plasminogen than in the absence of it (Fig. 2),

Table 1  Fibrinolytic activity of three enzymes in the presence or absence of plasminogen

<table>
<thead>
<tr>
<th>Lytic area (mm²)</th>
<th>Plasminogen</th>
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<tr>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Codium intricatum protease</td>
<td>50</td>
</tr>
<tr>
<td>Codium latum protease</td>
<td>491</td>
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<tr>
<td>Codium divaricatum protease</td>
<td>79</td>
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Fig. 1  Analysis of fibrinogenolytic activity of Codium latum protease (CLP) in the: (a) presence or (b) absence of plasminogen. Fibrinogen consists of three non-identical pairs of polypeptides (Aα, Bβ, γ)₂. Sodium dodecylsulfate-polyacrylamide gel electrophoresis was performed under reducing conditions.

The fibrinolytic activity of algal enzymes in the presence or absence of plasminogen is shown in Table 1. Codium latum protease hydrolyzed the fibrin plate more strongly in the presence of plasminogen than in its absence. This result suggested
demonstrating that plasminogen was activated to plasmin. Amidolytic activity of plasminogen was undetected in the assay (data not shown).

It has been of interest to investigate whether algal macromolecules that influence blood coagulation and fibrinolysis affect plasminogen activation. Plasminogen is effectively activated to plasmin by t-PA or urokinase in the presence of fucoidan,9 which is an anticoagulant polysaccharide isolated from brown algae. In the present experiment we found that CLP isolated from the marine green alga 

\[ C_{\text{latum}} \]

activated plasminogen to plasmin. We have reported previously the strong fibrinolytic activity of CLP,5 which is comparable to that of snake venom fibrinolytic enzymes. The strong activity of CLP was found to be due to the direct hydrolysis of fibrin and the activation of plasminogen. Its specificity for plasminogen, however, was not as high as that of t-PA or urokinase because CLP directly hydrolyzed fibrin and fibrinogen in the absence of plasminogen. A similar fibrinolytic enzyme (CK) isolated from a bacterium, Bacillus sp. strain CK 11-4, directly hydrolyzes fibrin and activates plasminogen;10 however, its specificity for a chromogenic substrate, pyro-Glu-Gly-Arg-pNA (S-2444), was quite different from that of CLP. The CLP hydrolyzes S-2444 effectively,9 whereas CK is inactive.10 A novel plasminogen activator (TSV-PA) from venom of the snake Trimeresurus stejnegeri has been reported to activate human plasminogen without showing any non-specific protease activities involved in blood coagulation and fibrinolysis.11 Although such a specific enzyme has not been found in marine algae, we have found a fibrinogen clotting enzyme in 

\[ C_{\text{divaricatum}} \]

and the plasminogen-activating activity of CLP. Therefore, marine algae are a unique resource for novel enzymes.

REFERENCES