Factor XIII-mediated cross-linking of NH_2-terminal peptide of α_2-plasmin inhibitor to fibrin

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The NH_2-terminal 12-residue peptide of α_2-plasmin inhibitor, Asn–Gln–Glu–Gln–Val–Ser–Pro–Leu–Thr–Gly–Leu–Lys–NH_2 · AcOH, was found to be a good substrate for plasma transglutaminase (activated blood coagulation factor XIII) and rapidly incorporated into fibrin by the enzyme. A high concentration of the peptide inhibited the enzyme-mediated cross-linking of α_2-plasmin inhibitor to fibrin probably by competing with the inhibitor for the same site of fibrin α-chain.

α_2-Plasmin inhibitor *Fibrin* *Factor XIII* *Plasma transglutaminase*
Cross-linking *Glutamine substrate*

1. INTRODUCTION

We have shown that α_2-plasmin inhibitor (α_2PI) is cross-linked to fibrin by plasma transglutaminase, activated coagulation factor XIII (XIIIa), when blood coagulation takes place [1–4]. The cross-linking of α_2PI renders fibrin clot more resistant to fibrinolytic process that occurs subsequently to fibrin formation and is caused by fibrin-associated plasminogen activation [5]. α_2PI serves only as a glutamine substrate for XIIIa in the cross-linking reaction [4], and the cross-linking occurs between lysine residues of fibrin α-chains and a glutamine residue of α_2PI molecule that is the second residue from the NH_2-terminal [4].

Here, the synthesized peptide which has the same amino acid sequence as that of the NH_2-terminal part of α_2PI is shown to be cross-linked to fibrin by XIIIa and thereby competetively inhibits the cross-linking of α_2PI to fibrin.

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Abbreviations: α_2PI, α_2-plasmin inhibitor; XIIIa, activated coagulation factor XIII; N-peptide, NH_2-terminal peptide of α_2PI

2. MATERIALS AND METHODS

2.1. *Proteins*

α_2PI, fibrinogen and blood coagulation factor XIII were purified from human plasma as in [6–8]. Contaminating plasminogen and fibronectin were removed from the fibrinogen preparation using lysine–Sepharose [9] and gelatin–Sepharose [10], respectively. The factor XIII present as a contaminant in the fibrinogen preparation (300 mg protein/100 ml) was 0.2 U/ml as assayed by antibody neutralization method [11] using the clotting factor XIII-test kit supplied by Behringwerke AG (Marburg). One unit of factor XIII is defined as the amount of factor XIII present in 1 ml normal pooled standard plasma. Purified thrombin was prepared from a bovine thrombin preparation (Mochida Pharmaceuticals, Tokyo) as in [12].

2.2. *NH_2-terminal peptide of α_2PI*

The NH_2-terminal 12-residue peptide of α_2PI (N-peptide), Asn–Gln–Glu–Gln–Val–Ser–Pro–Leu–Thr–Gly–Leu–Lys–NH_2 · AcOH [13], was synthesized by Protein Research Foundation (Minoo, Osaka).

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2.3. *Radioiodination of proteins and peptide*

Purified proteins (α_2PI and fibrinogen) were radioiodinated by the solid-state lactoperoxidase method [14] using lactoperoxidase (Calbiochem-Behring–American Hoechst Corp., San Diego CA) and $Na^{125}I$ (17 Ci/mg) (New England Nuclear, Boston MA). The labelled α_2PI and fibrinogen preparations had spec. act. 5.2×10^5 cpm/μg and 1.2×10^5 cpm/μg, respectively. N-peptide was radioiodinated as in [15] using N-succinimidyl 3-(4-hydroxy, 5-[^{125}I]iodophenyl) propionate (1.86 Ci/μmol) (Radiochemical Centre, Amersham). Free unconjugated radiolabelled compounds were removed by gel filtration using Sephadex G-10. The radioiodinated N-peptide had spec. act. 3.9×10^5 cpm/μg.

2.4. *Measurement of the cross-linking to fibrin*

Fibrinogen, N-peptide and/or α_2PI were mixed, clotted and cross-linked at 37°C by thrombin, factor XIII, $CaCl_2$ in Tris-buffered saline (0.05 M Tris–HCl/0.15 M NaCl, pH 7.4). Radioactively labelled N-peptide, α_2PI or fibrinogen was used for estimating the extent of cross-linking of each respective component. After allowing the cross-linking reaction to proceed for various lengths of time, the extent of cross-linking was examined as in [2,4].

3. RESULTS AND DISCUSSION

N-peptide was rapidly cross-linked to fibrin when the mixtures of N-peptide, factor XIII and fibrinogen were clotted with thrombin and calcium ions (fig. 1). This supports the finding that Gln-2 is the site where α_2PI is cross-linked to fibrin [4]. With 1 unit of factor XIII/ml, the cross-linking of N-peptide was completed within 5 min. This reaction rate is similar to that of α_2PI which is the most efficient substrate for XIIIa among known XIIIa substrate plasma proteins [16]. Although the reaction rate was dependent on the concentration of factor XIII, the maximum level of cross-linking achieved was independent of factor XIII and determined by the concentrations of fibrinogen and N-peptide (fig. 1,2). When N-peptide concentration was increased and became a large molar excess over fibrinogen, the increase of the cross-linking deviated from linearity and fell off to reach a plateau (fig. 3). At the maximum plateau level,

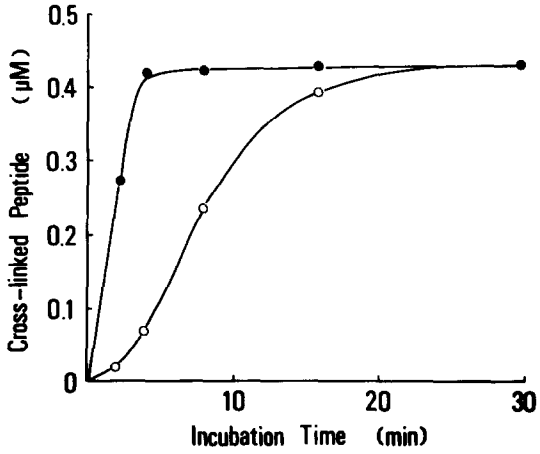

Fig. 1. Time-course of the cross-linking reaction of N-peptide to fibrin. Radioiodinated N-peptide (1 μM), fibrinogen (300 mg/100 ml), factor XIII, and thrombin (0.33 U/ml) were mixed and incubated at 37°C. Factor XIII was 0.2 U/ml (○) and 1 U/ml (●). The amount of N-peptide cross-linked to the formed fibrin after various times of incubation was determined.

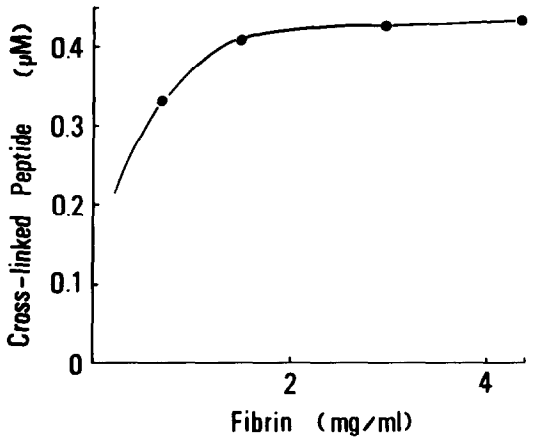

Fig. 2. The relationship between the concentrations of fibrin and the amounts of cross-linked N-peptide. The experimental conditions were the same as those in fig. 1, except for variation of fibrinogen concentration and a fixed length of incubation time (30 min). Factor XIII was 1 U/ml.

~17 molecules of N-peptide were bound to each α-chain of fibrin. These modes of reaction are the same as those observed in the α_2PI-fibrin(ogen) cross-linking reaction [2,3] and may be similarly explained by an equilibrium of the reaction [3].

The cross-linking of α_2PI to fibrin was decreased

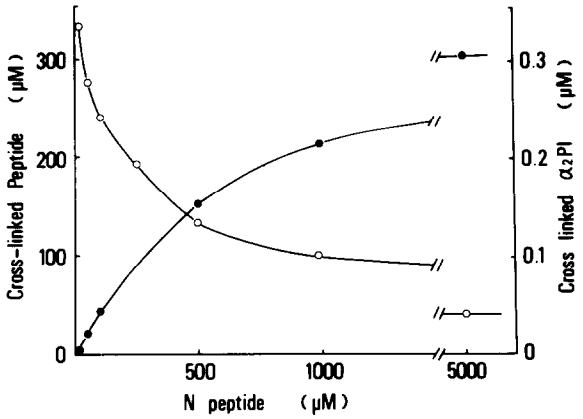

Fig. 3. Increase of cross-linked N-peptide and decrease of cross-linked α_2PI with increasing concentrations of N-peptide. Fibrinogen (300 mg/100 ml), α_2PI (1 μM), various concentrations of N-peptide, $CaCl_2$ (5 mM) and thrombin (0.33 U/ml) were mixed and incubated at 37°C. The amounts of the peptide (●---●) or α_2PI (○) cross-linked to the formed fibrin after 30 min incubation were determined using radiolabelled N-peptide or α_2PI, respectively.

by the presence of N-peptide. The decrease was in inverse relationship to the increase of cross-linking of N-peptide (fig.3). However, a very high concentration of the peptide was required to inhibit α_2PI cross-linking. The 50% reduction of α_2PI cross-linking in the presence of 1 μM α_2PI was achieved by an addition of ~350 μM of N-peptide (fig.3). At this level (50%) of inhibition of α_2PI cross-linking, the molar ratio of cross-linked N-peptide to cross-linked α_2PI was estimated to be about 1000:1. These findings suggest that α_2PI is cross-linked very efficiently to a specific site of fibrin α-chain, and N-peptide can not compete efficiently for this site with α_2PI. Probably some structure of α_2PI molecule other than the NH_2-terminal region is required for the efficient interaction of XIIIa with α_2PI of factor XIII-α_2PI intermediate complex [3] with its complementary site (lysine residue) of fibrin. A contribution to specificity of amino acid residues located some distance from the substrate glutamine was indicated in the study of synthetic glutamine substrates for XIIIa [17].

Cross-linked polymerization of fibrin α-chains was also decreased when the concentration of N-peptide was >1 mM. Cross-linked dimerization of γ-chains was not affected by N-peptide.

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