CONVERSION OF BOVINE PREKALLIKREIN TO KALLIKREIN. EVIDENCE OF LIMITED PROTEOLYSIS OF PREKALLIKREIN BY BOVINE HAGEMAN FACTOR (FACTOR XII)

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1. Introduction

Hageman factor, an intrinsic trigger of the sequence of proenzyme–enzyme transformations leading to blood coagulation [1, 2], has been found to trigger off liberation of hypotensive polypeptides called plasma kinins [3]. We recently obtained evidence using highly purified preparations that Hageman factor activates prekallikrein directly to kallikrein [4]. The activation was found to be of a catalytic nature [5], but no information has been obtained on the role of Hageman factor in the transformation of prekallikrein to kallikrein. This paper reports evidence indicating that the conversion of prekallikrein to kallikrein by Hageman factor may be accompanied by limited proteolysis of the precursor molecule.

2. Materials and methods

Prekallikrein was isolated from the pseudoglobulin fraction of bovine plasma as described previously [6]. Bovine Hageman factor was purified by the method of Komiya et al. [7], following its potency to activate prekallikrein, and the preparation was characterized by determining its ability to correct the coagulation defect of Hageman factor-deficient human plasma [7]. SDS-polyacrylamide gel disc electrophoresis was performed by the method of Shapiro et al. [8]. Gels were stained with 0.2 % Coomassie Brilliant Blue in methanol–acetic acid–water (5:1:5, v/v/v) and excess dye was removed by dialysis against 7.5 % acetic acid–5 % methanol solution [9]. Carboxyl-terminal amino acid was determined by the selective tritium labelling method of Matsuo et al. [10].

3. Results and discussion

Fig. 1 shows the electrophoretic patterns of prekallikrein and kallikrein activated with Hageman factor on SDS-polyacrylamide gel disc electrophoresis. In the absence of β-mercaptoethanol, they had the same mobility on the gel. This result supports our previous finding that the conversion of prekallikrein to kallikrein by Hageman factor does not involve a marked molecular change [5]. The electrophoretic mobility of prekallikrein on SDS-gel was also similar to that of prekallikrein treated with β-mercaptoethanol, suggesting that prekallikrein may be composed of a single polypeptide chain. However, when kallikrein activated with Hageman factor was subjected to electrophoresis in the presence of β-mercaptoethanol, the original protein band of kallikrein disappeared and new bands of rapidly migrating protein appeared. These results suggested that activation of prekallikrein was associated with limited proteolysis of peptide chains located between two cysteine residues which form the disulfide linkage in the prekallikrein molecule, just as observed on activation of plasminogen [11].

To identify the C-terminal amino acid of prekallikrein before and after activation by Hageman factor,

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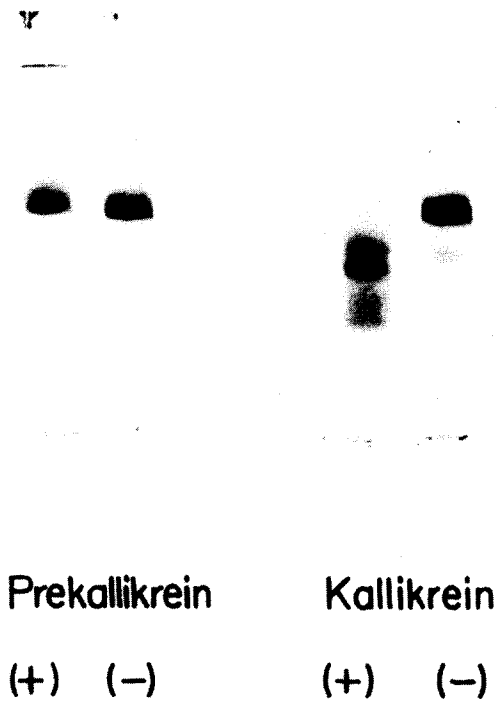

Fig. 1. SDS-polyacrylamide gel patterns of prekallikrein and kallikrein in the presence and absence of β-mercaptoethanol. Mixture containing bovine prekallikrein (30 μg) and bovine Hageman factor (1.7 μg) was incubated at 37° for 30 min. After incubation, the mixture or prekallikrein itself, with (+) or without (−) treatment with 0.1 M β-mercaptoethanol, was subjected to electrophoresis in 0.1 M phosphate buffer, pH 7.2, containing 0.1 % SDS, at 7 mA/tube for 4 hr.

about 1 mg each of prekallikrein and kallikrein activated with Hageman factor were treated separately with 50 mCi of tritium water in pyridine–acetic anhydride mixture. After standing for 12 hr at room temp., each mixture was dialyzed extensively against distilled water and lyophilized. The lyophilized material was hydrolyzed in an evacuated sealed tube with 6 N HCl at 105° for 24 hr. The hydrolyzate was then evaporated to dryness several times and finally dissolved in 0.2 ml of water. The sample was subjected to high voltage paper electrophoresis on Toyo filter paper No 51A (30 X 60 cm) at 3000 V for 70 min in pyridine–acetic acid buffer, pH 3.5. For measurement of radioactivity, the electropherogram was cut into small pieces and the radioactive material in each piece was extracted with 2 ml of 1% acetic

Table 1
Radioactive amino acids in hydrolyzates of prekallikrein and kallikrein after ^{3}H-labelling

Labelled amino acid	Prekallikrein	Kallikrein
	– Radioactivity (cpm) –	
Aspartic acid	25	67
Glutamic acid	23	83.5
Leucine (Isoleucine)	141	177.5
Arginine	48.5	306.5
Lysine	25.5	106

Mixture containing bovine prekallikrein (0.96 mg) and bovine Hageman factor (0.017 mg) was incubated at 36° for 4 hr. After incubation, the mixture of prekallikrein itself was trituated by the method of Matsuo et al. [10], and the labelled amino acid released after acid hydrolysis of the trituated proteins was separated, identified and counted.

acid. The extract was evaporated to dryness, and its radioactivity was counted in 10 ml of scintillation fluid (5 g of 2,5-diphenyloxazole and 0.1 g of 1,4-bis(2-(4-methyl-5-phenyloxazolyl))benzene per l of toluene) with a Beckman liquid scintillation spectrometer, model LS 250.

The radioactivity incorporated into prekallikrein was found to be mostly associated with the neutral amino acid region, but in case of kallikrein activated with Hageman factor, tritium was detected in the regions of neutral residues and arginine on the paper electropherogram (table 1). To identify the labelled neutral amino acid, the residues from vials corresponding to regions of neutral amino acids were washed several times with acetone and then dissolved in a minimum amount of water. These solutions were subjected to paper chromatography on Toyo filter paper No 51A (30 X 60 cm) in a solvent system of pyridine–*n*-butanol–acetic acid–water (10:15:13:12, by volume), with authentic specimens of neutral amino acids. The chromatogram was cut horizontally into 1 cm widths, and the radioactivity of each piece was determined with a liquid scintillation counter as described above.

Leucine (isoleucine) was identified as the radioactive amino acid released from prekallikrein. Thus leucine (isoleucine) must be located at the C-terminal end of the molecule. However, with kallikrein activated by Hageman factor, tritium was incorporated

predominantly into arginine besides the neutral amino acid observed with prekallikrein (table 1).

These results suggest that activation of prekallikrein by Hageman factor is accompanied by a specific cleavage of the prekallikrein molecule, probably of a single arginyl peptide bond. The finding that an arginine residue appeared as a new C-terminus during activation of prekallikrein may be correlated with the fact that bovine Hageman factor can hydrolyze a synthetic N-substituted arginine ester. Thus, the Hageman factor seems to function as a trypsin-like enzyme with restricted specificity.

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