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SHORT COMMUNICATIONS

Bradykininase activity of aloe extract

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Aloe (*Aloe arborescens* Mill var. *Natalensis* Berger) has been used as folklore medicine for centuries, especially for skin injury and burns. However, critical pharmacological evidence to justify its therapeutic use as an anti-inflammatory agent for the promotion of healing is still lacking[1]. As pharmacological evidence for the anti-inflammatory action of aloe, we have found that aloe extract contains bradykininase activity.

The exudate from fresh leaves of aloe was filtered through ultra-filtration membranes, and the fractions containing the components of molecular weights higher than 10,000 were lyophilized. The lyophilized powder was dissolved in water to make a concentration of 30 mg of the aloe powder/ml. Synthetic bradykinin was purchased from the Protein Research Foundation, Osaka.

Bradykininase activity was estimated by biological assay on the guinea pig ileum as described by Takeya *et al*[2]. Synthetic bradykinin (10 μg/ml in final concentration) was incubated in 10 mM phosphate buffer, pH 7·3, with aloe extract at 30°C. The reaction was terminated by boiling at 90°C for 10 min, and after centrifugation, 1 ml of the incubation mixture was added into the assay mixture for bradykinin.

A 1–2 cm segment of guinea pig ileum was suspended in a water bath containing 9 ml of Tyrode solution. The contractile response of the segment was recorded by a mechanoelectric transducer. The estimation of bradykinin in the reaction mixture was made by assaying the response against a standard solution of bradykinin.

The product from bradykinin after incubation with aloe extract was analyzed by high voltage paper electrophoresis at pH 3·5 in pyridine/acetic acid/water (1:10:89, by vol.) at 3,600 volts for 20 min. The amino acid composition was analyzed by two-dimensional paper chromatography (n-butanol/acetic acid/water (120:30:150, by vol.), and phenol/ammonia (200:1, v/v) as solvents).

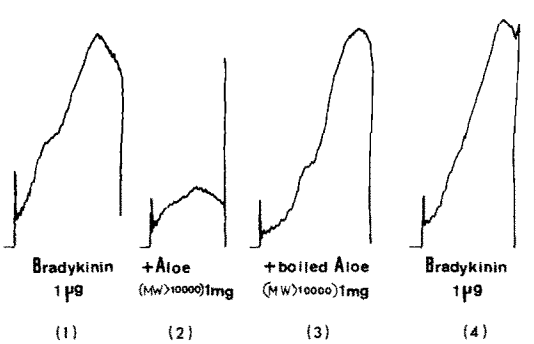

Fig. 1. Inactivation of bradykinin by aloe extract. Bradykinin was incubated with aloe extract (a fraction containing substances of molecular weights higher than 10,000) at 30°C for 60 min, and the remaining bradykinin was assayed as described in text. The contraction of guinea pig ileum was measured. (1) Standard, 1 μg bradykinin; (2) incubation with aloe extract (1 mg); (3) incubation with aloe extract boiled for 10 min; and (4) standard, 1 μg bradykinin.

As shown in Fig. 1, incubation of bradykinin with aloe extract containing the components of mol. wts. higher than 10,000 greatly reduced the amount of bradykinin. In contrast, boiled aloe extract did not reduce the bradykinin concentration during the incubation. The result suggests that aloe extract contains bradykininase activity. Aloe extract alone had no effect on the guinea pig ileum. However, the fraction of aloe extract containing substances of mol. wt. lower than 10,000 caused contraction of the ileum.

When bradykinin was incubated with aloe extract its activity decreased almost linearly with time for 60 min, after an initial lag period of about 5 min.

The products formed from bradykinin by incubation with aloe extract were analyzed by high voltage paper electrophoresis of the supernatant after boiling the incubation mixture for 10 min and centrifuging. In the reaction mixture with aloe extract, three ninhydrin-positive spots were observed. The middle spot migrated together with authentic bradykinin and was shown to have the same amino acid composition as bradykinin. This spot alone was observed with blank incubations without aloe or without bradykinin during incubation.

The other two ninhydrin-positive spots were eluted from unstained paper strips, hydrolyzed in acid, and the amino acid compositions were examined by paper chromatography. The fast-moving product was shown to be composed of Arg, Pro, and Gly, and thus corresponds to Arg^1-Pro^2-Pro^3-Gly^4, while the slow moving product was shown to be composed of Phe, Ser, Pro, and Arg, and thus corresponds to Phe^5-Ser^6-Pro^7-Phe^8-Arg^9. The results indicate that aloe bradykininase may hydrolyze bradykinin mainly between Gly^4 and Phe^5.

Stem bromelain, a plant peptidase from the pineapple stem, was also reported to have bradykininase activity, and it hydrolyzes bradykinin between Gly^4 and Phe^5 and between Phe^5 and Arg^6 [3, 4 and personal communication from Dr. T. Shigei]. Thus the enzyme in aloe may attack the peptide in a similar way as stem bromelain. The possible anti-inflammatory actions of the aloe bradykininase *in vivo* remains for further investigation.

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