RELEASE OF KININ ACTIVITY FROM HUMAN KININOGENS AND FRESH PLASMA BY CYANOGEN BROMIDE

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Received March 7, 1966

The hendecapeptide methionyllysylbradykinin has been isolated from the pseudoglobulin fraction of bovine plasma (Elliott and Lewis, 1965). Bovine kininogen has been highly purified (Habermann, 1963; Suzuki et al., 1966), and the structure of the peptic kinin-yielding fragments (PKF) has been studied (Habermann, 1965; Habermann, 1966). The methionyllysylbradykinin (1-11) is pictured as a unit within the polypeptide chain of the precursor protein:

BrCN BrCN

..Met—Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-Ser-Val-Gln-Val-Met—...

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

Two kininogens, designated as Kg I and Kg II, have been isolated from human plasma. They, like bovine kininogens, are glycoproteins with a molecular weight of about 50,000 (Habermann, 1963; Pierce and Webster, 1966). Studies with carboxypeptidase B suggest that Kg I has kallidin, i.e., lysylbradykinin, (2-11), as the COOH-terminal sequence, but that Kg II has kallidin within the protein chain. Amino acid analysis shows that both kininogens contain two or possibly three residues of methionine.

*Fellow in the Visiting Program of the USPHS 1965-1966.

The two human kininogens have now been treated with cyanogen bromide, a reagent which under acidic conditions selectively cleaves methionyl bonds in peptides and proteins (Gross and Witkop, 1962).

In the following experiments kinin activities were estimated by the muscle contraction of estric rat uterus. As a control the stability and recovery of kallidin were tested under the same experimental conditions as were used for cleavage of the kininogens. In a series of experiments 90-98% of the kallidin activity was recovered. Solutions of 100 γ of Kg I and Kg II, respectively, in 1 ml of 0.25 N hydrochloric acid containing 1 mg of cyanogen bromide were incubated at 35° for 20 h. The reaction products were then lyophilized and assayed for kinin activity. Table I lists for comparison the activities observed upon release of kallidin from human kininogens by human urinary kallikrein.

When Kg I and Kg II were incubated with 0.25 N hydrochloric acid alone under the above conditions no measurable kinin activity was observed.

Table I

Kininogen	Kinin Activity (expressed as γ kallidin/mg protein)	
	after cleavage with CNBr	after cleavage with human urinary kallikrein
Kg I	2.5	8
Kg II	0.8	6

Although under these preliminary conditions the cleavage of the kininogens by cyanogen bromide may be incomplete, this explains only in part the low kinin activity relative to that obtained by the action of kallikrein. A biologically active peptide, e.g., bradykinin, may be lengthened at the NH_2-terminal end with only a small effect on potency, whereas the addition of amino acid residues to the COOH-terminal end in general leads to drastic losses in activity (Stewart and Woolley, 1965). The cyanogen bromide cleavage is consistent with methionine preceding the kallidin in the human kininogens in analogy to bovine kininogen.

In addition the lower activity of the kinin released from Kg II is compatible with additional COOH-terminal amino acid residues.

The question whether treatment of unfractionated native human plasma with cyanogen bromide will release kinin activity is of interest in view of the projected isolation of the liberated peptides on a preparative scale. Table II summarizes our preliminary data. For these experiments human plasma was freshly collected in plastic vessels or silicon-treated glass containers. The plasma was treated with a large excess of cyanogen bromide in hydrochloric acid varying in strength from 0.25-5.0 N at 35-90° for 15-20 hr. In each experiment 0.1 ml of plasma was added to 0.5 ml of the hydrochloric acid, and excess cyanogen bromide (5 mg) added after 30 min. The reaction products were neutralized prior to lyophilization. These preliminary data reflect some variability from plasma to plasma. Nevertheless, the combination of acid and BrCN is capable of releasing kinin activity approaching 10% of that released by kallikrein.

Table II

Normality of HCl	Temperature	Kinin activity after BrCN cleavage expressed as ng kallidin/ml plasma [a]	"Blank activity" [b] expressed as ng kallidin/ml plasma
0.25	36°	110	< 60
0.25	60°	> 230	< 60
0.25	95°	> 230	110
0.5	35°	60, 150	< 60
1.0	35°	220, 360	< 70
2.0	35°	220, 530	< 70
3.0	35°	400	< 70
5.0	35°	400	Trace

(a) The enzymatic release of kinin activity by human urinary kallikrein is 3500-5000 ng/ml plasma.
(b) Controls were obtained by treating plasma in the same way without BrCN.

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