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Methionyl-lysyl-bradykinin release from plasma kininogen by plasmin

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EVIDENCE that incubation of plasmin with plasma globulins results in the release of vasoactive substance was given as early as 1950.[1] Later other authors also found that plasmin is involved in the release of kinin in plasma.[2–5] More recent experiments leave no doubt as to the kininogenase activity of plasmin.[6–9] Our previous work indicated that plasmin plays an important part in the release of kinin in plasma.[9] This observation led us to try and identify the kinins formed by this enzyme when incubated with a purified substrate. The results of these experiments are described in this paper.

Material and methods. Bradykinin acetate, synthetic, was obtained from Sigma Chemical Company, St. Louis, U.S.A.; Methionyl-lysyl-bradykinin was a gift from Dr. Schröder, A.G., Berlin; Kallidin was a gift from Sandoz, Ltd., Basel, Switzerland. Kininogen II[10] was prepared as previously described[11] from the supernatant of horse plasma precipitated at 6·7% (v/v) saturation with polyethyleneglycol, mol. wt. 6000.[12] Plasmin was prepared by the method of Robbins and Summaria[13] with minor modifications[9] from Fraction B (Cohn's II + III) from human plasma. Trypsin, crystalline, Spofa, Czechoslovakia, was used. The activity of the kinins released was assayed on the guinea pig ileum.[11] Incubation of kininogen with plasmin was made as follows: 20 ml solution of kininogen (15 mg protein) were incubated with 6 casein units[13] of plasmin for 150 min, as recommended by Budnitskaya *et al.*[9] The mixture was then immediately frozen and lyophilized. For the separation of

the peptides obtained the dry residue was dissolved in 1 ml water and samples of this solution were used for electrophoresis or chromatography or electrophoresis followed by chromatography as previously described.[14]

Results. Electrophoresis of the mixture of kinins obtained after incubation of kininogen II with plasmin revealed two spots ninhydrin-positive and Sakaguchi-positive, one slow-moving, very pale, with a mobility similar to that of kallidin and another fast-moving, sharply stained, with a mobility similar to that of methionyl-lysyl-bradykinin (MLB) and bradykinin. When only chromatography was performed also two spots were observed, one with an R_f similar to that of bradykinin, very weakly stained, and a second, very sharply stained, with an R_f similar to that of kallidin or a mixture of kallidin + MLB. These observations already suggested that the sharply stained peptide was MLB. Electrophoresis followed by chromatography gave the results shown in Fig. 1. From an unstained chromatogram similar to that shown in Fig. 1 the spot corresponding to MLB was eluted and to the eluate synthetic MLB was added. The mixture was rechromatographed and Fig. 2 shows that only one spot was found.

An eluate corresponding to the MLB spot obtained after electrophoresis-chromatography showed a kinin activity which increased 2·5 times after 15 min incubation with trypsin; an increase of about three times was found when synthetic MLB was exposed to trypsin under the same conditions (Fig. 3).

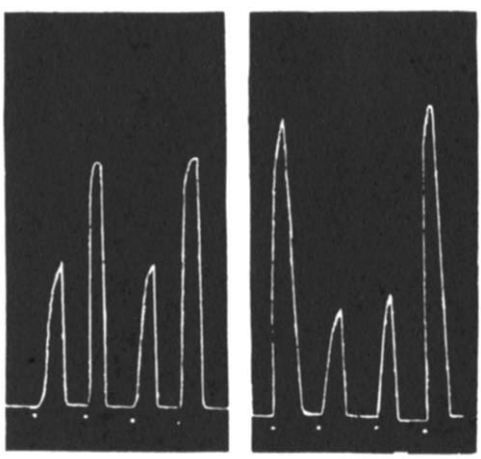

FIG. 3. Responses of guinea-pig ileum to synthetic bradykinin (B); to the eluate of the chromatogram spot (Fig. 1) corresponding to MLB (A); to a tryptic digest of this eluate (A_1); to synthetic MLB (M) and to a tryptic digest of synthetic MLB (M_1). The tryptic digest was obtained by 15 min incubation of the peptides with 25 μg crystalline trypsin.

Electrophoresis of a tryptic digest of a similar eluate is compared to a tryptic digest of synthetic MLB in Fig. 4. The fast moving spots which were ninhydrin-positive but Sakaguchi-negative corresponded to methionyl-lysine since in eluates of this zone from an unstained electropherogram, after hydrolysis by 6 N HCl, only methionine and lysine could be found on chromatographic analysis. The slower-moving spots, which were Sakaguchi-positive, moved like bradykinin.

Discussion. The present investigation shows that the kinin released by plasmin from kininogen II is mostly MLB, small amounts of peptide with the R_f of kallidin and bradykinin being observed. We have shown[9] that plasmin releases twice as much kinin, expressed in bradykinin, from fresh plasma than plasma kallikrein. Considering that most of the kinin released by plasmin is MLB

while the kinin released by plasma kallikrein is bradykinin[15, 16] and that MLB was shown to be 2–5 times less active than bradykinin on the guinea pig ileum[17] (the synthetic MLB we used in the experiments here described was five times less active than our synthetic bradykinin), the kinin released by plasmin seems to account for most of the total kinin that is released by the endogenous kininogenase.

The findings described in the present paper suggest that in the experiments of Elliott and Lewis[17] probably plasmin was the endogenous kininogenase responsible for the release of MLB in their serum incubates. This assumption is supported by the fact that the treatment to which serum was submitted in their experiments does not remove pre-plasmin or plasmin, since pre-plasmin is also precipitated between 0·25 and 0·45 saturation with ammonium sulphate;[18] it is very stable at low pH[19] and is rapidly activated in slightly alkaline medium at 37°.[19]

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Selective inhibition of thymidine incorporation into lymphocytes by cucurbitacins B and D

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THE CUCURBITACINS (elatericins) are tetracyclic triterpenoid bitter principles which have been isolated from *Cucurbitaceae*[1] and other sources, including begonias.[2] Cucurbitacins B and D are quite toxic