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DETECTION OF CATHEPSIN B-LIKE ENZYME ACTIVITY IN MOUSE EPIDERMIS

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In this study, we could detect cathepsin B-like, i.e., BANA-hydrolysing, enzyme activity in the cytosolic fraction of mouse epidermal lysate. The optimum pH and the optimum temperature of this enzyme activity were pH 6.0 and 37℃, respectively. The BANA hydrolysis occured dose dependently to the protein amount of the cytosolic fraction and, lupeptin, which is a specific inhibitor to cathepsin B, was stlongly inhibited BANA hydrolysis at pH 6.0 under 37℃ condition.

Key words: cathepsin / b-like enzyme

Cathepsin B (CB), which is a member of the cysteine proteinase family, is known to be present widely in lysosomes of mammalian cells (1). It is an endopeptidase to act specifically at the carboxyl side of some synthetic substrates, of which μ -N-benzoyl-DL-arginine β -naphthylamide (BANA) is the representative (2). This enzyme has been shown to participate in a variety of cell functions (3, 4). The activity of CB was detected also in the epidermis of several mammals. In the rodents, the CB activity was detected in the epidermis of the rat (5, 6), but never done in that of the mouse. Since the mice, as well as rat, are used frequently as experimental animals in many medical fields including dermatology, the knowledge on the CB in the epidermis of, not only the rat, but also mouse should be accumulated. Thus, in this study, we have tried to demonstrate the CB-like enzyme activity in the cell lysate from the epidermis of the mouse.

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MATERIALS AND METHODS

The epidermis was separated from the whole skin taken from the anesthetized Balb/c mice according to the method of Murozuka *et al.* (7). Approximately 1 g of the epidermis from 10 mice was homogenized in 9 volumes of 50 mM Tris-HCl buffer (pH 7.5) containing 250 mM sucrose, 1 mM ethylenediaminetetraacetic acid (EDTA, Wako Pure Chemical Industries, Ltd.) and 0.2 mM phenylmethylsulfonyl fluoride (PMSF, Sigma Chemical Co.) using a polytron homogenizer with 20 strokes. After stirring for 10 min, the mixture was ultracentrifuged at 105,000 xg for 30 min. All steps were carried out at 4℃. The supernatant thus obtained was kept at − 80℃ until use as the enzyme preparation of cytosolic fraction from the mouse epidermis.

The CB-like activity was assayed using BANA (Nakarai Tesque Inc.) as the substrate according to the method of Barrett (2, 8) with some modifications (9). The volume of the enzyme preparation containing 200 μg of proteins and 20 μl of 250 mM sodium phosphate buffer (pH 6.0) containing 1.25 mM BANA, 6.5 mM EDTA, 10 mM cysteine, 11.5 mM PMSF and 5 mM pepstatin (Boehringer Mannheim, GmbH) were mixed, and the final volume was adjusted to 100 μl by adding the phosphate buffer. To examine the effect of the inhibitor to CB, the reaction mixture containing 1 mM leupeptin (Boehringer Mannheim, GmbH) was made. Leupeptin has been known to be a specific inhibitor to CB (10). They were then incubated at 37℃ for indicated times. After the incubation, the reaction was stopped by adding the equal volume of coupling reagent. The released naphthylamine was measured using a microplate leader (Spectramax 250®, Wako Pure Chemical Industries, Ltd.).

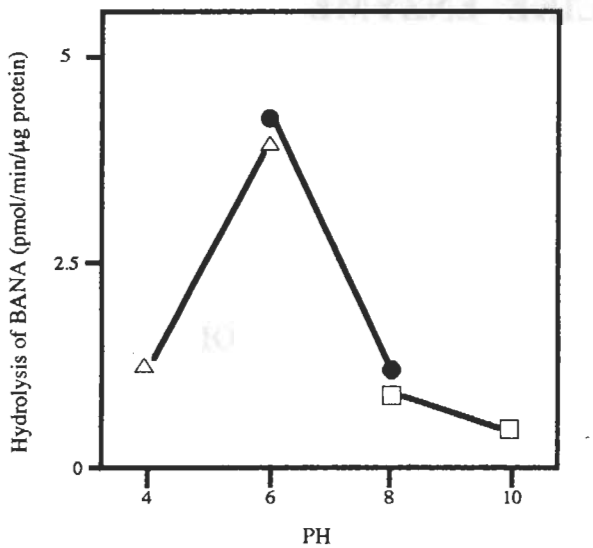

Fig. 1. Effect of pH on CB-like activity. The assays were carried out using the enzyme preparation as described under "Materials and Methods". △:acetate buffer, ●: phosphate buffer and □ : Tris-HCl buffer, respectively. The concentrations of buffers were adjusted to 50 mM.

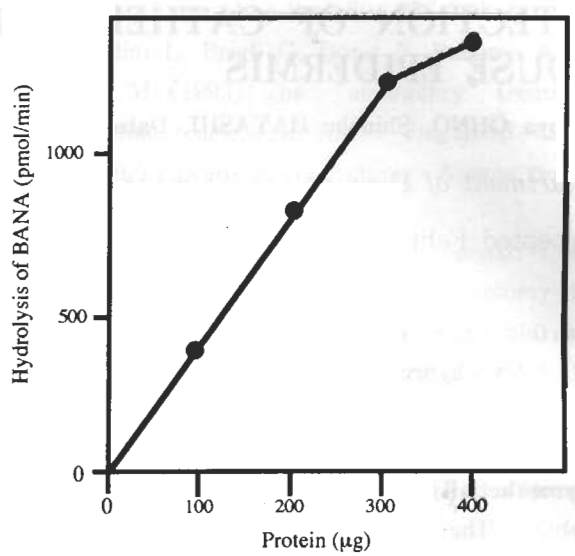

Fig. 3. Dose dependency on CB-like activity. The assays were carried out in the reaction mixtures containing various amounts of proteins described under "Materials and Methods".

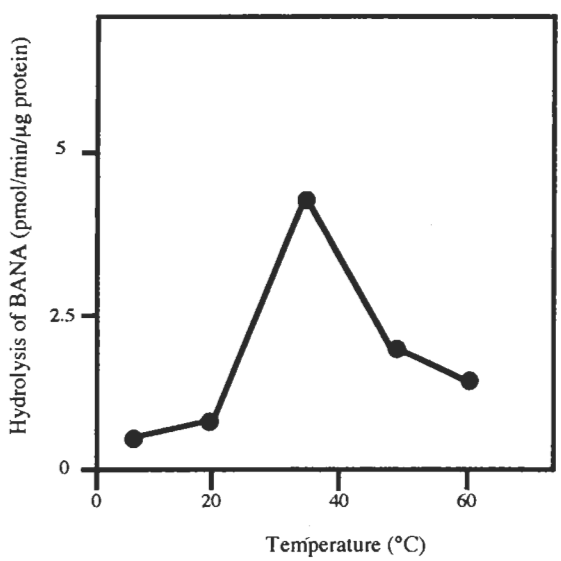

Fig. 2. Effect of temperature on CB-like activity. The assays were carried out under various temperatures described under "Materials and Methods.

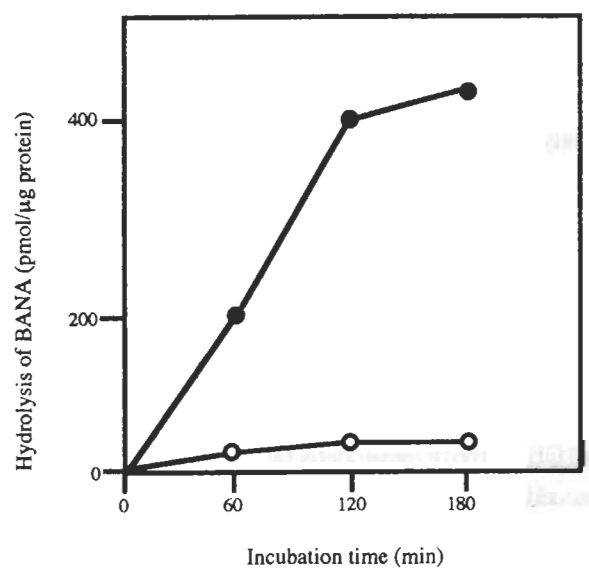

Fig. 4. Effect of leupeptin on CB-like activity. The assays were carried out using the reaction mixtures with (○) or without (●) leupeptin.

RESULTS

pH *optimum*

Effects of pH on the prepared enzyme activity from mouse epidermis were determined over a pH range of 5 units with acetate buffer (pH 4.0), sodium phosphate buffer (pH 6.0 to 8.0) and Tris-HCl buffer (pH 8.0 to 10) at a concentration of 50 mM. As shown in Fig. 1, the optimum pH was observed at pH 6.0 when 1.25 mM BANA were used as the substrate. The following experiments were carried out at pH 6.0 with sodium phosphate buffer at a concentration of 50 mM.

Effect of temperature on CB-like activity

To determinate the optimum temperature on the prepared enzyme activity from mouse epidermis, the reaction mixture containing 1.25 mM BANA

and 50 mM sodium phosphate buffer (pH 6.0) as described under "Materials and Methods" was incubated for 20 min at various temperature (4℃, 20 ℃, 37℃, 50℃ and 60℃, respectively). As shown in Fig. 2, the highest enzyme activity was observed at 37℃, and at high and low temperatures more than 37℃, the low enzyme activity compared with that at 37℃ was observed.

Effect of varying enzyme concentrations

BANA was hydrolyzed in the reaction mixtures containing various amounts of proteins at 37℃ under pH 6.0 for 20 min. As shown in Fig. 3, the BANA hydrolysis occurred dose dependently.

Effect of leupeptin on CB-like activity

To know the effect of leupeptin to CB-like activity, BANA was hydrolyzed by the enzyme preparation in 50 mM sodium phosphate buffer at 37° C under pH 6.0 for various incubation times. Two series of the experiments were performed; in one, the reaction mixture contained 1 mM leupeptin, and in another, it did not contain leupeptin at all. As the results, in the reaction mixture with leupeptin, BANA was little hydrolyzed at any incubation times. On the other hand, in the mixture without leupeptin, BANA was hydrolyzed usually throughout the experiments (Fig. 4).

DISCUSSION

In this study, the enzyme activity to hydrolyze BANA has been shown to be present also in the mouse epidermis. The optimum pH and optimum temperature of this CB-like enzyme from mouse epidermis were pH 6.0 and 37℃, respectively. Since the activity was inhibited very much by leupeptin, the BANA hydrolysis is concluded to have developed by the CB-like enzyme activity (10). These properties of the CB-like enzyme of the mouse epidermis are very similar to those of the CB of the epidermis of other mammals, such as the rat (5, 6) and human (9). We may guess that the CB-like activity in the mouse epidermis plays important roles in the regulatory mechanisms also in the skin of the mouse.

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