Trichorzin HA V, a Member of the Peptaibol Family, Stimulates Intracellular cAMP Formation in Cells Expressing the Calcitonin Receptor

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By a cell-based screening of an in-house natural product library, trichorzin HA V, belonging to a peptaibol family, was isolated from a strain of fungus Trichoderma as a calcitonin (CT) agonist. Like CT, trichorzin HA V elevated cAMP levels in T47D cells which endogenously express the human CT receptor. It also stimulated cAMP formation in cells expressing recombinant human CT receptor, but not in those that do not express the receptor, suggesting that it selectively interacts with the CT receptor. In contrast to trichorzin HA V, alamethicin, another well-characterized peptaibol, showed no cAMP-elevating activity at all. These results suggest that, although there was little amino acid sequence similarity between trichorzin HA V and CT, the biological activity of trichorzin HA V can mimic that of CT, acting via the CT receptor.

Key words trichorzin HA; peptaibol; calcitonin; cAMP; agonist

Calcitonin (CT), a 32-amino acid peptide hormone (Fig. 1) secreted mainly from the thyroid gland in response to an increase in blood calcium levels, plays an important role in maintaining bone homeostasis. The signal transduction of CT is initiated by its binding to the CT receptor, followed by activation of a stimulatory G protein, Gs. Gs in turn induces the activation of adenyl cyclase to generate a second intracellular messenger molecule, cAMP, which is biologically important in CT actions. To discover novel compounds that increase the intracellular levels of cAMP similarly to CT, we performed a cell-based screening of an in-house natural product library and isolated a series of trichorzins HA. One peptide, trichorzin HA V (Fig. 1), was identified and characterized. Peptaiboles are linear hydrophobic peptides containing a high proportion of α-amino isobutyric acid (Aib) and iso-valine (Iva), with an acetylated N-terminus and a C-terminal amino alcohol. Alamethicin is one of the best characterized peptides with a 20-amino acid residue (Fig. 1). Trichorzins HA were originally isolated as antibiotic peptides with 18 amino acid residues from Trichoderma harzianum in 1995. The six identified trichorzins HA (HA I through VII) have been reported, there is no report that they selectively interact with the CT receptor. In contrast to trichorzin HA V, alamethicin, another well-characterized peptaibol, showed no cAMP-elevating activity at all. These results suggest that, although there was little amino acid sequence similarity between trichorzin HA V and CT, the biological activity of trichorzin HA V can mimic that of CT, acting via the CT receptor.

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RESULTS

We isolated a series of antibiotic peptides (HF182a—d), trichorzins HA. 7) HF182a was found to be a mixture of trichorzins HA II and III. Three others were shown to be homogeneous by spectroscopy and found to be trichorzins HA V, VI and VII (data not shown). When we performed a cell-based cAMP assay with a human breast carcinoma cell line, T47D cells, which endogenously express human CT receptors on the cell surface,10) trichorzins HA (II, III, V, VI, VII) caused cAMP elevation (data not shown). One major peptide (HF182b), trichorzin HA V (Fig. 1), was further characterized. As shown in Fig. 2, trichorzin HA V and human CT stimulated the intracellular cAMP formation in T47D, CHO/hCTR and SaOS2/hCTR cells expressing endogenous and recombinant human CT receptors, respectively. However, they showed no activity in CHO/pKDEMSS or SaOS2 cells, which do not express the CT receptor.

Figure 3 shows the effects of trichorzin HA V and alamethicin on cAMP accumulation in CHO/hCTR cells. CHO/hCTR cells were incubated in 96-well plates with various concentrations of trichorzin HA V (a), alamethicin (c) and human CT (d). After incubation for 1 h, the intracellular cAMP was measured as described in Materials and Methods. The basal level of cAMP in CHO/hCTR cells was 0.637 pmol/well. Data are the mean±S.E.M. (n=4).

DISCUSSION

In the present study, we identified an antibiotic peptaibol, trichorzin HA V, which showed cAMP-elevating activities only in cells expressing the CT receptor. CT is known to activate adenyllyl cyclase to generate the intracellular second messenger molecule, cAMP, through interaction with the CT receptor. 3) The finding that, like CT, trichorzin HA V stimulated the pathway in cells expressing the CT receptor suggested that it interacts selectively with the CT receptor. On the other hand, alamethicin, another member of the peptaibol family, did not show any cAMP-elevating activity. Thus, the cAMP-elevating activity of trichorzin HA V is not a common feature of the peptaibol family, such as membrane perturbing activity or ionophoric activity, 5,8) but probably results from selective interaction with the CT receptor. As far as we know, the present study is the first to demonstrate that a particular member within the peptaibol family selectively acts on a receptor. Further detailed studies are certainly needed to clarify
which amino acids are responsible for the CT-like activities.

REFERENCES