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TOWARD ELUCIDATION OF THE INHIBITION MECHANISM OF PHOSPHOLIPASE A_2 BY MANOALIDE: SELECTIVELY MODIFIED AMINO ACID RESIDUES BY MANOALIDE ANALOGUES

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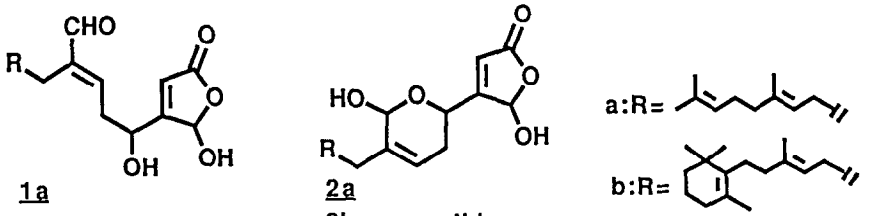
Abstract: Manoalide and seco-manoalide analogues synthesized from geranyl chloride selectively modified only two of eleven lysine residues of bovine pancreatic phospholipase A_2.

In the preceding paper[1], we described that simple analogues of seco-manoalide and manoalide, 1a and 2a, inhibited the enzymatic activity of bovine pancreatic phospholipase A_2(PLA_2) to the same extent as seco-manoalide and manoalide. In order to understand the inhibition machanism of PLA_2 by manoalide, it is important to characterize and identify the amino acid residues modified by manoalide analogue 1a or 2a in bovine pancreatic PLA_2. Two groups of researchers are attempting to elucidate the inhibition mechanism of cobra[2] and bee[3] venom PLA_2s by natural manoalide and its analogue. Although the catalytic mechanism is thought to be common to all kinds of PLA_2s, the apparent kinetic properties and substrate specificities of venom PLA_2s are somewhat different from those of mammalian pancreatic PLA_2s. In this paper, we describe that manoalide analogue 1a or 2a modified two out of eleven lysine residues of the native bovine PLA_2, while treatment of the enzyme with manoalide or seco-manoalide modified six lysine residues.



1a
1b seco-manoalide
2a
2b manoalide

The irreversibly modified PLA_2s by manoalides and their analogues were prepared as follows. One hundred times equivalent of inhibitors (manoalide, seco-manoalide, analogues 1a or 2a in dioxane-water) was incubated with purified bovine pancreatic PLA_2 in a Tris-HCl buffer at pH 8.0 and 40°C. At appropriate intervals, the residual enzymatic activity was measured with a part of the reaction mixture by the method described in the preceding paper, and the amino acid composition was determined with the rest of the mixture after gel filtration using a small column of Sephadex G-50. As shown in Figs.1 and 2, different numbers of lysine residues were found to be modified by manoalide and its analogues. Most interestingly, nine out of eleven native lysine

residues were recovered from the modified PLA_2s preparations obtained after 90 min incubation with a manoalide analogue of 1a or 2a, while five lysine residues were recovered from the sample treated with manoalide or seco-manoalide. After 15 min incubation with analogue 1a, the modified enzyme was separated from native PLA_2 by reversed phase high performance liquid chromatography. Amino acid analysis on this preparation showed the loss of two lysine residues.

Thus, the analogue, either 1a or 2a, might modify selectively only two lysine residues. It is of great interest that the modification of only two lysine residues by a manoalide analogue, 1a or 2a, lead to the enzymatic inhibition of bovine pancreatic PLA_2 to the same extent as manoalide or seco-manoalide, which modified about six lysine residues. An attempt to identify and characterize the modified two lysine residues is now in progress.

Fig 1 Amino Acid Analysis of Bovine Pancreatic PLA2 Modified by Analogue 1a

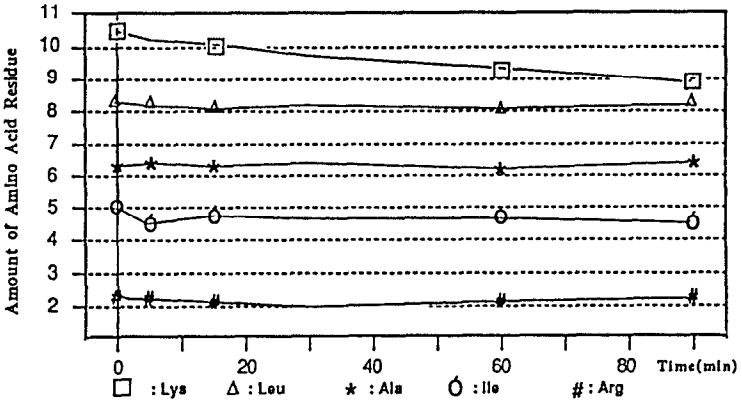
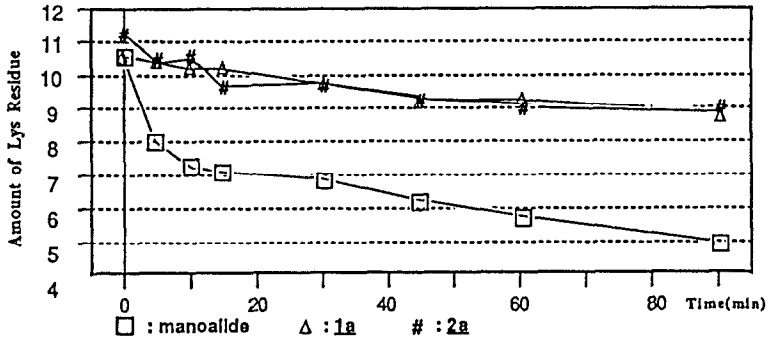


Fig 2 Amount of Lys Residues in Bovine Pancreatic PLA2 Modified by Manoalide and its Analogues



References

1. S.Katsumura, Q.Han, H.Kadono, S.Fujiwara, S.Isoe, S.Fujii, Y.Nishimura, and K.Ikeda, preceding paper of this journal, 1992.
2. S.Lambardo and E.A.Dennis, *J.Biol.Chem.*, **260**, 7234 (1985); L.J.Laynolds, E.D.Mikelich, and E.A.Dennis, *J. Biol. Chem.*, **266**, 16512(1991). It was reported that cobra venom PLA_2 modified by manoalog, which is dehydroxymanoalide analogue, contained 2.8 mol of Lys less than the native enzyme, and that one of modified Lys was Lys^6. In the bovine pancreatic PLA_2, the 6th position is not placed with Lys but with Asn. The inhibition mechanism of these PLA_2 with manoalide may be different from one another.
3. K.B.Glaser, T.T.Vedvick, and R.S.Jacobs, *Biochem. Pharmac.*, **37**, 3639 (1988).