

## Molecular Model of the Interaction of Bee Venom Phospholipase A<sub>2</sub> with Manoalide

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Received January 21, 1993

A molecular model of the interaction between manoalide (MLD) and bee venom phospholipase A<sub>2</sub> (bv-PLA<sub>2</sub>) has been derived making use of a combination of computational methods. MLD was built in its open form and simulated by using molecular dynamics techniques. It is shown that the polar part of the molecule, which is thought to be the reactive region, is endowed with considerable conformational flexibility whereas the apolar region is rather rigid. The proposed active conformation of MLD and the main putative binding site for MLD on this enzyme were identified by matching potential energy GRID maps for both ligand and receptor with the chemical structure of the respective counterpart. The binding site is found in the C-terminal region of bv-PLA<sub>2</sub>, forming part of the proposed interfacial surface for binding to aggregated substrates, and comprises two distinct regions: (i) a hydrophobic cavity delimited by the C-terminal  $\beta$ -sheet and the antiparallel  $\beta$ -sheet, which interacts with the apolar zone of MLD, and (ii) a cationic site made up of residues Arg-58 and Lys-94, which interacts with the polar zone. Molecular dynamics and molecular orbital calculations indicate that the most likely initial reaction between MLD and bv-PLA<sub>2</sub> is formation of a Schiff base between Lys-94 and the aldehyde generated upon opening of MLD's  $\gamma$ -lactone ring, supporting recent model reaction studies. The inhibition seems to be a consequence of the occupation by MLD of a site overlapping a phosphocholine binding site in bv-PLA<sub>2</sub> presumably involved in the interface desolvation process. The present model represents a starting point for further structural studies on the mechanism of phospholipases A<sub>2</sub> inactivation by MLD and MLD-like compounds.

### Introduction

Manoalide (MLD), a sesterterpenoid (Figure 1a) isolated from the sponge *Luffariella variabilis*,<sup>1</sup> has been shown to be a potent antiinflammatory agent, and in this activity phospholipase A<sub>2</sub> (PLA<sub>2</sub>) inhibition is believed to play a major role.<sup>2</sup> In fact, MLD is a potent inhibitor of PLA<sub>2</sub>s from several sources,<sup>3</sup> but bee venom PLA<sub>2</sub> (bv-PLA<sub>2</sub>) has been shown to be particularly sensitive to inactivation by this molecule.<sup>4</sup> Because of the important role of PLA<sub>2</sub> in the release of arachidonic acid, the biosynthetic precursor of proinflammatory eicosanoids, understanding the molecular mechanism of action of this compound is of considerable interest and can be of help in the design of novel PLA<sub>2</sub> inhibitors.<sup>5</sup>

Although some of the conditions necessary for MLD reactivity have been elucidated, the precise mechanism by which MLD irreversibly inactivates PLA<sub>2</sub>s remains largely unknown. Furthermore, even though bv-PLA<sub>2</sub> inhibition is accompanied by the modification of only three, and mainly one,<sup>6</sup> of the 11 lysine residues present in the enzyme, the exact location of the MLD binding site has not been identified as yet. Studies with a structural analogue of MLD called manoalogue have shown that this compound has no affinity for the catalytic site and that manoalogue-modified enzymes still contain a functional active site.<sup>7</sup> On the other hand, whereas a 1:1 stoichiometry of manoalogue to lysine has been demonstrated in cobra venom PLA<sub>2</sub>,<sup>8</sup> studies based on the reactivity of MLD with several lysine-containing peptides suggest that a peptide sequence in the enzyme containing a 1,4-Lys arrangement might be involved.<sup>6b</sup>

MLD contains two reactive ring structures, a hemiacetal ring and a  $\gamma$ -lactone ring, which become open at high pH

to generate two  $\alpha,\beta$ -unsaturated aldehydes<sup>2b,6,9</sup> (Figure 1b), and an apolar chain that might be involved in hydrophobic interactions with the enzyme.<sup>10</sup> Previous studies have demonstrated that both the opening of the lactone ring and the presence of the free aldehyde groups are required for irreversible inhibition.<sup>7a,9,10</sup> Only very recently have model reactions proved that the initial reaction between MLD and bv-PLA<sub>2</sub> involves the formation of a Schiff base, presumably with a lysine residue.<sup>11</sup>

All of the above-mentioned studies suggest the following sequence of events: (i) unmasking of two  $\alpha,\beta$ -unsaturated aldehydes upon opening of both rings in the MLD molecule and (ii) subsequent binding to a specific site on bv-PLA<sub>2</sub> separate from the active site. Yet the precise binding site and the actual mechanism of bv-PLA<sub>2</sub> inhibition by MLD remain unclear. On the other hand, the reported crystallographic structure of bv-PLA<sub>2</sub> complexed with a transition-state analogue in the active site solved at 2.0-Å resolution<sup>12</sup> has provided us with the foundation for a theoretical analysis of this reaction. In this paper, we use a combination of computational methods to present a molecular model of the interaction between this enzyme and the open form of MLD which accounts for most of the experimental results at the molecular level.<sup>13</sup>

### Results

**Rationale for Modeling the Open Form of Manoalide.** Inhibition studies have indicated that hydrocarbon derivatives containing just the 4-hydroxy-2-butenolide ring of MLD are inhibitory, although in this case the loss of enzyme activity is reversible.<sup>9</sup> On the other hand, methylation of the 4-hydroxy group of MLD's butenolide ring yields compounds that are no longer irreversible PLA<sub>2</sub> inactivators.<sup>7a</sup> These results suggest that the pharmacologically relevant form of MLD is the open form and that the two aldehydes present in this form are required for

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