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Functional characterization of a fatty acid double-bond hydratase from *Lactobacillus plantarum* and its interaction with biosynthetic membranes

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ABSTRACT

Hydrogenation of linoleic acid and other polyunsaturated fatty acids is a detoxification mechanism that is present in the *Lactobacillus* genus of lactic bacteria. The first stage in this multi-step process is hydration of the substrate with formation of 10-hydroxy-9-*cis*-octadecenoic acid due to fatty-acid hydratase activity that has been detected only in the membrane-associated cell fraction; however, its interaction with the cell membrane is unknown. To provide information in this respect we characterized the homotrimeric 64.7 kDa-native protein from *Lactobacillus plantarum;* afterwards, it was reconstituted in proteoliposomes and analyzed by confocal fluorescence microscopy. The results showed that hydratase is an extrinsic-membrane protein and hence, the enzymatic reaction occurs at the periphery of the cell. This location may be advantageous in the detoxifying process since the toxic linoleic acid molecule can be bound to hydratase and converted to non-toxic 10-hydroxy-9-*cis*-octadecenoic acid before it reaches cell membrane. Additionally, we propose that the interaction with membrane periphery occurs through electrostatic contacts. Finally, the structural model of *Lactobacillus plantarum* hydratase was constructed based on the amino acid sequence and hence, the putative binding sites with linoleic acid were identified: site 1, located in an external hydrophobic pocket at the C-terminus of the protein and site 2, located at the core and in contact with the FAD molecule. Interestingly, it was found that the linoleic acid molecule arranges around a methionine residue in both sites (Met154 and Met81, respectively) that acts as a rigid pole, thus playing a key role in binding unsaturated fatty acids.

KEYWORDS