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Gene Organization of *pld A* and *pld B*, the Structural Genes for Detergent-resistant Phospholipase A and Lysophospholipase L_2 of *Escherichia coli**

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In contrast to the advanced state of knowledge of the water-soluble phospholipases of mammalian pancreas and various snake venoms, the study of membrane-bound phospholipases is still in its infancy. These enzymes are of interest biochemically due to their possible roles in membrane physiology.

In *E. coli*, there are four kinds of phospholipases which hydrolyze acyl groups of phospholipids. Two of those are membrane-bound enzymes : detergent-resistant (DR-) phospholipase A located in the outer membrane and lysophospholipase L_2 in the inner membrane. Several mutants for DR-phospholipase A (*pld A*) or lysophospholipase L_2 (*pld B*) were isolated and characterized. For the further analysis of these enzymes from biochemical and genetical points of view, cloning of the *pld* genes was performed. The genes (*pld A* and *pld B*) were cloned together on the plasmid pKO1 (HOMMA et al (1983) *J. Biochem.* **94** 2079—2081). To study their gene organization, a transducing phage *pld A pld B*, carring both the *pld A* and *pld B* genes was constructed *in vitro* from plasmid pKO1. Viable deletion mutants of *λ pld A pld B* were isolated by EDTA killing, and their deleted DNA regions were determined by electron microscopic analysis of appropriate heteroduplexes. The activities of DR-phospholipase A and lysophospholipase L_2 were also measured in lysates of cells infected with the deletion phages. The DNA region essential for the expression of each lipolytic activity was determined. In addition, protein coded by the bacterial DNA on the plasmids containing the *pld A pld B* region to various extents were detected by the maxicell system. The results showed that the product of the *pld B* gene is a protein with molecular weight of 40,000. It was also shown that the *pld B* gene is located at a region about 3 kilobase from the *pld A* gene.

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