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Lysophospholipase L_1 from *Escherichia coli* K-12 Overproducer

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After screening 900 *E. coli* strains of the Clarke and Carbon collection for by lysophospholipase L_1 activities, we isolated a clone bearing the plasmid pLC6-34, which showed an increased level of lysophospholipase L_1 activity. Strains bearing the plasmid pC124, a subclone of pLC6-34 in plasmid vector pUC8, showed approximately 11.4 times higher lysophospholipase L_1 activity than that of the parental strain. Starting from those overproducing strains, the lysophospholipase L_1 was purified to near homogeneity by sequential use of ammonium sulfate fractionation, Sephacryl S-300, DEAE-cellulose, hydroxyapatite and Sephacryl S-200 column chromatographies. The apparent molecular weight of the purified lysophospholipase L_1 was estimated to be 20,500-22,000 both by SDS-polyacrylamide gel electrophoresis and by gel permeation chromatography. The specific activity of the homogeneous lysophospholipase L_1 was 10,400 nmol/min/mg protein when 1-acyl-sn-glycero-3-phosphoethanolamine was used as the substrate. The amino acid sequence of the amino-terminal portion of purified lysophospholipase L_1 was determined and was different from that of lysophospholipase L_2, which had previously been purified from the envelope fraction of *E. coli* strains bearing its cloned structural gene, *pldB* [Karasawa, K., Kudo, I., Kobayashi, T., Sa-eki, T., Inoue, K., & Nojima, S. (1985) *J. Biochem* 98, 1117—1125]. The gene responsible for overproduction of lysophospholipase L_1 activity was designated as *pldC* (*p*hospho*l*ipid *d*egradation *C*). Its restriction enzyme map was also different from that of cloned *pldB*. These results further confirmed that, in *E. coli*, there are two lysophospholipases with distinct characteristics.

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