Title:	Expression, purification, refolding and characterization of a putative lysophospholipase from Pyrococcus furiosus: Retention of structure and lipase/esterase activity in the presence of water-miscible organic solvents at high temperatures
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Abstract:	A putative lysophospholipase (PF0480) encoded by the Pyrococcus furiosus genome has previously been cloned and expressed in Escherichia coli. Studies involving crude extracts established the enzyme to be an esterase; however, owing presumably to its tendency to precipitate into inclusion bodies, purification and characterization have thus far not been reported. Here, we report the overexpression and successful recovery and refolding of the enzyme from inclusion bodies. Dynamic light scattering suggests that the enzyme is a dimer, or trimer, in aqueous solution. Circular dichroism and fluorescence spectroscopy show, respectively, that it has mixed beta/alpha structure and well-buried tryptophan residues. Conformational changes are negligible over the temperature range of 30-80 °C, and over the concentration range of 0-50% (v/v) of water mixtures with organic solvents such as methanol, ethanol and acetonitrile. The enzyme is confirmed to be an esterase (hydrolyzing p-NP-acetate and p-NP-butyrate) and also shown to be a lipase (hydrolyzing p-NP-palmitate), with lipolytic activity being overall about 18- to 20-fold lower than esterase activity. Against p-NP-palmitate the enzyme displays optimally activity at pH 7.0 and 70 °C. Remarkably, over 50% activity is retained at 70 °C in the presence of 25% acetonitrile. The high organic solvent stability and thermal stability suggest that this enzyme may have useful biodiesel-related applications, or applications in the pharmaceutical industry, once yields are optimized.
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