Title:	Refolding and functional assembly of the Vibrio cholerae porin OmpU recombinantly expressed in the cytoplasm of Escherichia coli
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Keywords:	Porin OmpU Vibrio cholerae
Issue Date:	2012
Publisher:	Elsevier B.V
Citation:	Protein Expression and Purification, 85 (2), 204-210.
Abstract:	OmpU is one of the major outer membrane porins of Vibrio cholerae. OmpU has been biochemically characterized previously for its ‘porin’-property. However, previous studies have used the OmpU protein extracted from the bacterial outer membrane envelope fractions. Such method of isolation imposes limitations on the availability of the protein reagent, and also enhances the possibility of the OmpU preparation being contaminated with lipid molecules of bacterial outer membrane origin, especially lipopolysaccharides (LPS). Here we report a strategy of purifying the V. cholerae OmpU protein recombinantly overexpressed in heterologous protein expression system in Escherichia coli, without its being incorporated into the bacterial membrane fraction. In our strategy, the majority of the protein was expressed as insoluble inclusion body in the E. coli cytoplasm, the protein was dissolved by denaturation in 8 M urea, refolded, and purified to homogeneity in presence of detergent. Our strategy allowed isolation of the recombinant OmpU protein with significantly enhanced yield as compared to that of the wild type protein extracted from the V. cholerae membrane fraction. The recombinant V. cholerae OmpU protein generated in our study displayed functional channel-forming property in the synthetic liposome membrane, thus confirming its ‘porin’-property. To the best of our knowledge, this is the first report showing an efficient refolding and functional assembly of the V. cholerae OmpU porin recombinantly expressed as inclusion body in the cytoplasm of a heterologous host E. coli.
URI:	http://www.sciencedirect.com/science/article/pii/S1046592812002148 (http://www.sciencedirect.com/science/article/pii/S1046592812002148) http://dx.doi.org/10.1016/j.pep.2012.08.005, (http://dx.doi.org/10.1016/j.pep.2012.08.005,)
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