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Use of n-Octyl- β -D-thioglucopyranoside in the isolation of a bacterial membrane protein: an improved method for immunological applications

J. M. PEULA-GARCÍA^{1*}, J. ROJAS², A. G. CAMACHO², J. A. MOLINA-BOLÍVAR¹, J. AGUIAR¹ and C. CARNERO RUIZ¹

¹Amphiphilic Systems and Structured Fluids Group, Department of Applied Physics II, Campus de Teatinos, University of Málaga, 29071, Málaga, Spain

²VIRCELL SL, Pol. Industrial Dos de Octubre, 18320, Santa Fe, Granada, Spain

Abstract Surfactants are essential in the isolation of integral membrane biomolecules from biological membranes of different microorganisms. Our objective is the isolation of the major outer membrane protein (MOMP) of *Chlamydia trachomatis*, to use it in the preferential and specific detection of immunoglobulins. We have improved a method using an alkylglycoside surfactant, n-Octyl- β -D-thioglucoside (OTG), which is a non-ionic detergent used in membrane solubilization due to its "weak" action preserving the biological and functional properties of solubilized biomolecules. Different solubilization conditions, such as surfactant and salt concentrations, temperature and the presence of additive (di-thiothreitol (DTT)), were tested. To know the influence of the parameters previously indicated on the micellar properties and the solubilizing ability of the surfactant, we have studied the micellization process of OTG under several conditions by using static fluorescence measurements. MOMP was isolated by a simplified method consisting of a two-step extraction using OTG and DTT with an optimization of the experimental conditions. In this way, MOMP turns out to be separated from other biomolecules (i.e., lipopolysaccharides) in order to avoid immunological cross-reactions among the different chlamydial species. Furthermore, gel electrophoresis experiments showed monomeric MOMP without multi-aggregation, even after removing DTT and OTG molecules by dialysis of the OTG DTT soluble fraction. The isolated protein (MOMP) is used to coat microplate wells to develop immunoenzymatic assays (ELISA). The preliminary immunological study shows the detection of specific IgG of *Chlamydia trachomatis*, with adequate values of the immunological parameters: sensibility and specificity. Moreover, these results show the role of surfactant molecules in the

*Author for correspondance.

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binding process of proteins to solid-liquid interface.

Keywords : n-octyl- β -D-thioglucopyranoside, bacterial membrane protein, immunological application, solubilization, immunoenzymatic assays.