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Expression, purification and preliminary crystallization of Schistosomal universal stress G4LZI3 protein towards new schistosomide discovery

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The formation of high quality and perfect crystals forms the bottleneck and rate-limiting step for structure determination by X-ray crystallography. Protein crystallography has formed an intricate part of the chemical, biotechnology and pharmaceutical industry as an important tool towards drug design, protein engineering and in understanding various biological systems. In this study, a Universal stress G4LZI3 protein, identified as a 'lead' molecule for the design of alternative treatment against schistosomiasis, was subjected to protein crystallization trials. Schistosoma mansoni, a parasitic helminth, is responsible for the neglected tropical disease schistosomiasis that ranks second to malaria in public health significance. It accounts for over 280 000 deaths per year and is equally to blame for the physical life-long disability and disfigurements associated with the infection with an estimated global prevalence of 200 million. Praziquantel, which has been the gold standard for treatment for over 3 decades, has now exhibited drug resistance. Over-expression of the G4LZI3 protein throughout the schistosome's lifecycle caused by conditions of stress, has warranted the need to determine its structure in a bid to design new schistosomides.
The gene sequence coding for the G4LZI3 protein was first cloned into a pQE-30 vector using BamH1 and HindIII restriction enzymes. The resultant pQE30-G4LZI3 construct was transformed into JM109 bacteria cells. Expression screening was done to determine the best expressing clone, and was used for heterologous expression of sufficient amounts of recombinant G4LZI3 protein, followed by purification on a Ni-NTA column. Thereafter, the G4LZI3 protein was purified to homogeneity using size exclusion chromatography; purified fractions under the chromatogram was pooled together and concentrated down to 10mg/ml, 15mg/ml, 17mg/ml, 20mg/ml and 24mg/ml. These various concentrations were subjected to various crystallization trials and various conditions yielded considerably sized and 3-dimensional shaped crystals. Future studies will aim to determine the structure and biological function of the protein, as well as perform virtual screening of identifying small molecule inhibitors that can serve as anti-schistosomals.
Keywords: Crystallization, G4LZI3, Praziquantel, gel filtration, Schistosomiasis

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