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A simple and high efficiency purification of His-tagged turnip yellow mosaic virus-like particle (TYMV-VLP) by nickel ion affinity precipitation

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ABSTRACT

Virus-like particles (VLPs) is one of the most favourable subjects of study, especially in the field of nano-biotechnology and vaccine development because they possess good immunogenicity and self-adjuvant properties. Conventionally, VLPs can be tagged and purified using affinity chromatography or density gradient ultracentrifugation which is costly and time-consuming. Turnip yellow mosaic virus (TYMV) is a plant virus, where expression of the viral coat protein (TYMVc) in *Escherichia coli* (*E. coli*) has been shown to form VLP. In this study, we report a non-chromatographic method for VLP purification using C-terminally His-tagged TYMVc ($TYMVcHis_6$) as a protein model. Firstly, the $TYMVcHis_6$ was cloned and expressed in *E. coli*. Upon clarification of cell lysate, nickel (II) chloride [$NiCl_2$; 15 μM or equivalent to 0.0000194% (w/v)] was added to precipitate $TYMVcHis_6$. Following centrifugation, the pellet was resuspended in buffer containing 1 mM EDTA to chelate Ni^{2+}, which is then removed via dialysis. A total of 50% of $TYMVcHis_6$ was successfully recovered with purity above 0.90. Later, the purified $TYMVcHis_6$ was analysed with sucrose density ultracentrifugation, dynamic light scattering (DLS), and transmission electron microscopy (TEM) to confirm VLP formation, which is comparable to $TYMVcHis_6$ purified using the standard immobilized metal affinity chromatography (IMAC) column. As the current method omitted the need for IMAC column and beads while significantly reducing the time needed for column washing, nickel affinity precipitation represents a novel method for the purification of VLPs displaying poly-histidine tags (His-tags).

1. Introduction

Virus like particles (VLPs) are nanoparticles which resemble viruses but are void of genetic materials. Unlike viruses, VLPs do not propagate in living cells. Therefore, countless studies have been performed on various VLPs to harness their potential in the medical field, particularly in vaccine research and targeted delivery of nanomedicines (Rohovie et al., 2017; Roldao et al., 2014; Syomin and Ilyin, 2019; Zdanowicz and Chroboczek, 2016). For instance, *Macrobrachium rosenbergii* nodavirus (MrNV) VLPs have been used to display influenza A virus (IAV) matrix 2 ectodomain (M2e), where immunization with the candidate vaccine induced strong immune responses which protected mice against lethal IAV challenges (Ong et al., 2019; Yong et al., 2015b). In addition, the VLPs have also been exploited as a carrier for delivery of plasmids into insect cells and shrimps (Jariyapong et al., 2015, 2014). Likewise, modified hepatitis E VLPs that displayed breast cancer-targeting ligand (LXY30) have shown tropism towards breast cancer tumour in vivo (Chen et al., 2016). Similarly, VLPs of different origins such as bacteriophage MS2 (Li et al., 2014; Sun et al., 2011; Wu et al., 2005), hepatitis B virus (Lee and Tan, 2008; Suffian et al., 2018; Zhang et al., 2019; Zhao et al., 2020), and canine parvovirus (Gilbert et al., 2004; Jin et al., 2016; Singh et al., 2006), have also been investigated.

Conventionally, VLPs can be purified using density ultracentrifugation, such as sucrose or caesium chloride density gradient ultracentrifugation. However, this method requires the use of expensive ultracentrifuge equipment and involves multiple pre-concentration

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