A Method for Removing Contaminating Protein during Purification of Human Papillomavirus Type 18 L1 Protein from Saccharomyces cerevisiae

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Human papillomavirus (HPV) types 16 and 18 are the main targets in the field of prophylactic vaccines for preventing cervical cancer. L1 protein, the major capsid protein of HPV, self-assembles into virus-like particles (VLP), which are the major component of prophylactic vaccines. To obtain highly purified L1 protein, contaminants must be removed by several chromatography steps. However, this requires a great deal of time and labor, and results in loss of large amounts of the target protein. Therefore, we have sought to develop an efficient method for removing contaminants prior to chromatography during the purification of HPV18 L1 protein from Saccharomyces cerevisiae. For this purpose the contaminating proteins were removed by an ammonium sulfate precipitation step and further removed by a removal of precipitated contaminants step. Purification of the L1 protein by chromatography was significantly improved by the removal of precipitated contaminants step. In the present work we developed two one-step chromatography methods (heparin and cation-exchange chromatography), and HPV18 L1 proteins purified by both methods self-assembled into VLP. The two chromatographic purification methods are simpler and more convenient than previous methods and are widely applicable to work with VLPs.

Key words: Cervical cancer, Papillomavirus, Virus-like particles, Heparin, Cation-exchange

INTRODUCTION

Human papillomavirus (HPV) is a double-stranded DNA virus, and over 100 types of HPV have been identified (Bosch et al., 1995; Jeong et al., 2009). Most cervical cancer is caused by HPV infection, and about 70% of all cervical cancer cases worldwide are associated with infections by HPV types 16 and 18 (Jeong et al., 2009; Moscicki, 2008; Schadlich et al., 2009). Therefore, these two types of HPV are the main targets in the development of vaccines to prevent cervical cancer.
can generate high neutralizing antibody titers and strong T cell responses against HPV (Garland et al., 2007).

The L1 protein has been expressed in E. coli, yeast, and insect and mammalian cell systems (Kirnbauer, 1996; Lowe et al., 1997; Park et al., 2008; Zhou et al., 1991). The yeast expression system has advantages in terms of cost-effectiveness, suitability for large-scale production, and low potential for contamination by toxins or viruses compared with bacterial and mammalian expression systems (Cook et al., 1999; Joyce et al., 1999; Neeper et al., 1996). Therefore, production of VLP-based vaccines via the yeast expression system has advantages over the other expression systems.

Infection by HPV essentially requires binding to heparan sulfate on the cell surface, and it is known that the HPV induces an immune response upon binding to heparan sulfate on dendritic cells, and that this step controls the immune response and is important for vaccine development (de Witte et al., 2007). Heparin, which is structurally similar to heparan sulfate, binds correctly folded HPV VLPs but not incorrectly folded HPV VLPs (Giroglou et al., 2001; Rommel et al., 2005; Wang et al., 2005). This suggests that it should be useful for selecting correctly folded VLPs. However, a heparin-based chromatography method has not been developed despite the fact that ten years have elapsed since the heparin-HPV VLP interaction was established.

Size-exclusion chromatography, ultracentrifugation on a sucrose cushion and cesium chloride density gradient centrifugation have all been used to purify HPV VLPs because the VLP is larger than most contaminants (Aires et al., 2006; Hofmann et al., 1995; Jeong et al., 2006; Kim et al., 2007; Park et al., 2008; Woo et al., 2008). However, these methods are limited to small scale purification because they cannot cope with large amounts of protein. In addition, further chromatography steps are required because the contaminants are not efficiently removed by these methods alone, and the additional chromatography steps result in large losses of the target protein. The demanding nature of the available methods for purifying HPV VLPs has been for a long time an obstacle to the study of HPV.

In the present work, we have developed a method that removes contaminating proteins efficiently prior to the chromatography step and have developed two one-step chromatography methods. One is heparin chromatography and the other, cation-exchange chromatography.

**MATERIALS AND METHODS**

**Expression of HPV18 L1 protein**

Vector YEGα-HPV18 L1 harboring the native HPV18 L1 gene was constructed and transformed into Saccharomyces cerevisiae (S. cerevisiae) Y2805 as described in Woo et al. (2008). To express HPV18 L1 protein, HPV18 L1-producing S. cerevisiae was cultured in YPDG medium (1% glucose, 3% galactose, 1% yeast extract and 2% peptone) at 30°C for 2 days. Growth was stopped when 1:10 dilutions in distilled water gave an OD of 1.4 at 600 nm.

**Preparation of cell lysates**

The yeast cells were harvested by centrifugation at 5000 g for 10 min, washed twice with phosphate-buffered saline (PBS) and frozen at -70°C. One hundred grams of wet cells were thawed at room temperature (RT) for 3 h and resuspended in 100 mL of cold break buffer (20 mM sodium phosphate, pH 7.2, 100 mM NaCl, 1.7 mM EDTA, 0.01% Tween 80). The resuspended cells were mixed 1:1 with glass beads (BioSpec Products) and broken in a Bead-Beater (BioSpec Products). Cell debris was removed by centrifugation at 12000 g for 10 min at 4°C, centrifugation being repeated until the debris was completely removed.

**Ammonium sulfate precipitation**

The HPV18 L1 protein in the clarified supernatant was recovered by ammonium sulfate precipitation (Fig. 1): the supernatant was slowly adjusted to 45% saturated ammonium sulfate at 4°C for 4 h, and precipitated protein was pelleted by centrifugation at 12000 g for 10 min at 4°C, centrifugation being repeated until the debris was completely removed.

**Removal of precipitated contaminants**

Frozen sample was thawed at RT and dialyzed against PBS + 0.01% Tween 80 for 4 h at 4°C. The dialyzed sample was diluted 1:20 in incubation buffer (10 mM sodium phosphate, pH 7.2, 0.15 M NaCl + 0.01% Tween 80, the protein concentration of dilute was adjusted to 2 - 5 mg/mL) and incubated at RT for 24 h to induce precipitation of contaminants. The solution was clarified by centrifugation at 12000g for 10 min and the supernatant recovered. The HPV18 L1 protein in the supernatant was then purified by heparin chromatography or cation-exchange chromatography as described in Fig. 1. To evaluate the solubility of L1 protein and the fraction of the protein precipitated as