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Review

Methods of isolation and purification of outer membrane vesicles from gram-negative bacteria

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ABSTRACT

Outer membrane vesicles secreted by gram-negative bacteria play an important role in bacterial physiology as well as in virulence and host–pathogen interaction. Isolated vesicles of some bacteria have also been studied for their immunomodulatory potential in the vaccine development. However, the production of vesicles in sufficient amount, purity and reproducibility remains a critical challenge for subsequent analyses in most bacteria. In the present review methods of production, isolation, purification and quantification of outer membrane vesicles are summarized and discussed.

Contents

Abbreviations: OMV, outer membrane vesicles; OM, outer membrane; MV, membrane vesicles; LPS, lipopolysaccharide; EDTA, ethylene-diamine-tetraacetic acid; UF, ultrafiltration; MW, molecular weight; AS, ammonium sulphate; SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis; CFU, colony forming unit; TEM, transmission electron microscopy; nOMV, native OMV; dOMV, detergent-derived OMV; sOMV, spontaneously released OMV; eOMV, extracted OMV.

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1. Introduction

A growing number of gram-negative bacteria has been reported to secrete outer membrane vesicles (OMV) whose functions are frequently involved in the delivery of active substances to a host and/or the modulation of a host immune response; for recent reviews see Amano et al. (2010), Ellis and Kuehn (2010), MacDonald and Kuehn (2012), Berleman and Auer (2013), and Acevedo et al. (2014). OMV production is often provoked by a variety of living conditions such as growth stage and nutritional, temperature and oxidative stress – conditions closely associated with the hostile environment during infection (MacDonald and Kuehn 2013).

1.1. OMV generation

OMV are formed when a portion of outer membrane (OM) separates from the bacterial surface and encapsulates inside part of the periplasmic space. These OM "blebs" or vesicles range in size from 50 to 250 nm in diameter and are composed of constituents of OM (lipopolysaccharide, phospholipids and proteins) and periplasm (Beveridge 1999; Kuehn and Kesty 2005; Mashburn-Warren et al. 2008). However, in many cases the content, or "cargo", of OMV may also contain nucleic acids and cytosolic or inner membrane proteins but the sorting mechanism for how the components are targeted to OMV remains unclear (Bonnington and Kuehn 2014; Kato et al. 2002; Mashburn-Warren and Whiteley 2006). On the other hand, inner membrane or cytosolic proteins constitute only a minor fraction of OMV, supporting the fact that OMV are not generated by cell lysis.

The gram-negative OM is bound to the subjacent peptidoglycan layer by specific proteins with more or less homogenous distribution. These include Lpp – a lipoprotein (in *E. coli*) that covalently binds OM to peptidoglycan, the Tol-Pal system which non-covalently binds inner membrane through peptidoglycan with OM, and OmpA – an OM protein non-covalently connecting OM to peptidoglycan (Schwechheimer et al. 2013). OMV biogenesis starts by OM outward bulging in the areas where these OM-peptidoglycan links are impaired either by their repositioning or by breaking the connection. Further budding of the bulge is then extended by accumulation of periplasmic proteins – a future cargo of OMV. Additionally, the membrane budding is activated by localized accumulation of curvature-inducing OM proteins in loosened areas (Kulp and Kuehn 2010). In *Salmonella*, the deletion of proteins that form OM-peptidoglycan or OM-peptidoglycan-inner membrane links leads to the discovery that these proteins control the OMV size distribution, vesiculation rate and localization during OMV biogenesis (Deatherage et al. 2009). New type of OMV has been described recently in *Shewanella vesiculosa* which show the presence of DNA in the OMV lumen. Apart from the conventional single-membrane OMV formed by OM and periplasm this bacterium is capable of releasing more complex double-membrane vesicles. During the formation of these vesicles a portion of inner membrane is dragged and strangulated to encapsulate some cytosolic content (Pérez-Cruz et al. 2013).

Compared to gram-negative bacteria, little attention has yet been focused on membrane vesicles (MV) secretion in gram-positive bacteria. This disproportion may be attributed to different architectures of the gram-positive cell envelope such as the lacking of OM. On the other hand, archaea and eukaryotic cells lack OM as well and produce MV independently (Deatherage and Cookson 2012). Shedding of MV has been described in several gram-positive bacteria, e.g. *Staphylococcus aureus*, *Bacillus anthracis*, *B. cereus* or *B. subtilis* (Dorward and Garon 1990; Lee et al. 2009; Rivera et al. 2010; Tashiro et al. 2010a; Gurung et al. 2011). Vesicles derived from *B. anthracis* have been observed to protect mice against challenge with the bacterium, suggesting the possibility that gram-positive MV preparations may also be developed into vaccines in the future (Rivera et al. 2010). Because majority of the published information about vesicle isolation deals with gram-negative OMV, the following chapters focus on this subject. Nevertheless, where gram-positive MV isolation and purification are described, same methods and protocols as for OMV also apply.

1.2. OMV functions

OMV serve countless functions in the bacterial life style. They act as a secretion system to deliver their content into the environment. The secretion of virulence factors and toxins into a host via OMV has been described in many bacterial species (Horstman and Kuehn 2000; Wai et al. 2003; Amano et al. 2010; Ellis and Kuehn 2010). For some bacteria OMV also serve as weapons in the interspecies environmental competition (Li et al. 1998) or as a decoy for bacteriophages and antimicrobial peptides (Manning and Kuehn 2011). The role of OMV in the intra- and inter-species communication lies also in the delivery of small signalling molecules and DNA (Berleman and Auer 2013). The release of misfolded and aggregated proteins via OMV has been presented as a novel stress response mechanism (McBroom and Kuehn 2007).

1.3. OMV in vaccine development

Study of the role of OMV in the host–pathogen interaction yields new information about the virulence mechanism of pathogenic bacteria. An immunomodulatory effect has already been employed in the development of acellular vaccines (Collins 2011; Acevedo et al. 2014). Nevertheless, the current production of OMV poses critical challenge to obtain sufficient amount, purity and reproducibility for subsequent studies of their protein and lipid compositions or their biological functions. Especially in the vaccine production the consistency of compositions between batches is a critical factor for vaccine safety and efficacy. In the present review we summarize and discuss methods that have been used for the production, isolation and purification of OMV and their subsequent analyses.

2. Production of OMV

A typical workflow of OMV preparation is depicted in Fig. 1. The procedure usually consists in the following steps: cultivation,