Purification and analysis of integrity and components of VLPs

Virus-like particles (VLPs) as vaccines, vectors and adjuvants

Fondation Mérieux Conference Center
Veyrier du Lac – France
April 1, 2014

Andris Zeltiņš
Latvian Biomedical Research and Study Centre,
Ratsupites 1, Riga, LV-1067, Latvia
anze@biomed.lu.lv
**Purification and analysis of integrity and components of VLPs**

**History: First VLPs at Latvian Biomedical Research & Study Centre**

**HBcAg VLPs (1987)**

**Qβ VLPs (1993)**

**Bacteriophage fr VLPs (1987)**


Kozlovska TM, Cielens I, Dreilina D, Dislers A, Baumanis V, Ose V, Pumpens P.

### Purification and analysis of integrity and components of VLPs

#### Examples of VLPs constructed at Latvian Biomedical research & study centre

<table>
<thead>
<tr>
<th>Bacterial VLPs</th>
<th>Plant VLPs</th>
<th>Mammalian VLPs</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP205</td>
<td>ACLSV</td>
<td>HbcAg</td>
</tr>
<tr>
<td>CB5</td>
<td>ASPV</td>
<td>HaPyV</td>
</tr>
<tr>
<td>fr</td>
<td>ASGV</td>
<td>E.coli / yeasts / mammalian cells</td>
</tr>
<tr>
<td>GA</td>
<td>PVX</td>
<td>E.coli / Drosophila S2</td>
</tr>
<tr>
<td>PP7</td>
<td>PVY</td>
<td></td>
</tr>
<tr>
<td>Q8</td>
<td>PVM</td>
<td></td>
</tr>
<tr>
<td>SP</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Bacterial VLPs**
- E. coli
- P. pastoris
- S. cerevisiae

**Plant VLPs**
- E. coli
- P. pastoris

**Mammalian VLPs**
- E. coli / yeasts / mammalian cells
- E. coli / Drosophila S2
How to obtain new VLPs?

- Cell disruption
- Early identification
- Purification
- Components of VLPs
- Stability
- Storage
Buffer systems preserving the integrity of VLPs

- Ionic strength (high/low salt content);
- Metal ions (e.g. Ca$^{2+}$, Mg$^{2+}$);
- Chelating agents (e.g. EDTA);
- Reducing agents (e.g. DTT, mercaptoethanol);
- Protease inhibitors (e.g., EDTA, PMSF, protease inhibition «cocktails»);
- Urea;
- Nucleases (DNAses, RNAses, nuclease benzonase etc.);
- Detergents (e.g. TX-100, Tween 20);
- Precipitation with ammonium sulfate or PEG;

- For new VLPs – buffer systems derived from isolation protocols of corresponding native viruses ensuring the structural integrity and/or infectivity;
Purification and analysis of integrity and components of VLPs

Cell disruption

Freezing / thawing grinding with Al₂O₃

French press

Ultrasonication focused, non-contact

Ultrasonication conventional

RYMV-K VLPs

RGMoV VLPs

RGMoV VLPs

RYMV VLPs

RYMV-K VLPs?

E.coli

P.pastoris

P.pastoris

E.coli

Cell disruption have to be efficient and preserve the integrity of VLPs
Procedures useful for early detection of VLPs

Analytical ultracentrifugation / SDS/PAGE

Visual test

Typical VLPs, no structures in background

No VLPs found

Some VLPs found, most CP in aggregates
Purification and analysis of integrity and components of VLPs

Procedures useful for early detection of VLPs

Agarose gels / Crude lysates

Agarose gel analysis can suggest the presence of VLPs in the samples
Purification and analysis of integrity and components of VLPs

Purification: ultracentrifuge gradient separation

ApMV VLP purification:
SW32 rotor (Beckman), 25 000 rpm, 6 h, discontinuous sucrose gradient (20-60%, 1xPBS, 0.5% TX-100), fraction size 5 ml

Second sucrose gradient (10-40%, 1xPBS, 0.5% TX-100), fraction size 4 ml
Purification and analysis of integrity and components of VLPs

Purification: column chromatography

Size-exclusion chromatography

Ion exchange chromatography

Purification and analysis of integrity and components of VLPs

Characterization of VLPs: mass spectrometry

SDS/PAGE  Western blot

1 - PVY VLPs

1 - PVY CP
Mw theor = 30 053

MGNDTIDAGGSTKKDAKQEIQPSLPNKEKEDVNVGSGTVTVPRIK...

Purification and analysis of integrity and components of VLPs

Characterization of VLPs: mass spectrometry

1 - HBc VLPs
E.coli

2 - phosphorylated HBc VLPs
P. pastoris

Purification and analysis of integrity and components of VLPs

Characterization of VLPs: dynamic light scattering

Dynamic light scattering can serve as an alternative tool for EM

Skrastina D, Petrovskis I, Petraityte R, Sominskaya I, Ose V, Lieknina I, Bogans J, Sasnauskas K, Pumpens P.
Purification and analysis of integrity and components of VLPs

Characterization of VLPs: 3D crystallographic models

Bacteriophage ϕCB5 VLPs

ϕCB5 3D structural model
VLP crystals

VLPs can serve as an alternative source of viral structures for crystallographic studies

Purification and analysis of integrity and components of VLPs

Stability studies: Sypro Orange / Real-time PCR (melting point determination)

**Principle:**
- Solution containing VLPs is not activating Sypro Orange fluorescence at room temperature (hydrophobic surfaces are hidden);
- Thermal denaturation expose the hydrophobic surfaces of VLPs and cause Sypro Orange binding and fluorescence;
- VLP stability curve and its midpoint value (melting temperature) can be obtained by changing the temperature gradually to unfold the protein and measure the change in fluorescence.

### 15 mM phosphate +50 mM DTT +25 mM EDTA +0.5 M NaCl

<table>
<thead>
<tr>
<th></th>
<th>RGMoV virions</th>
<th>RGMoV VLPs</th>
</tr>
</thead>
<tbody>
<tr>
<td>78°C</td>
<td>76°C</td>
<td>65°C</td>
</tr>
<tr>
<td>74°C</td>
<td>73°C</td>
<td>64°C</td>
</tr>
</tbody>
</table>

RGMoV virions

RGMoV VLPs from *P. pastoris*
Stability studies: storage of VLPs

- Optimal VLP concentration;
- Salt concentration
- Additives (e.g., glycerol, sucrose, trehalose, sorbitol, polysorbat);
- Freezing (-20°C/-80°C) / storage at +4°C

Aggregated VLPs after freezing

Disassembled VLPs after freezing
Characterization of VLPs: introduced epitopes

Mass spectrometry

Competitive ELISA

The surface localization of the preS1 epitope was demonstrated by competitive ELISA. Increasing amounts of competitor antigen (PVY-CP-preS1; PvyCP) were added to the preS1-specific mAb MA18/7 and the binding of antibodies to the immobilized preS1 peptide was measured.

Purification and analysis of integrity and components of VLPs

Characterization of VLPs: nucleic acid content

Next-generation sequencing (Ion Torrent system)

Total reads: 26,253
Identified sequences: 7,820

- RGMoV CP mRNA: 4,678
- pPIC3.5 CoE1 transcript: 1,102
- *P. pastoris* Dienoyl-CoA isomerase (mitochondrial): 966
- pPIC3.5 Km resistance: 563
- *P. pastoris* chromosome 2 UTR: 346
- Other (*P. pastoris* transcripts): 18,598
Typical workflow for purification and characterization of new VLPs

- «Knowledge-based» buffer systems
- «Correct» cell disruption procedure
  - Early identification:
    - analytical sucrose gradients
    - agarose gel analysis after nuclease treatment
    - electron microscopy
  - Purification:
    - sucrose gradients;
    - column chromatography
  - Characterization:
    - estimation of VLP concentrations;
    - mass spectrometry;
    - dynamic light scattering;
    - electron microscopy;
    - stability studies including storage conditions;
    - nucleic acid packaging studies;
Purification and analysis of integrity and components of VLPs

Acknowledgements:

Dr. Ina Bāķe
Gunta Reseviča
Ieva Kalnciema
Jeļena Šaripo
Vilija Zeltiņa

Dr. Kaspars Tārs
Dr. Andris Kazaks
Dr. Ivars Petrovskis
Dr. Velta Ose
Dr. Dace Skrastiņa
Dr. Dāvids Fridmanis
Prof. Dr. Pauls Pumpēns

Prof. Dr. Martin Bachmann (Zurich)