In Vitro Monitoring of the Formation of Pentamers from the Monomer of GST Fused HPV 16 L1

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1. Materials and Methods

1.1 Protein expression and purification.

The HPV 16 L1 coding sequences, in lacking 4 amino acids at the N-terminus and 30 amino acids at the C-terminus for better expression, were cloned into pGEX-6p-1 based vectors. It was expressed in *Escherichia coli* under the IPTG induce and with glutathione-S-transferase (GST) fusion. The protein from the cell lysate was purified by using the protocol reported by Chen *et al.*,[1] which could be summarized as follows. Cells from one liter culture were resuspended in 30 ml of buffer L (50 mM Tris-HCl, pH = 8.0, 0.2 M NaCl, 1 mM DTT, 1 mM EDTA) and then were lysed by sonification. Then the lysate was added ATP and $MgCl_2$ to the final concentrations of 2 and 5 mM, respectively. After one hour incubation at room temperature, urea (ultrapure grade) was slowly added to the lysate to a final concentration of 3.5 M under slowly stirring. The mixture was incubated at room temperature for one hour with gentle shaking, and then dialyzed against three changes of buffer L over 18h. The obtained GST-L1 was then separated from other proteins by using a glutathione-Sepharose column.

Then the column purified GST-L1 was treated in two ways for different purposes. One was eluted out by 10 mM GSH, after further dialysis to remove GSH the eluted GST-L1 was used for monitoring of pentamer formation. Another was cleaved directly by PPase on column to obtain L1 elution for further protein characterization. In following, the left small amount of GroEL in L1 elution was isolated as a by-product by using size-exclusion chromatography (SEC, Superdex-200, 26/60) and the further purified target protein L1 was used for further size and structural characterizations.

1.2 FPLC elution profiles.

Fast protein liquid chromatography (FPLC) system (GE, USA) was used to further purify the target proteins (GST-L1/L1) and analyze their relative molecular masses. An analytical protocol based on SEC (Superdex-200, 26/60) was used for this purpose. Sample loading and column washing were performed with Buffer L (mobile phase) and all samples were clarified by filtration through 0.22 μm filter unit. A flow rate of 3 ml/min was employed and a 50 ml sample loop was used for sample injection, and all the purification steps were conducted at 4 °C. The purified and separated proteins from FPLC were also analyzed by SDS polyacrylamide gel electrophoresis (SDS-PAGE) and further identified by Western-blot.

1.3 *In Vitro* Monitoring of Pentamer Formation from GST-L1 by SLS Measurements.

The solutions containing the purified GST-L1 were used for the time-dependent *in vitro* static light scattering (SLS) measurements, which were conducted at 25 °C with a fluorescence spectrophotometer, SHIMADZU (Japan) RF-5301. 2.0 ml GST-L1 fusion proteins (0.1 or 0.2 mg/ml) in buffer L (50 mM Tris-HCl, 0.2 M NaCl, 1 mM EDTA, DTT) were passed through 0.22 μm filter unit to remove any aggregates and dust particles firstly, and then was added into a 10×10 mm quartz cuvette. A static fluorescence intensity of it was recorded with an excitation wavelength of 350 nm. Under constant stirring, PreScission Protease (PPase, 200 IU/100 μl) was added to the cuvette and the moment was treated as the starting point (t = 0) of the measurement, continuously recording the SLS intensities by the spectrophotometer at 10s intervals for 12 h. In addition, to track the process of PPase cleavage on GST-L1, each 30 μl sample was taken out from the cuvette between 0-10 h at 1 h sampling intervals, respectively, for the synchronous Western-blot analysis.

In order to further explore the kinetics of the pentamer formation *in vitro*, we firstly controlled the pH of buffer L at 7.2, 8.0 and 8.8, respectively, to determine the optimal pH for the experiment. Then, we controlled each the initial concentration of GST-L1 (0.1, 0.2 mg/ml), DTT (2, 5, 10 mM) and the added volume of PPase (5, 10, 15 μL) on 0.2 mg/ml GST-L1 in following the optimal pH to monitor the pentamer assembly processes. The obtained static light scattering plots and the corresponding linear/curve-fitting plots were list as Fig. S4 for more clear comparison and mechanism consideration.

1.4 Dynamic Light Scattering (DLS) Measurements.

The solutions containing the purified GST-L1, L1 (either in monomer or pentamer) were prepared firstly by using the described method in **1.1**. After filtration to remove dust particles through 0.22 μm filter unit, the particle size analyzer (Malvern Zetasizer Nano-ZS 90) with a 4 ml cuvette and the Dispersion Technology Software (DTS, V6.01) were used for data collection and analysis. The graphs and statistical analysis for the particle size in solution were reported.

1.5 Transmission Electron Microscopy (TEM) Measurements.

For the TEM measurement, sample was spotted on carbon and formvar-coated copper grids for 2 min and dried in air. Then it was negatively stained with phosphotungstic acid for 2 min and air dried. The samples were examined using a H-7650 transmission electron microscope (Hatachi Japan) and was operated at 80 kV.

1.6 Circular Dichroism (CD) Spectrum Measurement.

CD spectral measurements were performed with PMS 450 spectropolarimeter (Biologic, France). Sample was put in a fused quartz cell with 0.1 mm path length. Data was expressed in terms of mean residue ellipticity [θ] (deg · cm^2/dmol) during measurement, and then it was calculated and reported for per mol of amide groups present by soft-ware Bio-Kine 32 V4.51.

1.7 Thermal Stability Determination.

The thermal stability of HPV L1, either in monomer or pentamer, was evaluated with a UV-cloud point-temperature ramping (CP-Temp) plot,[2] which was measured by using a UV-2450 spectrophotometer (SHIMADZU, Japan) equipped with a DC-0506 LOW-CONSTANTTEMP BATH temperature control system (HANGPING, China).

The CP-Temp determination was performed by observing the ultraviolet absorption signals as the optical density at 350 nm of the protein solution. All samples were measured at a scan rate of 0.8 °C/min with the scan temperature ranged from 30 to 70 °C to monitor the thermal denaturation and aggregation of HPV L1 in solution.

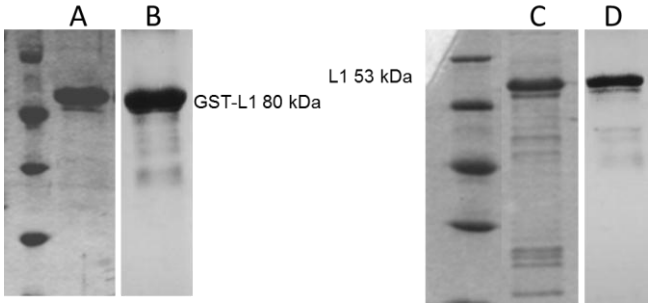

Fig. S1 SDS-PAGE (lane A, C) and Western-blot (lane B, D) of HPV 16 L1; lane (A, B) showed the proteins eluted from the resin before PPase digestion on GST; lane (C, D) showed the L1 proteins eluted from the GST column after PPase digestion on GST-L1.

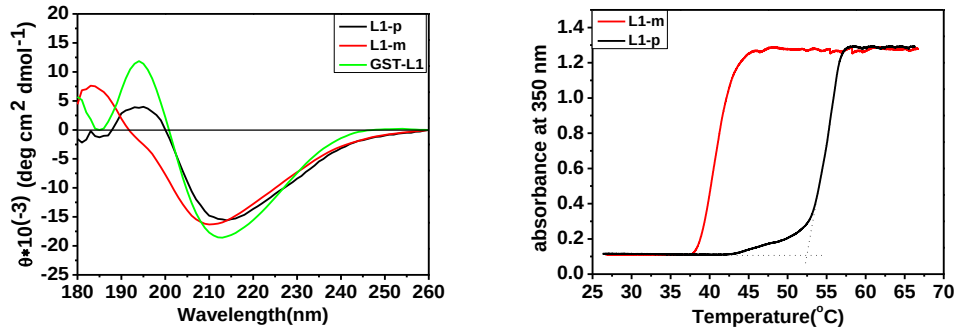

Fig. S2 CD spectra (left) and UV-cloud point-temperature ramping (CP-Temp) plot profiles (right) of HPV 16 L1-m and L1-p in 0.4 mg/ml, respectively. The CD spectra gave strong negative peak centered at around 210 nm, being typical for β-sheet dominance of HPV 16 L1. Further quantitative evaluation illustrated slight difference at the secondary structural proportion, for L1-p *vs* L1-m: α-helix (13.1% *vs* 16.0%), β-sheet (34.1% *vs* 27.3%), unordered structure (25.1% *vs* 33.8%) and β-turn (16.5% *vs* 22.5%). Further, the UV-cloud point with temperature ramping determination indicated that the L1-m was much less stable than the L1-p as the onset of its transition temperature (T_m=37.5 °C) was much lower than that of pentamer (T_m=52.5 °C).

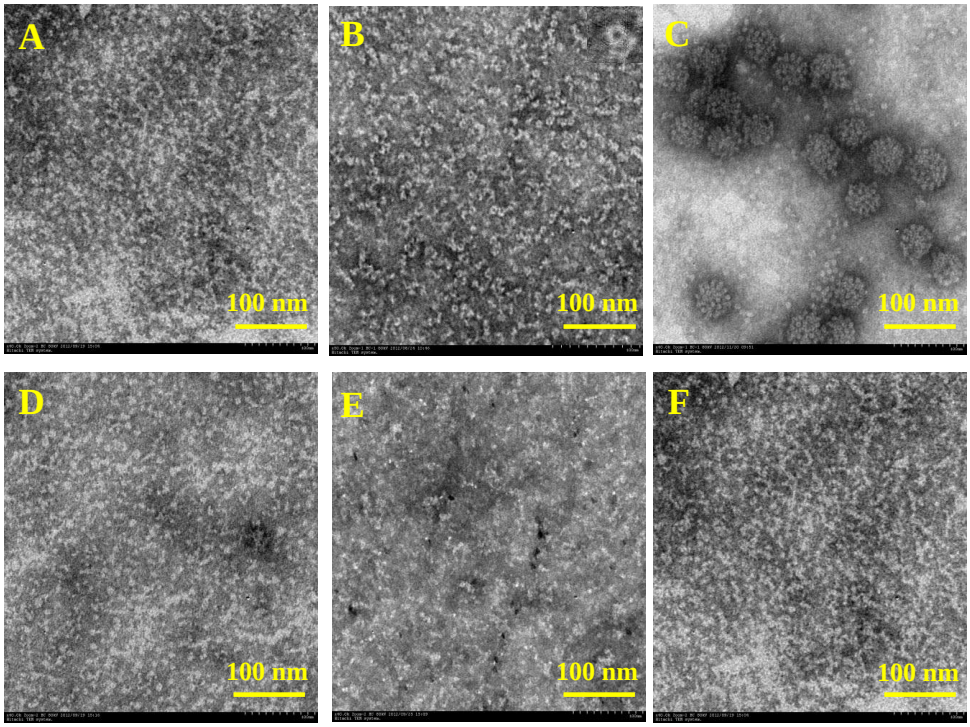

Fig. S3 Comparison of the electronic micrograms (EM) of HPV 16 L1 in monomer, L1-m; pentamer, L1-p; GST-L1 monomer and VLPs assembled from L1-p: (A) shows the purified GST-L1; (B) L1-p after removal GST and further purification by gel filtration chromatography. Typical "donuts" pentamer,[3] in five-pointed star and size of ~9 nm was observed there; (C) VLPs particles, the size of ~55 nm, assembled from the purified L1-p; (D) show the L1 monomers purified from gel filtration chromatography; (E) further assembly of (D) after incubated in assembly buffer solutions for 24h, no sign of VLP was observed; (F) further assembly of GST-L1 by dialyzed in assembly buffer solutions for 24h, either no sign of VLP was observed.

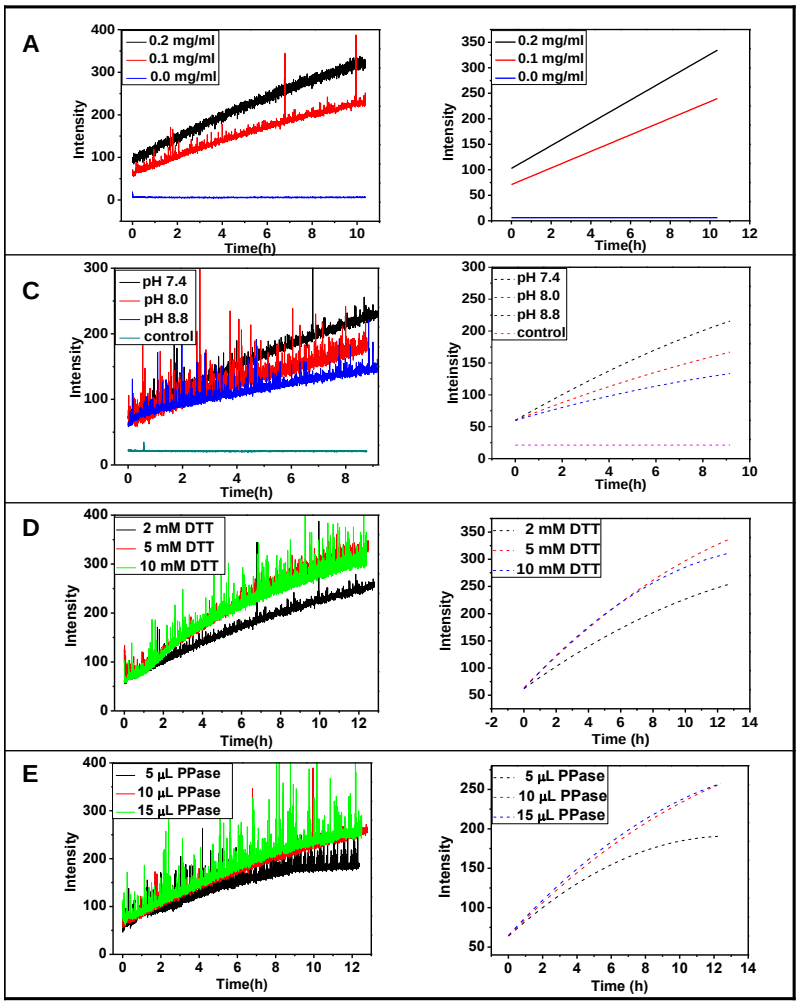

Fig. S4 Monitoring of the assembly of pentamer from GST-L1 monomer by time-dependent static light scattering plots (left) and the corresponding linear/curve-fitting plots (right), which were used for more clear comparison and mechanism consideration. The effect of the following parameters on the process was evaluated: (A) GST-L1 concentration; (C) Reaction pH; (D) The effect of DTT concentration; (E) The different amount of PPase (200 U / 100 μL), where large different increasing rate was observed when the unsaturated PPase (5 μL) was used.

References

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