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An efficient production and characterization of HIV-1 gp41 ectodomain with fusion peptide in *Escherichia coli* system

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ABSTRACT

We demonstrated a high level expression and purification of recombinant human immunodeficiency virus type 1 gp41 ectodomain (gp41e-FP) using glass bead approach with a final yield of 12 ± 2 mg/L bacterial culture. The proper folding of gp41e-FP encompassing the fusion peptide (FP) was ascertained by circular dichroism (CD) measurement and recognition by NC-1 antibody. The latter assay revealed stabilization of the gp41 coiled coil structure in the presence of liposome dispersion. The differential affinity of gp41e-FP and gp41e (devoid of FP) by NC-1 suggested an aggregated state for gp41e-FP and/or possible proximity of the fusion peptide domain to the coiled coil structure of gp41 ectodomain. Perfluorooctanoate (PFO)–PAGE electrophoresis experiment revealed the trimeric propensity of the recombinant gp41e-FP. In comparison to gp41e, the lipid mixing activity of gp41e-FP was two-fold higher suggesting a role of FP in promoting membrane fusion. The present approach to efficiently and quantitatively preparing the functional full-length recombinant gp41 ectodomain protein can be employed for structural and biomedical investigations and the extraction of other inclusion body-embedded recombinant proteins.

1. Introduction

To infect the human host, HIV-1 uses the envelope protein gp41 transmembrane subunit to mediate fusion with the target membrane following conformational change triggered by the surface subunit gp120 engagement to CD4-expressing cells. Because of its importance in the structural, functional, immunological and viral inhibitory studies (Chan et al., 1997; Caffrey et al., 1998; Weissenhorn et al., 1997), a reliable and quantitative protocol for gp41 protein production has been actively sought by the biomedical and pharmaceutical communities.

Previous preparations of gp41 constructs were featured by truncated gp41 proteins lacking FP (aa 1–23) in both eukaryotic and prokaryotic systems. Thus Weissenhorn et al. (1996) have expressed a soluble oligomeric gp4l ectodomain devoid of the fusion peptide domain in insect cells while other laboratories have elaborated via bacterial systems (Blacklow et al., 1995; Tan et al., 1997; Krell et al., 2004). With respect to the yield, the truncated gp41(24–157) by Krell and coworkers was 60 mg/L cell culture. The gp41(34–154) protein expressed by Tan et al. (1997) contained a shortened, flexible loop which, however, may modify the dynamic and antigenic properties of the protein.

Because the FP domain has been shown to interact extensively with the membrane (Nieva et al., 1994; Chang et al., 1997) and its structural influence on the gp41 core remains to be elucidated, incorporation of FP in the protein is necessary for the functional, anti-fusion inhibitor and immunogenic studies (Farzan et al., 1998). However, the FP sequence is highly hydrophobic, aggravating the difficulty in preparing membrane proteins due to their low solubility. Furthermore, toxicity to the expression host – likely resulting from the membrane-disrupting activity of FP – renders a large scale production of the gp41 ectodomain protein with the fusion peptide a challenging task (Yuan et al., 2004).

To circumvent this problem, Sackett and coworkers produced a gp41 protein (1–154) in which the FP region was chemically ligated to the recombinant truncated gp41 (Sackett et al., 2006, 2009). Expression of the soluble HIV gp140 consisting of gp120 and gp41 ectodomain in mammalian cells (Kirschner et al., 2006) has been attempted. Buzon et al. (2010) prepared a loop-truncated gp41

Abbreviations: HIV-1, human immunodeficiency virus; FP, fusion peptide; SHB, six-helix bundle; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DMPG, 1,2-dimyristoyl-*sn*-glycero-3-phosphoglycerol; DPPC, 1,2-dipalmitoyl-sn-glycero-3-phospho-L-choline; NBD-PE, N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-1,2-dihexadecanyol-*sn*-glycero-3-phosphoethanolamine; Rho-PE, Lissamine rhodamine B 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine; CD, circular dichroism; gp41e-FP, gp41 ectodomain encompassing the fusion peptide domain; mAb, monoclonal antibody; PFO, perfluorooctanoate; MBP, maltose binding protein; GST, glutathione S-transferase; ESI/LC/MS, electrospray ionization/liquid chromatography/mass; SPR, surface plasmon resonance.

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Table 1
Purification and extraction of gp41e-FP from GST fusion protein.

Protein	Treatment	Concentration	Recovery (%)
GST-gp41e-FP	Crude extraction[a,g]	19.1 ± 2.1 mg/g	100%
	IMAC[b,g]	15.8 ± 0.2 mg/g	82%
	Centrifugal concentration[c,g]	13.0 ± 1.7 mg/g	68%
	Dialysis[d,h]	12.8 ± 1.5 mg/g	67%
gp41e-FP	Prior to cleavage[e,h]	0.42 mg/mg	100%
	Thrombin cleavage[f,g]	0.32 ± 0.001 mg/mg	76%
	Centrifugal concentration[c,g]	0.26 ± 0.030 mg/mg	62%
	First dialysis[d,h]	0.25 ± 0.023 mg/mg	60%
	RP-HPLC (C4)[h]	0.10 ± 0.012 mg/mg	24%
	Lyophilization[i]	0.09 ± 0.011 mg/mg	22%
	Second dialysis[d,h]	0.09 ± 0.015 mg/mg	21%

[a] The amount of protein per gram of wet weight of bacteria from 1 L culture.
[b] Immobilized metal affinity chromatography.
[c] Using Amicon Ultra-15 filter (3000 MWCO).
[d] In sodium formate, pH 3, followed by ddH_2O.
[e] The mass of gp41e-FP out of 1 mg GST-gp41e-FP.
[f] Using 35 U for digesting 1 mg GST-gp41e-FP.
[g] Protein concentration was determined by densitometer from SDS–PAGE gel.
[h] Protein concentration was quantitatively determined by UV spectrophotometer.
[i] Protein concentration was quantitatively determined by analytical balance.

protein by assembly of the two core regions of gp41 (aa 11–70) and (aa 117–172). Alternatively, to boost the yield and to reduce the arduous purification steps (Hendrickson, 1996), expression in *E. coli* system was adopted. Previous procedures involved conjugating a tag protein such as GST (GST-gp41 aa 1–172; Penn-Nicholson et al., 2008) or MBP (Lay et al., 2004) to the N-terminus of gp41 to generate a chimeric gp41 protein to avert possible interference of the fusion peptide with the host and increase the solubility in the expression host. Yet it was difficult to remove the GST tag from GST-gp41 as a result of low solubility of the chimeric gp41 protein in the enzymatic reaction buffer. For the tag-free, full-length version, Lev et al. (2009) reported the expression of gp41 ectodomain (1–172) encompassing the FP domain and the pre-transmembrane sequence involved in neutralization and fusion pore expansion (Lorizate et al., 2006), as well as its shorter fragments (35–172 and 27–154). But the yield of the full-length construct was not detailed.

We have reported that a recombinant cysteine-free gp41 ectodomain (24–154, designated gp41e), in which the two cysteine residues (C87 and C93) were replaced by serine, had similar secondary and tertiary structures to its wild-type protein with a yield of 23 ± 3 mg/L LB culture (Lin et al., 2008). In the present study, we demonstrated a high yield protocol for the expression and purification of cysteine-free HIV-1 gp41 ectodomain (aa 1–154) with the fusion peptide in the *Escherichia coli* system, gp41e-FP. The protein can refold to a soluble form in aqueous environment. The present study also afforded a protocol to prepare membrane proteins in an expedient and quantitative manner.

2. Materials and methods

2.1. Expression construct

The expression constructs were prepared by a standard PCR cloning strategy and fully sequenced. The gp41 ectodomain coding sequences were amplified by PCR from plasmid pSVE7′-puro and subcloned into the *Bam*HI/*Eco*RI sites of pGEX-4T plasmid (GE Healthcare, UK). This amplified DNA product encodes 162-amino acid ectodomain that consists of 1st to 154th aa of gp41 from the HIV-1 HXB2 strain and eight histidines at the carboxyl terminus (Fig. S1) for purification.

2.2. Protein expression and immobilized-metal affinity chromatography (IMAC) purification

The gp41e protein was produced as previously described (Lin et al., 2008). For the gp41e-FP protein, pGEX-4T-gp41e-FP transformed *Escherichia coli* (*E. coli*) BL21 host was cultured in 1 L broth (LB with 100 mg/mL ampicillin). The protein expression was inducted by adding 1 mM isopropyl-D-1-thiogalactopyranoside (IPTG) to the BL21 host culture for 4.5 h. The cells were harvested by centrifugation at 10,000 × *g*, 4 °C for 20 min. The pellet was resuspended in 5 mL BugBuster protein extraction reagent (Novagen, Germany) per gram wet cell pellet, subsequently 1 μL (25 U) benzonase (Novagen, Germany) per mL of BugBuster was added and incubated on a shaking platform at room temperature for 20 min. One mg lysozyme (Sigma, St. Louis, MO, USA) per gram cell pellet was then added to the suspension and then shaken at room temperature for 20 min. The recombinant gp41e-FP was accumulated in the inclusion body and collected by centrifugation at 16,000 × *g*, 4 °C for 20 min. To reduce the loss of the protein, the pellet was resuspend in the binding buffer (20 mM Tris/HCl, 200 mM NaCl, 5 mM imidazole; pH 7.5, 6 M guanidine–HCl) with some glass beads and shaken at room temperature for 12 h. Strikingly, the yield was increased by 6-fold with the glass bead treatment to facilitate solubilization of the inclusion body pellet.

After centrifugation at 16,000 × *g*, 4 °C for 20 min, the supernatant containing dissolved recombinant ectodomain protein was purified under denaturing condition by 6 M guanidine–HCl with IMAC, followed by dialysis in acidic urea gradient solution from 8 M to 0 M with 50 mM sodium formate (pH 3), and finally equilibrated in deionized and distilled (dd) H_2O.

2.3. Thrombin cleavage

One mg GST-gp41e-FP chimera was digested by 35 U thrombin protease (Sigma, St. Louis, MO, USA). The lyophilized thrombin powder was dissolved and washed with pre-chilled 20 mM Tris–HCl, pH 8.8 and mixed with the protein. The reaction buffer was subsequently adjusted to pH 8.8 with 20 mM Tris–HCl in final volume of 1 mL. The enzymatic reaction solution was incubated at RT for 5 days with slow rotation. After the thrombin cleavage, the reaction solution was dialyzed with acidic urea gradient solution