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Unusual Features of Human Immunodeficiency Virus Type-1 Virion

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=국문초록=

면역결핍 바이러스 입자의 비특이적 성질

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본 연구는 인간면역결핍바이러스의 입자를 비이온성 계면활성제로 처리할 때 바이러스 입자구조에서 분리되어 방출되는 바이러스 구조단백질들의 분포를 sucrose gradient로 분석하여, 바이러스 입자를 구성하는 바이러스 구조단백질과 바이러스입자의 생물리학적 특성을 연구하였다.

바이러스입자들을 0.16% NP40 (Nonidet P-40)으로 처리할 때, 바이러스 capsid 단백질과 바이러스 막 단백질 (membrance protein)들은 다른 바이러스 구성성분들과 잘 분리되었다. 계면활성제처리에서 방출되지 않은 구성성분들은 matrix 단백질, nucleocapsid 단백질, reverse transcriptase, integrase 및 바이러스 RNA genome로써, 이들은 subviral 구조를 형성한다. 이러한 결과는 상대적으로 다른 바이러스들의 capsid 단백질과 면역결핍바이러스의 capsid 단백질 (p24)를 비교할 때, 면역결핍바이러스의 capsid 단백질은 바이러스핵을 형성할 때, capsid 단백질 사이의 결합력이 매우 약한 것으로 추정된다. 또한 바이러스 조절단백질의 하나인 vpr 단백질을 함유하는 바이러스입자를 NP40 처리하여 분석하였을 때, vpr 단백질은 subviral 구조에 존재하는 것으로 나타났다.

Key Words: Human immunodeficiency virus, Virion, Sucrose gradient, Vpr protein.

INTRODUCTION

Human immunodeficiency virus (HIV), a member of the lentivirus group of the retrovirus family, share many morphological and biological features with other retroviruses [1,2]. The mature HIV-1 virion consists of an outer lipid membrane and an condensed nucleoprotein core. The lipid envelope, derived from a host cell membrane, contains two viral glycoproteins (gp41 and gp120) as well as host cell-derived proteins [3]. The transmembrane glycoprotein, gp41, serves as anchorage for the surface glycoprotein, gp120, which binds to CD4 receptor on the target cells [4,5]. The nucleopotein core was known to have two copies of the viral RNA genome in association with the major structural components of the core, the nucleocapsid (NC) and capsid proteins [3,6,7]. Within the nucleoprotein core, the virion RNA and nucleoproteins are believed to be tightly associated by virtue of the affinity of the nucleoprotein for nucleic acids. The term "nucleocapsid" has been used to describe the subviral structure (the packaged form of the genome) consisting of viral RNA and nucleocapsid proteins [8]. The capsid proteins,

which have no direct affinity for nucleic acids, form a shell surrounding the nucleocap-sid. The word "core" has been used to define the subviral structure composed of the nucleocapsid surrounded by the capsid protein shell [8]. Many electron microscopic studies showed that p24 constructs cone-like core wrapping RNA and NC proteins in the HIV-1 virion [3,9-11]. Recently the HIV-1 core was reported to be a bilaterial, elongated structure having 100 nm in length with a 40-60 nm free end and a 20 nm narrow end. The narrow end of the core is attached to the envelope with a core-envelope link [12]. In addition to the viral RNA and structural proteins, the viral core contains the enzymatic functions required for the conversion of the viral RNA to DNA (reverse transcriptase/ribonuclease H) and the integration of newly synthesized DNA into the host chromosome (the integrase). The precise location and organization of these functions in the viral core is not known in any detail. However, since they are required for the conversion of the viral RNA to DNA, these enzymatic functions are presumably located in the nucleocapsid in association with the viral RNA. The matrix proteins (p17) was suggested to reside at the inner leaflet of the lipid membrane and to have a role in scaffolding the envelope structure during morphogenesis as well as the mature virion [3,13].

Most of the current knowledge known about the HIV-1 virion and core structures have been made by transmission electron microscopic studies in infected cells [3,10,12,14], which may not reflect exact bichemical and biophysical profiles of the virion or core. According to Gottlinger et al. [15], p24-p17 cleavage defective mutant virion was shown to have an eccentrically placed condense core which was circular in all cross-sectional views in electron micrographs. p24-p17 fusion protein was stretched out to the envelope in the mutant virion, resulting that the envelope shell was thicker than the wild type envelope. Therefore, it seemed that the circular core was the nucleocapsid composed of RNA and NC proteins. On the other hand, although the HIV-1 virion, like other enveloped animal viruses, has been known to be sensitive to the detergent treatment, little is known about structural changes caused by detergent. Recently, p 24 was observed to be easily removed from the HIV-1 virion by treatment of the HIV-1 virion with ionic and nonionic detergents but other structural proteins remained in the subviral structures [16].

In this study the fine structural changes of the HIV-1 virions caused by detergent were described to understand structures of the HIV-1 core and virion in biochemical and biophysical aspects. The detergent treatment of the HIV-1 virion caused complete loss of p24 and the membrane glycoproteins from the virion structure, but p17 and NC proteins remained with reverse transcriptase (RT, p66 and p51), integrase (IN) in the subviral structure. In addition, the considerable amounts of the vpr product remained in the detergent core when vpr^+-virions were treated with detergent, indicating that the vpr proteins are present in the HIV-1 core.

MATERIALS AND METHODS

Preparation of chronically HIV-1 producing T-cell line

In order to obtain large amounts of metabolically radiolabeled virions, chronically HIV-1 producing T-cell line was prepared as described by Farnet and Haseltine [17]. Molt3 cells, grown in RPMI 1640 media supplemented with 10 % fetal calf serum (Gibco Laboratories), were washed with serum-free media (SFM) 2 times. 1 ml of the cell solution in SFM (10^7 cell/ml) was transfected with HIV-1 proviral DNA of 10 ug, by using DEAE-dextran method [18]. Supernatants of the tansfected cells were collected every 3 day to measure virus production. Virus production was mon-

itored by measuring reverse transcriptase activity derived from virus particle in the culture supernatants as described elsewhere [19]. Cell line showing continuously high production of virus for more than one month was regarded as chronically HIV-1 producing cell line, which was used for preparation of large quantity of virus.

Cell culture and Metabolic labeling

Chronically HIV-1 producing Molt3 cells were cultured for 14 hr in cysteine-free RPMI 1640 medium containing 10 % FCS and phorbol 12-myristate 13 acetate (10 ng/ml) in the presence of 100 uCi of [^{35}S] cysteine per ml. The clarified culture supernatant was obtained by 10 min centrifugation at 17,000g. The pure virions were prepared from the clarified supernatant by pelleting through a layer of 20 % sucrose in 10 mM Tris (pH 8.0) and 1 mM EDTA for 90 min at 100,000g. The virions were resuspended in indicated buffer and stored at 4℃ until use.

To determine location of the vpr protein in virion, the detergent-treated vpr$^+$-virion were analyzed in sucrose gradient. 107 Jurkat cells were transfected with 10ug of proviral DNA (HXBRU$^+$, kindly provided by Dr. E. Terwiliger, Harvard Mdical School) by modified DEAE-dextran method [20] and labeled with [^{35}S] cysteine and [^{35}S] methionine (50 uCi/ml for each) for 14 hr at 15th day of transfection.

Detergent treatment and fractionation in sucrose gradient

Virions were treated with Nondiet P-40 (NP 40, Sigma Co.) at indicated concentrations for 10 min at room temperature. The disrupted virion suspension was layered onto a 4-ml linear sucrose gradient (15 to 60 % (w/v) in indicated buffer) and centrifuged at 45,000 rpm for 3 hr. 10 to 12 fractions of about 330 ul were collected from the bottom of the gradient. The distribution of viral proteins in each fraction was analyzed on the 10 to 20 % gradient gels of sodium dodecyl sulfate polyacrylamide (SDS-PAGE) [21]. Autoradiographs of gels were made as described previously [19].

Dot blot hybridization

Presence of viral RNA genome in each fraction was analyzed using dot blot hybridization. A complementary oligonucleotide for the region of sequence #4284-4303 in HXBc2 geneomic DNA (5'-TCACTAGCCATTGCTCTCCA-3') was synthesized as DNA probe. The oligonucleotide of 100 pmol was labeled with [γ-^{32}P] ATP of 250 uCi and bacteriophage T4 polynucleotide (T4 PNK) kinase of 10 unit for 15 min at 37℃ in 0.05 M TrisCl (pH 7.6), 0.01 M $MgCl_2$, 5 mM dithiothreitol, 1 mM spermidine and 1 mM EDTA. Unincorporated nucleotides were removed by Sephadex G25. For hybridization, 100 ul of each fraction was applied to the nitrocellose membrane (Schleicher & Schuell, BA85) and probed for viral RNA with the labeled oligonucleotide.

Immunoprecipitation

In order to identify the viral proteins fractionated in sucrose gradient, specific viral proteins were immunoprecipated from viral lysates or thier fractions using specific antisera. For immunoprecipitation, 200 ul of the fractions was centrifuged for 30 min at 16000g to eliminate any insoluble protein aggregates. Clear supernatant of 200 ul was mixed with 50 ul of 10X RIPA (1.4 M NaCl, 80 mM Na_2HPO_4, 20 mM NaH_2PO_4, 10 % Nonidet P40, 5 % sodium deoxycholate and 0.5 % SDS) and 250 ul of H_2O. 3-10 ul of specific antisera were added to each reaction. Except antiserum to HIV-1 integrase or to the vpr protein, the specific antisera were obtained through the AIDS Research and Reference program, Division of AIDS, National Institute of Allergy and Infectious Diseases. Antiserum to HIV-1 integrase was kindly donated by Dr. C. Farnet (Harvard Medical School, USA), and antiserum to the vpr protein was obtained from

Drs. Eric Cohen and Claude Lavallee (University of Montreal, Canada). The mixture was incubated 4℃ overnight on a rocking platform. Separately, Next morning, 100 ul of 10 % protein A-Sepharose 4B (Pharmacia Co.) was mixed with equal volume of 10 % BSA and rocked 4℃ for 1 hr for pretreatment of protein A-Sepharose 4B to block nonspecific binding. By brief centrifugation, protein A-Sepharose 4B pellet was collected. Mixture of the viral lysate and antisera was added to protein A-Sepharose 4B pellet and incubated 1 hr at 4℃. The Sepharose 4B pellet was precipitated and the supernatant was discarded. The Sepharose 4B pellet was washed with 1 ml of RIPA-buffer (140 mM NaCl, 8 mM Na_2HPO_4, 2 mM NaH_2PD_4, 1 % NP40, and 0.05 % SDS) 5 times. Finally, the Sepharose 4B pellet was washed with 1 ml of 140 mM NaCl, 8 mM Na_2HPO_4 and 2 mM NaH_2PO_4. 50 ul of 2X SDS gel loading buffer (120 mM Tris-HCl, pH 6.8, 4 % SDS, 20 % glycerol, 10 % 2-mercaptoethanol and 0.02 % bromophenol blue) was added to the final Sepharose 4B pellet. The sample was boiled for 5 min and centrifuged briefly. About 40 ul of the sample was loaded onto SDS-PAGE.

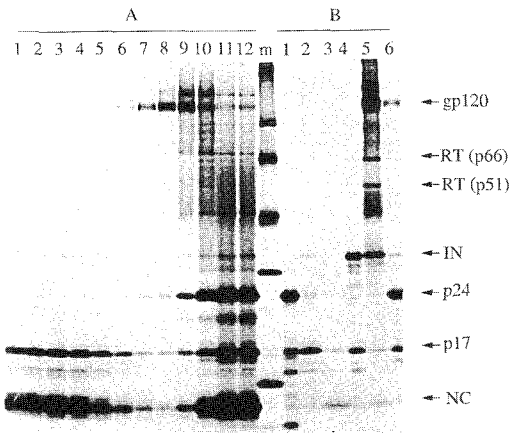

Figure 1. SDS-PAGE of sucrose gradient fractions of 0.16% NP40-treated HIV-1 virion. **A,** [^{35}S] cysteine-labeled HIV-1 virions were treated with 0.16% NP40 for 10 min at room temperature and analyzed in a 15 to 60 (w/v) % sucrose gradient. Twelve fractions were collected and 30 ul at each fraction was analyzed in a 10 to 20% gradient gel of SDS-PAGE. **B,** Spectic viral proteins were immunoprecipitated from the virion lysates, by using specific antisera; rabbit antiserum to HIV-1 p24 (lane 1), sheep antiserum to HIV-1 p17 (lane 2), sheep antiserum to HIV-1 p15 (lane 3), rabbit antiserum to HIV-1 integrase (lane 4), human polyclonal immunoglobulin G to HIV-1 reverse transcriptase (lane 5), an AIDS patient serum (lane 6). m, molecular marker; lysozyme (MW 14,300), carbonic anhydrase (MW 30,000), ovalbumin (MW 46,000), bovine serum albumin (MW 69,000), phosphorylase b (MW 92,500) myosin (MW 200,000).

RESULTS AND DISCUSSIONS

When intact virions were run on a 15 to 60 % sucrose gradient, virions were sedimented in the middle of the gradient as described by Cohen et al. [22], while all the viral proteins in the lysates prepared by 15 min boiling of virion in 2 % SDS were localized at the top of gradient (Data not shown).

When the virion suspension treated with 0.16 % NP40 was run on a sucrose gradient, most of p24 were localized at the top of the gradient, indicating that p24 is easily released from the virion (Fig. 1A, lanes 10-12). This observation is consistent with that of Bukrinskaya and Sharova [16]. Complete loss of p24 from virion was observed in wide ranges of NP 40 concentration from 0.01 to 10 % (Data not shown). The easy dissociation of p24 may explain abundance of free p24 in the blood of AIDS patients. In addition, the easy dissociation of p24 makes it difficult to isolate intact HIV-1 core, while cores of other retroviruses such as equine infecious anemia virus (EIAV) [23], avian myelobastosis virus [24], and murine leukemia virus [24] were well isolated and characterized because their capsid proteins are tightly assembled enough to endure disssociation caused by detergent.

Most of the envelope proteins were present in the top of the gradient (Fig. 1A, lanes 8-12), indicating that the detergent can efficiently remove the HIV-1 envelope and the envelope

proteins from the virion. The detergent treatment of EIAV [23] was shown to remove the lipid envelope and membrane glycoproteins efficiently from the EIAV virions.

Other viral proteins were sedimented in lower part of the gradient (Fig. 1A, lanes 2-4). They were abundant NC protein, matrix protein (p17), IN, RT and trace amount of gp120. p17 and NC proteins were very resistant to dissociation caused by the detergent, resulting that most of those proteins remained in the subviral structure which was designated as detergent core to distinguish from conventional viral cores which are composed of capsid protein, NC protein, viral enzymes and viral genome. Among the proteins remained in the detergent core, presence of p17 was unexpected because p17 has been known to locate between envelope and core. Therefore, p17 was expected to be disappeared in the detergent core when the membrane glycoproteins (gp120) and capsid proteins (p24) were removed from the virion structure. Therefore, it is likely that there are two possibilities for the presence of p17 in the detergent core. First, p 17 may be nonspecifically reassociated with viral RNA present in the detergent core. p17 was suggested to bind to complexs containing viral RNA in cell nucleus [25]. Therefore, it is possible that free p17 released from the detergent-treated virions binds nonspecifically to the RNA complexes. But this possibility can be ruled out by the facts that treatment of the virions with up to 10 % NP40 showed same results (Data not shown) and that much less p17 was observed in the detergent core derived from vpr+-virion treated with detergent (Fig. 3). Second, p17 may form the crosslinking network from the envelope glycoproteins to the complexes containing RNA and nucleocapsids through cone-like core constructed with p24. Under this possibility, p17 can remain with nucleocapsid proteins associated with genomic RNAs in the detergent-disrupted virions even though p24 is completely disappeared. Also, this possibility is well corresponding with recent observations of interactions between p17 and the viral envelope glycoproteins during viral assembly [26] and between p17 and complexes containing RNA in cell nucleus [25]. There was an unidentified band between p17 and NC in the detergent core. It is believed that it was proteolytic cleavage product of gag proteins as immunoprecipitated with antisera against HIV-1 p24 or p17 (Fig. 1B, lanes 1 and 2).

To determine identities of the viral proteins fractionated in sucrose gradient, lysates of labeled virions were separately prepared. Viral proteins were immunoprecipitated with specific antisera [20] and analyzed on the same gel (Fig. 1B). Immunoprecipitation of the specific viral proteins shown in Figure 1B supported that major proteins present in the detergent core are p17, NC, IN, and RT.

To test viral genomic RNA in the virions treated with detergent, unlabeled HIV-1 virions were prepared, disrupted, and fractionated in sucrose gradient as described above. Fractions were tested for viral RNA, by using radiolabeled complementary oligonucleotide [18]. Dot blot analysis showed that majority of viral RNA was localized in lower part of the gradient, indicating that the detergent core has viral RNA (Fig. 2).

In addition to gag, pol and env genes, HIV-1 has tat, vif, vpu, nef and vpr of which proteins are known to modulate viral replication. Among 6 regulatory proteins, only the vpr protein was found in HIV-1 virion [22]. To find position of the vpr protein in virion, the radiolabeled vpr+-virions were prepared as described in Materials and Methods. The virions were disrupted with 0.16 % NP40, and analyz-

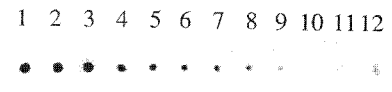

Figure 2. Dot-blot analysis for HIV-1 RNA in sucrose gradient fractions.

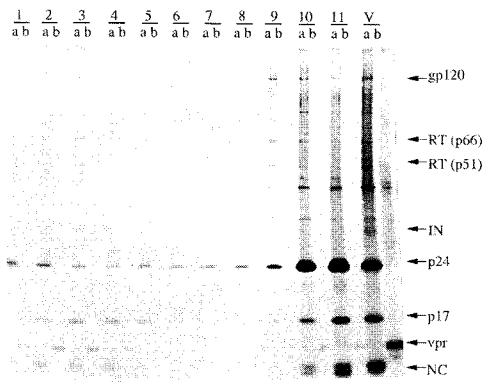

Figure 3. Detection of the vpr protein in HIV-1 core.10^7 Jurkat cells were transfected with 10 ug of proviral DNA (HXBRU$^+$) and labeled with [^{35}S] cysteine and [^{35}S] methionine for 14 hr at 15th day of transfection. The virions were treated with 0.16% NP40, and analyzed in a 15 to 60% sucrose gradient as described in Fig. 1.
1-11, 20 ul of each fraction was derectly analyzed in a 10 to 20% gradient gel of SDS-PAGE (a). The vpr proteins were immunoprecipitated with antisera againt HIV-1 vpr from 200 ul of each fraction (b).V, vpr$^+$-virions were directly analyzed in SDS-PAGE (a). The vpr proteins were immunoprecipitated from the virion lysate (b).

ed in sucrose gradient as described above. Eleven fractions were collected. 20 ul of each fraction was directly analyzed in SDS-PAGE (Fig. 3, lanes 1a-11a). The vpr proteins were immunoprecipitated with antiserum against HIV-1 vpr (a generous gift of Dr. E Cohen, University of Montreal, Canada) from 200 ul of each fraction (Fig. 3, lanes 1b-11b). Considerable amounts of the vpr protein were immunoprecipitated in lower part of the gradient, suggesting that the vpr proteins are present in the HIV-1 core, not in an outer envelope. Also, some p24 retained in the dertergent core, but significant amounts of p17 and NC proteins were relatively released from the detergent core, compared to figure 1. Therefore, it is likely that the presence of the vpr protein in virion affects the stability of core structure in unknown mechanisms.

Although the vpr protein is not essential for viral replication, the vpr protein was shown to play important roles in viral replication by modulating viral expression [27]. The vpr protein has transactivation activity to increase expression of viral proteins and also stimulate expression of heterologous genes driven by the HIV-1 long terminal repeat as well as other promoters [27]. To understand exact roles of vpr in viral infection and replication, further studies are required. To determine location of the vpr protein in virion may provide crucial clues in understanding its role in viral infection and replication.

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