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MAS NMR of HIV-1 protein assemblies

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ABSTRACT

The negative global impact of the AIDS pandemic is well known. In this perspective article, the utility of magic angle spinning (MAS) NMR spectroscopy to answer pressing questions related to the structure and dynamics of HIV-1 protein assemblies is examined. In recent years, MAS NMR has undergone major technological developments enabling studies of large viral assemblies. We discuss some of these evolving methods and technologies and provide a perspective on the current state of MAS NMR as applied to the investigations into structure and dynamics of HIV-1 assemblies of CA capsid protein and of Gag maturation intermediates.

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1. Introduction

Since the discovery of the human immunodeficiency virus (HIV) as the causative agent for acquired immunodeficiency syndrome (AIDS) in 1983 [1,2] three decades of intense research have followed. Current estimates reveal that 35 million people are living with HIV, a global prevalence rate of 0.8%. In some Sub-Saharan African nations, the HIV prevalence rate exceeds 20% [3]. Current treatment options for those infected with HIV are based on antiretroviral therapies from several different classes of drugs. While effective, they are often limiting due to prohibitive cost, debilitating short- and long-term side effects, and the ability to give rise to drug-resistant mutants (www.AIDS.gov). A better understanding of important atomic-level structural information of the various macromolecules and macromolecular assemblies comprising HIV and the molecular processes that govern the virus' function could lead to enhanced therapies and hopefully an eventual cure.

The three major genes comprising the HIV genome, gag, pol, and env, encode the main structural proteins (synthesized as polyproteins) and enzymes [4]. Six additional genes, tat, rev, nef, vpr, vif, and vpu, encode regulatory proteins (Tat and Rev) and auxillary proteins (Nef, Vpr, Vif, and Vpu). The HIV's gag gene encodes the precursor Gag polyprotein, which contains the major structural proteins of the virus (Fig. 1). These include matrix (MA), capsid (CA), spacer peptide 1 (SP1), nucleocapsid (NC), spacer peptide 2 (SP2), and P6 [5]. Immature, noninfectious viruses contain intact, uncleaved Gag polyprotein, see Fig. 1. Proteolytic cleavage of the Gag polyprotein into its constituent protein domains results in an infectious virion through a process termed viral maturation [6]. Upon maturation, a characteristic capsid core is formed which encloses the viral RNA as well as complementary viral proteins, as shown in Fig. 1 [7]. This perspective article discusses the recent structural and dynamics characterization of HIV-1 CA capsid protein assemblies, by magic angle spinning (MAS) NMR spectroscopy.

The molecular structure of mature HIV-1 capsid has been extensively studied at different levels of resolution using various methods, including X-ray crystallography [8–10], cryo-electron microscopy [7,11–13], solution NMR spectroscopy [14,15] and, most recently, MAS NMR spectroscopy [16–19]. The HIV-1 capsid is assembled from 1200 copies of the 25.6 kDa CA protein, a cleavage product of the Gag polyprotein [7]. The capsid encloses two copies of viral RNA and a small complement of proteins for replication [20]. On the basis of the early cryo-EM and electron cryo-crystallography studies, an idealized "fullerene cone" model [11,21] was developed to describe the structural organization of the mature capsid. In this model, 250 hexameric CA subunits are

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organized in a hexagonal lattice, with 12 pentameric CA subunits incorporated to close up the structure [7,11,21]. This model incorporated atomic-resolution NMR and X-ray structures of the individual N- and C-terminal CA domains (NTD and CTD, respectively), which were docked into the electron densities [8,9,14,22–24]. Subsequently, high-resolution structures of capsid subunits were obtained by examining disulfide-stabilized CA hexamers and pentamers using X-ray crystallography, yielding more detailed structural information for the CA model [10,13]. Most recently, a pseudoatomic capsid structure has been solved using a hybrid approach combining cryo-EM, solution NMR spectroscopy, and molecular dynamics simulations, as illustrated in Fig. 2 [7].

MAS NMR spectroscopy is an emerging technique for structural investigations of HIV protein assemblies. It is complementary to other structural biology techniques in that it is well suited for both structural and dynamic analysis of native-like protein assemblies, and possibly intact viral particles in the future, at atomic resolution. In contrast, solution NMR studies are limited to solubilized monomers or dimers of CA and other Gag maturation intermediates [25–28]. Higher order oligomers, such as cross-linked hexamers and pentamers (as well as smaller constructs) can be characterized with X-ray crystallography but the native-like assemblies are not amenable to X-ray analysis as they do not crystallize [9,29,30]. Cryo-electron microscopy (cryo-EM) is currently limited in resolution (~5 Å), even though recent advances in instrumentation and data acquisition and analysis may push the resolution limits [31]. Furthermore, both X-ray crystallographic and cryo-EM microscopic structural analyses typically require non-physiological cryogenic temperatures, nor do they provide direct insights into internal dynamics of the HIV-1 protein assemblies, which have been found to be important for their biological function [7,17,25]. In contrast, MAS NMR experiments can be conducted at physiological or close to physiological temperatures [16–19,25]. Using MAS NMR, many important features of CA and other HIV-1 proteins can be understood at atomic resolution, including the structure of the CA molecule in the assembled state, the inter-hexamer CTD–CTD contacts as well hexamer-forming NTD–NTD and CTD–NTD contacts. Tubular CA assemblies in particular appear to exhibit the same hexameric lattice and to mimic the curvature and intermolecular contacts as the native HIV-1 capsid core. Furthermore, MAS NMR experiments yield detailed insights into conformational dynamics on multiple timescales in HIV-1 protein assemblies, as we and others have demonstrated [17–19,25]. Finally, interactions of HIV-1 protein assemblies with small molecules can be readily probed by MAS NMR, as has been demonstrated in other protein assemblies [32,33].

Below, we discuss the recent methodological advances that have enabled atomic-resolution analysis of viral assemblies and provide a perspective on the current body of MAS NMR investigations into assemblies of HIV-1 CA protein and Gag maturation intermediate CA-SP1.

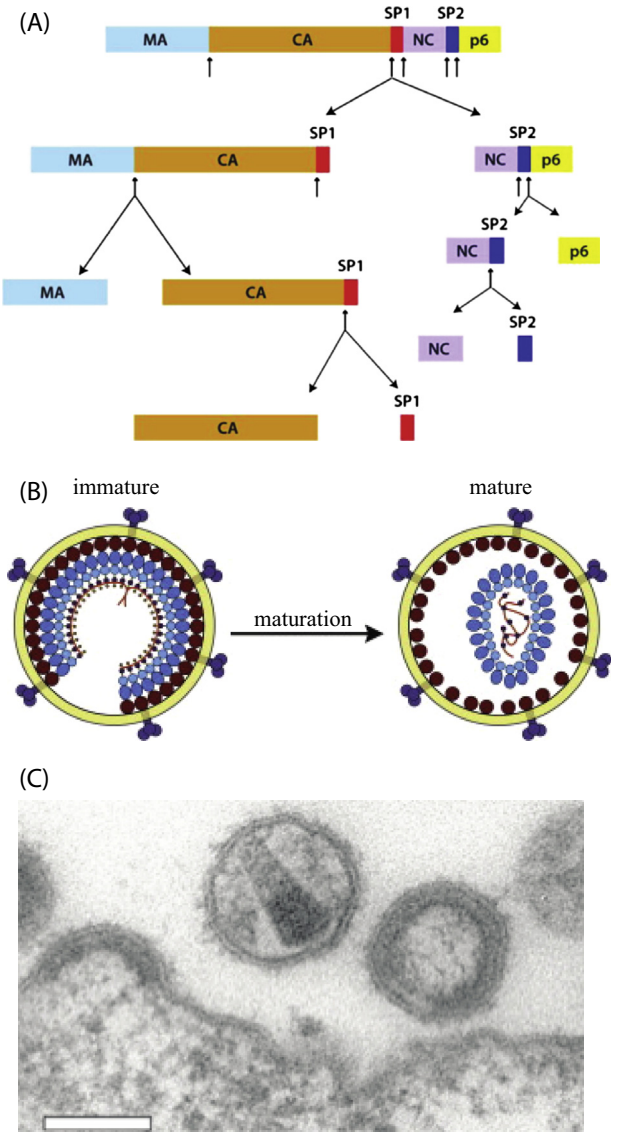

Fig. 1. (A) Schematics of Gag polyprotein domain organization, including the cleavage pathway leading to viral maturation. Reprinted with permission from Han et al., *J. Am. Chem. Soc.*, **2013**, 135, 17793–17803 [17]. Copyright 2013 American Chemical Society. (B) Artistic representation of structural differences between immature and mature HIV-1 virions. Reprinted with permission from Monroe et al., *Structure*, **2010**, 18, 1483–1491 [111]. Copyright 2010 Cell Press. (C) Electron micrograph image of HIV-1 virions. Both immature and mature virions are visualized here. Reprinted from open access Baumgartel et al., *Viruses*, 4, **2012**, 777–779 [112].

2. Sample preparation and MAS NMR methods for structural analysis of HIV-1 protein assemblies

2.1. Preparation of HIV-1 protein assemblies for MAS NMR

CA and CA-SP1 assembly conditions compatible with MAS NMR have been developed by us and by others [16–19,25]. These conditions yield morphologically homogeneous assemblies of desired morphologies that exhibit high-resolution NMR spectra and are stable under magic angle spinning for extended periods of time [16–19]. Notably, the preparation of morphologically homogeneous assemblies typically requires high concentrations of NaCl, of the order of 1–2 M in the sample [17]. As we have demonstrated, the use of EFree or low-E probes enables multidimensional MAS NMR spectroscopy in these samples, with high-quality data readily attainable [17]. The typical ^{13}C line widths in the HIV-1 CA and CA-SP1 assemblies of tubular morphologies are 40–100 Hz at the magnetic field strengths of 20.0–21.1 T, in fully protonated samples acquired at MAS frequencies of 14–20 kHz. It is important to corroborate the morphology of the samples prepared for the NMR experiments, and transmission electron microscopy (TEM) is used by us and by others for this purpose [16–19,25].

It is interesting to note that there is some variability in the morphology of tubular assemblies of CA and the CA-SP1 maturation intermediate, depending on the primary sequence and the preparation method. Tubular assemblies of CA and CA-SP1 have the same overall morphology, with diameters ~40–50 nm [17]. In another study, dark field transmission (tilted-beam) TEM has been used

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