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Interaction between the C-terminal domains of N and P proteins of measles virus investigated by NMR

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ABSTRACT

In this paper we investigate the interaction between the C-terminal domains of the measles virus phosphoprotein (XD) and nucleoprotein (N_{TAIL}) by using nuclear magnetic resonance chemical shift perturbation experiments. Using both N_{TAIL} constructs and peptides, we show that contrary to the conserved Box2 region ($N^{489–506}$), the C-terminal region of N_{TAIL} ($N^{513–525}$) does not directly interact with XD, and yet affects binding to XD. We tentatively propose a model where the C-terminus of N_{TAIL} would stabilize the N_{TAIL}–XD complex either via a functional coupling with $N^{489–506}$ or by reducing the entropic penalty associated to the binding-coupled-to-folding process.

Structured summary:
MINT-7009780, MINT-7009793, MINT-7009808: *N-tail* (uniprotkb:Q89933) and *P* (uniprotkb:P03422) *bind* (MI:0407) by *nuclear magnetic resonance* (MI:0077)

1. Introduction

Measles virus (MV) possesses a non-segmented, negative-sense, single-stranded RNA genome that is encapsidated by the viral nucleoprotein (N) within a helical nucleocapsid. This latter is used as the substrate for transcription and replication by the viral polymerase, which consists of a complex between the large protein (L) and the phosphoprotein (P).

The P protein is an essential subunit of the viral polymerase complex as it tethers the L protein onto the nucleocapsid template (Fig. 1A). P is a modular protein consisting of an intrinsically unstructured N-terminal region (PNT) [1,2], and of a C-terminal region (PCT) containing alternating disordered and structured regions [2] (Fig. 1B). In particular, it possesses a coiled-coil domain referred to as PMD (P multimerization domain) responsible for both oligomerization and binding to L [3], and a C-terminal globular region ($P^{459–507}$), referred to as X domain (XD), that constitutes the nucleocapsid-binding domain (for reviews see [4–6]). We have previously reported the crystal structure of XD and shown that it consists of an anti-parallel three-helix bundle [7].

The MV N protein consists of a globular N-terminal moiety, N_{CORE} ($N^{1–400}$), which contains all the regions necessary for self-assembly and RNA binding [8], and a C-terminal domain, N_{TAIL} ($N^{401–525}$) that is intrinsically unstructured [9] (Fig. 1C) and that undergoes α-helical folding upon binding to XD [7].

Within a conserved region of MV N_{TAIL} (Box2, $N^{489–506}$), an α-helical molecular recognition element (α-MoRE) undergoing induced folding upon binding to MV XD was identified [10] and modeled in the hydrophobic cleft delimited by helices α2 and α3 of XD [7]. This model was thereafter confirmed by the crystal structure determination of a chimeric construct composed of MV XD and $N^{486–504}$ (pdb code 1T6O) [11]. Using small angle X-ray scattering (SAXS), we have obtained a low-resolution structural model of the complex between MV XD and full-length N_{TAIL}, which showed

Abbreviations: MV, Measles Virus; N, nucleoprotein; L, large protein; P, phosphoprotein; PMD, P multimerization domain; PNT, P N-terminal domain, PCT, P C-terminal domain; XD, X domain; N_{TAIL}, C-terminal unstructured domain of N; MoRE, MOlecular Recognition Element; NMR, nuclear magnetic resonance; HSQC, heteronuclear single quantum correlation; nOe, nuclear Overhauser effect; DMSO, dimethyl sulfoxide

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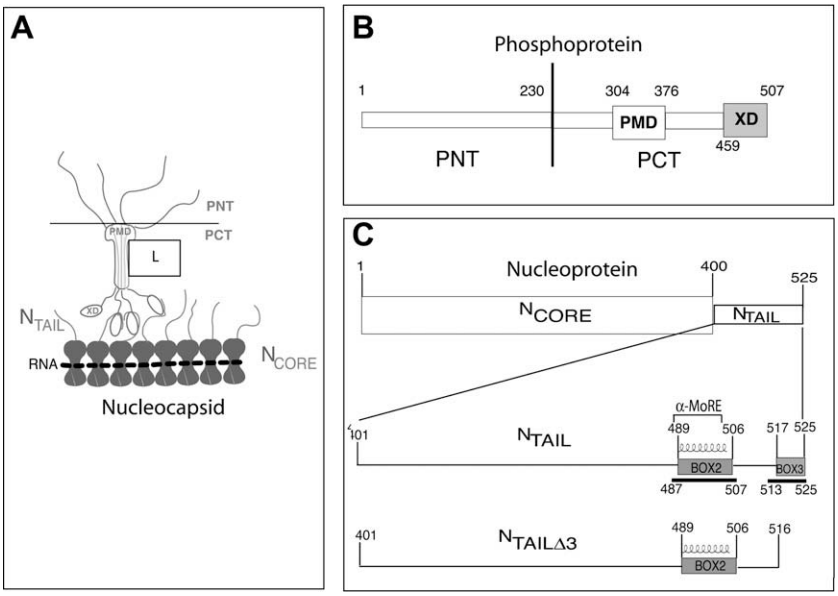

Fig. 1. (A) Schematic representation of the polymerase complex (L–P) bound to the nucleocapsid template. The disordered N_{TAIL} ($N^{401–525}$) and PNT ($P^{1–230}$) regions are represented by lines. The encapsidated RNA is shown as a dotted line embedded in the middle of N according to Albertini et al. [23] and Green et al. [24]. The multimerization domain of P ($P^{304–375}$, PMD) is represented with a dumbbell shape by analogy with the Sendai virus PMD structure [25]. The segment connecting P multimerization domain (PMD) and X domain (XD) is represented as disordered according to Longhi and co-worker [2,9]. The L protein is shown as an oval contacting P through PMD. (B) Schematic organization of P, where globular and disordered regions are represented by large and narrow boxes, respectively. The PMD and XD regions are shown. (C) Schematic organization of N, N_{TAIL} and $N_{TAIL-\Delta3}$ highlighting Box2, Box3 and the α-MoRE. Box2 and Box3 peptides are shown by a black bar.

that most of N_{TAIL} (residues 401–488) remains disordered in the bound form and does not establish contacts with XD, in contrast to the 489–525 region [12]. Using surface plasmon resonance (SPR) and nuclear magnetic resonance (NMR) titration of N_{TAIL} constructs with XD, we showed that beyond Box2 ($N^{489–506}$), Box3 ($N^{517–525}$) also contributes to binding [12]. Albeit previous experiments have clearly established that the region downstream the α-MoRE (i.e. $N^{505–525}$) also participates to the binding to XD, [12–14], the molecular mechanisms by which this region contributes to the stabilization of the complex are not fully understood. Previous spectroscopic studies have shown that in contrast to Box2, $N^{505–525}$ does not gain any regular secondary structure [12–14]. Using EPR spectroscopy, we have recently shown that $N^{505–525}$ does not directly contact XD and have proposed that it may rather establish transient, tertiary contacts with Box2 [14]. However, this model is merely speculative and no direct structural information is available. Indeed, crystallographic data have been obtained on a chimera construct containing only Box2 [11], and the only available structural data on the N_{TAIL}–XD complex are based on a low-resolution model derived by SAXS studies, which only indicates that the C-terminus of N_{TAIL} does not constitute a flexible appendage exposed to the solvent [12].

The aim of the present work is to unambiguously identify within XD and the C-terminus of N_{TAIL} the precise regions that mediate the association. To this endeavor, we have carried out NMR chemical shift perturbation experiments using uniformly ^{15}N-labeled XD and unlabeled N_{TAIL} deletion constructs and peptides.

2. Materials and methods

2.1. Sample preparation

Unlabeled N_{TAIL}, $N_{TAIL-\Delta3}$ ($N^{401–506}$) and uniformly ^{15}N-labeled XD were expressed and purified as described previously [7,12]. All samples contained 50 mM sodium phosphate buffer at pH 8.0, 95/5% v/v H_2O/D_2O and a protease inhibitor cocktail (complete, Roche Applied Science©). Various concentrations (from 0.125 mM to 1.13 mM) of ^{15}N-labeled XD were used for the titration experiments. Due to poor solubility in water, the synthetic peptide DSRRSADALLRLQAMAGISEE (Genemed Synthesis, Inc.©), corresponding to $N^{487–507}$ and referred to as Box2 peptide, was dissolved in 100% dimethylsulfoxide (DMSO) to yield a final concentration of 4.25 mM, and peptide DTPTVYNDRNLLD (SIGMA Genosys©), corresponding to $N^{513–525}$ and referred to as Box3 peptide, was dissolved in 5% DMSO leading to a final concentration of 2.6 mM.

2.2. NMR experiments

All spectra were recorded at 300 K on a Bruker DRX 500 MHz spectrometer. The sequential assignment of free XD was obtained using homonuclear and heteronuclear standard approaches [15]. The ^{1}H–^{15}N resonances of XD alone and bound to N_{TAIL} were checked via the measurement of a 2D ^{1}H–^{15}N HSQC–NOESY that was recorded with a mixing time of 100 ms and acquired with the fast HSQC scheme [16]. The interaction between XD and various N_{TAIL} constructs or peptides was studied by NMR chemical shift perturbations in a titration of ^{15}N-labeled XD with unlabeled N_{TAIL} constructs or peptides. For each titration experiment, we first recorded a ^{1}H–^{15}N HSQC spectrum of free ^{15}N-labeled XD, and then in the presence of increasing amounts of the unlabeled partner. The XD:partner molar ratios used were: 1:0.2, 1:0.4, 1:0.8, 1:1, 1:1.3, 1:1.4 and 1:1.7 for the interaction with N_{TAIL}; 1:0.25, 1:0.5, 1:1, 1:1.6, 1:2.9 for the interaction with $N_{TAIL-\Delta3}$; 1:0.1, 1:0.3, 1:0.5, 1:0.75, 1:2.5, 1:4 for the interaction with Box2 peptide and 1:0.6, 1:1.2, 1:2.4, 1:4.2 for the interaction with Box3 peptide. Saturation (i.e. no changes in chemical shift upon further addition of the partner to ^{15}N-labeled XD) was achieved with XD:partner molar ratios of 1:1.3, 1:1.6 and 1:2.5 for N_{TAIL}, $N_{TAIL-\Delta3}$ and Box2 peptide, respectively. For titration experiments with Box2 peptide, the chemical shift perturbation induced by DMSO was accounted for by subtracting from the HSQC spectra of ^{15}N-labeled XD-Box2 mixtures the HSQC spectra of mixtures containing ^{15}N-labeled XD and