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OLIGOMERIZATION OF A RETROVIRAL MATRIX PROTEIN IS FACILITATED BY BACKBONE FLEXIBILITY ON NS TIMESCALE

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Abstract

Oligomerization capacity of the retroviral matrix protein is an important feature that affects assembly of immature virions and their interaction with cellular membrane. A combination of NMR relaxation measurements and advanced analysis of molecular dynamics simulation trajectory provided an unprecedentedly detailed insight into internal mobility of matrix proteins of the Mason-Pfizer monkey virus. Strong evidences have been obtained that the oligomerization capacity of the wild type matrix protein is closely related to the enhanced dynamics of several parts of its backbone on ns timescale. Increased flexibility has been observed for two regions: the loop between α -helices $\alpha 2$ and $\alpha 3$ and the C-terminal half of α -helix $\alpha 3$ which accommodate amino acid residues that form the oligomerization interface. On the other hand, matrix mutant R55F that has changed structure and does not exhibit any specific oligomerization in solution was found considerably more rigid. Our results document that conformational selection mechanism together with induced fit and favorable structural pre-organization play an important role in the control of the oligomerization process.

Keywords

¹⁵N NMR relaxation; molecular dynamics; isotropical reorientation eigenmode analysis (iRED); program for analysis of internal motion (PAIN); Mason-Pfizer monkey virus; conformational selection

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Supporting information

The RMSD versus starting structure of both WT and R55F MA molecules during MD simulation trajectory is shown in Fig. S1. Figure S2 shows examples of correlation functions and $S^2(w)$ for time window length ranging from 0 to 100 ns. The experimental NMR relaxation data are provided for WT MA (Tab. S1) and R55F MA (Tab. S2). The complete sets of the resulting experiment-based motional parameters are shown for two types of the fitting procedures: with monomer-dimer equilibrium included (Tables S3 and S4) and without considering the monomer-dimer equilibrium presence (Tables S5 and S6). Also the full sets of dihedral angle distribution from MD trajectories of WT MA for monomer, dimer and trimer are shown (Fig. S3 – S5). This material is available free of charge at <http://pubs.acs.org>.

1. Introduction

Retroviral matrix protein (MA) is the N-terminal part of Gag structural polyprotein, which determines the location where Gag molecules self-assemble. The assembly occurs either on the plasma membrane (PM), which is the case of the human immunodeficiency virus (HIV), or in the pericentriolar region, which is the case of the Mason-Pfizer monkey virus (M-PMV). Dynein molecular motor recognizes the cytoplasmic targeting/retention signal (CTRS) sequence in M-PMV MA and transports the Gag molecules to the assembly site.¹ We have shown that the accessibility of the CTRS sequence in WT MA is the important prerequisite of an effective pericentriolar transport of Gag.² This observation was supported by parallel study of R55F MA mutant. The mutation resulted in a large change of MA structure and burying of the CTRS sequence, which in consequence impaired the pericentriolar targeting of the mutated Gag. Instead, R55F MA Gag molecules were transported directly to and assembled at the PM, similarly to HIV and other retroviruses.³

Self-oligomerization capacity of several MAs has been described and suggested to play a role in the interaction of Gag molecules with the PM and also in the stabilization of the outer layer of virions.^{4–7} Trimerization of HIV-1 MA is mediated mostly by the myristoyl moiety, while oligomerization is very weak or non-detectable in the naturally non-myristoylated MAs of equine infectious anemia virus, Rous sarcoma virus and also in the non-myristoylated form of HIV-1 MA.^{5–8} We have recently characterized oligomerization of non-myristoylated forms of the wild type MA and R55F MA of M-PMV.⁹ While WT MA exists in a pronounced monomer–dimer–trimer equilibrium in solution, R55F MA does not form specific oligomers. This behavior is again a consequence of the changed structure of R55F MA that abolished the oligomerization interface, present in the structure of WT MA.

NMR spectroscopy is an outstanding method to study protein dynamics using relaxation properties of nuclear spins. The well established protocol involves determination of the longitudinal (R_1) and transverse (R_2) relaxation rates of ^{15}N , and the $^{15}\text{N} - \{^1\text{H}\}$ nuclear Overhauser enhancement (NOE).^{10, 11} Experimental data are then conventionally interpreted using the Lipari-Szabó model-free approach,^{12–16} which characterizes molecular motions by the correlation time of global reorientation τ_M and the local internal motions by the generalized order parameter S^2 and the correlation time of local motions τ_{loc} . The assumptions of the approach are an isotropic global reorientation of the molecule and a statistical independence of the global and local motions, which is ensured by a large difference of their correlation times ($\tau_{\text{loc}} \ll \tau_M$). This protocol is well established although it has certain caveats; the principal one is the applicability of the oversimplified Lipari-Szabó model to potentially quite complicated motions of a protein. Moreover, it is often difficult to justify the application of the chosen motional model with a limited set of experimental data. Nevertheless, reliable theoretical approaches based on the molecular dynamics (MD) simulation can counterbalance the above-mentioned drawbacks and enhance significantly the interpretation of experimental data.

The isotropic reorientational eigenmode analysis of MD trajectory (iRED) correlates motions of the N-H bond vectors of the protein backbone.¹⁷ It relies on the principal component analysis of the isotropically averaged covariance matrix of lattice functions describing spin interactions responsible for spin relaxation. Another very recently presented tool for analysis of MD trajectory is the program PAIN. It enables a separation of contributions to the generalized order parameter of each NH vector according to the timescale of a relevant local motion.¹⁸ This is a very important feature since an experiment provides information on local motions occurring on timescale that is significantly shorter than the correlation time of the global reorientation. Consequently, the motions on the timescales longer than approximately 1 ns are overlapped by the global protein reorientation

that occurs on the timescale of 10 ns in the case of a protein of the size comparable with M-PMV MA.

Here, we present the results of a detailed study of picosecond–nanosecond dynamics of both the WT MA protein of M-PMV and its R55F mutant. Our results contribute to better understanding of the mechanisms by which oligomeric species of WT MA are formed. We argue that the enhanced dynamics on nanosecond timescale is a profound demonstration that the conformational selection together with structural pre-organization and induced fit are important components of the oligomerization mechanism.

2. Materials and methods

2.1 NMR spectroscopy

Uniformly ^{15}N -labelled samples of WT MA and R55F MA were prepared and purified to the final concentration of 1.1 mM as published previously.^{9, 19} All NMR experiments were measured on Bruker DRX 500 Avance NMR spectrometer (basic frequency 500.13 MHz for ^1H) at 25 °C. Spectral widths were set to 7 kHz for ^1H and 1.52 kHz for ^{15}N , with 2048 and 200 complex points respectively. Experiments were measured with 16 dummy scans and relaxation delay of 2 s.

Standard HSQC-based NMR pulse sequence¹⁰ were utilized for the determination of longitudinal (R_1) and transverse (R_2) ^{15}N relaxation rates of amide groups (R_1 mixing times in ms: 20.2, 30.2, 56.2, 104.2, 200.2, 392.2, 776.2 and 1544.2; R_2 mixing times in ms: 15.0, 29.9, 59.9, 104.8, 164.7, 239.5, 479.1 and 958.2). $\{^1\text{H}\}$ - ^{15}N heteronuclear NOE was measured using an enhanced reverse INEPT pulse sequence.²⁰ The NOE enhancements were obtained as the ratio of the peak volumes from experiment measured with 8 s irradiation period and the reference experiment with the 8 s delay instead.

Measured data were processed using NMRPipe.²¹ Peak volumes were determined in NMRView, version 5.22²² by integrating manually-defined elliptical regions. Relaxation rates were then obtained by single-exponential fitting of the volumes within NMRView.

2.2 Motional analysis of the experimental data

Apparent global correlation times were determined from the trimmed mean of R_2/R_1 ratios of the residues from rigid parts of the molecules.^{14, 16} Because this procedure does not account for oligomerization, the program Dynamics developed by D. Fushman²³ was used to derive the parameters of global as well as internal motions from the experimental data. According to our previous data,⁹ the chemical exchange related to monomer–dimer equilibrium was assumed to be fast on the ^{15}N chemical shift timescale, leading to the averaging of the relaxation parameters over populations of the two states. It was further assumed that internal motions were the same in monomer and dimer. This assumption was reasonable especially for the residues outside the oligomerization interface and generally also due to relatively weak MA–MA interactions reflected in a dynamic equilibrium rather than formation of stable oligomers.

We utilized three LS models that differ in the treatment of internal motions, i.e. the truncated, standard and extended ones. The truncated model describes local motions by the generalized order parameter S^2 and is applicable for rigid residues with only small amplitude of motion. The standard LS model uses two parameters S^2 and the local correlation time τ_{loc} to describe the motion occurring on a single timescale much shorter than the global reorientation. This model may be insufficient when a more complicated motion of the protein backbone occurs on two different timescales. Such situation is treated by the extended LS model¹⁵ which introduces separate generalized order parameters for fast (S^2_f)

and medium-fast (S_{mf}^2 , originally referred to as “relatively slow”) motions, with corresponding local correlation time τ_{mf} . Selection of the appropriate model for a given residue is driven by Monte-Carlo analysis of the distributions of differences between experimental and back-calculated relaxation rates.^{16, 23}

2.3 MD simulations

MD simulations of both MA molecules were performed in Amber.²⁴ The starting structures (PDB codes 2F76 for WT MA and 2F77 for R55F MA) were prepared in the Leap module of Amber 8 by adding 3 Cl⁻ anions to neutralize the overall charge of the molecules. The molecules were placed into a rectangular solvent box (TIP3P) extending the edges of the molecules by 8 Å. The size of the water box was $51.73 \times 62.92 \times 58.91$ Å with 4256 water molecules for WT MA, and $48.87 \times 47.31 \times 52.55$ Å and 3522 water molecules for R55F MA. Before the production runs, the potential energy of the molecules was minimized both in vacuo and in water and the systems were equilibrated in three runs with the overall length of 120 ps, during which the temperature and pressure were adjusted to 300 K and 101 325 Pa, respectively, and kept constant during the simulations. The simulations were performed with the Sander module using ff99 force fields. Non-bonded electrostatic terms were treated according to the particle-mesh Ewald method²⁵ and the SHAKE algorithm enabled us to use the integration step of 2 fs.²⁶ The total length of both simulations was 118 ns, during which 59 000 snapshots were taken, each every 2 ps. 20 ns simulations were run on models of WT MA dimer and trimer. Here, 500 snapshots were taken, each every 25 ps.

2.4 Motional analysis of the MD data

The iRED approach relies on the direct evaluation of fluctuations of the physical interactions that influence relaxation. All relevant information is contained in an $n \times n$ real symmetric covariance matrix M (where n is the number of residues) calculated according to Eq. (1).²⁷

$$M_{ij} = \langle P_2(\cos(\Omega_i - \Omega_j)) \rangle, \quad (1)$$

where P_2 is the Legendre polynomial of rank 2, $(\Omega_i - \Omega_j)$ is the angular difference between the directions of two distinct NH vectors from the same snapshot and angular brackets denote an averaging over all trajectory snapshots. The correlation coefficient of the vectors i and j , r_{ij} , can be calculated from the elements of the covariance matrix according to Eq (2).

$$r_{ij} = \frac{M_{ij}}{(M_{ii}M_{jj})^{0.5}} \quad (2)$$

In order to distinguish how various motions occurring on different timescales contribute to the generalized order parameter, the program PAIN was employed.¹⁸ The dependence of the generalized order parameter on the length of the time window w was calculated from the whole MD trajectories. The order parameter $S^2(k, w)$, characteristic for the k -th interval starting at time t and with duration w , was obtained according to Eq. (3)

$$S^2(k, w) = \frac{1}{(w+1)^2} \sum_{l=k}^{k+w} \sum_{m=k}^{k+w} P_2(\cos(\Omega_l - \Omega_m)), \quad (3)$$

where k is a time step which runs from N/w to 1 and N is the number of snapshots. The beginning of the time window t was incremented in order to sample the whole trajectory of MD simulation. Therefore, the procedure gave a set of order parameter values for all periods of durations w . As discussed by Macek et al.¹⁸, the most representative value is $S^2(w)$ i.e. the *maximum* of the order parameter set for a particular time window w . In this way, the motions occurring on a timescale longer than the particular time window w are filtered out. Such slow motions are not properly statistically sampled during the period w , but they lower the resulting order parameter if they accidentally occur. Therefore, the period with the highest order parameter $S^2(w)$ is least affected by the motions occurring on slower timescales.

A plateau in $S^2(w)$ is observed for a chain of rigid residues, which typically belong to a regular secondary structure, while a stepwise decrease of $S^2(w)$ indicates the presence of two or more motional modes¹⁸ (See Fig. S2 C, D in Supporting information for examples).

2.5 Other techniques

All images of protein structures were prepared in PyMOL 1.2 (<http://www.pymol.org>). Solvent-accessible surfaces were calculated using NACCESS software²⁸ for WT MA and proteins with PDB IDs 1VE3, 1W33, 1YF2, 2O1O and 2P3X.

3. Results

3.1 Experimental relaxation data

Longitudinal (R_1) and transverse (R_2) relaxation rates and $\{^1\text{H}\}$ - ^{15}N heteronuclear NOEs were determined for the backbone ^{15}N nuclei of 78 residues of both WT and RF MAs (Fig. 1). A statistical overview of the parameters is provided in Table 1 and the full set of the experimental values is available as Supporting information (Tables S1, S2). There are five prolines (43, 46, 72, 76 and 97) and two N-terminal residues (M1, G2) for which the relaxation data were not available. Other peaks are either missing in the spectra (R10, Y11, K20, Y28, V59 (in WT), Y82) or their volumes couldn't be determined due to spectral overlap (K16, F35, F37, V38, C62, D65, A79, N84, I90). The relaxation data of a sufficient quality were obtained for the protein samples with the concentration of 1.1 mM. At this concentration a dynamic equilibrium between monomeric and oligomeric species exists in the case of WT MA.⁹

Longitudinal relaxation rates R_1 show quite uniform values for both molecules while R_2 and NOE display rather broad distributions. It clearly demonstrates that the relaxation occurs close to the limit of the extreme narrowing regime where R_1 reaches its maximum. Relaxation of the rigid parts of the proteins is thus outside while the relaxation of the flexible parts falls within the extreme narrowing regime. R_2 and NOE are significantly more sensitive to the mobility of the proteins than R_1 . NOE reflects sensitively internal motions (negative values are significant for high internal mobility). Situation is more complex for R_2 that generally includes contributions from global as well as local motions (on ps–ns timescale) and also from the chemical exchange, i.e. the motions occurring on μs –ms time scale.

3.2 Overall reorientation

We used several approaches to determine the overall rotational correlation times τ_M of the MA molecules. As the first step we estimated τ_M by HydroNMR program.²⁹ The construction of a hydrodynamic model of monomeric proteins and subsequent hydrodynamic calculations were carried out on a set of 20 (WT MA) or 18 (R55F MA) structures taken from the ensemble of NMR structures (PDB IDs 2F76 for WT MA and 2F77 for R55F MA). This procedure was adopted in order to account for internal flexibility

of the proteins since the hydrodynamic model in HydroNMR is always internally rigid. Individual structures within each set differed mainly in the conformations of their flexible regions (the $\alpha 2\alpha 3$ loop and both termini). The resulting τ_M values were in the range of 9–10 ns (Table 2). Somewhat larger variation of τ_M of WT MA is probably caused by a higher internal flexibility of its backbone compared to R55F MA (*vide infra*).

We also analyzed anisotropies of rotational diffusion tensors of both molecules. R55F MA displays isotropic reorientation with a small anisotropy of 0.9, while WT MA shows a mild anisotropy of 1.3, which is caused mostly by a poor definition of both termini. Because the anisotropies are rather limited, we will further consider the overall reorientation as isotropic for both molecules.

Experimental τ_M values, derived from relaxation data, were firstly estimated from the trimmed mean R_2/R_1 ratio. Only the NH vectors from rigid parts of the molecules and without apparent contribution of chemical exchange to R_2 were included.¹⁶ This procedure yielded τ_M values significantly longer than the values predicted by HydroNMR (Table 2). This result is, however, in agreement with the specific WT MA oligomerization manifested as a monomer–dimer–trimer equilibrium in which approximately a half of monomeric units is engaged in oligomers at 1.1 mM concentration.⁹ In order to keep the number of motional parameters as low as possible, we describe the oligomerization by a simpler monomer–dimer model in this work. Such simplification can be justified by a small sensitivity of the parameters of local motions (especially the generalized order parameter) to the oligomeric state of the protein. Including oligomerization into the treatment of experimental data was necessary mainly in order to obtain realistic global correlation times. The situation was different for the R55F MA mutant because it does not undergo a specific oligomerization.⁹ Nevertheless, the longer τ_M obtained from the R_2/R_1 ratios suggested that a limited, non-specific oligomerization of R55F MA occurs in solution.

Next, LS analysis accounting for monomer–dimer equilibrium²³ was utilized to obtain motional parameters of both proteins (Table 2). Global correlation time τ_M of 8.6 ns was found for WT MA using the known K_D of 0.76 mM⁹. In the case of R55F MA, the calculation was carried out for several K_D values and the lowest χ^2 between the fitted and experimental data was found for K_D of 1.5 mM, yielding τ_M of 7.9 ns. The fitted parameters of R55F MA internal motions remained unchanged in a relatively broad range of K_D (1–10 mM), while the optimal τ_M was directly proportional to K_D . The experimental τ_M data assuming the monomer–dimer equilibrium agreed well with the theoretical τ_M values obtained from HydroNMR.

3.3 Internal mobility derived from experimental data

We describe the internal motions of WT and R55F MAs by means of generalized order parameters of the amide groups from the protein backbones. They were obtained from the experimental NMR relaxation data by computer script Dynamics²³ that offered a selection of several motional models, which differ in complexity and number of adjustable parameters. Fitting of the experimental data of each amide group started with the simplest model and utilization of more complex ones had to be subsequently justified by statistical analysis of errors. Truncated or standard LS models (see Materials and Methods) were sufficient for the majority of residues and only 14 and 12 residues of WT and R55F MAs, respectively, required the extended model (Table 3).

The calculated values of the generalized order parameters are shown in Figure 2 and Tables S1 and S2 (Supplementary information). Additionally, the motional data of the residues that displayed more complicated local dynamics and most often were subjected to the extended LS model are shown in Table 4. Both proteins are fairly rigid, which is documented by

average order parameters of 0.76 and 0.78 for WT and R55F MAs, respectively. The $\alpha 2\alpha 3$ loop region is the only interior part of both proteins with a higher mobility as is demonstrated by lower order parameters, especially within the stretch Q47-D52. Internal dynamics of three residues 47–49 consists of two modes: while amplitudes of their fast motions (on the picosecond timescale) are only moderate, a large-scale dynamics occurs on the nanosecond timescale. We also included E48 of R55F MA into this group although it was subjected only to the standard LS model. The fitting resulted in a large χ^2 but the error analysis did not lead to the preference of the extended LS model. Therefore, we suspected the model selection procedure to be affected by the experimental error of the E48 NOE value.

We also performed the analysis of internal motions using a protocol that did not account for the formation of oligomers and employed the global correlation time as determined from R_2/R_1 ratios. The determined order parameters of internal motions from both methods were very similar (see Tables S5, S6 in Supporting information). The influence of the oligomeric equilibrium on the accuracy of determined motional parameters is further discussed in section 4.2.

3.4 Influence of chemical exchange on relaxation data

In some cases, transverse relaxation rates R_2 were increased by the contribution of chemical exchange R_{ex} . The motional model involving the chemical exchange was found appropriate for two residues of WT MA, F45 and Y67 ($R_{ex} = 9 \text{ s}^{-1}$ and 7 s^{-1} , respectively). Furthermore, significant differences between experimental R_2 values and their best theoretical fits were obtained for residues L15, T41, W44, I53, Y66 and T78 of WT MA and E48, T50, D52, I53 and T78 of R55F MA. In the case of WT MA, this is perfectly consistent with the previously described oligomerization interface. The kinetics of oligomerization was previously found to be fast on ^1H as well as ^{15}N chemical shift timescales.⁹ However, because this paper is primarily concerned with the internal dynamics of the protein backbone, we did not perform a deeper investigation of the oligomerization kinetics.

3.5 Analysis of MD data

The structures of both proteins were stable during the 118 ns unconstrained MD simulations (see Fig. S1 in Supporting information). The positional RMSDs of the backbone atoms in well ordered parts of the molecules (residues 7–20, 28–41, 54–70, 78–90), calculated as an average over the whole trajectory, were $4.5 \pm 0.52 \text{ \AA}$ for WT MA and $3.5 \pm 0.26 \text{ \AA}$ for R55F MA. The larger structural variation of WT MA corresponds with the higher degree of internal mobility of this molecule.

We performed the iRED analysis of the MD trajectories in order to reveal pair-wise statistical correlations between the internal motions of different amino acid residues along the protein backbones. Correlation coefficients r were calculated according to Eqs. (1) and (2) (see *Materials and Methods*) and the results summarized in the form of correlation maps in Figure 3. A high occurrence of motional correlations within a compact block of amino acids reflects its significant internal rigidity while the lack of such correlations is a strong evidence of an enhanced segmental flexibility. Therefore, most correlations are observed between amino acids within helices, especially between the i^{th} and $(i+4)^{\text{th}}$ residues. The number of such correlations for helical secondary structure elements is significantly higher for R55F MA than WT MA (Table 5).

A remarkable difference between both proteins is observed in helix $\alpha 3$ (Fig. 3). There are almost no correlations among the C-terminal residues of helix $\alpha 3$ in WT MA structure (Q64–F70), which contrasts with a high number of correlations in the same region of R55F

MA. The rigid helix $\alpha 3$ of R55F MA is, nevertheless, divided into two motionally uncorrelated parts by residue V59. The flexibility of this residue is well supported by NMR relaxation data, which are best fitted by the extended LS model evidencing a complex dynamics on ps – ns timescale.

Additional differences between WT and R55F MAs are found in the central part of helix $\alpha 1$. This region displays decreased motional correlations in WT MA, which is in contrast to rather flexible N-terminus and fairly compact C-terminus of helix $\alpha 1$ in R55F MA.

When considering pair-wise motional couplings between whole helices, we generally observe moderate to weak correlations except for the strong correlation between helices $\alpha 3$ and $\alpha 4$ of R55F MA. Taken together, the observed amounts of motional couplings in the structural motifs of both proteins are indicative of a higher flexibility of WT MA over R55F MA.

Next, PAIN software¹⁸ was utilized to assess the timescale of internal motions of protein backbone that contribute to the generalized order parameters. The program introduces the concept of time-dependent order parameter that is obtained by correlation analysis of NH vector directions over a time window of the length w . Only the motions which occur on a timescale significantly shorter than the window w contribute to the value of the order parameter $S^2(w)$. We calculated six order parameters $S^2(w)$ for each residue using six different time windows w of 2, 10, 20, 40, 60 and 80 ns (Fig. 2). The motions that contribute to $S^2(w)$ in the particular time window w (e.g. 80 ns) will be further ascribed to the physical motional timescale of $w/10$ (i.e. 8 ns) in the further discussion.

A remarkable agreement was obtained between experimental order parameters and those from the PAIN analysis with the shortest time window of 2 ns (Fig. 2). Notable time window dependence of the order parameter $S^2(w)$ as an evidence of more complex dynamics was observed for several internal regions of WT MA (except the protein termini): the C-terminal part of helix $\alpha 1$ and the linker $\alpha 1\alpha 2$, the N-terminal part of the loop $\alpha 2\alpha 3$ (residues I51 – I53), the C-terminal part of helix $\alpha 3$, and the linker $\alpha 3\alpha 4$. R55F MA displayed generally much lower time window dependence of $S^2(w)$: only the N-terminus of helix $\alpha 1$ and especially the loop $\alpha 2\alpha 3$ were found flexible.

3.6 Geometry of motions of NH vectors

Geometry of the motions of backbone NH vectors was visualized by the angular distributions of backbone dihedral angles Φ and Ψ provided by PAIN (Fig. 4). A single distribution maximum which is possible to approximate with a Gaussian curve of the width of approximately 25 degrees is characteristic for rigid residues. On the other hand, the distribution that is much broader or displays several distinct maxima matches the residues experiencing enhanced mobility (see Fig. 4 and 5). The number of such dynamic residues is twice higher for WT MA (29) than R55F MA (15). Importantly, seven residues of the C-terminus of helix $\alpha 3$ of WT MA show several maxima in the angular distributions, which is in concert with the unusual degree of motional freedom of this region.

3.7 MD simulations of WT MA dimer and trimer

Shorter, 20 ns MD simulations of WT MA dimer and trimer were carried out in order to compare distributions of the backbone dihedral angles with monomer. Both oligomers were stable during the MD simulations; the average RMSDs calculated over the backbone heavy atoms (N, C $^\alpha$, and C') of the well structured regions were 1.42 and 1.08 Å per subunit for WT MA dimer and trimer, respectively. These values were much lower than those obtained from MD of the monomer (4.50 Å), which indicates a higher stability of WT MA unit in oligomers.

The MD simulations showed that the individual subunits within an oligomer were not conformationally equivalent (Fig. 6), which somewhat complicated the comparison with the monomer. A careful inspection of the structures, however, provided an important insight into mechanisms of the oligomerization. V59 adopts a different conformation in one of the dimer subunits (Fig. 4, Supporting information Fig. S4), which together with conformational changes of F63 and Y66 in the second subunit leads to the stabilization of the dimer. Although experimental data were not available for V59 of WT MA, increased flexibility of this residue was found in the generally more rigid mutant R55F [$S^2_{mf} = (0.68 \pm 0.04)$, $S^2_f = (0.40 \pm 0.06)$; see also Fig. 2]. The residues of helix $\alpha 3$ belonging to the oligomerization interface, C62, Q64, D65, Y66, Y67, T69, F70 (with exception of Y66 in the dimer), experience a significant narrowing of their conformational space when compared to the dihedral angles distributions of monomer. Most typical examples demonstrating changes of distributions of dihedral angles are shown in Figure 4. Full sets of dihedral angles distributions for monomer, dimer and trimer are available as Supporting information (Fig. S3–S5).

4. Discussion

4.1 Methodology

The combination of experimental and computational methods afforded an unprecedentedly broad and detailed insight into the internal dynamics of the retroviral MA proteins on the ps–ns timescale. Well established NMR relaxation measurements provided a solid basis and a quantitative reference for the computational methods. MD simulations and the methods of advanced analysis of MD trajectory, i.e. iRED and PAIN, provided two types of information that differ mainly in time resolution. Correlation coefficient maps and the distributions of the backbone dihedral angles belong to the first type of information which lack any time resolution. Motional correlations, especially the long-range ones, occurring between residues very distant in the amino acid sequences of the proteins, are unique parameters that are impossible to obtain from an experiment. They are, however, very informative concerning the motions of the regular secondary structure elements such as α -helices or their major parts. The distribution of backbone dihedral angles is a particularly descriptive representation of the physical nature of motions that are otherwise characterized only by the values of generalized order parameter, which is difficult to relate directly to a particular geometry of motion.

The time window-dependent order parameters $S^2(w)$ belong to the second type of information which is time-resolved. Only this approach allows a direct comparison of theoretical and experimental parameters describing the motions that take place on rather short timescales (less than 1 ns in this work). The information about internal motions occurring on the timescale comparable to the global correlation time (10 ns in this work) is not generally accessible from NMR experiments in the liquid state. Thus, the PAIN analysis extends the accessible range of timescales of internal motions. When the time window reaches the total length of MD simulation, the results must be taken with caution due to insufficient statistical sampling. The concept of window-dependent order parameter $S^2(w)$ works with the maximal value of the order parameter observed for a window length w . The maximum is taken from the set of N/w time windows, where N is number of snapshots. This is a very robust approach. The choice of $S^2(w)$ is in fact equivalent to the selection of the time window that is least affected by infrequent motions.

4.2. Effect of the oligomerization on NMR data

The formation of oligomers affected experimental NMR data in two ways. First, it lead to a significant increase of the global correlation time, τ_M , of WT MA (12.6 ns), which was

longer than the expected theoretical value calculated by HydroNMR (10 ns). Second, it was the contribution of chemical exchange R_{ex} to transverse relaxation rates of residues belonging to the oligomerization interface. Importantly, the strong correlation between τ_M and S^2_{LS} is an intrinsic property of the Lipari-Szabó approach. Therefore, only analysis based on correctly determined τ_M can provide unbiased parameters of local motions. We determined previously that roughly 50 % of monomeric units of WT MA were involved in oligomers. Thus, the experiment provided us with parameters of internal motions effectively averaged between monomer and oligomers. A similar situation was recently reported by Baryshnikova and Sykes on SDF-1 α protein.³⁰ From concentration-dependent relaxation measurements they found that while τ_M showed a clear concentration dependence, the generalized order parameters S^2_{LS} changed only within experimental errors. Therefore, we claim that the effect of dynamic oligomeric equilibrium on the internal motional parameters, especially S^2 , is negligible and that the effect can not be characterized experimentally due to quite large errors resulting from NMR measurements at low concentrations.

4.3 Internal dynamics of WT MA

WT MA consists of four α -helices, closely packed into the canonical structural motif of retroviral matrix proteins.^{31–33} This motif is stabilized by a central hydrophobic core which ensures the conservation of MA structures among retroviruses of various genera despite their very low sequential homology.^{8, 34} According to our data, WT MA is fairly rigid on the sub-nanosecond timescale. This is documented by a remarkable agreement of experimental and theoretical generalized order parameters calculated for the shortest time window of 2 ns, which corresponds to the timescale of motions of approximately 200 ps (Fig. 2). There are three regions, for which the experimental data display more complicated internal dynamics: both termini and the $\alpha 2\alpha 3$ loop. For these regions the extended LS model provides order parameters for very fast internal motions, S^2_f (within extreme narrowing limit), and for about one order of magnitude slower motions, S^2_{mf} . They are in concert with the calculated values of $S^2(w)$ (Fig. 2): S^2_f of approximately 0.8, which reflects relatively high restrictions of fast motions, agrees well with $S^2(w=2\text{ ns})$, while the significant decrease of $S^2(w=80\text{ ns})$ agrees with high amplitudes of slow motions reported experimentally by S^2_{mf} . Minor exception is the stretch of amino acids 47–49 where the MD simulation probably somewhat underestimates rates of medium-fast motions.

As follows from the pattern of $S^2(w=40–80\text{ ns})$, the C-terminal half of the $\alpha 3$ helix and the attached $\alpha 3\alpha 4$ loop display an increased mobility on the timescale of around 10 ns. Such motions can not be monitored by NMR experiments due to the overlap with the overall reorientation (12.6 ns). These motions are also observable in the correlation coefficient map (Fig. 3) displaying a surprising lack of sequential correlations for the C-terminal half of $\alpha 3$ helix of WT MA when compared with the R55F MA mutant. The decrease of the order parameter $S^2(w)$ is also frequently (for the total of 27 residues in this work) correlated with the appearance of several distribution maxima in the histograms of backbone dihedral angles (Fig. 4). The histograms explain the nature of the motions: fast motions are represented by fluctuations within a limited range around the local maximum, while slower motions are consistent with jumps between distinct potential wells.

4.4 Internal dynamics of R55F MA and comparison with WT MA

Similarly to WT MA, the structure of the R55F MA mutant consists of a bundle of four α -helices. The mutual orientations of helices $\alpha 1$ and $\alpha 2$ (N-terminal part) as well as $\alpha 3$ and $\alpha 4$ (C-terminal part) do not differ between WT and R55F MAs. However, the substitution of arginine 55 in helix $\alpha 3$ for phenylalanine led to a large change of the mutual orientation of the N- and C-terminal parts of the molecule.³¹ The reorientation was also accompanied by the rotation of helix $\alpha 3$ along its long axis by 100°.

The experimental order parameters S^2_{LS} document a substantial internal rigidity of R55F MA, with the exception of the terminal and loop residues which are more mobile. NMR experiments thus showed a similar degree of internal mobility of both proteins. However, the analyses of MD trajectories revealed a large difference between R55F and WT MAs, especially a higher rigidity of the C-terminal half of helix $\alpha 3$ of the R55F mutant. This is evident from the comparison of the $S^2(w)$ time windows of 2 ns and 80 ns where no decrease is seen in the case of the mutant. Furthermore, a large number of correlations in the iRED maps of this region is observed.

Higher rigidity of the C terminus of helix $\alpha 3$ in the mutant may be easily explained by the stabilization of this region due to the described structural change. The rotation of helix $\alpha 3$ buried and hence stabilized four aromatic residues (F63, Y66, Y67, and F70) that are exposed in WT MA (Fig. 7).

4.5 Mechanism of WT MA oligomerization

The oligomerization of WT MA is mediated mainly by residues located in the $\alpha 2\alpha 3$ loop (T41, W44, F45) and the C terminus of helix $\alpha 3$ (D61, C62, D65, Y66, Y67, T69 and F70), which form a continuous oligomerization interface on the protein surface.⁹ We found that the C-terminal part of helix $\alpha 3$ experiences a substantial degree of internal mobility on the ns timescale. This region contains four aromatic amino acids (F63, Y66, Y67, and F70) whose side chains are exposed on the surface of the monomeric structure (Fig. 7), which is energetically unfavorable. In order to quantify the exposure of the aromatic amino acid residues, we calculated their average relative solvent-accessible surface (SAS) and found a surprisingly large value of $(64 \pm 18) \%$. The value was then compared with the accessibility of the same FXXYYXXF motif (X stands for any amino acid residue) present in α -helices of other proteins. Five such proteins from the PDB structural database gave an average accessibility of the aromatic amino acids of $(10 \pm 1.3) \%$, which is much smaller value than what we have found for WT MA. α -Helices in these proteins are in all cases oriented so as the aromatic side chains are buried within the protein core and help to stabilize the surrounding parts of the molecules.^{35, 36} We conclude that the lack of such interactions in the monomeric WT MA structure leads to the increased flexibility of the C-terminal half of helix $\alpha 3$ and, subsequently, to favorable interactions upon oligomerization. Such mechanism of WT MA oligomerization is supported by the observed stabilization of the end of helix $\alpha 3$ in dimer and trimer, in which the aforementioned aromatic amino acids from the individual subunits form a stable cluster during the whole MD runs.

During the past decade, there has been an intense debate on generalization of the mechanisms of protein–protein and/or protein–ligand interactions. First, the well established Koshland's model of induced fit was developed from the key–lock principle by addition of certain allowed flexibility to interacting molecules.^{37, 38} Thus, the complex formation leads according to this model to appreciable changes of subunits structures. The model, however, failed to explain certain phenomena such as molecular recognition.³⁹ Therefore, the theory of the conformational selection was developed.^{40,41} It is based on the assumption that free constituents of a complex occupy certain conformational space and the favorable conformations are selected upon complex formation. There is still limited but quickly growing amount of experimental evidence that the conformational selection is an important mechanism of protein–protein interactions.^{42–46} Our data on internal dynamics of M-PMV WT MA protein provide strong evidence that the conformational selection plays a key role in its oligomerization. The residues of the C-terminus of helix $\alpha 3$ display increased internal mobility on the timescale of around 10 ns. The presented distributions of dihedral angles clearly document that only a limited subspace of the monomeric conformational space is populated in the oligomers. The timescale of transitions between individual conformers is in perfect agreement with recent works of Lange et al.⁴³ and Wlodarski and Zagrovic,⁴⁴ who

reported that increased internal mobility of ubiquitin occurred on the time scale comparable to or longer than the global correlation time. Moreover, the timescale of internal mobility which is necessary for successful molecular recognition was previously estimated to 0.4 – 10 ns.^{39,47} The timescale of the internal motions of the WT MA oligomerization residues falls exactly into this range.

5 Conclusions

Recently, we have suggested that the increased propensity of M-PMV MA to form specific oligomers helps to stabilize the outer layer of the virions.⁹ Our current results support such conclusion and show that the virus has developed an efficient mechanism of the formation of oligomers. Oligomerization mechanism of M-PMV MA protein includes three major attributes: 1) structural pre-organization, 2) induced fit and 3) conformational selection. The structural pre-organization is demonstrated by the exposure of two WT MA regions – the $\alpha 2\alpha 3$ loop and especially the patch of hydrophobic residues in the C-terminus of $\alpha 3$ helix. Several residues of the $\alpha 2\alpha 3$ loop as well as few residues of $\alpha 3$ helix (“joint residues”) experience large changes of conformation upon oligomerization that are characteristic for the induced fit mechanism. Furthermore, most of the residues of the C-terminus of $\alpha 3$ helix populate multiple conformational states in monomer with transitions occurring on 10 ns timescale. The conformational selection leads to greatly narrowed dihedral angles distributions in oligomeric species. Our results thus support the model of protein – protein interactions developed recently by Grünberg et al.,⁴⁸ which effectively combines both previously antagonist mechanisms, i.e. the induced fit and conformational selection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Experimental backbone amide ^{15}N relaxation rates R_1 , R_2 , steady state $\{^1\text{H}\}$ - ^{15}N NOE values and R_2/R_1 ratios together with primary amino acid sequence of WT and R55F MAS.

Figure 2.

Generalized order parameters for WT and R55F MAs obtained from the experiments and MD simulations. The experimental S^2_{LS} values are shown by asterisks and for the residues subjected to the extended LS model, asterisk corresponds to S^2_{mf} and circle to S^2_f . The calculated $S^2(w)$ are plotted by bars and colored according to the window length w as indicated.

Figure 3. Correlation coefficients $|r| > 0.5$ shown in the form of two-dimensional maps for WT and R55F MAs. The value of each correlation is color-coded according to the scale and helical regions are highlighted by thick lines.

Figure 4.

Distributions of backbone torsion angles Φ and Ψ for five selected residues of helix $\alpha 3$ with characteristic behavior as obtained from MD simulations of monomer, dimer and trimer of WT MA. W56 is rigid in monomer and remains unchanged upon oligomerization, V59 shows a different conformation in dimer and the residues D65-Y67 narrow the distributions upon oligomerization. The occurrences are normalized with respect to the total number of snapshots in each trajectory.

Figure 5. Characteristics of $S^2(w)$ behavior and number of maxima in the histograms of dihedral angles Φ and Ψ shown for residues of WT and R55F MAs. Colors have the following meaning: green - converged $S^2(w)$; red - step-wise decrease of $S^2(w)$; yellow - a single maximum; orange - several maxima of dihedral angles.

Figure 6. Representative structures of WT MA dimer (A) and trimer (B) during MD simulation trajectory. Helices are colored as follows: $\alpha 1$ orange, $\alpha 2$ green, $\alpha 3$ red, $\alpha 4$ blue. $\alpha 2\alpha 3$ loop is shown in cyan. The presence of structural irregularities of helix $\alpha 3$ helps to optimize hydrophobic interactions within oligomerization patch.

Figure 7. Accessibility of aromatic amino acids F63, Y66, Y67 and F70 from the oligomerization interface are shown in two views for WT (A, B) and R55F (C, D) MAs. Surfaces of the neighboring helix $\alpha 2$ and $\alpha 3\alpha 4$ loop are shown in green and magenta mesh, respectively.

Table 1

Statistical overview of experimental ^{15}N relaxation rates and NOEs.

	$R_1[\text{s}^{-1}]$				$R_2[\text{s}^{-1}]$				NOE	
	min	max	average	median	min	max	average	median	min	max
WT MA	0.93	1.7	1.4 ± 0.1	1.4	2.0	26.2	14.7 ± 5.8	16.4	-1.5	1.1
RF MA	1.04	1.9	1.6 ± 0.1	1.6	2.5	22.3	12.6 ± 4.1	13.7	-1.1	0.9

Table 2

Global correlation times of WT and R55F MAs

	τ_M^a (HYDRONMR)	τ_M^b (R ₂ /R ₁)	τ_M^c (M/D equilibrium)
WT MA	10.0 ± 0.7	12.6 ± 0.2	8.6 ± 1
R55F MA	9.2 ± 0.3	10.4 ± 0.2	7.9 ± 1

^a hydrodynamic calculations based on sets of structures,

^b R₂/R₁ ratios ¹⁶,

^c the LS analysis accounting for monomer-dimer equilibrium.

Table 3

Number of residues assigned to each of three utilized motional models

	Motional model		
	truncated LS	standard LS	extended LS
WT MA	40	24	14
R55F MA	28	38	12

Table 4

Amino acid residues belonging to the protein core that display more complicated internal dynamics interpreted by the extended LS model (except for E48 of R55F).

	Residue	S^2_{mf}	τ_{mf} [ns]	S^2_f
WT MA	Q47	0.06 ± 0.02	1.04 ± 0.03	0.78 ± 0.04
	E48	0.07 ± 0.02	0.61 ± 0.06	0.80 ± 0.11
	G49	0.06 ± 0.02	1.22 ± 0.03	0.55 ± 0.04
R55F MA	Q47	0.14 ± 0.03	0.99 ± 0.05	0.74 ± 0.05
	E48	0.78 ± 0.03	0.30 ± 1.00	-
	G49	0.09 ± 0.03	0.83 ± 0.06	0.62 ± 0.08
	V59	0.39 ± 0.06	2.23 ± 0.50	0.68 ± 0.04

Table 5

Numbers of motional correlations between amino acid residues within α -helices of WT and R55F MAs ($|r| > 0.5$).

Helix	WT MA	R55F MA
$\alpha 1$	48	59
$\alpha 2$	63	73
$\alpha 3$	40	96
$\alpha 4$	32	44