Impact of ion valency on the assembly of vimentin studied by quantitative small angle X-ray scattering

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The assembly kinetics of intermediate filament (IF) proteins from tetrameric complexes to single filaments and networks depends on the protein concentration, temperature and the ionic composition of their environment. We systematically investigate how changes in the concentration of monovalent potassium and divalent magnesium ions affect the internal organization of the resulting filaments. Small angle X-ray scattering (SAXS) is very sensitive to changes in the filament cross-section such as diameter or compactness. Our measurements reveal that filaments formed in the presence of magnesium chloride differ distinctly from filaments formed in the presence of potassium chloride. The principle multi-step assembly mechanism from tetramers via unit-length filaments (ULF) to elongated filaments is not changed by the valency of ions. However, the observed differences indicate that the magnesium ions free the head domains of tetramers from unproductive interactions to allow assembly but at the same time mediate strong inter-tetrameric interactions that impede longitudinal annealing of unit-length filaments considerably, thus slowing down filament growth.

1 Introduction

The multigene family of intermediate filament proteins (IFs) constitutes one of the major elements of the cytoskeleton found in metazoan cells^{1–3}. In the cell, these biopolymers build complex and dynamic networks that are extraordinarily resilient to denaturation^{4,5}. Vimentin is one member of the IF family⁶ that is found in cells of mesenchymal origin such as fibroblasts. *In vitro*, vimentin forms tetramers (diameter about 5 nm, length 60 nm) in buffers of low ionic strength such as 2 mM sodium phosphate. The addition of monovalent ions (K⁺, Na⁺) at a concentration of at least 50 mM initiates the self-assembly to extended filaments (diameter 10 nm, length up to several μ m), involving several distinct sub-steps^{7–12}. (See figure 4a for a schematic of the monomer and the principle assembly steps; for atomic models see, e.g., Ref.¹³). In contrast, the effect of the addition of divalent ions is more complex. Stromer et al. reported that desmin filaments formed by adding either calcium or magnesium ions resemble each

other and are slightly thicker than filaments formed by the addition of sodium ions or both sodium and magnesium ions. The results by Stromer further indicate that these differences are not only due to changes in the ionic strength of the buffer, but also due to differences of the interaction of mono- and divalent ions with the protein¹⁴. Also, with different divalent ions rather strong differences may be observed. Hence, Hofmann et al. noted for recombinant vimentin that, whereas structures induced with MgCl₂ were indistinguishable from IFs assembled with monovalent ions, CaCl₂-induced structures were much more heterogeneous with diameters ranging from 15 nm to 40 nm and a mean diameter of 24.2 nm compared to 12.2 nm for NaCl-induced IFs¹⁵. Notably, the axial repeat and cross-striation pattern along the filament in 5 mM CaCl₂ is very similar to the one observed in NaCl-assembled filaments (18.4 nm versus 20.3 nm).

Along these lines, Kooijman et al. demonstrated by transient electric birefringence (TEB) experiments that vimentin tetramers could be triggered to form increasingly longer and thicker filaments by increasing the magnesium concentration from 0.5 to 2.5 mM in the presence of 0.7 mM magnesium phosphate^{16,17}. However, these structures appeared less regularly organized than standard filaments as they stopped to grow at rather short length and formed comparably thick filaments whose mass per length was determined to be between two- and threefold increased as compared to standard IFs¹⁸. Hence, it was suggested, that magnesium alone hinders and thereby slows down the assembly considerably, whereas in

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the presence of excess monovalent ions it may affect the filament surface or substructure in a coordinated way. This is in agreement with the observation that in the presence of monovalent ions, both magnesium and calcium ions induce identical cross-linking of the networks as indicated by rheology studies^{19–21}. Similarly, keratin intermediate filaments (K8/K18) have been reported to form bundles in buffers of physiological ionic strength containing divalent ions^{22–24}.

The molecular details for the basic structural unit, the tetramer, are now available as the structure of a coiled-coil dimer has recently been determined by crystallography and the mode of association of two coiled-coils into a tetramer is known^{13,25–29}. However, the exact lateral organization of tetramers in a cross-section is not known at this high resolution. Accordingly, as the vimentin dimer consists of two α -helical coiled-coil rods which are flanked by flexible non- α -helical amino-terminal (“head”) and (“tail”) domains, the orientation of these eight non-structured domains within each tetramer is still being discussed. Importantly, headless vimentin does not form tetramers under low salt conditions, indicating that the head domains are instrumental to assemble two coiled-coil dimers in an anti-parallel, half-staggered fashion^{11,30}.

Sokolova et al. performed small angle X-ray scattering (SAXS) experiments on vimentin to model the arrangement of vimentin dimers in early assembly stages, including low-salt tetramers, octamers and unit length filaments (ULFs)³¹. While they find an elliptical cross-section for the ULFs, there are also many indications in the literature, that the cross-section is actually radially symmetric^{32,33}. SAXS is a label-free solution technique that is sensitive to structures on the nanometer scale³⁴. For fibrillar proteins, it can provide insights into the fiber cross-section, like compactness or inner structure that are inaccessible by conventional imaging techniques^{35,36}. Additionally, it can provide information about typical spacings in bundles and networks, as shown on the example of neurofilament gels^{37,38}.

Here we take this approach further by introducing true assembly conditions, thus avoiding overshoot phenomena otherwise observed at the relative high protein concentration needed for SAXS experiments, and investigate the central question of intermediate filament biology how salt ions influence filament assembly. Importantly, we keep the buffer conditions, in particular the pH value, in the physiological range. We systematically and quantitatively investigate how changes in the concentration of potassium and magnesium ions affect the cross-section of vimentin filaments assembled for extended time periods. To reach this goal, we collect SAXS data at different salt and protein concentrations. The SAXS curves show qualitative structural differences between the filaments formed by different cation species. We quantify these differences by fitting a reduced parameter model to all curves, which, however, needs to include polydispersity in the filament diameters. We

find that increasing the ion concentration results in thicker filaments. The effect is more pronounced for MgCl_2 than for KCl. Increasing particularly the MgCl_2 concentration additionally reduces the smoothness of the filaments.

2 Experimental

2.1 Protein purification and assembly

Human vimentin protein was expressed in bacteria (*Escherichia coli*, strain TG1) and purified from inclusion bodies³⁰. The protein was stored at $-80\text{ }^\circ\text{C}$ in 8 M urea, 5 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.1 mM EGTA, 1 mM DTT, and 10 mM methyl ammonium chloride (MAC). The purity of the protein was verified by SDS-polyacrylamide gel electrophoresis. For assembly, the protein was dialyzed into 2 mM sodium phosphate buffer, pH 7.5 (dialysis buffer) in a stepwise manner (8, 4, 2, 1, 0 M urea) using membranes with a 50 kDa cut-off. The protein concentration was determined by measuring the absorption at 280 nm (Nanodrop ND-1000, ThermoScientific Technologies, Inc., Wilmington, USA). Protein concentrations above 3.5 mg/mL were achieved by using a Nanosep 10K centrifuge concentrator (Nanosep Centrifugal Devices, PALL, Ann Arbor, Michigan, USA). After about half an hour at room temperature, assembly was initiated by addition of a salt solution buffered with 2 mM sodium phosphate to the vimentin solution at a 1:9 ratio¹¹. Samples were filled immediately into capillaries for SAXS (mark-tubes made of quartz glass, outer diameter 1.5 mm, wall thickness 0.01 mm; Hilgenberg, Malsfeld, Germany). The capillaries were then sealed with wax and spun down briefly at room temperature to remove remaining air bubbles (acceleration to 1500 rpm, Eppendorf centrifuge 5810 R, Eppendorf, Hamburg, Germany). Afterwards, all samples were stored at about $4\text{ }^\circ\text{C}$ for at least 24 hours before the measurements. Extended storage (up to 5 days) did not change the scattering signal (data not shown).

2.2 Small angle X-ray scattering (SAXS) measurements and analysis

The SAXS measurements were performed at the beamline B1 at DORIS III of DESY (Hamburg, Germany)³⁹. For each sample the scattering was recorded at 885 mm and 3585 mm distance from the sample using a Pilatus 1M detector (981×1043 pixels, pixel size: $172 \times 172\text{ }\mu\text{m}^2$; Dectris, Baden, Switzerland) at a photon energy of 9 keV, unless otherwise specified. At these sample-to-detector distances we could access real-space length scales up to about 100 nm. At the shorter distance the total exposure time per sample was up to 40 min, at the longer distance up to 60 min. The time chosen depended on the signal-to-noise ratio and beam stability. The exposure time was split up in 5 or 10 min intervals depend-

ing on the beam stability. A semi-transparent beamstop enabled measurements of the intensity of the transmitted beam directly with the Pilatus detector, and in combination with a pre-sample ionisation chamber the transmission of each sample was automatically determined. On-site tools were used for the averaging of the data as well as for the unification of the data for the two different sample-to-detector distances (<https://github.com/uvainio/Beamline-B1-macros>). The averaging includes correction for the sample transmission and beamline background. The background scattering of capillaries filled with buffer was subtracted from the resulting SAXS curves. Finally, all curves were normalized by division by the respective protein concentration c and the scattering intensity I was plotted against the scattering vector q using MatLab R2009b (The MathWorks, Inc., Natick, USA). Test for protein concentration effects by varying the protein concentration from 0.5 mg/ml to 5 mg/ml at fixed salt concentrations showed no changes in the scattering signal (data see supplemental info). Pair distance distribution functions $p_c(r)$ were calculated with the program GNOM⁴⁰ which as additional output provides the values for the maximum r -value D_{max} , and normalized such that the height of the main peak equals 1. Fits to the cylindrical micelle model were performed using the “lsqnonlin” function of MatLab.

2.3 Transmission electron microscopy (TEM)

The proteins (3.5 mg/ml) were dialyzed into 2 mM sodium phosphate buffer (pH 7.5) and assembly was started with the corresponding buffers as described for the SAXS experiments. The assembly reaction was stopped by addition of nine volumes of assembly buffer containing 0.1 % glutaraldehyde. A 10 μ L sample was applied to a freshly glow-discharged carbon-coated copper grid, allowed to attach for one minute followed by negative staining of protein structures with uranyl acetate. Storage of filaments at 4 °C during and after the assembly process (for transport to the synchrotron) did not influence their appearance as judged by EM analysis of parallel samples. For better retention of material on the grids and observation of individual filaments, assembly was performed also at 0.2 mg/ml. In principle, identical filaments were formed (see also Ref.⁴¹ for filaments assembled at higher protein concentration). Washed and dried samples were recorded in a transmission electron microscope, EM912 (Carl Zeiss, Oberkochen, Germany).

3 Results and discussion

3.1 Electron microscopy

Extended filaments are formed for KCl concentrations between 10 mM and 100 mM and MgCl₂ concentrations be-

Fig. 1 Temporal evolution of filaments in the presence of 2.5 mM MgCl₂. a) After 1 h, b) after 3.5 h c) after incubation overnight and d) 1 h 2.5 mM MgCl₂ plus additional 50 mM NaCl. The scale bar indicates 250 nm.

tween 0.5 mM and 4 mM when assembled for 2 h at 23 °C. In both cases, increase of the ionic strength accelerates assembly. However, the MgCl₂ filaments are not as long as the KCl filaments, reaching up to only about 1 μ m in length. Upon further incubation for 24 h, also the MgCl₂ filaments grow very long similar to the KCl filaments at 1 h of assembly (data not shown). The MgCl₂ filaments exhibit the 21.5 nm repeat more clearly than the KCl filaments under the TEM conditions. However, the same appearance has been noted early on with IFs assembled in monovalent salt upon visualization by glycerol spraying/rotary metal shadowing^{15,42,43}. We therefore conclude that regular filaments are formed under both conditions except that with MgCl₂ assembly is significantly slower. The polymorphism with regard to filament diameter and mass as observed by STEM measurements is as strong as with monovalent ions^{8,18}. The fact that IF structures are practically indistinguishable by TEM after induction with magnesium and with monovalent cations, respectively, was actually realized early on¹⁵ and is confirmed in this study.

Fig. 1 shows a snapshot series of vimentin assembly in the presence of magnesium at room temperature. Between 1 h and 3.5 h (Fig. 1a, b) filaments grow more slowly than measured in the monovalent assembly regime^{9,44}, but eventually grow to long filaments after overnight incubation (Fig. 1c). Interestingly, the addition of monovalent ions after 1 h of assembly with 2.5 mM MgCl₂ boosts the elongation reaction dramat-

Fig. 2 The small angle scattering signal of vimentin recorded at different concentrations of a single cation species. a) Changes in the small angle scattering and b) corresponding $p_c(r)$ of vimentin upon variation of the KCl concentration. The black curve was recorded at low salt buffer conditions. c) Changes in the small angle scattering of vimentin upon variation of the MgCl_2 concentration and d) corresponding $p_c(r)$. Again, the black curve was recorded at low salt buffer conditions.

ically and filaments grow very long within a 1 h incubation (Fig. 1d). Hence, magnesium as compared to potassium does not lead to principally different structures, but the elongation of the filaments proceeds much more slowly, suggesting that the presence of magnesium ions interferes with dynamic rearrangements within the unit length filaments needed for their longitudinal annealing into longer filaments¹¹.

3.2 Small angle X-ray scattering

3.2.1 Filaments in the presence of KCl. Complementary to TEM, SAXS provides a method to investigate the internal structure of the filaments. For the SAXS experiments presented here, the vimentin-ion mixtures have been filled into capillaries and then stored for extended time at 4 °C, and the measurements were performed at room temperature. The conditions are therefore well comparable to the TEM experiments presented above. We first address the question whether and to which extent the structure of the filaments and thereby their SAXS signal depend on the KCl concentration during assembly. When comparing the scattering signal for different concentrations, we find that the increased addition of KCl to the protein solution leads to an increase of the

scattering at low q -values ($\lesssim 0.1 \text{ nm}^{-1}$), accompanied by a steepening of the scattering curve, resulting in less scattering at higher q (figure 2a). Also, whereas the scattering curve in low salt buffer (i.e. no additional salt, black) levels off towards small values of q , and even slightly decreases, the curves at higher salt concentrations continue to rise. Furthermore, at a scattering vector of about 0.7 nm^{-1} a kink in the scattering curve, consisting of a very slight local minimum and maximum, emerges. A weaker second kink emerges at $q \approx 1.2 \text{ nm}^{-1}$. All these features are more prominent at higher salt concentrations (e.g. 100 mM, magenta) than at lower salt concentrations (e.g. 10 mM, red). At higher q , above 2 nm^{-1} , all scattering curves are parallel. Taken together, these changes indicate that higher KCl concentrations result in slightly thicker filaments that are also more defined in their diameter. For large q the slope of the curves is approximately constant when comparing different ion concentrations, showing that the filament structure is well defined for all concentrations. For smaller q a transition to q^{-1} can be observed, which is typical for rod-like particles³⁴. Figure 2b shows the corresponding pair distance distribution functions $p_c(r)$. In the absence of any additional ions, i.e. in low salt buffer, $p_c(r)$ has one narrow peak at $r = 1.5 \text{ nm}$ with one additional small shoulder at about $r = 4 \text{ nm}$. These features hint to the existence of coiled coils in the tetramers as the peak positions correspond to favored characteristic distances in the sample. Upon the addition of 10 mM KCl the shoulder becomes the main peak and moves to $r = 3.5 \text{ nm}$ and the peak at $r = 1.5 \text{ nm}$ is reduced to a shoulder. With increasing KCl concentration, the shoulder at smaller r is suppressed and eventually vanishes and the second peak moves to slightly larger $r = 5.5 \text{ nm}$ for 100 mM. The development of this peak is likely to correspond to assembling filaments. Most curves display a long tail towards large distances D_{max} . With the exception of the data for 10 mM KCl the systematically increasing values for D_{max} can be interpreted increasing particle sizes. The changes in the form of $p_c(r)$ support the assumption above that higher potassium concentrations result in slightly thicker filaments but the shoulder at 1.5 nm also indicates a remaining pool of tetrameric vimentin at 10 mM KCl and 20 mM KCl.

3.2.2 Filaments in the presence of MgCl_2 but in the absence of KCl. As shown by TEM, adding MgCl_2 to vimentin tetramers in the range of 0.5 mM to 4 mM instead of KCl also leads to the formation of filamentous structures. In the SAXS signal, the changes observed at low salt concentrations appear to be similar (figure 2c): We observe an increase of scattering at small q and an overall steeper scattering curve. At higher salt concentrations the slope at small q continues to become steeper and a kink in the scattering curve emerges at

about $q = 0.6 \text{ nm}^{-1}$. In contrast to the kinks found in presence of KCl, this kink is not linked to local maxima and minima, and after the kink, all curves assume a similar, constant slope. Note that the concentration of divalent magnesium ions, at which these changes occur, are about one order of magnitude smaller than for monovalent ions.

In the pair distance distribution function $p_c(r)$, the addition of 0.5 mM MgCl_2 in the absence of KCl shifts the peak to $r = 2.5 \text{ nm}$ with a shoulder at $r = 3.5 \text{ nm}$ (figure 2d). At higher MgCl_2 the shoulders vanish, the peaks broaden and their position moves to larger distances ($r = 11.5 \text{ nm}$ at 4 mM MgCl_2). The height and length of the tail of the distribution towards large distances as well as the values for D_{max} increase systematically with the MgCl_2 concentration. These changes again imply that the filaments broaden as the cation concentration increases, yet not in exactly the same manner as for added KCl. Comparing the data for KCl and MgCl_2 , for KCl the ionic strength is equal to the concentration and for MgCl_2 it corresponds to the threefold concentration. Therefore, for the ion concentrations studied here, the ionic strengths are lower for MgCl_2 but with some overlap to KCl. Note, however, that this is an estimate for the ionic strength in the bulk. Locally on the surface of the filaments it might be considerably higher, especially in the case of MgCl_2 (due to counterion condensation).

3.2.3 Filaments in the presence of both MgCl_2 and KCl. The TEM results shown in figure 1d reveal a competitive behavior of monovalent and magnesium ions. In order to elucidate the mechanisms behind the assembly with added monovalent or magnesium ions, we therefore finally investigate the situation with both ionic species present. We add different MgCl_2 concentrations in the presence of 20 mM and 80 mM KCl, respectively (figure 3). At 20 mM KCl, 0.5 mM MgCl_2 induces a kink that resembles the kinks found in the presence of only KCl. At 2.5 mM the kink position shifts to $q \approx 0.5 \text{ nm}^{-1}$ and its shape more closely resembles those found in the presence of only MgCl_2 . Due to the different shapes of the kinks, it is likely that they do not stem from the same type of structural features. We therefore assume that at low MgCl_2 concentration the effect of the monovalent ions is stronger, whereas an increasing MgCl_2 concentration outperforms the influence of KCl.

This assumption is confirmed by the data for 80 mM KCl, where we observe fewer changes for different MgCl_2 concentrations in the scattering curves than at 20 mM KCl. Upon the addition of 0.5 mM MgCl_2 , the second local minimum (at $q \approx 1.2 \text{ nm}^{-1}$) vanishes and the first one moves to slightly smaller q . Simultaneously, the scattering at low q increases. These trends continue for 1.5 mM MgCl_2 , but the shape of the kink comes to resemble the shape observed in the absence of KCl. At 2.5 mM and 4 mM MgCl_2 the shape of the kink and

Fig. 3 The small angle scattering signal of vimentin recorded at different concentrations of two cation species. a),c) Changes in the small angle scattering and b),d) corresponding $p_c(r)$ of vimentin upon variation of the MgCl_2 concentration in the presence of a),b) 20 mM KCl and c),d) 80 mM KCl. The black curve was collected at low salt buffer conditions, the green and cyan curves in the absence of magnesium.

the subsequent slope of the curve closely resembles the features observed in the absence of KCl.

When we compare the pair distance distribution functions $p_c(r)$ (figure 3b,d), we see that in the presence of 20 mM KCl, the addition of MgCl_2 removes the shoulder at $r = 1.5$ nm. The peak moves to larger distances and broadens. In the presence of 80 mM KCl, the position of the peak moves less when MgCl_2 is added but the peak still broadens. These observations indicate that the presence of KCl reduces the effect, which MgCl_2 has on the filament structure and that this influence is stronger for higher KCl concentrations.

Taken together, we observe an increased diameter of the filaments upon increasing the concentration of added ions (monovalent or magnesium). We assume that the assembly mechanism (tetramers forming ULFs and then elongated filaments) remains the same regardless of the ion species. However, the different shapes of the curves point to slightly different organization of the tetramers relative to each other in the presence of magnesium as compared to standard filaments. The competitive addition of potassium ions reduces this effect.

3.3 Modeling of vimentin filaments

3.3.1 Determination of the form factor. The persistence length of vimentin filaments is on the order of 1-2 μm ⁴⁵ and therefore much larger than the accessible length scale of up to about 100 nm. For a tetramer the persistence length is smaller but even tetramers can be assumed as stiff rods on the measured length scales. Therefore, in order to quantify the structural changes in the vimentin filaments which lead to the changes in the SAXS curves, we model the radial electron density ρ_c of the vimentin filaments as long cylinders with a Gaussian electron density (corona) added to the boundary of the cylinder (figure 4b). The Gaussian electron density serves as a minimal model approach to roughen the filament surface¹³. The form factor for this kind of structures corresponds to the form factor of cylindrical micelles and has been derived by Pedersen⁴⁶. For a monodisperse solution (both in length and radius) it is given by

$$F(q) = N^2 \beta_s^2 F_s(q) + N \beta_c^2 F_c(q) + 2N^2 \beta_s \beta_c S_{sc}(q) + N(N-1) \beta_c^2 S_{cc}(q)$$

Here N is the number of Gaussian chains per filament and β_s and β_c are the total excess scattering lengths per chain of the core and the corona, respectively.

The normalized self-correlation term $F_s(q)$ for a filament with radius R is given by

$$F_s(q) = \left(\frac{2B_1(qR)}{qR} \right)^2 F_L(q, L)$$

where $B_1(qR)$ is the first order Bessel function of first kind and F_L , which depends only on q and the length L of the filament,

Fig. 4 a) Top: Domain organization of vimentin: boxes: α -helical segments with the property to form coiled-coil dimeric complexes; lines: mostly unstructured, non- α -helical segments; the amino-terminal segment is named "head", the carboxyl-terminal segment is termed "tail". Bottom: Schematic (not to scale) of the principle assembly steps for vimentin from tetramers to unit length filaments and extended filaments.

b) The radial electron density ρ_c of the IF model: The electron density within the central cylinder ($r < R$, light blue) is constant. At the edges of the cylinder, a Gaussian distribution with width R_G describes the flexible tail regions (dark blue). The total electron density is shown by a red line. c),d) Exemplary fits at 80 mM KCl and 4 mM MgCl_2 , respectively.

describes the scattering parallel to the filament axis. F_c is the scattering term of a single Gaussian chain whose radius of gyration is R_G as derived by Debye⁴⁷

$$F_c(q) = \frac{2(\exp(-(qR_G)^2) - 1 + (qR_G)^2)}{(qR_G)^4}.$$

The cross-term between the core and the chains is given by

$$S_{sc}(q) = \Psi(qR_G) \frac{2B_1(qR)}{qR} B_0(q(R+dR)) F_L(q, L)$$

where dR is the distance of the centre of the corona from the cylinder boundary, $\Psi(qR_G) = \frac{1 - \exp(-(qR_G)^2)}{(qR_G)^2}$ is the form factor amplitude of a Gaussian chain⁴⁸ and B_0 the zeroth order Bessel function of first kind.

Finally, the scattering between different chains in the corona is given by

$$S_{cc}(q) = \Psi(qR_G)^2 B_0(q(R+dR))^2 F_L(q, L)$$

Both the length of intermediate filaments and their persistence length are significantly longer (μm) than the structural range accessible by the recorded q -range⁴⁵. Therefore

$$F_L = \frac{\pi}{qL}$$

describes the longitudinal contributions well^{34,49–51}. As the length L of a filament is linear in the number of ULFs N_{ULF} ($L \approx lN_{ULF}$, where l is about 43 nm)⁴⁴, assuming a fixed number n of Gaussian chains per ULF we can rewrite

$$F_L N^2 = \frac{\pi}{qL} N^2 = \frac{\pi}{qlN_{ULF}} N^2 = n^2 N_{ULF} \frac{\pi}{ql}.$$

The complete form factor therefore becomes linear in N_{ULF}

$$F(q) = N_{ULF} \left[n^2 \beta_s^2 \frac{\pi}{lq} \left(\frac{2B_1(qR)}{qR} \right)^2 + n \beta_c^2 F_c(q) + 2 \cdot n^2 \beta_s \beta_c \frac{\pi}{lq} \Psi(qR_G) \frac{2B_1(qR)}{qR} B_0(q(R+dR)) + n^2 \beta_c^2 \frac{\pi}{lq} \Psi(qR_G)^2 B_0(q(R+dR)) \right]$$

For an ensemble of filaments of different length, the scattering hence does not depend on the length distribution of filaments but only on the total protein concentration.

To model the radial polydispersity, we estimate the standard deviation of the filament thickness distribution obtained by Mücke et al. using TEM at 100 mM KCl to 13.4 % of the average radius¹¹. We apply this distribution to the core radius and consider R_G monodisperse. Based on $p_c(r)$ for low KCl

concentrations, we extend the model by adding scattering from tetrameric vimentin based on the actually recorded scattering signal at low salt.

Additionally, we reduce the number of free parameters by introducing the total scattering from the filament core per length $\tilde{\beta} = N_{ULF} n^2 \beta_s^2 / l$, the ratio of scattering from the corona to scattering from the core $b = \beta_c / \beta_s$, and the average distance between Gaussian chains, $\lambda = l/n$. As we assume the Gaussian chains to be centered at the rod surface, we set $dR = 0$. Therefore, the scattering of the filaments can be described by six parameters: The mean filament radius R , the radius of gyration of the Gaussian chains R_G , the total scattering intensity of the core $\tilde{\beta}$, the scattering from a side chain normalized to the scattering from the core b , the average distance between Gaussian chains λ and the remaining amount of tetrameric vimentin. In the absence of MgCl_2 , we further need to fix the value of λ , as S_{sc} and S_{cc} contribute considerably less to the scattering than the other terms. Therefore, the fit result is weakly dependent on the prefactors of S_{sc} and S_{cc} resulting in an ill-posed problem. We choose $\lambda = 1.34$ nm, where we set the number of Gaussian chains equal to the number of vimentin monomers per ULFs to 32¹⁸.

Figure 4c,d show two representative fits at 80 mM KCl and 2.5 mM MgCl_2 , respectively. These fits describe the data very well, whereas attempts to reduce the complexity of the model, such as using a mono-disperse model or removing Gaussian chains, fail to reproduce the general shape of the scattering curves. All fit curves can be found in the supplemental info.

3.3.2 Quantification of structural parameters. Figures 5a,c and 6a,c show how the radius R and the radius of gyration R_G of the Gaussian chains depend on the KCl and MgCl_2 concentrations. For increasing potassium concentration, we find an initial strong increase in the filament radius followed by a slight continued increase at 100 mM KCl. For small potassium concentrations (10 and 20 mM) the radius of gyration is about as large as the actual radius, but for higher concentrations it is only about half as large. In contrast, the core radius changes much more strongly between different MgCl_2 concentrations. This change is accompanied by a continuous increase in the radius of gyration of the corona. Adding KCl reduces the effects of the MgCl_2 concentration: At 20 mM KCl the radius increases only slightly and the radius of gyration also remains smaller. At 80 mM KCl, the difference in radius is similar. When we consider the scattering intensities in terms of the forward scattering per length of the rod contribution $\tilde{\beta}$ and the relative (as compared to the rod) scattering intensity of a Gaussian chain b (figures 5b,d and 7b,d), we see that neither term shows a strong dependence on the KCl concentration. In contrast, from 0.5 mM to 2.5 mM MgCl_2 , the scattering from

Fig. 5 a),c) Radii of the core cylinder (rod contribution, red circles) and radii of gyration of the corona (blue stars) as obtained from fits to the model in the case of a) different KCl concentrations in the absence of MgCl_2 and c) different MgCl_2 concentrations in the absence of KCl. b),d) Scaling factors of the rod (red circles) and intra-chain scattering (blue stars) in dependence of the b) KCl and d) MgCl_2 concentration.

Fig. 6 a),c) Radii of the core cylinder (rod contribution, red circles) and radii of gyration of the corona (blue stars) as obtained from fits to the model for different magnesium concentrations in the presence of a) 20 mM KCl and c) 80 mM KCl. b),d) Scaling factors of the rod (red circles) and intra-chain scattering (blue stars) in dependence of the MgCl_2 concentration in the presence of b) 20 mM KCl and d) 80 mM KCl.

the rod increases by a factor of four while the forward scattering of the side chains increases by a factor of two. At 4 mM, the scattering from the corona decreases by 50 % whereas the contribution from the corona increases to about five times as high as the highest value found in the presence of KCl. Increasing the MgCl_2 concentration at 20 mM KCl results in a similar dependence, but the scattering from the unstructured regions increases continuously. At 80 mM KCl, the scattering from the rod stays mostly constant between 0.5 mM and 4 mM MgCl_2 and the contribution from the chain scattering rises steadily.

These quantitative results reveal a distinct change in the sub-filamentous organization of subunits between filaments formed in the presence of KCl and those formed in the presence of MgCl_2 . Moreover, the effect of magnesium decreases as KCl is added, indicating that the monovalent cations dominate the interaction of the coiled-coils within an IF.

3.4 Discussion

Our findings must be interpreted with respect to previously published information about vimentin assembly. In SAXS, we observe an increase of filament thickness for higher ion concentrations. This effect is stronger for magnesium ions than for monovalent ions. In TEM, this increase in thickness and difference between monovalent and magnesium ions is less pronounced. Absolute length scales such as filament diameters should be treated carefully when comparing SAXS studies and TEM studies due to differing sample treatment: TEM requires heavy chemical fixation with bifunctional cross-linkers, deposition to a charged solid support, drying and staining of the sample with heavy metal ions. By contrast, SAXS is a solution technique. We do not expect the numerical values of the diameter for TEM and for SAXS measurements to be exactly equal. Whereas TEM visualizes the surface of the filaments directly, but after staining and drying, SAXS is sensitive to the electron density distribution. The aqueous environment, in which the SAXS measurements take place, are likely to strengthen the influence of dissolved ions which interact with the filament surface and the tail regions. In agreement with this reasoning and in comparison to TEM studies at identical buffer conditions, the filament diameter we observe at 100 mM KCl is larger: 12.6 nm as opposed to 9.4 ± 2.7 nm¹¹. Moreover, it has to be noted that the diameter of filaments obtained from negatively stained samples depends on the respective staining procedure and on the experimentalist doing the measurements. Correspondingly, the numbers reported in the literature for intermediate filament diameters vary considerably^{52,53}.

By combining two complementary techniques, TEM and SAXS, we show that internal structural differences that are visible in the electron density distribution may not be

detectable by imaging the surface of the filaments only. The work by Lin et al., who found no change in filament morphology by TEM between 0 mM and 4 mM MgCl_2 ,¹⁹ also shows that such differences may not be visible in direct imaging. However, it has to be noted, that high concentrations of monovalent ions reduce the effect of magnesium ions on the structure which might explain why Lin et al. saw no effect, as they used an even higher monovalent ion concentration than the highest concentration used here (160 mM NaCl).

In addition to revealing that filaments formed in the presence of magnesium have an increased radius, we see that the size of their unstructured regions is slightly larger and their contribution to the SAXS signal increases with the magnesium concentration. This indicates that magnesium, which scatters more strongly than oxygen, carbon and nitrogen, locally accumulates in the unstructured regions. A similar idea has been presented by Lin et al.¹⁹ who attribute the cross-linking of vimentin networks by MgCl_2 to the negatively charged “tip-of-the-tail” in the vimentin monomer by employing progressively “tail”-shortened vimentin variants. The fact that we do not observe a change in the relative contributions of the Gaussian chains and the core region for KCl, which scatters even more strongly, indicates that there is no such local accumulation for potassium. This observation is in agreement with the finding that multivalent counter ions condense on polyelectrolytes, whereas for monovalent ions the electrostatic screening effect largely dominates.

The model-based quantitative description of the differences of the cross-section of vimentin filaments under different assembly conditions allows us to address the question how differences in the ionic environment affect filament assembly. Summing up, we can base our explanation on the following observations: (i) both monovalent and magnesium cations induce filament assembly where for magnesium ions lower concentrations are sufficient but assembly is much slower and in both cases a higher ion concentration results in thicker filaments; (ii) the sub-filament structure and compactness of the filaments, however, is specific for the ion species; (iii) magnesium, but not potassium, accumulates in the outer regions (the Gaussian corona in our model) of filaments, as also supported by previous rheology studies¹⁹; (iv) assembly consists of at least two, possibly concurrent, sub-steps: lateral association and elongation.

We conclude from these and previous observations which employed short, basic “head” peptides in the absence of salt that in order to start assembly the head domains have to be “unblocked” from strong, unproductive interactions acquired during renaturation of monomers from 8 M urea. In fact, tetrameric complexes form already in 5 M urea and further dialysis into low ionic strength buffers such as 2 mM sodium phosphate (pH 7.5) preserves this assembly state^{8,11}. Hence, in the absence of salt, these peptides compete for

binding sites on the acidic “rod” domains, unlock the heads and thereby mediate explosive assembly of tetramers into ULFs⁵⁴, filaments and filament networks⁵⁵

. In the work presented here, the unblocking is mediated by high concentrations of monovalent and low concentrations of magnesium ions, respectively. Thus, for monovalent ions we suggest an opening of the head region, mediated by electrostatics, which makes the elongation reaction of the filaments possible. At the same time higher monovalent salt concentrations screen the repulsion between individual tetramers and thereby facilitate the lateral assembly. This leads to thicker filaments, whereas at lower monovalent ion concentration the kinetics of the elongation dominate. Probably both the lateral assembly via electrostatic screening and the elongation via the opening up of the head region, depend on the monovalent ions, resulting in an overall similar filament structure. However, the lateral assembly is clearly influenced more strongly, resulting in thicker filaments for higher ion concentrations.

For magnesium ions we assume a similar effect, yet at much lower concentration. Furthermore, we propose an additional interaction: Just as magnesium can cross-link different vimentin filaments, we suggest that it also cross-links tetramers within an individual filament. This opens an additional pathway for lateral association resulting in thicker, but also less well ordered and less compact filaments. In addition, cross-linking magnesium ions increases the local magnesium concentration, which corresponds to the observed increase of scattering in the outer regions of the filaments. Also, a local increase in salt concentration may explain the much lower bulk concentration necessary in the case of magnesium ions as compared to monovalent ions.

When adding both monovalent and magnesium cations, an increasing overall ionic strength of the buffer due to higher monovalent ion concentrations reduces the relative importance of the “cross-linking” pathway for magnesium ions and possibly even reduces its effectiveness due to increased electrostatic screening. Both phenomena result in a shift to filament structures formed in the absence of magnesium cations, in agreement with our observations.

Finally, we note that in contrast to other biopolymer systems, such as actin, microtubules or DNA^{56–58}, neither the TEM images nor the scattering from vimentin filaments formed in the presence of both potassium and MgCl_2 at these concentrations show any indication of bundle formation. However, as MgCl_2 alone induces filament formation, it is not possible to draw a clear distinction between filament assembly inducing ions and bundling ions. Therefore, experiments on the potential bundling of vimentin by higher valency cations need to ensure the presence of structurally stable filaments before adding the higher valency ions^{22,59}.

4 Conclusions

Using SAXS, we investigate the association state of dimers and tetramers within a vimentin filament. We observe that the structure of vimentin filaments depends critically on the ionic environment, and we can quantify these changes using a simple, yet complete model. Our findings indicate that the ordering of vimentin tetramers in a filament depends on the valency of the assembly-inducing cations. We suggest that unblocking of the head-domains is the critical step and can be realized in different ways. In the presence of monovalent ions, the heads are electrostatically unblocked. If magnesium ions are present, elongation takes much longer, but the end-state is similar (yet not exactly the same), since the cross-linked tetramers have to be transferred to the productive state first. In a competitive setting with both monovalent and magnesium ions present, the monovalent ions are able to “rescue” the productive state. Importantly, intermediate filaments form polymorphic structures with a different number of monomers per unit-length segment along one and the same filament, i.e. 32, 40, 48 and even more^{8,52}. This property easily explains why filaments assembled first in the divalent ion regime are compatible with further and very fast assembly after shift to the monovalent ion regime (see 1d). This structural “flexibility” may indeed be important for properties exhibited by intermediate filaments *in vivo*.

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