Lutetium(III)-dependent self-assembly study of ciliate 
Euplotes octocarinatus centrin

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Abstract

Ciliate Euplotes octocarinatus centrin (EoCen) is a member of the EF-hand superfamily of calcium-binding proteins, which often associated with the centrosomes and basal bodies. To explore the possible structural role of EoCen, we initiated a physicochemical study of the self-assembly properties of the purified protein in vitro. The native PAGE results indicate that only the integral protein shows multimers in the presence of Lu^{3+}. The dependence of Lu^{3+} induced self-assembly of EoCen on various chemical and physical factors, including temperature, protein concentration, ionic strength and pH, was characterized using resonance light scattering (RLS). Control experiments with different metal ions suggest that Ca^{2+} and Lu^{3+} bindings to the N-terminal domain of EoCen are all positive to the self-assembly of the protein, and Lu^{3+} exhibits the stronger effect, however, Mg^{2+} alone cannot take the same effect. The experiments of 2-p-toluidinylnaphthalene-6-sulfonate (TNS) binding and ionic strength demonstrate that the lutetium(III)-dependent self-assembly is closely related to the exposure of hydrophobic cavity. Control experiment on pH value with EoCen and the fragments of it, N-terminal domain of EoCen (N-EoCen), indicates that the electrostatic effect is of small tendency to be served as the main driving force in the self-assembly of EoCen. The specific oligomerization form of the protein was exhibited by cross-linking experiment.

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Keywords: Centrin; Lutetium(III); Calcium(II); Self-assembly

1. Introduction

Centrins are small acid proteins (about 170 residues and a molecular mass of 20 kDa) from the highly conserved Ca^{2+} binding EF-hand superfamily, which were identified in a variety of species from the eukaryote kingdom of protists, fungi, plants, and animals. Since the discovery of its first member, caltractin, in the green alga Tetraselmis striata [1], spatial and time distribution within the mitotic organizer centers (MTOC) and the critical role of these proteins in cell cycle control has been the subject of intensive investigation in cellular biology for the last decade [2–6]. Although it is apparent that centrins from different organisms have different functions, it is thought that there may be a common underlying molecular mechanism. This may involve a change in conformation of centrin on binding calcium with the specificity of the reaction being determined, and the signal being conducted, by interacting proteins [4].

Ciliate Euplotes octocarinatus centrin (EoCen) is first reported by our laboratory [7], which is cloned from E. octocarinatus, and the detailed biological function is unclear. EoCen is a protein of 168 residues, which shares about 60, 62 and 66% sequence identity with human centrin 1, human centrin 2 and human centrin 3, respectively, and shares approximately 50% sequence identity with the well studied EF-hand protein calmodulin (CaM). Like CaM, centrin consists of two independent domains tethered by
a flexible linker, each domain comprising a pair of EF-hand motifs of helix–loop–helix that can potentially bind two calcium ions [8]. Sequence analysis and the available experimental data suggest that a large contribution to the sequence variability among various centrins originate from the first 20-residue fragment and this N-terminal fragment constitute distinctive features among the members of the large CaM superfamily [9]. Wiech et al. [10] have reported that self-assembly exists in centrins from various sources, including green algae, yeast and human but in different levels. Based on the reports of Hu et al. [11], one can infer that the interactions in self-assembly of cenrin are similar with the interactions between protein and peptide, namely the hydrophobic interaction and the electrostatic interaction are all contributing to the polymerization of cenrin, but the specific mechanism and the predominant driving force are still not much clear. In the calcium-dependent self-assembly of human cenrin 2 (HsCen2) [6], a truncated form of HsCen2, lacking the first 25 residues (A25HsCen2), shows no detectable self-assembly, pointing to the critical role played by the N-terminal fragment in the supermolecular reorganization of HsCen2, namely the basic amino acids of the N-terminal domain interacting with the negative charge of C-terminal of another subunits may be essential for the self-assembly of HsCen2.

The lanthanide ions (Ln$^{3+}$), including La$^{3+}$, Tb$^{3+}$, and Lu$^{3+}$, due to the similarity to Ca$^{2+}$ in coordination chemistry, can compete with Ca$^{2+}$ at calcium-binding sites [12], and have usually been used to monitor the sensitized emission of them at 545 nm as the protein is irradiated at 280 nm in the tyrosine absorption band [13–17]. In the light of our previous work (unpublished results), the order of metal ion induced conformational change of EoCen is Ca$^{2+}$ > La$^{3+}$ > Tb$^{3+}$ > Lu$^{3+}$. In order to observe clearly, in this paper, we mainly characterize the Lu$^{3+}$ ions dependent self-assembly properties of EoCen, and further indicate that N-terminal domain may be the important portion of EoCen due to the conformational change induced by Lu$^{3+}$. Besides, our experiments using isolated domains or a truncated form of EoCen, through contrasting with the other centrins which belongs to different species, suggests that hydrophobic interactions may take a more significant effect than electrostatic interactions in the self-assembly of EoCen.

2. Materials and methods

2.1. Reagents and stock solutions

TNS (2-potluidinyl-naphthalene-6-sulfonate) and Hepes (4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid) were purchased from Sigma. Salts, EDTA and other chemicals utilized in protein purification were of reagent grade. The purchased from Sigma. Salts, EDTA and other chemicals (4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid) were added to the culture medium. The calcium stock solution (0.1 M) was prepared by dissolving CaCl$_2$ in a small volume of distilled water and was diluted into a certain volumetric flask with deionized water. The Mg$^{2+}$ stock solution (0.1 M) was prepared by a similar way. The pH buffers (0.01 M) were prepared respectively by the common analytical chemistry methods.

2.2. Construction and cloning of the domain N-EoCen (residues 1–101) and Δ23EoCen (residues 24–168)

The sequence and the cloning strategy of the full-length gene of EoCen have been described earlier [7]. Using the full-length gene as template, Δ23EoCen was obtained by PCR technique, primers are 5'-GCC GGATCC GAG AAG CAG AAG CAA GAG AT-3' and 5'-GCC CTC-GAG TTA GTA TAT TGA TGT CTG T-3'. The resultant PCR product was subcloned into a PGEX-6p-1 expression vector. The sequence of the clone was confirmed by commercial company. N-EoCen was obtained in the similar way and the primer has been reported previously [18].

2.3. Protein expression and purification

Recombinant proteins or domains of EoCen were over-expressed off a PGEX-6p-1 plasmid construct in Escherichia coli BL21 (DE3) induced with isopropyl-D-thiogalactopyranoside (IPTG) to yield milligram quantities of the desired protein as reported previously [7]. Briefly, transformed E. coli cells were grown in LB media containing 100 µg/mL ampicillin and incubated at 37 ºC while monitoring its growth via optical density (OD) measurements at 600 nm. Once OD600 reaches 0.6, a final concentration of 0.5 mM IPTG was added to the culture, and 3 h later, the cells were harvested and frozen. Frozen cells were thawed in PBS buffer and sonicated with a macro probe at mediate power on ice. This solution was centrifuged at 15,000g for 25 min at 4 ºC. The supernatant was applied to a Glutathione–Sepharose TM 4B column which has been equilibrated with PBS buffer. After the initial purification by washing the supernatant with PBS buffer, prescissor proteinase was added at 4 ºC for reacting about one night. Primary target proteins were washed out and eluted with PBS buffer. Then the proteins were applied to a superdex 75 column to be further purified. Fractions containing cenrin were identified via 15% SDS-PAGE. After purification, the proteins were kept in –80 ºC. The stock protein solutions were conserved in 0.01 M Hepes.

2.4. Native PAGE analysis

A 1 mg/ml solution of each protein sample in Hepes (0.01 M pH 7.4) was prepared for all of the native PAGE experiment. Polyacrylamide gels contained 390 mM Tris (pH 8.8), 10% ammonium persulfate, 15% acrylamide/bis (29:1), and 0.1% TEMED. Tris-glycine electrophoresis running buffer contained 25 mM Tris (pH 8.3), 250 mM...
glycine. All electrophoreses were run at room temperature. Gels were run at a constant current of 11–12 mA for 2 h. Gels were fixed in 50% methanol, 7% acetic acid for 1 h, washed in distilled water for 1 h, stained with Coomassie blue R-250 for 2 h, washed in distilled water for 2 h.

2.5. Resonance light scattering measurements

Solution turbidity changes were monitored by the change of fluorescence at a wave length 430 nm of the protein samples in quartz cells of 1 cm optical path. The slit width of excitation and emission was set at 10 nm. In our experiment, the resonance light scattering (RLS) signals were recorded using synchronous scanning from 250 to 700 nm. Using the same excitation and emission wave-lengths of 430 nm, time scanning of RLS was performed once the Lu$^{3+}$ was added to the protein solution at the given temperature. The dilution effect was correct in the similar way with fluorescence emission spectroscopy. The protein concentration is about 4.4 μM except for the experiment conducted on different protein concentration.

2.6. Fluorescence emission spectroscopy

TNS fluorescence experiments were measured by Hitachi F-2500 or Hitachi 850 at 37 °C, with all slit widths set to 10 nm. Excitation wavelength was set at 320 nm. Fluorescence emission spectra were recorded with a single scan over the range 350–600 nm. To correct the dilution effect, the fluorescence intensities were converted to molar fluorescence intensity by dividing the fluorescence intensity via the analytical concentration of EoCen in the titration curve. The protein solutions were prepared by dilution of the stock solution with 0.01 M Hepes pH 7.4. The protein concentration is about 4.4 μM.

2.7. Cross-linking analysis

Cross-linking experiment was carried out as following: 2 mg/ml solution of each protein sample in PBS was incubated with glutaraldehyde of different ratio at 37 °C for 10 min. Reactions were stopped by adding SDS sample buffer and boiling for 2 min. Cross-linked products were analyzed under reducing condition on 15% SDS-PAGE and detected by Coomassie blue R-250 staining.

3. Results

3.1. Lu$^{3+}$ induced self-assembly of EoCen

In our research, Lu$^{3+}$ induced self-assembly of EoCen was observed on the native PAGE (Fig. 1A), which is probably the most highly resolving electrophoretic method yet developed for separating proteins. When the [Lu$^{3+}$]/[protein] ratio was up to 3:1 or 4:1, a clear area band above the monomers was exhibits that corresponded to the multimers. In order to further explore the self-assembly, we overexpressed and purified two truncated EoCen variants: the isolative N-terminal domain of EoCen (N-EoCen) and the fragment which lacks the first 23 residues of EoCen (D$_{23}$EoCen). It had been reported that the N-terminal domain of human centrin 2 has a closed structure, binds calcium with a very low affinity, and plays a role in the
protein self-assembly [9]. In addition, the members of the centrin family are characterized by an additional N-terminal segment of about 20 residues with a basic character and high sequence variability [6]. In our experiment, Fig. 1D Lane 3 indicates that on the native PAGE, the position of D23EoCen (E24-Y168) is much closer to the bottom of the gel (the positive pole), exhibiting the much more negative net charge of it that is obviously different from the SDS-PAGE (Fig. 1C 3), in which the area band position of different proteins should be coherent with their molecular weight. While for N-EoCen (M1-E101), by contrasting with the SDS-PAGE (Fig. 1C 1), native PAGE (Fig. 1D 1) result showed the more positively charged property of it. However, in the same conditions, we could not detect any multimers of N-EoCen and D23EoCen on the native PAGE (Fig. 1B). These observations suggest strongly that electrostatic interaction truly exists in the Lu3+ induced self-assembly of EoCen, and the oligomerization requires the integral protein.

3.2. The temperature increase is positive to the self-assembly of Lu3+ saturated EoCen

Resonance light scattering (RLS) is a valuable technique for detecting and characterizing self-assemblies and extended aggregates of chromophores since the assemblies or aggregations lead to the formation of large fractal structures exhibiting strong RLS signals [19]. The thermal induced self-assembly of Lu3+ oversaturated EoCen (0.01 M Hepes, pH 7.4, 20 mM KCl, [Lu3+]/[EoCen] = 10:1) was performed at the given temperature using the time scanning of RLS. The relatively low KCl concentration was used in the buffer (20 mM) to keep the working temperature range under the physiological values. As clearly seen in Fig. 2A, with the increasing temperature, RLS at 430 nm reaches the higher and higher plateau, indicating the larger and larger size of the scattering objects. At the same time, the first order rate constant k was obtained using standard fitting procedures (Table 1). As shown in Fig. 2A inset, the constant k enhances with the increase of temperature, and exhibits a sharp change ranging from 298 K to 308 K, suggesting the most active self-assembly process in the physiological range. Meanwhile, Fig. 2B shows the influence of temperature on the enhanced RLS intensity (ΔRLS). It can be seen that apoEoCen is nearly not affected by the temperature. As for the more physiologically relevant Ca2+, it exhibits the similar results as Lu3+, but the intensity is far lower than the Lu3+ saturated EoCen.

3.3. Dependence on protein concentration

Increasing the protein concentration, while keeping the other physicochemical parameters (0.01 M Hepes, pH 7.4,

Table 1

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Fig. 2. (A) Resonance light scattering intensity at 430 nm as a function of time after the rapid addition of Lu3+ (44 μM) to the protein solution (4.4 μM EoCen in 0.01 M Hepes, pH 7.4, 20 mM KCl) at given temperature. Inset, the first order rate constant k (10^-3 s^-1) as a function of temperature. (B) The plot of the enhanced resonance light scattering of Lu3+ saturated EoCen ([Lu3+]/[EoCen] = 10:1) (c); Ca2+ saturated EoCen ([Ca2+]/[EoCen] = 100:1) (b); apoEoCen (a) at 730 s ex. temperature.
20 mM KCl, [Lu$^{3+}$]/[EoCen] = 10:1 at 37°C) constant, results in a faster self-assembly process and a higher plateau (Fig. 3). In these conditions, a concentration of approximately 5 μM appears as the minimum requirement for the experimental observations and is mainly used throughout this work.

3.4. The metal ions (Ca$^{2+}$/Lu$^{3+}$) binding to the N-terminal domain is more favorable to the oligomerization of EoCen

Lu$^{3+}$ as well as other metal ions induced polymerization was also performed in our experiment. When a solution of EoCen, buffered at physiological conditions (pH 7.4, 0.01 M Hepes, 37°C, 20 mM KCl), was added into the first 2 equivalent Ca$^{2+}$, RLS increases slightly, and with combining the additional 2 equivalent Ca$^{2+}$, RLS enhances remarkably (Fig. 4A). As for Lu$^{3+}$, in agreement with the native PAGE result (Fig. 1A), the similar results were observed when Lu$^{3+}$ took place of Ca$^{2+}$ as a probe to further detect self-assembly of EoCen. Fig. 4B showed that Lu$^{3+}$ induced polymerization of EoCen is about five times higher than Ca$^{2+}$. Being clearly contrasted with Ca$^{2+}$ and Lu$^{3+}$, it is nearly impossible to detect any increase in the RLS at the Mg$^{2+}$/protein ratio up to 7 (Fig. 4B), although both Ca$^{2+}$ and Mg$^{2+}$ are small, closed-shell, spherical metal ions, and are most physiologically relevant metal ions that interact with EF-hand proteins [20].

As a result of the comparatively weaker effect of Ca$^{2+}$, the multimer band of Ca$^{2+}$ induced oligomerization cannot be detected on the native PAGE. EoCen has two tight Ca$^{2+}$ binding sites (III/IV site) in the C-terminal domain and two weak binding sites (I/II site) in the N-terminal domain [21]. Therefore the results above indicate that the last two equivalent metal ions (Ca$^{2+}$/Lu$^{3+}$), namely the metal ions binding to the N-terminal domain, favor the polymerization of EoCen, furthermore, the Lu$^{3+}$ take more effect than Ca$^{2+}$. These results may be derived from the larger conformational change of N-terminal domain with binding metal ions (Ca$^{2+}$/Lu$^{3+}$) [21].

3.5. TNS binding

We applied 2-pterolidinylnapthalene-6-sulfonate (TNS) to confirm the hypothesis that the different levels of Ca$^{2+}$/Lu$^{3+}$ induced self-assembly are closely related the conformational change, namely the exposure of the hydrophobic
cavity. TNS is a hydrophobic fluorescence probe generally used to detect exposed apolar surfaces in protein structures [22,23]. Interaction of the probe with a hydrophobic sites result in an increased fluorescence intensity, usually accompanied by a blue-shift of the maximum. Fig. 5A shows the change of TNS fluorescence intensity induced by EoCen in the absence or presence of metal ions. Based on our reported results, the addition of TNS does not affect the metal ion binding characteristic of EoCen and full occupancy of the four binding sites by the similar ions, Tb³⁺, in the presence of saturated Ca²⁺ [21]. In our experiment, we compared the metal ion induced conformational change of EoCen by adding Lu³⁺ to the Ca²⁺ saturated solutions. Fig. 5B shows the TNS fluorescence intensity increases and the maximum shifts from 500 nm to 440 nm, indicating the probe transfers from the polar to the apolar environment. Moreover, for the [TNS]/[EoCen] = 1:1 ratio, the TNS fluorescence enhancement of Lu³⁺ saturated protein is remarkably higher than Ca²⁺ saturated protein and the enhancement is mainly originated from the last 2 equivalent Lu³⁺ binding to N-terminal domain of EoCen, suggesting strongly that the Ca²⁺/Lu³⁺ induced polymerization is closely related to the exposure of large
hydrophobic surface in N-terminal domain. N-terminal domain of centrin is more compact and better structured even in the absence of Ca$^{2+}$ [9]. Binding of the metal ions change the conformation of N-terminal domain from close or compact to open, and expose the hydrophobic cavity that constitute the binding site for the apolar residues that take effect in the process of self-assembly. On the same protein concentration, the RLS fluorescence enhancement for the 2 equivalent Lu$^{3+}$ binding to the isolative N-terminal domain of EoCen (N-EoCen) is equal with such Ca$^{2+}$ binding to the EoCen (Fig. 5A inset), which may be an explanation of the above results that the multimers of the N-EoCen cannot be detected on the native PAGE, reinforcing that although the hydrophobic cavity of N-terminal domain of EoCen plays an essential role in oligomerization, N-terminal half cannot take effect isolately, and the oligomerization requires the integral protein.

To investigate the role of the Lu$^{3+}$ induced hydrophobic surface in the EoCen self-assembly process, we assessed whether binding of a fluorescent probe interfere with the protein/protein interaction. As shown in Fig. 5B, in the presence of saturated Lu$^{3+}$ ([Lu$^{3+}$]/[EoCen] = 4:1, pH 7.4, 20 mM KCl at 37 °C), the increase of the TNS fluorescence intensity is accompanied by a considerable decrease in RLS. In addition, experiment conducted at different temperatures shows the similar decreasing tendency of RLS (Fig. 5C) and temperature is a positive factor (Fig. 5C inset) which is in agreement with the thermal induced self-assembly of Lu$^{3+}$ saturated EoCen. However, with the increase of the temperature, RLS exhibits the different increasing slope at different [TNS]/[protein] ratios, namely the higher ratio of [TNS]/[protein] the solution contains, the lower oligomerization the protein exhibits. The inhibitory effect of the hydrophobic probes strongly suggests that the primary binding sites of TNS are identical or close to the intermolecular interaction sites inducing oligomer formation. Moreover, the hydrophobic binding sites of TNS are largely exposed by the Lu$^{3+}$ binding to the N-terminal domain of EoCen, so we can demonstrate the idea that the hydrophobic binding sites which is exposed by the Lu$^{3+}$ are positively related to the interaction sites of polymerization.

The temperature profiles of the two observed parameters were further explored and are clearly different. Revealed by Fig. 5D, in the presence of saturated Lu$^{3+}$ ([Lu$^{3+}$]/[EoCen] = 4:1, EoCen/TNS (4.4 μM/22 μM), 20 mM KCl, pH 7.4, at 37 °C), the binding affinity of TNS at higher temperatures may be smaller than those driving the intermolecular associations, in agreement with the data in Fig. 5C inset.

3.6. Ionic strength experiment

The influence of the ionic strength on the enhanced RLS (ΔRLS) was studied by titrating KCl to the solution of Lu$^{3+}$ saturated EoCen (Fig. 6c, [Lu$^{3+}$]/[EoCen] = 4:1, pH 7.4, at 37 °C), Ca$^{2+}$ saturated EoCen (Fig. 6b, [Ca$^{2+}$]/[EoCen] = 100:1, pH 7.4, at 37 °C) and apoEoCen (Fig. 6a, pH 7.4, at 37 °C). With the increase of the KCl concentration, Lu$^{3+}$ saturated protein exhibits the sharply enhanced RLS (Fig. 6c). As for the physiologically relevant Ca$^{2+}$ (Fig. 6b) it shows a similar result as Lu$^{3+}$, and the final intensity is lower although the [Ca$^{2+}$]/[EoCen] ratio is up to 100:1. The high ionic strength was advantageous to the hydrophobic process but disadvantageous to the electrostatic binding [24]. The result demonstrates again that the self-assembly of Lu$^{3+}$ saturated EoCen is mainly the hydrophobic process. When the ionic strength is up to 150 mM, there exhibit an obvious decreasing tendency (data not shown), supporting the existence of electrostatic component in self-assembly. However, for apoEoCen (Fig. 6a), ΔRLS is not very detectable.

3.7. pH control experiment

pH dependence of Lu$^{3+}$ saturated EoCen ([Lu$^{3+}$]/[EoCen] = 4:1, 20 mM KCl, at 37 °C), in the range of common physiological values, was pointed out by our experiment. Fig. 7a shows that the RLS exhibits a peak at pH 7.4, indicating that the polymerization induced by Lu$^{3+}$ has the most effect at physiological pH. As well known, the neutral pH is the most suitable condition for the protein to carry on many physiological activities, so the result suggests that the Lu$^{3+}$ induced self-assembly of EoCen is closely related to the biological function of centrin. In addition, the self-assembly mechanism of apoEoCen and N-EoCen was pointed out by the pH control experiments. The result suggests that apoEoCen and N-EoCen exhibit completely different pH dependence. Fig. 7c indicates that the acid pH favors N-EoCen polymerization, whereas basic pH is an unfavorable factor. However, for apoEoCen
(Fig. 7b), an exactly opposite tendency is observed. ApoEoCen is an acidic protein, with a pI around 4.5, whereas the N-EoCen is comparatively basic (pI, 4.8) derived from richly containing basic amino acids. In fact, the more the pH value of the buffers deviates from pI, namely the more basic pH for our protein, the more the net charge of the proteins contains and the smaller is the effect of the electrostatic interactions in polymerization. Therefore, the result demonstrates again that although the pI of the two proteins are much close to each other, the self-assembly mechanism of N-EoCen is indeed different from apoEoCen in the electrostatic interaction component proportion. Namely, electrostatic attraction plays an essential role in the polymerization of N-EoCen, but for the integral protein apoEoCen, the electrostatic effect is comparatively weaker.

3.8 Cross-linking analysis

Fig. 8A exhibits tetramer band when the cross-linker, glutaraldehyde, in the extremely low concentration, and the figure shows dimer band gradually with increasing concentration of the cross-linker, indicating that for apoEoCen, the tendency of forming tetramer is more common than dimer. The conclusion is consistent with the result that EoCen exist by tetramer’s form in vivo, which has been demonstrated by western blotting analysis (X.J. He, unpublished results). Fig. 8B shows that for N-EoCen, the dimer hold the main position in multimers and a slight transition of dimer form of N-EoCen to high molecular mass multimers took place with the change of concentration gradient, which is different from the apoEoCen, namely N-EoCen polymerization is weaker than apoEo-

4. Discussion

On the basis of a number of physicochemical techniques, we characterized the self-assembly properties of the highly purified Ciliate *E. octocarinatus* Centrin (EoCen). Lu$^{3+}$ binding, especially to the N-terminal domain of EoCen, is necessary for the self-assembly process, which is closely related to the metal ion-binding properties of it. Additionally, the increases of temperature and protein concentration are all positive to the Lu$^{3+}$ induced EoCen self-assembly. It may be derived from the more opportunities of the protein subunit interact with others when the protein concentration increases, and the temperature effect may be attributed to the severer molecular mobility at high temperature and similarly increase the interaction opportunities. For other metal ions, Ca$^{2+}$ exhibits similar results as Lu$^{3+}$ but in lower levels, maybe due to the ion-potential of Ca$^{2+}$ ($e/r = 0.0202$) being much less than Lu$^{3+}$ ($e/r = 0.0353$), namely the more the ion-potential of the metal ion is, the larger conformation change of the protein the binding process induces and in the light of the above
results, this larger conformation change makes the larger exposure of the hydrophobic cavity, which is related to the Lu$^{3+}$ induced self-assembly of EoCen. As for the other biological ion, Mg$^{2+}$, it is different from Ca$^{2+}$ in many aspects, because of its much smaller ionic size. Obviously, a question was raised: the ion-potential of Mg$^{2+}$ is more than Ca$^{2+}$, however, Mg$^{2+}$ cannot take much more or even the same effect in the self-assembly of EoCen. Based on our unpublished results, Mg$^{2+}$ exhibits a binding constant to the N-terminal domain of EoCen on the order of 10$^2$ mol$^{-1}$ L$^{-1}$, whereas Ca$^{2+}$ has an obviously higher affinity (on the order of 10$^3$ mol$^{-1}$ L$^{-1}$) [12], which can be an explanation of the different self-assembly behaviors of the two similar ions on one hand. On the other hand, self-assembly of EoCen may have selectivity for the divalent cations binding to it.

TNS binding experiment and ionic strength experiment demonstrated that the Lu$^{3+}$ induced self-assembly of EoCen is dominated by the hydrophobic interaction. PAGE result suggests that electrostatic interaction truly exists in the self-assembly of EoCen, and the oligomerization requires the integral protein. Finally, control experiment affected by pH value indicates that in the process of EoCen self-assembly, although molecular interactions are mediated by both electrostatic and hydrophobic forces, the electrostatic interaction has the smaller tendency to be served as the main driving force. It may be quite different from HsCen2. Martine Tourbez et al. [6] demonstrate that the N-terminal fragment, with its +5 net charge, is essential for HsCen2 assemblies, and the interactions between N- and C-terminal domains from different sub-units play a crucial role in the observed process. The primary structure prediction analysis of EoCen about hydrophobic profile shows that in general the distribution of hydrophobic amino acids of EoCen is much more dispersing, and in the process of polymerization, the opportunity of apolar amino acids to interact with one another will be high due to the van der Waals force. As for HsCen2, the hydrophobic amino acids, which are nearly located in the N-terminal half of HsCen completely, are more concentrating. However, the polymerization of HsCen2 is not induced mainly by the hydrophobic interactions between the N-terminal half of two subunits. Maybe that is comparatively difficult to form the contacting surface between the two molecules, and the hydrophobic interactions does not occupy the dominant position in the polymerization of HsCen2. Considering the net charge (based on the amounts of Arg, Asp, Glu, and Lys, as may be clearly seen in Table 2) at the physiological pH, the net charge of HsCen2 is −6, and the removal of the first 25 amino acids makes the net charge of the Δ25HsCen2 up to −11, nearly two times higher than HsCen2. So the electrostatic interaction was destroyed intensely and polymerization was inhibited to a large extent, suggesting that the electrostatic interactions play an essential role in the self-assembly of HsCen2. Comparatively, at the physiological pH, EoCen, with the −13 net charges, even much more than the net charge of Δ25HsCen2, therefore has smaller tendency to form multimers by taking electrostatic interaction as the main driving force.

In addition, the biological significance of the present results is that Lu$^{3+}$ induced self-assembly of EoCen happened acutely within the range of cell parameters, including the sharp enhancement of the first order rate of self-assembly at 37 °C as well as the promoting effect on RLS at pH 7.4, the relatively lower concentration of KCl (<20 mM), suggesting the Lu$^{3+}$-dependent self-assembly may be closely related to kinds of cellular functions in the physiologically active phase.

As is well known, oligomerization is mediated by Ca$^{2+}$ which, of course, is more physiologically relevant. As a similar ion like Ca$^{2+}$, similar results can be observed with Lu$^{3+}$ which exhibits a stronger effect than Ca$^{2+}$, such as temperature, ionic strength, and the N-terminal effect. The results can provide us a chemical foundation to further research the biological effect of lanthanide ions.

We carried out the experiment to elucidate the main driving force in the process of polymerization and tried to evaluate the equilibrium that may exist between the self-assembly tendency of centrins and the interaction with specific target proteins [25]. A better understanding of the driving force in self-assembly and a better description of the polymerization mode may help us to find the modulate mechanism of centrins in the centrosomes and basal bodies.

### 5. Abbreviations

- **EoCen**: ciliate *Euplotes octocarinatus* centrin
- **N-EoCen**: N-terminal domain of EoCen
- **Δ23EoCen**: a truncated form of EoCen which lacks the first 23 residues
- **HsCen2**: human centrin 2
- **CaM**: Camodulin
- **RLS**: resonance light scattering
- **TNS**: 2-ptoluidinylnaphthalene-6-sulfonate
- **PBS**: phosphate-buffered saline
- **Hepes**: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
- **LB**: Luria Bertzani
- **IPTG**: isopropyl-p-thiogalactopyranoside
- **TEMED**: N,N',N''-tetramethylethylenediamine

### Acknowledgements

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References