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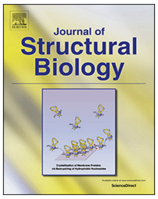

Reductive power of the archaea right-handed coiled coil nanotube (RHCC-NT) and incorporation of mercury clusters inside protein cages

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ABSTRACT

Coiled coils are well described as powerful oligomerization motifs and exhibit a large diversity of functions, including gene regulation, cell division, membrane fusion and drug extrusion. The archaea S-layer originated right-handed coiled coil –RHCC-NT- is characterized by extreme stability and is free of cysteine and histidine moieties. In the current study, we have followed a multidisciplinary approach to investigate the capacity of RHCC-NT to bind a variety of ionic complex metal ions. At the outside of the RHCC-NT, one mercury ion forms an electrostatic interaction with the S-methyl moiety of the single methionine residue present in each coil. We demonstrate that RHCC-NT is reducing and incorporating metallic mercury in the large-sized interior cavities which are lined up along the tetrameric channel.

1. Introduction

The α-helical coiled coil is the most frequently encountered subunit oligomerization motif found in proteins (Burkhard et al., 2001; Lupas and Bassler, 2017; Lupas and Gruber, 2005). Typically, coiled coils consist of two to five right-handed amphipathic α -helices that are intertwined to a left-handed superhelix. Based on the presence of undecad repeats, a right-handed coiled-coil structure has been proposed for the C-terminus of the surface layer glycoprotein tetrabrachion from the hyperthermophilic archaebacterium *Staphylothermus marinus* (*S. marinus*) (Peters et al., 1996; Peters et al., 1995). The 1.7 MDa tetrabrachion protein complex consists of four identical subunits that form an α -helical coiled-coil stalk of 70 nm length, which is anchored to the cell membrane at its C-terminal end and branches into four arms of 24 nm length at its N-terminus. The arms form a canopy-like meshwork by end-to-end contacts, enclosing a “quasi-periplasmic space” (Mayr et al., 1996). *S. marinus*, isolated from the periphery of black smoker vents, is a strict anaerobic sulfur reducing archaeon that employs these extremely stable proteinaceous S-layer as protective shield arranged in a 2D-network coating the archaeon (Hao and Ma, 2003; Fiala et al., 1986).

A 24 kDa segment of this tetrabrachion forms the tetrameric right handed coiled-coil nanotube (RHCC-NT) (Stetefeld et al., 2000). RHCC-NT is a cysteine -and histidine free protein segment that has extreme stability with respect to temperature, pH and salinity (McDougall et al., 2017). The tetramer structure is stabilized by a complex pattern of complementary hydrophobic interactions between neighboring helices Fig. 1). The side chain packing of the core residues at the undecad **a** and **h** positions point into the center of the tetramer and are packed in a perpendicular and parallel manner, respectively. As a consequence, four large cavities of the tetramer are connected to a continuous central channel, which is exclusively lined with aliphatic side chains. The cavities lay in between the middle of the **d** and **e** layers and have sizes in between 150 $Å^3$ and 360 $Å^3$. In the native structure, the cavities are occupied with water molecules. A large buried surface of ∼9.500 $Å^2$ provides further support that the hydrophobic interactions are likely the main cause for the extraordinary thermal stability of the tetrabrachion stalk domain which resists denaturation at 130 °C in the presence of strong denaturants such as 1% (w/v) dodecyl sulfate or 6 M guanidine (Peters et al., 1995; Mayr et al., 1996).

A characteristic feature of the RHCC-NT sequence is the high content (∼27%) of charged residues. On the basis of distance criteria, all charged residues with the exception of Glu 6 and Asp 43 appear to form intra- and/or interhelical salt bridges. The three intra- and four

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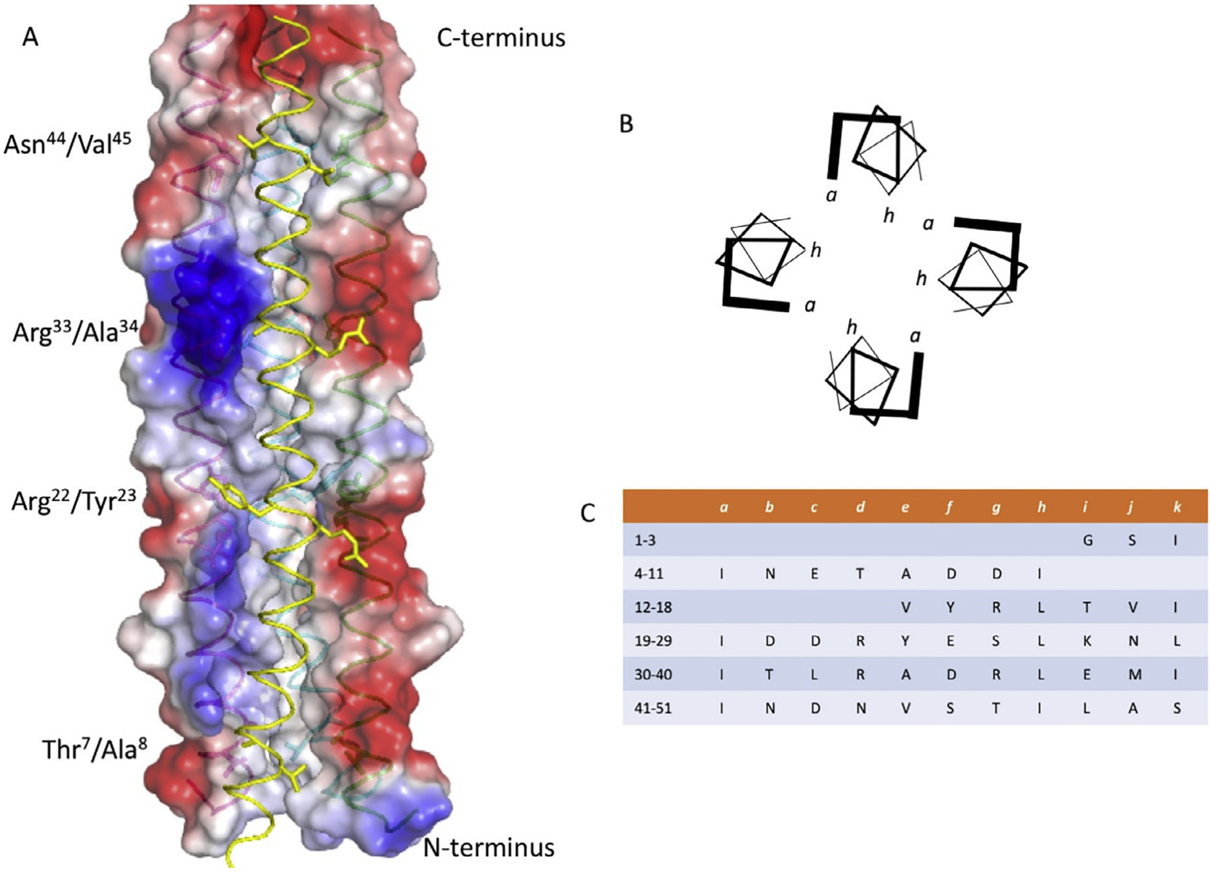

	a	b	c	d	e	f	g	h	i	j	k
1-3									G	S	I
4-11	I	N	E	T	A	D	D	I			
12-18					V	Y	R	L	T	V	I
19-29	I	D	D	R	Y	E	S	L	K	N	L
30-40	I	T	L	R	A	D	R	L	E	M	I
41-51	I	N	D	N	V	S	T	I	L	A	S

Fig. 1. The right-handed coil (RHCC-NT). (A) Overall presentation (open book) of the tetrameric channel. Amino acid residues in de-layers are highlighted. The surface presentations is reflective for an electrostatic presentation with positively and negatively charged residues colored in blue and red, respectively. (B) Helical wheel presentation of the 11-residue repeats. Only residues in a and h position form the knobs-into-holes packing, equivalent to a 7,4 –pattern. (C) Sequence of RHCC-NT aligned to the undecad repeat pattern.

interhelical salt bridges are occupied in all chains of the tetramer crystal structure. This frequency of favorable electrostatic interactions has not been observed previously in any other coiled-coil structure solved to atomic resolution and may be explained in terms of the hyperthermophilic nature of the protein.

The four large cavities of RHCC-NT were identified originally as highly efficient binders for heavy atom derivatives to determine the first crystal structure of a naturally occurring right-handed coiled coil (Stetefeld et al., 2000; Ozbek et al., 2005). In subsequent studies, the uptake of anti-cancer drugs such as cis-Pt/PTCl$_4$ was exploited to develop targeted Carrier-Pathfinder systems (Eriksson et al., 2009; Thanasupawat et al., 2015; McFarlane et al., 2009). In addition, QM/MM studies revealed unique gas phase properties for the hydrophobic cavities upon coordination of uranyl complexes (Odoh et al., 2011). Most recently, the uptake of elemental sulfur S8 crowns has been shown by mimicking black smoker conditions. In conjunction with atomistic MD simulations it is clear, that filling the large-sized cavities has a highly favorable transfer free energy (Yin et al., 2007).

The incorporation of metals into *de novo* proteins has been reported extensively in the literature (Peacock, 2016). Most of the reported studies concentrate on artificially designed coiled coils or helical bundles with a focus on the insertion of cysteine and histidine side chains to allow for mono –and multinuclear chelation of different metal compounds (Matzapetakis et al., 2002; Peacock, 2013; Zastrow et al., 2011). The design of aspartate and asparagine moieties in trimeric coiled coil motif has been proven to be efficient for sequestration of ionic metals in the hydrophobic core of the peptide elements (Berwick et al., 2014; Hartmann et al., 2009). On the other hand, the sulfur-containing side chain of methionine is unbranched and provides considerable conformational flexibility combined with unusually large polarizability of the sulfur atom itself (Gellman, 1991). There are different studies showing, for example, a water-shielding effect of thioethers of methionine residues (Orriss et al., 2010) or the coordination of Platinum (IV) via Met39 of RHCC-NT (Thanasupawat et al., 2015). The thioether moiety provides a common qualitative feature for binding metal ions by providing a center of high hydrophobicity contrast (Yamashita et al., 1990).

In this study, the capability of the naturally occurring RHCC-NT to interact with bivalent and trivalent cations, such as Hg $^{2+}$, Cu $^{2+}$ and Au $^{3+}$ was investigated. The research goal was to determine what if any type of bivalent cation is capable of binding to RHCC-NT, in a thiol -and imidazole-free environment. The authors have performed an integrated approach combining anomalous dispersion experiments at synchrotron sources with determinations of the reductive power via X-ray photoelectron spectroscopy. The data presented indicate that RHCC-NT has not only a strict reductive power for bivalent cations, it also incorporates reduced mercury clusters inside the hydrophobic cavities.

2. Results

To study the uptake of heavy metal complexes by RHCC-NT we followed an integrated approach combining X-ray photoelectron spectroscopy (XPS) and synchrotron-based X-ray crystallography (XRD).

2.1. XPS to study reduction behavior

X-ray photoelectron spectroscopy was undertaken to determine the oxidation state of the metal atoms incorporated into the RHCC-NT