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Apo and Calcium-Bound Crystal Structures of Cytoskeletal Protein Alpha-14 Giardin (Annexin E1) from the Intestinal Protozoan Parasite *Giardia lamblia*

Puja Pathuri[1], Emily Tam Nguyen[1], Gabriel Ozorowski[1], Staffan G. Svärd[2,3] and Hartmut Luecke[1,4,5,6*]

[1]*Department of Molecular Biology and Biochemistry, University of California, Irvine, CA 92697, USA*

[2]*Department of Cell and Molecular Biology, Uppsala University, SE-751 24 Uppsala, Sweden*

[3]*Microbiology and Tumor Biology Center, Karolinska Institute, SE-171 77 Stockholm, Sweden*

[4]*Department of Physiology and Biophysics, University of California, Irvine, CA 92697, USA*

[5]*Department of Information and Computer Sciences, University of California, Irvine, CA 92697, USA*

[6]*Center for Biomembrane Systems, University of California, Irvine, CA 92697, USA*

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Alpha-14 giardin (annexin E1), a member of the alpha giardin family of annexins, has been shown to localize to the flagella of the intestinal protozoan parasite *Giardia lamblia*. Alpha giardins show a common ancestry with the annexins, a family of proteins most of which bind to phospholipids and cellular membranes in a Ca^{2+}-dependent manner and are implicated in numerous membrane-related processes including cytoskeletal rearrangements and membrane organization. It has been proposed that alpha-14 giardin may play a significant role during the cytoskeletal rearrangement during differentiation of *Giardia*. To gain a better understanding of alpha-14 giardin's mode of action and its biological role, we have determined the three-dimensional structure of alpha-14 giardin and its phospholipid-binding properties. Here, we report the apo crystal structure of alpha-14 giardin determined in two different crystal forms as well as the Ca^{2+}-bound crystal structure of alpha-14 giardin, refined to 1.9, 1.6 and 1.65 Å, respectively. Although the overall fold of alpha-14 giardin is similar to that of alpha-11 giardin, multiwavelength anomalous dispersion phasing was required to solve the alpha-14 giardin structure, indicating significant structural differences between these two members of the alpha giardin family. Unlike most annexin structures, which typically possess N-terminal domains, alpha-14 giardin is composed of only a core domain, followed by a C-terminal extension that may serve as a ligand for binding to cytoskeletal protein partners in *Giardia*. In the Ca^{2+}-bound structure we detected five bound calcium ions, one of which is a novel, highly coordinated calcium-binding site not previously observed in annexin structures. This novel high-affinity calcium-binding site is composed of seven protein donor groups, a feature rarely observed in crystal structures. In addition, phospholipid-binding assays suggest that alpha-14 giardin exhibits calcium-dependent binding to phospholipids that coordinate cytoskeletal disassembly/assembly during differentiation of the parasite.

Keywords: Giardia; annexin; calcium binding; flagella; cytoskeleton

*Corresponding author. Department of Molecular Biology and Biochemistry, University of California, Irvine, CA 92697, USA. E-mail address: hudel@uci.edu.

Abbreviations used: PEG, polyethylene glycol; EDTA, ethylenediaminetetraacetic acid; PI, phosphatidylinositol; PtdIns(4)P, phosphatidylinositol 4-phosphate; PtdIns(3,4,5)P_3, phosphatidylinositol (3,4,5) trisphosphate; PS, phosphatidylserine; PA, phosphatidic acid; PE, phosphatidylethanolamine; DAG, diacylglycerol; PC, phosphatidylcholine; PG, phosphatidylglycerol; GST, glutathione *S*-transferase; EGTA, ethylene glycol bis(β-aminoethyl ether) *N*,*N*′-tetraacetic acid; FOM, figure of merit; BSA, bovine serum albumin; MAD, multiwavelength anomalous dispersion.

Introduction

Giardia lamblia (syn. *Giardia intestinalis*, *Giardia duodenalis*) is a flagellated intestinal protozoan that triggers a form of diarrhea called giardiasis in humans and other mammals worldwide.[1,2] Its life cycle alternates between a dormant, water-resistant cyst that is able to survive the acidity of the stomach and an actively swimming trophozoite that attaches, colonizes and divides in the upper small intestine of the host.[3] Infection typically starts when cysts are ingested with contaminated water, food or through direct fecal–oral transfer.[4] After the cysts pass through the stomach, the parasite undergoes excystation, a process whereby the cysts transform into flagellated trophozoites.[3] The trophozoites attach themselves to the epithelial cells lining the small intestine with the help of an adhesive disk that functions as a suction cup.[5] To ensure transmission, *Giardia* completes its life cycle when it undergoes encystation into cysts and is excreted through the feces.[6]

Giardia is able to maneuver within the small intestine to locate an optimal environment with the aid of four pairs of flagella (anterior, posterior-lateral, caudal and ventral) and subsequently attaches itself to the epithelial cells in the intestine to avoid being swept away *via* the adhesive disk.[7] Since the flagella and adhesive disk are essential for survival, there is a strong connection between the cytoskeleton and *Giardia* virulence.[7] Previous studies have identified the association of a class of cytoskeletal proteins called alpha giardins to the *Giardia* cytoskeleton.[6] Alpha giardins, a multigene family consisting of 21 members, show a common ancestry with the annexins, a family of cytosolic proteins that bind to phospholipids in a Ca^{2+}-dependent manner and are implicated in numerous membrane-related processes.[6–9] A phylogenetic analysis of all alpha giardin sequences showed that the alpha giardins are early representatives of the annexins; therefore, it is expected that key structural motifs are conserved between the alpha giardins and the rest of the annexin family, suggesting that they share similar biological functions.[6] Alpha giardins have been shown to localize to cytoskeletal components such as the flagella, the adhesive disk and the plasma membrane and may be active participants in the highly organized membrane and cytoskeletal rearrangements during excystation and encystation.[6] At

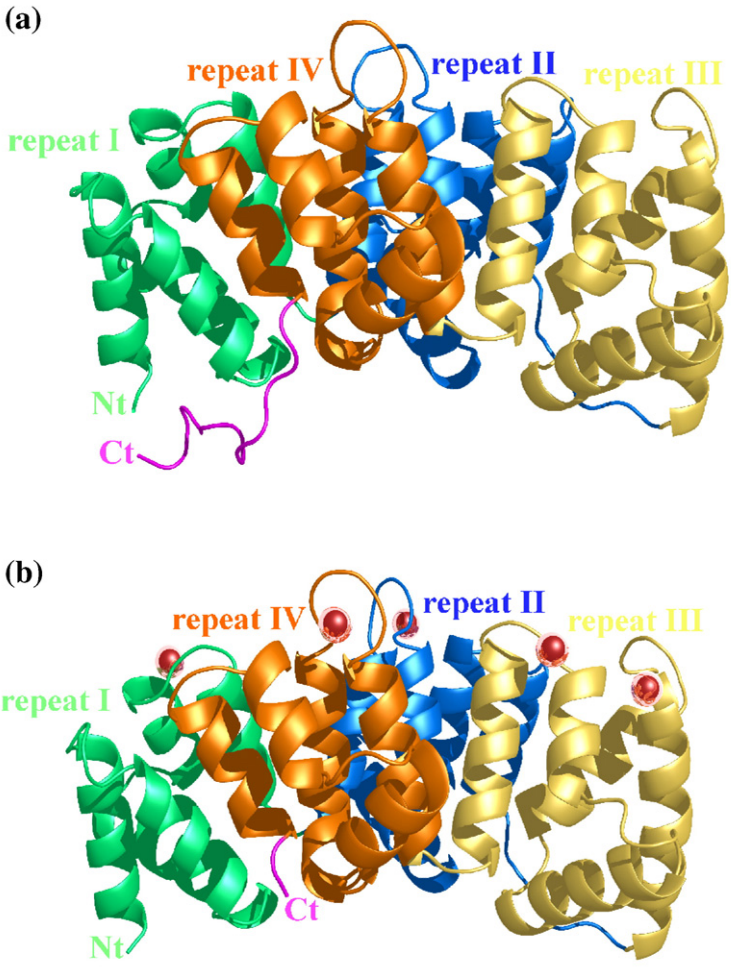

Fig. 1. Ribbon diagrams of the apo *R*3 crystal form (a) and Ca^{2+}-bound form of alpha-14 giardin (b). Repeat I is shown in green, repeat II in blue, repeat III in yellow, repeat IV in orange and the C-terminus in magenta. The N- and C-termini are labeled as Nt and Ct, respectively. Calcium ions bound to the AB and DE loops in the Ca^{2+}-bound alpha-14 giardin structure are illustrated as red spheres. The figures were produced with the programs PyMOL (http://pymol.sourceforge.net/) and POVRAY (http://www.povray.org/).