Journal of Inorganic Biochemistry 115 (2012) 28–35

Contents lists available at SciVerse ScienceDirect

Journal of Inorganic Biochemistry

journal homepage: www.elsevier.com/locate/jinorgbio

Femtomolar Zn^{2+} affinity of LIM domain of PDLIM1 protein uncovers crucial contribution of protein–protein interactions to protein stability

Małgorzata Sikorska, Artur Krężel *, Jacek Otlewski *

Department of Protein Engineering, Faculty of Biotechnology, University of Wrocław, Tamka 2, 50–137 Wrocław, Poland

ARTICLE INFO

Article history:
Received 7 March 2012
Received in revised form 24 May 2012
Accepted 28 May 2012
Available online 2 June 2012

Keywords:
LIM domain
Zinc finger
PDLIM1
Protein stability
Zinc buffering

ABSTRACT

An individual LIM domain has approximately 55 amino acids with 8 highly conserved residues responsible for binding of two Zn^{2+} into two distinct zinc finger motifs. We examined LIM domain stability of PDLIM1 protein (known also as Elfin protein), its C-terminally extended constructs as well as separate zinc fingers, and several full domain mutants in terms of Zn^{2+} affinity and domain stability. Thermal denaturation, mass spectrometry, limited proteolysis, protein oxidation and circular dichroism techniques were used to determine a set of thermodynamic stability parameters. The results demonstrate unambiguously very high (femtomolar) affinity of both Zn^{2+} to the conserved LIM domain ($K_d^{av} = 2.5 \times 10^{-14}$ M) and its additional elevation in the C-terminally extended domain construct ($K_d^{av} = 3.1 \times 10^{-15}$ M). We demonstrate in the example of PDLIM1 using a set of LIM protein constructs and its zinc finger peptides that stability of the entire zinc-containing domain is not only defined by the Zn^{2+} coordination environment but significantly depends on the set of protein–protein interactions with the C-terminus of the protein. We discuss structural similarities of LIM domains and suggest the prolongation of the conserved LIM sequence to its C-terminal helix that has a significant impact on domain stability. We also discuss the functionality of LIM domains in terms of different physiological zinc and redox buffering capacity.

1. Introduction

The majority of fundamental biological processes are based on proper cellular signaling pathways. One of the key requisites that is important for accurate signal transduction is correct assembly of the signaling protein complex. Among modular domains constructed in a cassette-like fashion that specifically interact with target protein are LIM domains [1]. Due to their capacity for specific protein interactions, frequently in tandem mode, LIM domains can place targets in discrete subcellular locations, modulate function and participate in a highly complex protein system [2].

PDLIM1 protein (known also as Elfin, PDZ and LIM domain protein 1, Clim1) is a 329 amino acid protein built with PDZ, ZM and LIM domains, all important in protein–protein interactions. It is also known as PDZ and LIM protein 1, belongs to the Enigma protein family, and is an important player in cytoskeleton organization, neuronal signaling, cell lineage specification, organ development, and oncogenesis [3]. It was found at high levels in nonmuscle cells, where PDLIM1 localizes to actin stress fibers and associates with α-actinin [4].

An individual LIM domain typically conforms to the consensus $CX_2CX_{16\text{-}23}HX_2CX_2CX_2CX_{16\text{-}21}CX_2(C/H/D)$ with highly conserved eight amino acid positions required for Zn^{2+} coordination [1]. These residues – mostly Cys and His – form two distinct zinc fingers (ZF1 and ZF2) in tandem topology, significantly different from those in other double zinc finger domains such as PHD, RING, and MYND domains [5]. Two zinc ions, crucial for the stability and proper structure of the LIM domain, are bonded tetrahedrally (Fig. 1A). Surprisingly, so far there are no systematic studies that compare biophysical data of double and single zinc finger domains that participate in protein–protein or protein-DNA interactions. Their various biological functions may reflect different protein stability and Zn^{2+} affinity of the protein. The dissociation constant value (K_d) established experimentally in vitro for Zn^{2+} binding to single ZF peptides varies usually from 10^{-8} M to 10^{-13} M and corresponds to their biological functions [6,7]. However, a recent single zinc finger stability reinvestigation shed more light on the experimental methodology, showing that some of the values known to date might be underestimated [8]. Stability of small zinc domains such as zinc fingers is not exclusively controlled by their direct coordination environment built mostly with sulfur and nitrogen donors (i), but also depends on ligand interactions with the second coordination sphere (ii) [9]. This inter-sphere network may significantly modulate stability of the protein as well as its functionality [10]. The third factor that affects entire domain stability and has an important impact on metal ion affinity is the electrostatic or hydrophobic network within the domain (iii). The hydrophobic core in classic $\alpha\beta\beta$ zinc fingers formed with Phe, Tyr and Leu residues is responsible for both stable domain structure formation and elevation of Zn^{2+} affinity [11]. The latter is mostly due to a rigid structure that hinders the access of water molecules to Zn^{2+} and decreases its exchange.

* Corresponding authors. Tel.: +48 71 375 2824; fax: +48 71 375 2608.
E-mail addresses: krezel@biotech.uni.wroc.pl (A. Krężel), otlewski@protein.pl (J. Otlewski).

0162-0134/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.jinorgbio.2012.05.009

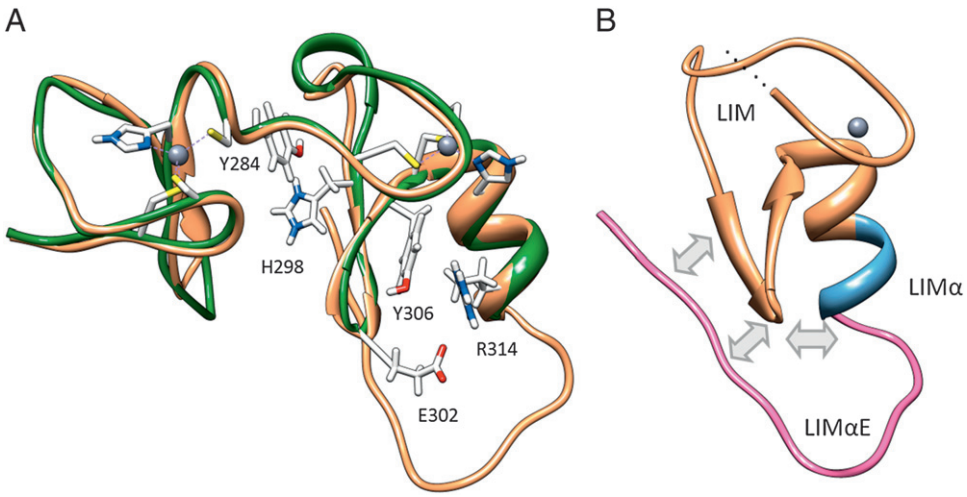

Fig. 1. (A) The comparison of NMR structure (PDB: 1×62) of LIM domain of PDLIM1 protein (green ribbons) with I-TASSER best model of 73 amino acid C-terminal tail of the protein (brown sandy ribbons). Residues discussed herein are indicated with relevant amino acid sequence number. (B) Presentation of C-terminus of LIM domain (zinc finger ZF2) and its extended constructs (I-TASSER model). Arrows indicate regions of hydrophobic core and hydrogen bond formation.

Here, we present thermodynamic studies of the highly conserved double zinc finger domain of PDLIM1 protein and critical impact of the C-terminal tail of the domain on entire LIM domain stability and Zn^{2+} affinity.

2. Materials and methods

2.1. Materials

Trizma base (Tris), N-2-hydroxy-ethylpiperazine-N′-2-ethanesulfonic acid (HEPES), 2,2′-dithiodipyridine (DTDP), 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), D,L-dithiothreitol (DTT), ethylenediaminetetraacetate disodium salt dihydrate (EDTA), tris(2-carboxyethyl)phosphine hydrochloride (TCEP), 4-(2-pyridylazo)-resorcinol monosodium salt (PAR), β-D-thiogalactopyranoside (IPTG), phenyl methyl sulfonyl fluoride (PMSF), reduced glutathione (GSH), $ZnSO_4 \cdot 7H_2O$, $MgSO_4$, NH_4HCO_3, NaOH, and NaCl were purchased as highest purity from Sigma-Aldrich. Chelex 100 resin (100–200 mesh, sodium salt) was from Bio-Rad. All pH buffers used in this studies were treated with Chelex resin to chelate contaminated heavy metal ions. Stock solution of Zn^{2+} was calibrated spectrophotometrically with PAR [12]. Single zinc finger peptides (ZF1 and ZF2) were custom-synthesized by CASLO Laboratory ApS (Lyngby, Denmark).

2.2. Cloning and mutagenesis

Three DNA fragments encoding LIM domain (256–312 amino acid residues of human PDLIM1 protein), LIMα (256–315), LIMαE (256–329) and ZF2αE (283–329), were prepared by two polymerase chain reactions (PCRI and PCRII) using the plasmid pOTB7 [RZPD ID: IRAUp969D1176D6] containing PDLIM1 cDNA as a template in the first reaction and the oligonucleotides with att*B* sites necessary for a subsequent Gateway recombination reaction. All used oligonucleotide primers are listed in Supplementary data (Table S1). After the PCRII, products were cloned into plasmid pDONR201. The ligated constructs were amplified in *Escherichia coli* DH5α cells. Multiplied plasmids were further isolated using Qiagen Plasmid Mini Kit and recloned into the plasmid pDEST15. The ligated constructs were amplified in *E. coli* DH5α cells, and plasmids were isolated as earlier using Plasmid Mini kit (A&A Biotechnology). All mutants were generated using the modified Quickchange Site-Directed Mutagenesis method [13]. Template products of PCR were digested with DpnI enzyme and the mutated constructs were amplified in *E. coli* DH5α cells. Multiplied plasmids were isolated using Qiagen Plasmid Mini Kit and their accuracy was verified by DNA sequence analysis (Genomed, Poland).

2.3. Protein expression and purification

pDEST15 expression vectors were transformed into *E. coli* strain BL21(DE3)CodonPlus-RIL competent cells. For large-scale production of GST-tagged protein cells transformed with the appropriate plasmid were grown at 37 °C in LB medium containing 100 μg/ml ampicillin until optical density 0.8. Then protein production was induced with 0.5 mM IPTG for 20 h at 17 °C. The final cell pellet was resuspended in 50 mM Tris–HCl, 150 mM NaCl, 1 mM PMSF, pH 7.5 (buffer A), and sonicated followed by centrifugation. The lysate was loaded on 10 ml of glutathione-Sepharose 4B beads (GE Healthcare Life Sciences) and incubated for 1 h at 4 °C. The beads were then washed with buffer A. The protein was finally eluted with 15 mM GSH in buffer A. The resulting protein solution was subjected to rTEV protease cleavage (48–72 h, 4 °C) to separate the protein from the GST-tag and concentrated using Vivaspin (3000 MWCO) centrifugal filters (Millipore). The protein was purified by gel filtration on a Superdex-75 FPLC column equilibrated in buffer A at 4 °C. The purity and identity of the proteins was confirmed by 15% SDS gel electrophoresis, as well as by API 2000 ESI-MS spectrometer or MALDI TOF/TOF 488 Plus (Applied Biosystems). All buffers were treated with Chelex resin before they were used to eliminate Zn^{2+} contamination from chemicals.

2.4. Zn^{2+}-depleted protein preparation

In order to obtain the apoform of a particular protein construct, a sample containing the concentrated protein from the above purification was incubated overnight at room temperature with 0.2 M DTT at pH 8.6 and then acidified to pH ~2 using 1 M HCl and loaded onto a Sephadex G-25 (Bio-Rad) gel filtration column as described previously [14]. The column was equilibrated with degassed 0.01 M HCl and 0.5 ml fractions were collected into 50–60 microtubes. The elution profile was analyzed by absorption detection at 220 and 280 nm. The concentration of apoprotein was determined using Ellman [15] and Bradford [16] protein assays.

2.5. Zn^{2+} reconstitution of LIM and ZF constructs

Aliquots of reduced and purified apoform of a particular LIM or ZF2αE protein constructs were mixed with $ZnSO_4$ at pH ~2 under argon atmosphere in molar ratio 1:2.5 and 1:1.5, respectively. pH