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Metal ion – Dehydrin interactions investigated by affinity capillary electrophoresis and computer models

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ABSTRACT

Dehydrins are specialized proteins which are related to environmental stress tolerance in plants. The proteins can bind different metal ions and have versatile other functions such as reduction of reactive oxygen species and acting as transcription factor. The structure determination of proteins from this family is challenging, since they have a high number of disordered structure elements. Consequently, to determine the functionality of these proteins on a molecular basis a computed model is helpful. This work focuses on a model for the *Arabidopsis thaliana* dehydrin AtHIRD11. To develop a model which reflects experimental data from literature and own binding data from affinity capillary electrophoresis experiments, a more rigid state of this protein was chosen. The Cu^{2+}-complex of this protein was formed and evaluated. The model explains some of the properties of the complexes. Possible Cu^{2+}-bindings site were found and the change of conformations were investigated via molecular dynamics simulation. The AtHIRD11-Cu^{2+}-complex is a first approach towards a complex model for a structural versatile protein, which is already sufficient to explain binding data and possible structure elements.

1. Introduction

Plants have to adapt to environmental changes such as e.g. extreme temperatures, drought, and high salinity.

To accomplish the protection of its cells, higher plants developed the late embryogenesis abundant proteins like the dehydrins (Hong-Bo et al., 2005). Dehydrins have manifolds of functions during drought conditions or other circumstances with high ion concentrations. They have been reported to bind to phospholipids, reduce reactive oxygen species by complexing Cu^{2+}-ions, bind other ions and water, complex Ca^{2+}-ions after phosphorylation, protect enzymes, interact with the cytoskeletons and bind DNA using Zn^{2+}-ions as cofactor (HARA, 2010).

In order to fulfill all these different functions, the protein has to be very flexible to interact with other involved proteins, DNA segments, phospholipids and ions. This is achieved by a lack of higher order protein structures (HOS). This concept is wide-spread likewise in other organisms e.g. in the human heat shock protein β-6 and the Alzheimer's disease associated tau protein. This type of proteins, also known as intrinsically disordered proteins (IDPs), do form HOS in presence of a functional binding partner (Ithuralde et al., 2016; Hara et al., 2011). Furthermore, the structure in aqueous solution for many of these proteins is still not solved and the binding sites have mostly not been described yet.

This work focuses on the KS-type dehydrin AtHIRD11 (Uniprot identifier: Q9SLJ2) originally derived from *Arabidopsis thaliana*. The main purpose was to characterize metal ion binding sites and use information from literature and own mobility-shift affinity capillary electrophoresis (ACE) data to create a model which describes the known facts for an AtHIRD11-Cu^{2+}-complex, such as the change in structural rigidity by metal ions. For this reason a dual approach has been performed. During the experimental approach the binding behavior of AtHIRD11 towards the metal ions Ca^{2+}, Mg^{2+}, Ba^{2+}, Sr^{2+}, Mn^{2+}, Au^{3+}, Ni^{2+}, SeO_3^{2-} and Zn^{2+} was investigated. On the other hand, a theoretical attempt was made to compute a Cu^{2+}-complex in order to visualize the findings and support the interpretation of new experimental results.

Abbreviations: ACE, affinity capillary electrophoresis; CGE, capillary gel electrophoresis; HOS, higher order (protein) structures; IDP, intrinsically disordered protein; MD, molecular dynamics; RMSD, root-mean-square-deviation

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2. Material and methods

2.1. Instrumentation

The computational work was performed on a Hewlett-Packard computer consisting of a 64 Bit system with four Intel® Core™ i5-3750 CPUs (3.4 GHz), 8 GB RAM and a GeForce GT630 graphics adapter with 6 GB RAM.

The ACE experiments were performed on an Agilent 1600A instrument (Agilent Technologies, Waldbronn, Germany). The capillary gel electrophoresis (CGE) separations were done using an Agilent 7100 CE (Agilent Technologies, Waldbronn, Germany). Both instruments used a DAD for detection.

2.2. Used software and force fields

The homology model was created using the software MOE (Chemical Computing Group Inc., 2013). The creation of the complex and first geometry optimizations were carried out by HyperChem Professional 8.0.10 (Froimowitz, 1993) using the AMBER96 force field. Subsequently, GROMACS 5.1 with the GROMOS 54a8 force field was used to determine the complex stability by molecular dynamics (MD) simulations (Van Der Spoel et al., 2005). For the visualization and the determination of RMSD-values (deviations from optimal bond angels and lengths of the complex) UCSF Chimera 1.8.1 (Pettersen et al., 2004) was used.

The net charge of the protein at the investigated pH was estimated using ProteinCalculator v3.4. This was developed by Chris Putnam at the Scipps Reseach Institute (http://protcalc.sourceforge.net).

2.3. Computational methods

The first step was the creation of a 3D-structure based on the amino acid sequence of the dehydrin AtHIRD11. For this reason MOE was used to align the sequence to a protein with a similar sequence. After finding a suitable structure, AtHIRD11 was adjusted to its structure and the geometry optimized with HyperChem 8.0.10.

The next step was finding suitable complexation sites for Cu^{2+}-ions. For this reason the structure was examined for residues which commonly form complexes with Cu^{2+}, such as histidine, glutamic acid, glutamine, aspartic acid, asparagine, cysteine and methionine (Zheng et al., 2008; Harding, 1999). Then a Cu^{2+}-ion was fitted in a potential binding site and the geometry was adjusted to its typical form (square-planar or tetrahedral) using HyperChem 8.0.10 and the AMBER96 force field. Subsequently, the stability was investigated using MD simulations. The first simulations were parameterized to equilibrate the complex. Therefore, the temperature was coupled to the pressure but the box size was kept flexible for a short time period. These settings were used due to Boyle's law. Since the box was heated up from 0 K to 300 K and the pressure should not change. The main MD simulation was set up for a short time period in aqueous solution (Table 1). The complex was considered as stable if it did not decompose during this 100 ps long simulation. This procedure was repeated until 7 Cu^{2+}-ions were added to the protein.

The complex stability of the whole complex was also evaluated for longer periods after a similar equilibration simulation. One MD simulation lasted for 2 ns and was repeated twice, while a further one lasted for 14 ns.

At the end the geometries of the metal ion centers and their deviation from the optimal bond angles as well as length were determined. In addition, a cluster analysis for the 14 ns MD simulation was performed.

2.4. Materials for the experimental part

In order to determine interactions between AtHIRD11 and different metal ions acetanilide, manganese(II) chloride, ethylenediaminetetraacetic acid (EDTA), barium chloride, sodium dodecyl sulfate (SDS) were purchased from Fluka (Steinheim, Germany). Gold(III) chloride, nickel(II) chloride, selene (IV) chloride, 2-amino-2-hydroxy-methyl-propane-1,3-diol (Tris) were acquired from Sigma-Aldrich (Steinheim, Deutschland). Zinc(II) chloride, strontium nitrate, calcium chloride and 37% hydrochloric acid were purchased from Merck (Darmstadt, Germany). Copper(II) chloride was obtained from Riedel-de Haën (Seelze, Germany).

The AtHIRD11 protein was expressed in *Escherichia coli* and provided by the group of M. Hara (Shizuoka University, Shizuoka, Japan) (Hara et al., 2011).

The CGE experiments were performed using the Beckman Coulter™ Proteome Lab™ SDS MW Gel Buffer (Beckman Coulter, Brea, USA).

The capillary electrophoretic separations were carried out using bare-fused silica capillaries obtained from Polymicro Technologies (Phoenix, USA). For ACE and CGE an inner diameter of 50 µm were chosen. For the capillary gel electrophoresis experiments the total length was adjusted to 33 cm with an effective length of 24.5 cm (length to the detector window). In contrary, the ACE experiments used a shorter capillary with only 30 cm total length (effective length: 21.5 cm).

Table 1
Metadata for the used molecular dynamics simulations.

	Short-time stability testing	2 ns simulations	14 ns simulation
Time step	2 fs	2 fs	2 fs
Sampling frequency	1 ps	1 ps	1 ps
Total numbers of frames	101	2001	14001
Software package	GROMACS 5.1	GROMACS 5.1	GROMACS 5.1
Ensemble	*NpT* (isothermal – isobaric)	*NpT* (isothermal – isobaric)	*NpT* (isothermal – isobaric)
Thermostat	V-rescale, 300 K	V-rescale, 300 K	V-rescale, 300 K
Thermostat relax time	0.1	0.1	0.1
Barostat	Parrinello-Rahman (isotropic), 1 bar	Parrinello-Rahman (isotropic), 1 bar	Parrinello-Rahman (isotropic), 1 bar
Barostat relax time	2.0	2.0	2.0
Boundary condition	Periodic	Periodic	Periodic
Unit cell	Cuboid	Cuboid	Cuboid
Force field	GROMOS 54a8	GROMOS 54a8	GROMOS 54a8
Solvent	Water	Water	Water
Solvent force field	SPC	SPC	SPC
Electrostatics treatment	Cutoff, 1 nm	Cutoff, 1 nm	Cutoff, 1 nm

2.5. Preparation of the used solutions

2.5.1. Affinity capillary electrophoresis (ACE) solutions

The metal ion-containing solutions were made by dissolving the metal salts in 30 mmol/L Tris buffer. This buffer was prepared by dissolving 3.63 g Tris in 200 ml demineralized water. Subsequently, the pH was adjusted to 7.4 by adding HCl and filled up to 1000 ml with demineralized water.

This buffer solution was also used to dissolve acetanilide and the protein AtHIRD11. For preparing the 60 µmol/L acetanilide solution, 6 mg of the EOF-marker was dissolved in 100 ml 30 mmol/L Tris buffer.

Before the sample solution was made, the used amount of Tris buffer was filtrated via a 0.22 µm syringe. Then the protein was dissolved to create a 1 mg/mL AtHIRD11 solution, which was used for 60 h before the sample solution was changed due to strong alterations in the electropherograms.