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Magnesium and manganese binding sites on proteins have the same predominant motif of secondary structure

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HIGHLIGHTS

- BCH and BCB motifs are predominant for both Mg^{2+} and Mn^{2+} binders.
- "Asp-Xaa-His" motif is used for Mn^{2+} binding but avoided by Mg^{2+} ions.
- Number of amino acid residues necessary for Mg^{2+} ions binding is lower than for Mn^{2+}.
- Usually already bound Mg^{2+} and Mn^{2+} ions attract phosphates to BCH and BCB regions.
- Some BCH and BCB regions can coordinate Mn^{2+} with the help of phosphates only.

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ABSTRACT

Manganese ion (Mn^{2+}) can substitute magnesium ion (Mg^{2+}) in active sites of numerous enzymes. Binding sites for these two ions have been studied in two sets of protein 3D structures from the Protein Data Bank with the homology level lower than 25%. The structural motif "beta strand – binder – random coil" is predominant in both Mn^{2+} and Mg^{2+} coordination spheres, especially in functionally relevant ones. That predominant motif works as an active binder of those divalent cations which can then attract additional ligands, such as different phosphate-containing compounds. In contrast, such Mg^{2+} and Mn^{2+} binding motif as "GK(T/S)T" being the N-terminal part of alpha helices works as an active binder of phosphates which can then attract divalent cations. There are few differences between Mg^{2+} and Mn^{2+} coordination spheres responsible of the cation specificity. His residues are underrepresented in certain positions around Asp and Glu residues involved in Mg^{2+} coordination, while they are overrepresented in certain positions around Asp and Glu residues coordinating Mn^{2+}. The random coil region in the "beta strand – random coil – alpha helix" motif for Mg^{2+} binding is usually shorter than that in the same motif for Mn^{2+} coordination. This feature is associated with the lower number of binding amino acids (and lower levels of usage of such "major" binders as Asp and Glu) for Mg^{2+} (which is a hard Lewis acid) in comparison with those for Mn^{2+} (an intermediate Lewis acid).

1. Introduction

Magnesium is an essential chemical element for living organisms. Mg^{2+} cations serve as co-factors for numerous enzymes (Piovesan et al., 2012). Even though it is known that Mg^{2+} cations are usually bound by Asp and Glu residues (namely, by oxygen atoms from their carboxyl groups and sometimes also by their carbonyl groups from the main protein chain) (Brylinski and Skolnick, 2011; Dudev and Lim, 2007), there is a lack of information about amino acids situated around them (on their microenvironment). It is known that Mg^{2+} coordinating amino acid residues are overrepresented in random coil regions (in loops) (Dudev and Lim, 2007), while the predominant structural motif for Mg^{2+} binding has not been described yet.

Currently some information on the predominant structural motif for Mn^{2+} ions has become available (Khrustaleva, 2014). That motif was described as "beta strand – binder – random coil" (Khrustaleva, 2014). Interestingly, both alpha helix and other beta strand may follow that motif. So, there is a kind of contrast between Ca^{2+} binding EF-hands motif (alpha helix – loop – alpha helix) (Yáñez et al., 2012) and Mn^{2+} binding motifs (beta strand – loop – beta strand; beta strand – loop – alpha helix) (Khrustaleva, 2014). In this study we approved that Mg^{2+} ions follow the trend

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for such transition metal cation as Mn^{2+} and do not follow the trend for the cation of the element from the same alkali earth group. However, Mn^{2+} ions are usually bound by imidazole Nitrogen atoms from side chains of His residues (along with Asp and Glu), unlike Mg^{2+} cations (Brylinski and Skolnick, 2011). Results of this study showed that the presence of His in certain positions around Asp and Glu residues may be the criterion of Mn^{2+} specificity for a given binding site.

Indeed, Mg^{2+} and Mn^{2+} ions can replace each other in active centers of many enzymes including DNA-polymerases (Lakhin et al., 2013; Lakhin et al., 2014), RNA-polymerases, primases, adenylate and guanylate cyclases (Bahre et al., 2014), phosphatases (Ciancaglini et al., 1997) and kinases (González et al., 1984). Some part of evidences about the possibility of Mg^{2+} to Mn^{2+} transition in active centers of enzymes came from the results of *in vitro* experiments. Other evidences came from crystallographic experiments. So, it is currently unknown which cation is normally present in many of those active centers. Even the information from the Uniprot data base should be taken with some skepticism: probably, there is a kind of activity regulation mechanism working via metal cation replacement. For example, replacement of Mg^{2+} by Mn^{2+} in gyanylate cyclase increases the velocity of that reaction and makes the level of such secondary messenger as cGMP higher (Bahre et al., 2014). Consequences of the last event are numerous. On one hand, such cGMP level promotes releasing hormones production in hypothalamus that leads to the increase of sexual hormones production (Lee et al., 2007). On the other hand, cGMP level increase in neurons caused by manganese intoxication finally results in the development of neurological symptomatic similar to that during Parkinson disease (Sikk et al., 2013).

Obviously, both manganese insufficiency and manganese intoxication are dangerous conditions. If we consider manganese insufficiency (which is very rare in human but can be developed in domestic animals) than we would have to suggest that other ions (such as Mg^{2+}) may substitute Mn^{2+} in active centers of some enzymes in that condition and affect their activities (Hohle and O'Brian, 2014). During manganese intoxication Mn^{2+} ions may replace Mg^{2+} ions in active centers of other enzymes (Hohle and O'Brian, 2014). So, it is important to know features of coordination spheres making them more or less specific for Mg^{2+} and Mn^{2+}. Manganese intoxication can be observed in miners, metallurgy industry workers and welders, as well as in people who live near metallurgic factories and places where manganese ore is extracted by the way of mining (Spangler, 2012). Manganese compound (methylcyclopentadienyl manganese tricarbonyl) is used as a gasoline additive that makes the risk of manganese intoxication development higher for people living in areas with intensive traffic (Spangler, 2012). Some drugs for intravenous injections used by addicts (such as ephedron) contain high concentration of Mn^{2+} (left from chemical reaction between pre-drug and potassium permanganate in acidic medium) (Sikk et al., 2013).

One of the enzymes which can be activated by Mn^{2+} ions is human DNA polymerase iota (Lakhin et al., 2013). According to the results of *in vitro* experiments Mn^{2+} ion replaces Mg^{2+} ion in its active center and increases activity of the enzyme (Lakhin et al., 2014). This switch from one divalent cation to another may be a kind of regulatory process. Indeed, DNA polymerase iota is needed when other DNA polymerases are unable to continue replication because of the presence of chemically modified nucleotides in the template strand. DNA polymerase iota in these circumstances introduces a mutation into the growing strand and allows other DNA polymerases to continue replication. There are several 3D structures of human DNA polymerase iota with Mg^{2+} ions in the Protein Data Bank, while there are no such structures with Mn^{2+} ions yet. To suggest a possible Mn^{2+} binding site we used our "VVTAK Mn(II)" algorithm (http://chemres.bsmu.by) (Khrustaleva et al., 2014).

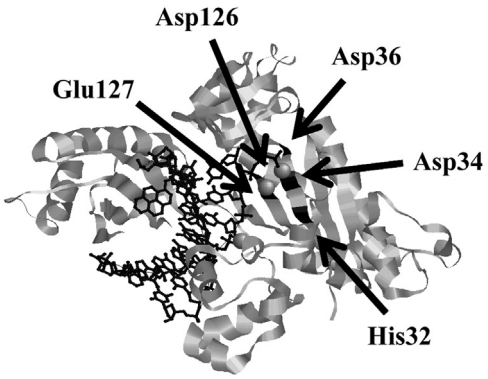

Fig. 1. The active site of the DNA polymerase iota with three Mg^{2+} ions and Mn^{2+} binding residues predicted by the "VVTAK Mn(II)" algorithm.

There are three Mg^{2+} ions coordinated by the DNA polymerase iota (PDB ID: 4EYH) (Kirouac et al., 2013). All of those ions are coordinated not just by atoms from amino acid residue of the enzyme, but also by oxygen atoms from phosphate anions of DNA and CDP (cytosine diphosphate). The first Mg^{2+} ion is coordinated by Asp34 and Leu35 residues together with CDP. The second one is coordinated by Asp34, Glu127, CDP and DNA. According to the results of Mn^{2+} binding site prediction, Asp34 is an "active binder" of those ions (Fig. 1). That residue is situated in the His-Xaa-Asp-Xaa-Asp motif frequently used for Mn^{2+} coordination (Khrustaleva, 2014). Moreover, Asp34 residue is situated in the end of the beta strand. It is known that such "beta strand – random coil – alpha helix" structural motifs are favorable for Mn^{2+} coordination (Khrustaleva, 2014). There are 4 possible "passive binders" which can complete the coordination sphere for Mn^{2+} (His32, Asp36, Asp126 and Glu127) at the distance less than 6 Angstroms around oxygen atoms from the Asp34 side chain. It is likely that Mn^{2+} ion will form a coordination bond with imidazole nitrogen atom from His32 residue unlike Mg^{2+} ions. This will cause a little change in cation position in the active center of the enzyme. The coordination sphere for Mn^{2+} will likely include Asp34, His32 and Glu127 together with phosphates from DNA and dinucleotides. Such subtle change in divalent cation and, probably, substrate position in the active center may be the cause of DNA polymerase iota activation.

Coming back to the third Mg^{2+} ion from 4EYH structure we can say that it is coordinated by amino acids rarely involved in cation binding (Lys237, Ile239 and Ile242) together with DNA. Probably, the third Mg^{2+} is primarily bound by phosphates from DNA itself, while some suitable atoms from the protein are just currently situated near that cation. Indeed, there are many enzymes which can coordinate both Mg^{2+} and Mn^{2+} together with phosphate containing compounds (Hirata et al., 2014; Gardner et al., 2014; Gao et al., 2012).

The situation described above raises several important questions on Mg^{2+} and Mn^{2+} coordination. Does the predominant structural motif for Mn^{2+} binding (beta strand – binder – random coil) also predominant for Mg^{2+} binding residues? Are there some structural features of binding sites which are specific for Mg^{2+} or Mn^{2+} ions only, or can Mn^{2+} ions replace Mg^{2+} in all the binding sites? Can active binders situated in the "beta strand – binder – random coil" motif bind cations without any help from additional ligands or is that motif a primarily phosphate binding site which can serve as Mg^{2+} and Mn^{2+} binding site if those cations have already been bound by phosphates? The current study is concentrated on the questions written above.