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In silico investigation of extracellular domain of RAGE receptor interaction with A-box and B-box of HMGB1 protein

In silico raziskava zunajcelične domene receptorja RAGE v interakciji z A-box in B-box proteina HMGB1

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Abstract: HMGB1 protein which is a non-histone chromosomal protein with two functional domains named A-box and B-box can also act as a signaling molecule after releasing from the cell and binding to the cell surface receptors such as RAGE. HMGB1 through its B-box domain binds to extracellular domain of RAGE and activates the signaling pathways involved in various pathological conditions like sepsis and tumor growth and metastasis. Interaction of recombinant HMGB1 A-box with RAGE antagonizes the RAGE activation by HMGB1. In the present study, interaction of human RAGE (hRAGE) extracellular domain (VC1C2) and B-box and A-box of human HMGB1 (hHMGB1) was investigated using a protein-protein docking software, HADDOCK. The results obtained were analyzed by PyMOL and LigPlot softwares. The results show B-box and A-box bind to different sites on the VC1 domain of RAGE and one of the B-box binding points is a positively charged groove located on the V domain surface which is also a major binding site for another RAGE ligand, Advanced Glycation End products (AGEs). The obtained results can be utilized to design new potent drugs for treatment of HMGB1-RAGE-related diseases such as cancer and sepsis.

Keywords: human HMGB1, human RAGE, HADDOCK software, cancer, sepsis

Izvleček: Protein HMGB1 je nehistonski kromosomski protein z dvema funkcionalnima domenama, A-box in B-box, ki lahko po sprostitvi iz celice deluje tudi kot signalna molekula in se veže na celično površino preko receptorjev kot je RAGE. HMBG1 se preko domene B-box veže na zunajcelično domeno RAGE in aktivira signalne poti, ki so vključene v različna patološka stanja kot so sepsa, rast tumorja in metastaze. Interakcija rekombinantnega proteina HMGB1 A-box z RAGE deluje antagonistično. V raziskavi smo preučevali interakcijo ekstracelularne domene (VC1C2) humanega RAGE (hRAGE) z B-box ter A-box humanega HMHB1 (hHMGB1). Uporabili smo računalniško orodje HADDOCK, pridobljene rezultate smo analizirali s programoma PyMOL in LigPlot. Rezultati so pokazali, da B-box in A-box vežeta na različna mesta domene VC1 na RAGE. Eno od vezavnih mest B-box je pozitivno nabita vdolbina na površini domene V in je hkrati glavno vezavno mesto za druge RAGE-ligande (Advanced Glycation End products – AGE). Rezultati raziskave so uporabni za načrtovanje novih zdravil za zdravljenje bolezni povezanih z interakcijami HMGB1-RAGE, kot sta rak in sepsa.

Ključne besede: humani HMGB1, humani RAGE, računalniški program HADDOCK, rak, sepsa

Introduction

Receptor for Advanced Glycation End products (RAGE) is a cell surface receptor belonging to the immunoglobulin superfamily (Neeper et al. 1992, Schmidt et al. 1992). RAGE receptor is involved in various pathological processes including inflammation (Orlova et al. 2007, Ramasamy et al. 2016), diabetic complications (Yamagishi et al. 2003, Litwinoff et al. 2015), cancer (Taguchi et al. 2000, Malik et al. 2015) and neurodegenerative disorders (Deane et al. 2003, Yan et al. 2003). The receptor structure is composed of 404 amino acids and three domains: an extracellular domain, a helical transmembrane domain and a short cytoplasmic domain. The extracellular domain which itself consists of three domains including a V-type immunoglobulin-like domain (V domain) and two tandem C-type immunoglobulin-like domains (C1 and C2 domains) is capable of binding to various biological ligands (Koch et al. 2010).

One of the RAGE ligands is High Mobility Group B1 (HMGB1) protein which binds to the V domain of extracellular part of RAGE receptor (Sorci et al. 2013, Musumeci et al. 2014). HMGB1 with 215 amino acids which also called HMG1 and amphoterin belongs to the superfamily of HMG proteins. The HMG proteins which were first isolated from calf thymus in 1973 are DNA-binding non-histone proteins with high content of acidic and basic amino acid residues (Goodwin et al. 1973). The highly evolutionary conserved HMGB1 protein in addition to the roles playing in the nucleus (Gerlitz et al. 2009, Lotfi et al. 2013), can act as a signaling molecule (cytokine) after releasing from the cell (Lotze and Tracey 2005). Today, the involvement of extracellular HMGB1 protein interaction with cell surface RAGE receptor in the various pathological states such as inflammation, sepsis (Zhu et al. 2010), growth and metastasis of tumor cells (Palumbo et al. 2004, Palumbo et al. 2009, Tang et al. 2010) and angiogenesis (Taguchi et al. 2000, Todorova and Pasheva 2012, He et al. 2017) has been confirmed.

HMGB1 structure consists of two tandem DNA-binding domains called HMG-box A and B (A-box and B-box) respectively and a long carboxylic tail rich in acidic residues. HMG-box domain which also exists in some other proteins, is consists of three alpha helices that are folded in the form of L, with an approximate angle of 80°. Although A and B boxes of HMGB1 have high similarity in structure, are functionally independent. As previously reported that the cytokine properties and the RAGE-binding site are located on the HMGB1 B-box (Huttunen et al. 2002, Tang et al. 2011). Surprisingly, the recombinant HMGB1 A-box can also bind to RAGE and antagonize the activation of RAGE by HMGB1 (Yang et al. 2004, LeBlanc et al. 2014).

In this study, the interaction of human RAGE (hRAGE) extracellular domain (VC1C2) with A-box and B-box of human HMGB1 (hHMGB1) has been investigated using protein-protein docking method. The results of this work show that B-box and A-box bind to distinct sites on the hRAGE VC1 domain and one of the binding pointes of B-box is a groove located on the V domain surface which also constitutes a main binding site for advanced glycation end products (AGEs). The results obtained from this work can be useful for designing new efficient drugs to treat HMGB1-RAGE-related diseases like sepsis and cancer.

Materials and methods

Searching PDB database to find the PDB structures

In order to study the interaction of A and B boxes of hHMGB1 with extracellular domain of hRAGE receptor (VC1C2) by protein-protein docking method, the PDB structures of three proteins were required. Thus, PDB website was searched to find the PDB structures corresponding to these proteins. The chain A of PDB entry 4LP5 (Yatime and Andersen 2013) which is the crystal structure of VC1C2 domain of hRAGE was selected as the receptor for the interaction study. The solution structure of hHMGB1 A-box (residues of 1 -84) has been also determined experimentally (PDB entry 2RTU) (Wang et al. 2013). 2RTU was used

as a ligand to study the interaction of A-box with extracellular domain of RAGE receptor.

Determination of three dimensional (3D) structure of B-box of hHMGB1 by I-TASSER web server

The I-TASSER server (Zhang-server) which operates based on the homology modeling method was ranked as No 1 protein structure prediction web server in CASP7, CASP8, CASP9 and CASP10 experiments (Yang and Zhang 2015). CASP (Critical Assessment of Techniques for Protein Structure Prediction) is a worldwide experiment for prediction of protein structure happening every two years since 1994 (Moult et al. 1995). The previous published data indicate that the residues of hHMGB1 which constitute the major site for hHMGB1-hRAGE interaction are located at the end of B-box and between B-box and C-terminal acidic tail (residues 150-183) (Huttunen et al. 2002). Although the structure of hHMGB1 B-box (residues 95-163) has been experimentally determined, there is no PDB structure covering all the residues responsible for the hHMGB1-hRAGE interaction. Thus, the 3D structure of residues 95-193 of hHMGB1 was determined by I-TASSER web server. For this purpose, the amino acid sequence corresponding to this part of hHMGB1 (UniProt entry: P094290) in FASTA format retrieved from UniProt website was given to the server. For each protein target, I-TASSER generates tens of thousands conformations (or decoys). I-TASSER clusters all the decoys according to the pairwise structure similarity and reports a total of five models corresponding to the five largest structure clusters. According to the Monte Carlo theory the largest clusters are related to the states of the largest partition function (lowest free energy) and thus possess the highest confidence. The confidence of each model created using I-TASSER is quantitatively measured by C-score. Although in most cases, the first model has higher C-score, since the top five models are ranked based on the cluster size, there is a possibility that the lower-rank models own a higher C-score. It is well known that the cluster size is more robust than C-score to rank the predicted models and therefore the first model is on average the most reliable and should be considered the best model unless there are certain biological and experimental reasons. C-score values vary between -5 to 2 (Yang et al. 2015). Between the five 3D models generated by I-TASSER, the first model (with highest C-score) was selected as the best model and applied for the interaction study as the B-box.

Study of interaction of hRAGE extracellular domain (VC1C2) with A-box and B-box of hHMGB1 using HADDOCK web server

HADDOCK 2.2 web server which is the most-cited data-driven protein-protein docking software was used for the protein-protein interaction study. In the data-driven docking methods such as HADDOCK, the available experimental data are used to set up the docking procedure and therefore the results obtained from these docking methods have more validity than *ab initio* docking methods (van Zundert et al. 2016). To perform the protein-protein docking process with HADDOCK, the easy interface of the web server was used. In addition to the PDB files of the interacting proteins, HADDOCK requires the active residues of them to perform the docking process. The HADDOCK software automatically finds the passive residues according to the given active residues. The active residues are directly contributing to the interaction process and surrounded by the passive residues which are not directly involved in this process. The active residues can be determined using the data obtained from the experimental methods or by means of CPORT web server (de Vries and Bonvin 2011). CPORT is an algorithm to predict the protein-protein interface residues. In fact, the CPORT is a combination of six interface prediction methods. For each HADDOCK run (Abox-VC1C2 and Bbox-VC1C2), the active residues of A-box and B-box were assigned according to the previously published experimental data (Huttunen et al. 2002, LeBlanc et al. 2014). Considering that there is no experimental data indicating that which residues of RAGE V domain are involved in the interaction with A-box and B-box, the active residues of 4LP5 chain A were predicted by CPORT server and only the active residues located in the V domain were selected for the docking process.

In each protein-protein docking run, HADDOCK generates hundreds of protein-protein conformations and classifies them into several clusters.

Each cluster specifies with a HADDOCK score. The HADDOCK score is a linear combination of various energies (van der Waals, electrostatic, desolvation and restraint violation) together with buried surface area (van Zundert et al. 2016). For each protein-protein docking (Abox-VC1C2, Bbox-VC1C2), the best conformation was selected according to the HADDOCK score and cluster size and analyzed by appropriate softwares.

The analysis of protein-protein interaction data

PyMOL software was applied to view and analyze the protein-protein complexes (Bbox-VC1C2 and Abox-VC1C2) obtained from HADDOCK software and LigPlot software (Wallace et al. 1995, Laskowski and Swindells 2011) was used to determine the amino acid residues involved in the protein-protein interaction process.

Results

The first I-TASSER model was selected as the B-box for the interaction study

As implied in the Materials and Methods, there is no PDB structure covering all the residues of hHMGB1 protein which are involved in the interaction with hRAGE receptor. Therefore, the 3D structure of residues 95-193 of hHMGB1 was determined by I-TASSER web server and the best model created by the web server was applied as the B-box for the interaction study. I-TASSER generated a total of five 3D models for B-box of hHMGB1. The model No. 1 with highest C-score (-1.16) was considered as the best model (Fig. 1). As shown in the Fig, B-box structure is composed of three alpha helices (I, II and III) connected with two loops and a long carboxylic tail.

hHMGB1 B-box through the residues located at helix III and C-terminal tail interacts with VC1 domain of hRAGE

As mentioned in the Materials and methods section, the Protein-Protein Docking Software, HADDOCK, was used to study the interaction of hHMGB1 Bbox with extracellular domain of hRAGE (VC1C2). To determine the residues involved in VC1C2-Bbox interaction, the results from HADDOCK were analyzed using DIMPLOT which is a part of the LIGPLOT software. DIMPLOT determines the residues of a protein-protein complex contributing to the formation of intermolecular hydrogen bonds. The results of this analysis are presented in Fig. 2. The surface representations of VC1C2 and B-box and the binding interface residues of two proteins (predicted by LigPlot) are shown in Fig. 3A. Figs. 3B and 3C represent the structure of HADDOCK VC1C2-Bbox complex in the surface and ribbon/sticks formats, respectively. As well seen in the Fig, the B-box binds to VC1C2 via the residues belonging to helix III and C-terminal tail. Although some Bbox-interacting residues of VC1C2 are located in C1 domain, most of the residues involved in the interaction belong to V domain.

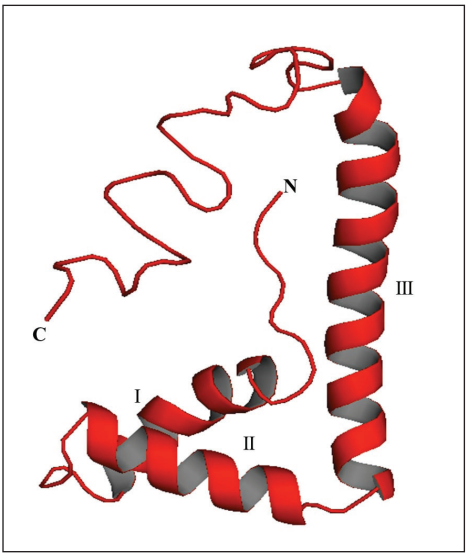

Figure 1: The best model created of residues 95-193 of hHMGB1 (referenced as the B-box in the text) by I-TASSER server.

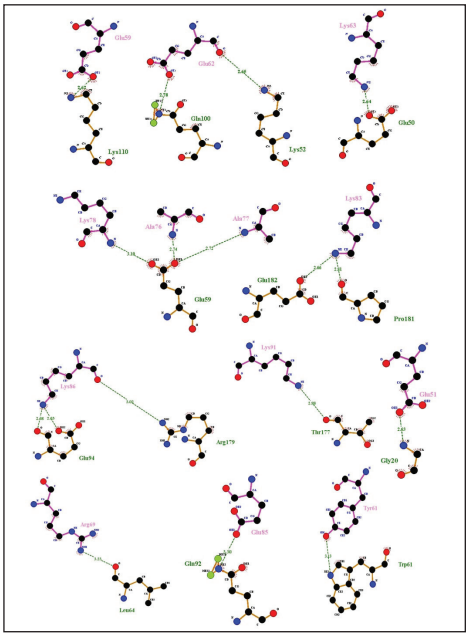

Figure 2: The amino acid residues of hHMGB1 B-box (titled with pink) and hRAGE VC1C2 (titled with green) interacting through hydrogen bonds in Bbox-VC1C2 HADDOCK complex predicted by LigPlot software. The hydrogen bonds are specified as green dotted line.

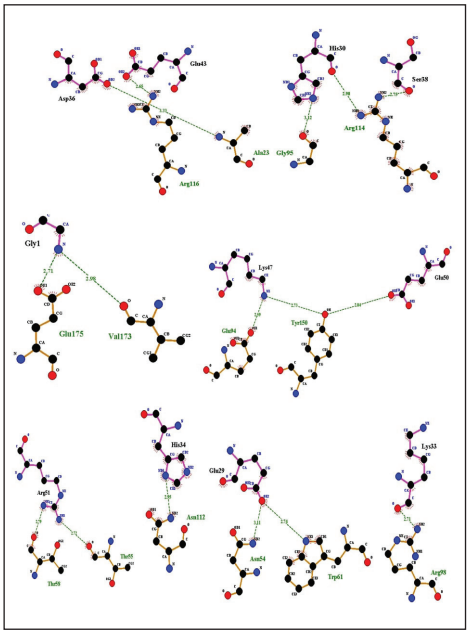

Figure 4: The amino acid residues of hHMGB1 A-box (titled with black) and hRAGE VC1C2 (titled with green) interacting through hydrogen bonds in Abox-VC1C2 HADDOCK complex predicted by LigPlot software. The hydrogen bonds are shown as green dotted line.

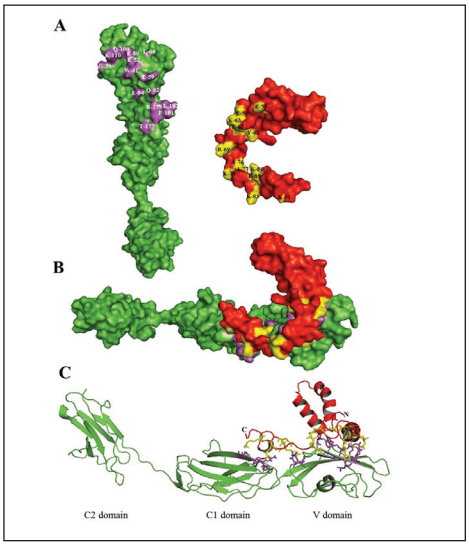

Figure 3: Illustration of VC1C2-Bbox interaction based on the protein-protein docking performed by HADDOCK and visualized by PyMOL. (**A**) The surface images of VC1C2 (green) and B-box (red) and the names and positions of their binding interface residues (predicted by LigPlot). The interfacial residues of VC1C2 and B-box are colored in purple and yellow, respectively. The surface (**B**) and ribbon/sticks (**C**) representations of the binding mode of VC1C2 protein (green) with B-box protein (red). The interfacial residues of two proteins (shown as sticks in the Figure 3C) are specified in purple and yellow, respectively.

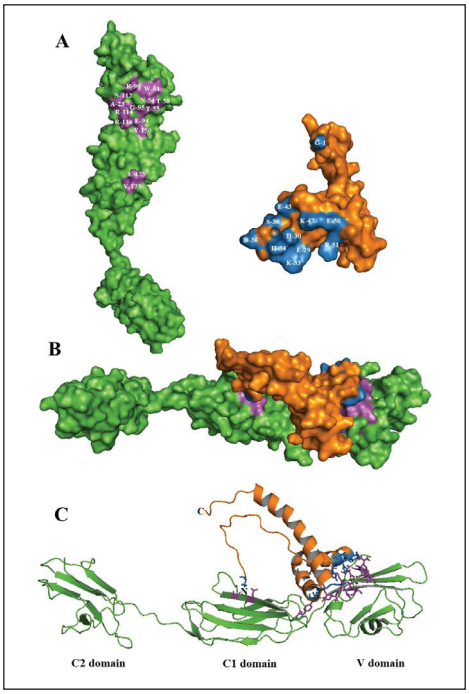

Figure 5: Illustration of VC1C2-Abox interaction based on the protein-protein docking performed using HADDOCK and viewed by PyMOL. (**A**) The surface presentations of VC1C2 (green) and A-box (orange) and the names and positions of their binding interface residues (predicted by LigPlot). The interfacial residues of VC1C2 and A-box are specified in purple and blue, respectively. The surface (**B**) and ribbon/sticks (**C**) representations of the binding mode of VC1C2 protein (green) with A-box protein (orange). The interfacial residues of two proteins (shown as sticks in the Figure 5C) are colored in purple and blue, respectively.

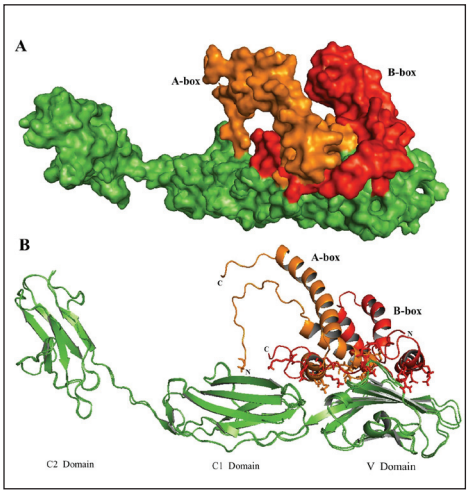

Figure 6: The superimposition of Bbox-VC1C2 and Abox-VC1C2 HADDOCK complexes using PyMOL software in surface (**A**) and ribbon/sticks (**B**) representations. In ribbon representation, the interfacial residues of B-box (red) and A-box (orange) are shown in sticks.

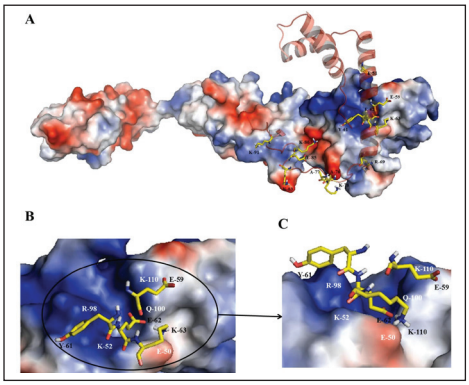

Figure 7: (**A**) The electrostatic potential surface of VC1C2 interacting with B-box (ribbon/sticks presentation). The names and positions of the binding interface residues of B-box (shown as sticks) are specified. (**B**) A close-up view showing that the VC1C2 surface groove created by Lys110, Arg98, Lys52, Glu50 and Gln100 constitutes one of the main B-box binding points. The names of the interfacial residues of B-box (shown in sticks) and VC1C2 are written in black and white, respectively. (**C**) The side chain of Glu62 fits into the groove.

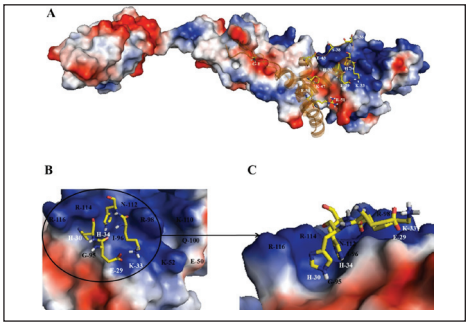

Figure 8: (**A**) The electrostatic potential surface of VC1C2 bound to A-box (ribbon/sticks format). The names and locations of the binding interface residues of A-box (shown in sticks) are specified. (**B**) A close-up view demonstrating that the groove generated by Lys110, Arg98, Lys52, Glu50 and Gln100 located on the VC1C2 surface does not constitute a binding point for A-box. The names of the interfacial residues of A-box (shown in sticks) and VC1C2 are written in white and black respectively. (**C**) The imidazole rings of His30 and His34 are located into the groove formed by the VC1C2 residues Arg114, Arg116, Asn112, Ile96 and Gly95.

Two first α-helices of A-box have a central role in the interaction with VC1C2

As mentioned before, the interaction of hHMGB1 Abox with VC1C2 domain of hRAGE was also investigated by means of HADDOCK software. The results of LigPlot analysis of VC1C2-Abox complex are shown in Fig. 4. In Fig. 5A, VC1C2 and Abox in the surface representation and the binding interface residues of two proteins (predicted by LigPlot) are shown. The structure of VC1C2-Abox complex obtained from HADDOCK software in the surface and ribbon/sticks formats are illustrated in the Figs. 5B and 5C, respectively. As it is clear from the results, in A-box, helix I, the first part of hellix II and the loop between them constitute the main site for biding to VC1C2 domain. The VC1C2 residues involved in the interaction are mostly from V domain, although a few residues from C1 domain participate in the interaction process.

A and B boxes of hHMGB1 interact with different regions of extracellular domain of hRAGE

Fig. 6 shows the VC1C2-Bbox and VC1C2-Abox complexes from HADDOCK software which are superimposed using PyMOL software in the surface (section A) and ribbon (section B) format. In the ribbon format, the residues of A-box and B-box involved in the interaction are shown as sticks. As seen in the Fig, A and B boxes of the hHMGB1 protein bind to different parts of the extracellular domain of hRAGE. Although the main binding site for both B-box and A-box is located on the V domain of hRAGE, the residues of this receptor interacting with these two proteins (Figs. 2 and 4), except for Trp61, are completely different. The B-box via its C-terminal half interacts with VC1C2 domain of hRAGE, whereas N-terminal half of the A-box participates in the interaction with this domain.

Discussion

As implied before in the text, residues 150-183 of hHMGB1 constitute the main binding site for the extracellular domain of hRAGE and the resulting activated signaling pathways contribute to the development of various cellular responses, such as inflammation and tumor growth and metastasis (Huttunen et al. 2002). In fact, the equivalents of these residues in the B-box created by I-TASSER are the residues 56-89. As it is clear in the Fig. 2, all the B-box residues contributing to the formation of intermolecular hydrogen bonds except for two residues (Glu51, Lys91) are compatible with the experimental published data. Analysis of the VC1C2 residues involved in the B-box interaction (Fig. 2) demonstrates that V domain constitutes the main interaction site, although some residues from C1 domain participle in this process.

According to the data published by LeBlanc et al. the A-box residues 23-50 are the main binding site for RAGE receptor and Abox-RAGE interaction antagonizes the activation of the receptor by HMGB1 protein (LeBlanc et al. 2014). The results obtained from investigation of Abox-VC1C2 interaction by HADDOCK are consistent with these experimental data. As mentioned in the

Materials and Methods, 2RTU which is the solution structure of hHMGB1 A-box, was selected as the A-box for the interaction study performed by HADDOCK. Of 87 residues of 2RTU, 1-3 are the protein expression tag and 4-87 are residues 1-84 (A-box) of hHMGB1 protein. Therefore, the RTU residues 26-53 are equivalent to the residues 23-50 (RAGE-binding site) of hHMGB1. The LigPlot analysis of the Abox-VC1C2 complex (Fig. 4) shows that all the A-box residues involved in the protein-protein interaction are located in this region (with the exception of Gly1). As mentioned above, this residue is not a major structural component of A-box and therefore, all the A-box residues participating in the interaction process are consistent with the experimental results already obtained (LeBlanc et al. 2014).

The electrostatic potential surface of VC1C2 protein created using the PyMOL software shows that the V domain molecular surface is mostly covered with positive charges. These positive charges in some areas are densely gathered and constitute a cationic center (Fig. 7A). One of these cationic centers is formed by Lys110, Arg98 and Lys52. These three residues together with Glu50 and Gln100 create a groove on the V domain surface (Figs. 7B and C). As obvious in the Fig. 7, this region of VC1C2 domain constitutes one of the main B-box binding points. The LigPlot analysis of the VC1C2-Bbox complex (Fig. 2) demonstrates that which residues of two proteins in this area contact with each other through hydrogen bonds. As it is clear, Glu59 of B-box makes a side chain-side chain hydrogen bond with Lys110 of VC1C2, Glu62 of B-box interacts with Gln100 and Lys52 of VC1C2 through a side chain-side chain and a backbone-side chain hydrogen bond respectively and Lys63 of B-box binds to Glu50 of VC1C2 by a side chain-side chain hydrogen bond. As seen in the Figs. 7B and C, the side chain of Glu62 is located inside the groove, while the side chains of Glu59 and Lys63 are in contact with the surface of the groove. It is worth noting that the experimental published data indicates that this groove is a major binding site for AGEs (Xue et al. 2011, Xue et al. 2014). AGEs are a heterogeneous class of compounds which are generated as a result of nonenzymatic protein Glycation. Today, it is well known that this group of RAGE ligands is linked to the complications of diabetes, chronic inflammation, cancer and Alzheimer's disease (Sorci et al. 2013).

Comparison of B-box and A-box binding sites on the extracellular domain of RAGE reveals that although both proteins interact with VC1 region, they bind to different locations of VC1C2 domain (Fig. 6). The groove created by Lys110, Arg98, Lys52, Glu50 and Gln100 on the surface of V domain does not play a role in the A-box-binding to the RAGE receptor. Although A-box binds to the vicinity of the groove and Arg98 makes a side chain-backbone hydrogen bond with Lys33 of A-box (Fig. 4), the side chain of any A-Box residues does not fit into the groove. However, the imidazole rings of His30 and His34 of A-box are located into the groove formed by the VC1C2 residues Arg114, Arg116, Asn112, Ile96 and Gly95 (Fig. 8).

Today, the design of protein-protein interaction inhibitors (PPIs) has been attracted much attention in the drug discovery. These inhibitors can include small molecules or peptides. The first step in PPI design process is to determine the binding interface of two proteins (Laraia et al. 2015). Given the fact that the role of HMGB1-RAGE interaction in various pathological states such as growth and metastasis of tumors (Palumbo et al. 2004, Palumbo et al. 2009, Tang et al. 2010) and sepsis (Zhu et al. 2010) is confirmed, the design of HMGB1-RAGE interaction inhibitors can lead to the development of promising drugs for the treatment of HMGB1-RAGE-related diseases. The results obtained from this study provide good information on HMGB1-RAGE interaction sites and the amino acid residues involved in the interaction process. Therefore, these results can be helpful to design the inhibitors which can efficiently block the interaction of these two proteins.

All amino acid residues involved in the protein-protein interaction do not have the same importance in the interaction process. A number of certain amino acid residues called hotspot residues participate more significantly in the binding affinity. Identification of these residues can help to design more specific and powerful inhibitors for the protein-protein interactions (Laraia et al. 2015). Nowadays, in addition to more costly and time-consuming experimental methods like point mutation, there are some computational methods such as molecular dynamics (MD) to detect the

hotspot residues (Morrow and zhang 2012). However, the performance of these additional researches will require more time and extensive studies in this area.

Conclusions

The results obtained from this work demonstrate that the A and B boxes of hHMGB1 bind to different locations of VC1 part of the extracellular domain (VC1C2) of cell surface RAGE receptor. Surprisingly, one of B-box binding points is a groove created by Lys110, Arg98, Lys52, Glu50 and Gln100 on the surface of VC1C2 which also constitutes a binding site for another ligand of RAGE receptor, AGEs. It should be noted that since the interaction of A-box and B-box with RAGE receptor leads to the activation of different signaling pathways, it is quite reasonable that these two proteins bind to different sites of the extracellular domain of the receptor. Considering that the involvement of HMGB1-RAGE interaction in the various pathological conditions has been confirmed, the results of this study can provide useful information to help design new potent drugs for HMGB1/RAGE-related pathologies.

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