Redox-sensitive structural change in the A-domain of HMGB1 and its implication for the binding to cisplatin modified DNA

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ABSTRACT

HMGB1 (high-mobility group B1) is a ubiquitously expressed bifunctional protein that acts as a nuclear protein in cells and also as an inflammatory mediator in the extracellular space. HMGB1 changes its functions according to the redox states in both intra- and extra-cellular environments. Two cysteines, Cys23 and Cys45, in the A-domain of HMGB1 form a disulfide bond under oxidative conditions. The A-domain with the disulfide bond shows reduced affinity to cisplatin modified DNA. We have solved the oxidized A-domain structure by NMR. In the structure, Phe38 has a flipped ring orientation from that found in the reduced form; the phenyl ring in the reduced form intercalates into the platinated lesion in DNA. The phenyl ring orientation in the oxidized form is stabilized through intramolecular hydrophobic contacts. The reorientation of the Phe38 ring by the disulfide bond in the A-domain may explain the reduced HMGB1 binding affinity towards cisplatinated DNA.

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1. Introduction

Cells use an elaborate mechanism to control protein function in response to oxidative stress through the formation of disulfide bonds between thiol groups [1]. For example, various types of transcription factors exhibit redox-dependent switches that regulate transcriptional activities [2,3]. Recent studies list the proteins regulated by thiol-disulfide exchange [4,5]. Intriguingly, most of the proteins were abundant structural proteins, molecular chaperones and others that are not directly involved in the gene regulation. HMGB1 (high-mobility group B1) is identified as a representative protein with a redox-dependent functional switch [6].

HMGB1 is a ubiquitously expressed nuclear protein, which has two tandem DNA-binding domains, high-mobility group boxes A and B, and a highly acidic C-terminal tail [7]. HMGB1 functions as an architectural chromatin-binding factor that binds to DNA in a structure-specific, yet sequence-independent manner, with higher affinity for unusual DNA structures, including bent, kinked or unwound duplexes [8]. HMGB1 also functions as an extracellular signaling molecule during inflammation, cell differentiation, cell migration and tumor metastasis, and is released from necrotic cells or secreted by activated immune cells [9]. The released HMGB1

oxidizes in the oxidative extracellular space [9–11]. HMGB1 has three cysteine residues. Cys23 and Cys45 in the A-domain are located in close spatial proximity [13], and form a disulfide bond under mild oxidative conditions [11]. The other cysteine, Cys106, in the B-domain is less reactive to oxidative modification; its thiol is buried within the structure [6]. The redox-dependent disulfide bond formation between Cys23 and Cys45 in the A-domain switches the HMGB1 functions in the extracellular space; the HMGB1 in the reduced form functions as a chemoattractant, while the protein with the disulfide bond functions as a proinflammatory cytokine [12].

The redox state of HMGB1 should be functionally relevant also in the intracellular environment, because the redox potential of the A-domain is within the range of the physiological intracellular redox potential [11]. A fraction of HMGB1 should exist in the oxidized form in cells, which could explain the variety of the cellular responses to the HMGB1 engaging gene regulations [13]. The high affinity of HMGB1 to the cisplatinated DNA lesions is expected to enhance the efficacy of cisplatin, a widely used anticancer drugs [14]. HMGB1 binding to the cisplatinated DNA impedes nucleotide excision repair (NER) by shielding the lesions from the repairing proteins [15,16], which thus inhibits replication and transcription in aggressively growing cancer cells, thereby leading to their cell death [17,18]. However, an improvement of the efficacy of cisplatin as a function of HMGB1 protein levels in cells has not been observed [19]. The failure to correlate cell sensitivity to cisplatin with the amount of HMGB1 in cells can be ascribed to

the co-existence of the oxidized HMGB1 in cells [13]; the oxidized HMGB1 with the disulfide bond between Cys23 and Cys45 has 10-fold reduced affinity to the cisplatinated DNA, thus reducing the inhibition of the NER process [13]. The oxidization to HMGB1 in cells is, therefore, a factor for the cisplatin resistance of certain tumors [13].

The functional switch according to the redox state of HMGB1 in cells and in the extracellular space has sparked interest to discover new biological roles of this ubiquitously expressed protein. However, the structure of the oxidized A-domain in HMGB1 remains unknown. Therefore, the molecular mechanisms that control functional changes in response to the redox states of HMGB1 remain elusive. To advance our understanding of the redox-dependent functional changes of HMGB1, we have solved the oxidized A-domain structure by NMR. The A-domain in the oxidized form has an unexpectedly large structural change in the loop between helices I and II. In particular, the flipped phenyl ring at Phe38, at the C-terminal edge of the inter-helix loop, relative to that in the structure of the reduced form was remarkable. Phe38 is the key residue interacting into cisplatin-[d(GpG)] intrastrand crosslink sites, as shown in the X-ray complex structure of the reduced A-domain of HMGB1 with the cisplatinated DNA [20]. The significant structural change associated with the disulfide bonding in the A-domain could explain the reduced affinity of the oxidized HMGB1 to the cisplatinated DNA.

2. Materials and methods

2.1. Expression and purification of the oxidized A-domain of HMGB1

HMGB1 A-domain (residues from 1 to 84 of human HMGB1 protein; SwissProt accession IDP09429) was purified from *Escherichia coli,* BL21 (DE3), harboring the corresponding cDNA cloned into pET28a (Merck Chemicals). The purification of the isotopically labeled sample was carried out according to a previously published protocol [21]. His$_6$-tag at the N-terminus of the HMGB1 A-domain was cleaved by thrombin in 30 units (GE Healthcare) during dialysis against the buffer A solution (50 mM Tris–HCl, pH 8.0) at 4 °C for 16 h. The tag-cleaved A-domain was further purified by Heparin-Sepharose (GE Healthcare) with an NaCl gradient (from 0 to 1 M) in buffer A. Disulfide bond formation in the A-domain was done according to a method published previously [13]; the protein was dialyzed overnight against buffer B (50 mM potassium phosphate with 150 mM KCl, pH 6.4) containing 5 μM CuCl$_2$ at 4 °C and then redialyzed against buffer B without CuCl$_2$. The protein concentration was determined by the OD$_{280}$. Disulfide bond formation in the final sample was confirmed by electrophoretic mobility in an SDS–PAGE and also by the ^{13}Cβ NMR chemical shifts for the Cys23 and Cys 45 residues [22] (Fig. S1).

It is noted that throughout this manuscript, residues in HMGB1 are numbered according to the immature form that retains the initial methionine [23]. The present HMGB1 A-domain fragment has an additional three residues at its N-terminus (i.e., GSH) from the expression vector.

2.2. NMR spectroscopy

A standard set of NMR spectra [24] for resonance assignments of the HMGB1 A-domain in buffer B were collected at 293 K on a Bruker AvanceII spectrometer equipped with a triple resonance cryogenic probe operating at the ^{1}H resonance frequency of 700 MHz. The ^{15}N–{^{1}H} heteronuclear NOE experiments [24] were performed at 293 K. The experiments for the anisotropic sample using liquid crystals were carried out at 300 K to keep the liquid

crystalline media stably aligned. NMR data were processed by NMRPipe [25]. Spectral analyses were done using the KUJIRA suite [26] running on the NMRview [27] platform and SPARKY (T.D. Goddard and Kneller, D.G. SPARKY 3, UCSA). The chemical shift data for the oxidized HMGB1 A-domain have been deposited in the Biological Magnetic Resonance Data Bank (accession code, 11532) (Fig. S2).

2.3. Dipolar coupling measurements

Dipolar couplings ($^1D_{NH}$, $^1D_{C'N}$ and $^2D_{C'H}$) for the ^{13}C/^{15}N labeled HMGB1 A-domain in buffer B were simultaneously measured using IPAP-HSQC spectra [28] under selective decoupling to ^{13}Cα spins. For aligning the protein, a 6% C$_{12}$E$_5$/hexanol ($r = 0.96$) liquid crystalline medium was used at 300K, which gave a residual deuterium quadrupolar coupling of 8.5 Hz and the magnitudes of the axial alignment tensor component (D_a) and rhombicity (R) were −8.7 Hz and 0.43, respectively.

2.4. Structure determination of the oxidized HMGB1 A-domain

Structure calculations were done with the CYANA program using the automated NOE assignment suite, CANDID [29,30]. The backbone dihedral angle restraints were generated by the TALOS + program [31,32]. The 40 lowest-energy CYANA structures calculated with the distance and the backbone torsion angle restraints were subjected to explicit water refinement with XPLOR-NIH [33,34]. In this refinement, the three types of RDCs ($^1D_{NH}$, $^1D_{C'N}$, and $^2D_{C'H}$) were added to the distance and backbone torsion angle restraints; the RDCs were used by normalizing to the $^1D_{NH}$ data based on the bond lengths and gyromagnetic ratios [35]. The final 20 XPLOR-NIH structures were validated using the program PROCHECK-NMR (Table S1) [36]. The final set of ensemble structure coordinates were deposited in the Protein Data Bank, under the accession code 2RTU.

2.5. Model building of the complex of the oxidized A-domain in HMGB1 with cisplatinated DNA

The complex of the oxidized A-domain in HMGB1 with cisplatinated DNA was modeled on the basis of the X-ray complex structure of cisplatinated DNA and the reduced form of the A-domain (PDB code 1CKT) [20]. The modeling was done as follows. Initially, the minimal essential pairs of the inter-molecular contacting residues were extracted from the coordinates, which enabled us to reproduce the original complex structure using HADDOCK [37]. Subsequently, the same inter-molecular contact residues were used to model the oxidized HMGB1 A-domain with the cisplatinated DNA using HADDOCK [37]. The contacting residues were selected through the analysis of the inter-molecular interactions with the LIGPLOT software [38]; the residues with more than one intermolecular hydrogen bond or more than five non-bonded interactions were chosen. In the complex structure of the cisplatinated DNA with the reduced A-domain, the identified contacting residues were Lys12, Tyr16, Ala17, Arg24, Phe38, Ser42, Lys43, Ser46 and Trp49 in the A-domain and G$_8$, G$_9$, A$_{10}$, C$_{11}$, C$_{12}$ and T$_{13}$ in one strand, and C$_{15}$/T$_7$, C$_8$ and C$_9$ in the other strand of the cisplatinated DNA. The docking modeling calculation was done on the HADDOCK web server [39] with the above contacting residues input as the "active" ambiguous interaction residues (AIRs) [37]. For comparison, the same docking calculation was done using the reduced HMGB1 A-domain structure solved under reduced conditions containing 1 mM DTT (PDB code 2YRQ).