High-Quality NMR Structure of Human Anti-Apoptotic Protein Domain Mcl-1(171-327) for Cancer Drug Design

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Abstract

A high-quality NMR solution structure is presented for protein hMcl-1(171–327) which comprises residues 171–327 of the human anti-apoptotic protein Mcl-1 (hMcl-1). Since this construct contains the three Bcl-2 homology (BH) sequence motifs which participate in forming a binding site for inhibitors of hMcl-1, it is deemed to be crucial for structure-based design of novel anti-cancer drugs blocking the Mcl1 related anti-apoptotic pathway. While the coordinates of an NMR solution structure for a corresponding construct of the mouse homologue (mMcl-1) are publicly available, our structure is the first atomic resolution structure reported for the ‘apo form’ of the human protein. Comparison of the two structures reveals that hMcl-1(171–327) exhibits a somewhat wider ligand/inhibitor binding groove as well as a different charge distribution within the BH3 binding groove. These findings strongly suggest that the availability of the human structure is of critical importance to support future design of cancer drugs.

Introduction

The malfunctioning of cellular apoptosis [1] is a major hallmark of cancer. The regulation of apoptosis depends on the family of Bcl-2 proteins which contain one or several Bcl-2 homology (BH) sequence motifs. Based on their function and the similarity of their respective BH sequence motifs, these proteins can be grouped into three classes [2],[3]: (i) multi-domain pro-apoptotic proteins such as Bax and Bak, (ii) anti-apoptotic (i.e., pro-survival) proteins such as Mcl-1, Bcl-1, Bcl-xL, Bcl-w and Bfl-1/A1, all of which exhibit a similar architecture as Bax and Bak, and (iii) several pro-apoptotic proteins comprising only a single BH3 sequence motif such as Bid, Bad, Bim, Puma, Noxa and Bak. Hence, Mcl-1 plays an early role in response to signals directing either cell survival or cell death [2] and has been shown to be up-regulated in numerous malignant tumors. Approaches abrogating the Mcl-1’s anti-apoptotic function either by reducing its abundance or by inactivating its functional BH3-binding groove show great promise for the cancer treatment [2],[4],[6],[7]. Here we present the high-quality NMR solution structure of polypeptide segment 171–327 of human Mcl-1 (hMcl-1) which comprises the three BH motifs deemed to be crucial for structure based drug design.

Results and Discussion

A high-quality NMR structure of hMcl-1(171–327) was obtained (Table 1) and the coordinates were deposited in the PDB [8] (accession code 2mhs). The structure comprises seven ß-helices ß1–ß7 (residues 173–191, 204–235, 240–253, 262–280, 284–301, 303–308 and 311–319) arranged to form a characteristic ‘Bcl-2 core’ structure [9] (Figure 1). The helices are locally clustered into two groups, ß1–ß3 and ß4–ß7, and the loops connecting, respectively, helices ß1 and ß2, helices ß3 and ß4, and helices ß4 and ß5 are flexibly disordered. The

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Labeled and colored differently, and the N- and C-termini are labeled as of the lowest energy conformer of hMcl-1(171–327). A green (BH3), red (BH1) and blue (BH2), respectively. (B) Helices for minimal rmsd. The three BH sequence motifs are colored in MOLMOL [36] and PYMOL [37].

Figure 1. NMR structure of hMcl-1(171–327). (A) Backbone of the 20 CYANA conformers representing the solution structure of hMcl-1(171–327) after superposition of backbone N, C' and C atoms of the $\alpha$-helices for minimal rmsd. The three BH sequence motifs are colored in green (BH3), red (BH1) and blue (BH2), respectively. (B) Ribbon drawing of the lowest energy conformer of hMcl-1(171–327). $\alpha$-helices $\alpha$1–$\alpha$7 are labeled and colored differently, and the N- and C-termini are labeled as “N’” and “C’”. The figures were generated using the programs MOLMOL [36] and PYMOL [37]. doi:10.1371/journal.pone.0096521.g001

Central helix $\alpha$4 is surrounded by the other six helices, with $\alpha$1, $\alpha$2, $\alpha$3 and $\alpha$5 packed around one side, and $\alpha$6 and $\alpha$7 packed against its N-terminus. Helices $\alpha$2, $\alpha$3, $\alpha$4 and $\alpha$7 participate in forming the BH3 binding groove. The electrostatic protein surface potential is positive at both ends of the BH3 binding groove (due to the presence of Arg 233, Lys 234, Arg 248 and Arg 263) and negative at the side of helix $\alpha$3 side (due to Asp 256) (Figure 2). This shows that the charge distribution in the BH3 binding groove of hMcl-1(171–327) differs distinctly from other anti-apoptotic proteins [10].

Including our hMcl-1(171–327) structure, twenty atomic resolution structures containing different Mcl-1 constructs are currently deposited in the PDB. In addition to the two ‘apo’ proteins hMcl-1(171–327) and mouse mMcl-1(152–308) [10] [PDB accession code 1wxs, 89% sequence identity with the human protein], the structures for nineteen protein-ligand complexes were deposited (Table 2) [9], [11-18]. Clearly, the large number of available structures reflects the outstanding interest in Mcl-1 as a target for the development of new cancer drugs. Superposition of the $\alpha$-helices reveals, as expected, close structural similarity for all Mcl-1 proteins structures (Figure 3); the root mean square deviation (rmsd) values range from 1.05 to 1.54 Å relative to hMcl1-1(171–327) (Table 2). However, comparison of the two apo protein structures of hMcl-1(171–327) and mMcl-1(152–308) with the complex structures shows that the binding pocket is widened upon complex formation (Table 2); the distances between the C’-atoms of residues His 224 in helix $\alpha$2 (His 205 in mMcl-1) and His 252 (His 235 in mMcl-1) at the C-terminus of helix $\alpha$3 are, respectively, $\sim$16 Å and $\sim$14 Å in hMcl-1(171–327) and mMcl-1(152–308), and $\sim$18–21 Å in the complexes.

The fact that the human apo protein exhibits a somewhat wider binding groove than the mouse homologue (Table 2) can be, at least partially, ascribed to the side chain of Leu 246 in the human protein which is not buried as deeply as the corresponding Phe side chain in the mouse protein. Furthermore, when comparing the human and the mouse protein, differences are observed for the charge distributions in the BH3-binding groove (Figure 2): the human protein is negatively charged on the side of helix $\alpha$3, while the corresponding surface of mouse protein is positively charged. This difference arises from Ser 255 corresponding to Lys 236 in the mouse protein. Remarkably, hMcl-1(171–327) is structurally more similar to the hMcl1(171–327)-hBim BH3 complex (Figure 3) than to apo mMcl-1(152–308) (Table 2).

Taken together, structural comparisons show that, in spite of the 89% sequence identity between human and mouse protein, the availability of the human hMcl-1(171–327) structure can be expected to be of critical importance for supporting future design of cancer drugs.

Materials and Methods

NMR Sample Preparation

Preliminary studies showed that hMcl-1(171–327) (UniProtKB/Swiss-Prot ID Q07820/MCL1_HUMAN) is not stable in solution. However, the mutant Cys 286 → Ser is stable for several weeks at concentrations $\sim$0.7 mM, and both wild-type and mutant bind the Bim-BH3 peptide with the same affinity ($K_{d}$ $\sim$ 60 pM) in a Biacore assay. Hence, we solved the NMR structure of hMcl-1(171–327) Cys 286 → Ser referred to as hMcl-1(171–327) in this publication.

hMcl-1(171–327) was cloned, expressed, refolded and purified following standard protocols to produce a uniformly $^{13}$C, $^{15}$N-labeled protein sample [19]. Briefly, the gene was cloned into a pET21d (Novagen) derivative yielding plasmid pSR482-21.1. The resulting construct contains seven nonnative residues at the C-

Figure 2. Electrostatic surface potentials. (A) For human hMcl-1(171–327) in the orientation shown in Figure 1 (left) and after rotation by 180° about the vertical axis (right). Surface colors (blue for positively charged; red for negatively charged) indicated the electrostatic potential calculated by using PYMOL [37] and its default vacuum electrostatics protocol. (B) Same as in (A) but for mouse mMcl-1(152–308).

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Table 1. Statistics of hMcl-1(171–327) NMR Structure.

| Completeness of stereo-specific assignments [%] | 55 (6/11) |
| 10CH2 of Gly | 38 (27/71) |
| Val and Leu methyl groups | 100 (27/27) |

Conformationally restricting distance constraints

| Intra-residue [i = j] | 1052 |
| Sequential [i−j = 1] | 1062 |
| Medium range [1 < |i−j| < 5] | 1197 |
| Long range [|i−j| ≥ 5] | 1058 |
| Total | 4369 |

Dideiral angle constraints

| φ | 113 |
| ψ | 113 |

Number of constraints per residue (170–327) | 29.1 |
Number of long range constraints per residue (170–327) | 6.7 |

CYANA target function (Å²) | 0.88±0.12 |
Number of distance violations per CYANA conformer 0.2−0.5 Å | 0 |
> 0.5 Å | 0 |
Number of dihedral-angle constraint violations per CYANA conformer > 5 | 0 |

Average rmsd to the mean CNS coordinates (Å) | 0.42±0.05 |
A-helices, backbone heavy atoms N, C', C'' | 0.88±0.07 |
A-helices, all heavy atoms | 0.65±0.13 |
Residues 172–321, backbone heavy atoms N, C', C'' | 1.05±0.10 |

PROCHECK [38] G-factors raw score (φ and ψ/all dihedral angles)² | 0.34/0.22 |
PROCHECK [38] G-factor Z - score (φ and ψ/all dihedral angles)² | 1.65/1.30 |
MOLPROBITY [39] clash score (raw/Z - score)² | 20.86/2.06 |
AutoQF R/P/F/DP scores [40] (%) | 96/97/96/81 |
Ramachandran plot summary² | 92.7 |
most favorable regions | 92.7 |
additionally allowed regions | 7.3 |
generously allowed regions | 0.0 |
disallowed regions | 0.0 |

*Related to pairs with non-degenerate chemical shift.
*Regular secondary element: α-helical residues 173–191, 204–235, 240–253, 262–280, 284–301, 303–308 and 311–319.
*Ordered residues: 172–192,194–198, 204–235, 238–255, 262–321 with dihedral angle order parameters S(φ) and S(ψ) > 0.9. Z-scores were computed relative to corresponding structure quality measures for high resolution X-ray crystal structures [42].

NMR spectroscopy

NMR spectra were recorded at 25°C. Five G-matrix Fourier transform (GFT) NMR experiments [22],[23] and a simultaneous 3D 15N/13C-glycine/13C-carboxyl-resolved NOESY [24],[25] spectrum (mixing time 60 ms; measurement time: 48 hours) were acquired on a Varian INOVA 750 MHz spectrometer equipped with a conventional probe. 2D constant-time [15N, 1H]-HSQC spectra (18 hours) were recorded for the 5% biosynthetically directed fractionally 13C-labeled sample on a Varian INOVA 600 MHz spectrometer equipped with a cryogenic probe as was described [21],[26]. Spectra were processed and analyzed using the programs NMRPipe [27] and XEASY [28].

Sequence specific backbone (Hα, HN, N, Cα) and Hα/Cα resonance assignments were obtained by using (4,3)D HN(C)N/Cα/Cα/CαN (63 hours)/4,3)D CαCα/CαN/CαN (62 hours), and (4,3)D Hα/Cα/Cα/CαN (69 hours) [23] along with the program AUTOASSIGN [29]. More peripheral side chain chemical shifts were assigned with aliphatic (3,4)D HCCH (67 hours) [23] and 3D 15N/13C-glycine/13C-carboxyl-resolved [1H, 15N]-NOESY [24],[25].
Table 2. Rmsd values for comparison of the NMR structure of hMcl-1(171–327) with the structures of mouse mMcl-1(152–308) and Mcl-1 complexes.

| Mcl-1 structures | 172–193, 203–321 | α1–α7 | 209–321 | α2–α4 | α2–α7 | dCA_{224,252} |
|------------------|------------------|-------|---------|-------|-------|-------------|
| mMcl-1 Bx      | 1.60±0.09        | 1.52±0.06 | 1.61±0.10 | 1.21±0.07 | 1.53±0.06 | 13.2–14.6 |
| hMcl-1-hBim     | 1.76±0.10        | 1.41±0.10 | 1.87±0.11 | 1.52±0.09 | 1.53±0.09 | 19.9       |
| hMcl-1-hMcl-1-mNoxaB | 1.46±0.12 | 1.05±0.08 | 1.53±0.14 | 1.00±0.10 | 1.08±0.09 | 19.9       |
| mMcl-1-mNoxa     | 1.52±0.11        | 1.16±0.07 | 1.59±0.13 | 1.11±0.11 | 1.18±0.08 | 18.8–20.2 |
| hMcl-1-Bim       | 1.57±0.09        | 1.30±0.08 | 1.59±0.11 | 1.24±0.11 | 1.30±0.09 | 18.3–19.9 |
| mMcl-1-mNoxaB    | 1.46±0.09        | 1.13±0.05 | 1.53±0.11 | 1.12±0.08 | 1.18±0.06 | 18.3–19.6 |
| rMcl-1-HMcl-1-hBim | 1.75±0.09 | 1.44±0.06 | 1.86±0.11 | 1.58±0.09 | 1.56±0.07 | 19.9       |
| rMcl-1-HMcl-1-hBimL62A | 1.80±0.09 | 1.44±0.06 | 1.90±0.11 | 1.53±0.08 | 1.55±0.07 | 19.8       |
| mMcl-1-mPuma     | 1.38±0.16        | 1.06±0.07 | 1.43±0.18 | 0.93±0.10 | 1.09±0.09 | 19.4       |
| mMcl-1-BimL12Y   | 1.75±0.13        | 1.50±0.08 | 1.85±0.15 | 1.48±0.12 | 1.59±0.09 | 20.2       |
| mMcl-1-BimBH3   | 2dA'             | 1.75±0.13 | 1.50±0.08 | 1.84±0.14 | 1.46±0.12 | 1.57±0.09 | 19.7       |
| hMcl-1-BimBH3   | 1.73±0.14        | 1.46±0.08 | 1.83±0.11 | 1.42±0.11 | 1.54±0.08 | 19.6–19.9 |
| hMcl-1-BimBH3F4ae | 1.47±0.16 | 1.21±0.09 | 1.48±0.18 | 1.08±0.15 | 1.24±0.11 | 20.3       |
| hMcl-1-BimBH3F7  | 1.69±0.12        | 1.46±0.08 | 1.79±0.13 | 1.43±0.12 | 1.54±0.09 | 19.4       |
| hMcl-1-Compound5 | 1.45±0.13        | 1.22±0.08 | 1.48±0.14 | 1.19±0.10 | 1.28±0.08 | 18.7       |
| hMcl-1-Compound6 | 1.45±0.13        | 1.22±0.08 | 1.47±0.12 | 1.12±0.09 | 1.25±0.08 | 17.9–19.6 |
| hMcl-1-BimBH3   | 1.51±0.16        | 1.22±0.09 | 1.57±0.18 | 1.06±0.12 | 1.27±0.10 | 20.3       |

*Average pairwise rmsd values (Å) were calculated for backbone heavy atoms N, Cα, and Cα between the 20 conformers of Mcl-1(171–327) and corresponding polypeptide segments in the other structures. The distances dCA (in Å) between the Cα-atoms of residues His 224 in helix α2 (His 205 in mMcl-1) and His 252 (Hs 233 in mMcl-1) at the C-terminus of helix α3 are provided as a measure for the width of the BH3 binding groove.

1Residue numbers are for hMcl-1(171–327); residues 194–202 were excluded since one structure (2n9p) does not contain the corresponding residues; residues 172–193 and 203–321 correspond to residues 153–174 and 184–302 in mMcl-1, and residues 209–321 correspond to residues 190–302 in mMcl-1.

2Helices α1–α7 in hMcl-1 comprise residues 173–191, 204–235, 240–253, 262–280, 284–301, 303–308 and 311–319; the corresponding residues in mMcl-1 are: 155–172, 185–216, 221–234, 243–261, 265–282, 284–289 and 292–300.

3Helices α2–α7 in hMcl-1 and residues 204–208 (numbers in hMcl-1) were excluded

4Mouse mMcl-1(152–308), PDB accession code 1wsx (the mean NMR coordinates were used) [10].

5Human hMcl-1 complexed with human hBim BH3, 2pjk [11].

6Chimeric rat-human fMcl-1(171–208)/mMcl-1(209–327) complexed with mouse mMcl-1 BH3, 2nla [9].

7Mouse mMcl-1 complexed with mouse mMcl-1 BH3, 2moc [12].

8Mouse mMcl-1 complexed with mouse mBim BH3, 2roc [12].

9Mouse mMcl-1 complexed with mouse mMcl-1 BH3, 2jnm [6].

10Chimeric rat-human 1Mcl-1(171–208)/mMcl-1(209–327) complexed with mouse mMcl-1 BH3, 2nla [9].

11Mouse mMcl-1 complexed with mouse mMcl-1 BH3, 2rod [12].

12Human hMcl-1 complexed with human Bid BH3, 2kbu [15].

13Human hMcl-1 complexed with human Bid BH3, 2kbf [15].

14Human hMcl-1 complexed with human Bid BH3 mutant 12dA, 3k0j [11].

15Human hMcl-1 complexed with human L62A, F68A, 3d7v [13].

16Human hMcl-1 complexed with human L62A, 3k0j [11].

17Human hMcl-1 complexed with human L62A, F68A, 3d7v [13].

18Human hMcl-1 complexed with human L62A, F68A, 3k0j [11].

19Human hMcl-1 complexed with human L62A, F68A, 3d7v [13].

20Overall, assignments were obtained for 96% of backbone and 1H,13C,15N resonances and for 93% of the side chain resonances which are assignable with the NMR experiments listed above (excluding the N-terminal NH3+, Pro 13N, 13C, preceding prolyl residues, Lys NH3+, Arg NH2, OH, side chain 13C and aromatic 13C). Furthermore, 100%/100% of Val and Leu isopropyl moieties with non-degenerate proton chemical shifts were stereo-specifically assigned (Table 1). Chemical shifts were deposited in the BioMagResBank [30] (accession code 19654). 1H–1H upper distance limit constraints for structure calculations were obtained from NOESY (Table 1). In addition, backbone dihedral angle constraints were derived from chemical shifts using the program TALOS [31] for residues located in well-defined secondary structure elements (Table 1). The programs CYANA [32],[33] and AUTO_STRUCTURE [34] were used in parallel to assign long-range NOEs [24]. The final structure calculations were
performed using CYANA followed by explicit water bath refinement using the program CNS [35].

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Author Contributions

Conceived and designed the experiments: GL LP TS. Performed the experiments: GL LP KA HY JL. Analyzed the data: GL LP KA HY JL TS. Contributed reagents/materials/analysis tools: GL LP KA HY JL TS. Wrote the paper: GL LP TS.