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Solution NMR structure of MED25(391–543) comprising the activator-interacting domain (ACID) of human mediator subunit 25

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Abstract

The solution NMR structure of protein MED25(391–543), comprising the activator interacting domain (ACID) of subunit 25 of the human mediator, is presented along with the measurement of polypeptide backbone heteronuclear $^{15}\text{N}\{-^1\text{H}\}$ NOEs to identify fast internal motional modes. This domain interacts with the acidic transactivation domains of Herpes simplex type 1 (HSV-1) protein VP16 and the Varicella-zoster virus (VZV) major transactivator protein IE62, which initiate transcription of viral genes. The structure is similar to the β -barrel domains of the human protein Ku and the SPOC domain of human protein SHARP, and provides a starting point to understand the structural biology of initiation of HSV-1 and VZV gene activation. Homology models built for the two ACID domains of the prostate tumor overexpressed (PTOV1) protein using the structure of MED25(391–543) as a template suggest that differential biological activities of the ACID domains in MED25 and PTOV1 arise from modulation of quite similar protein–protein interactions by variable residues grouped around highly conserved charged surface areas.

Keywords

ACID; MED25; Mediator complex; PTOV; Structural genomics

Introduction

The Mediator complex [1] has been identified in all eukaryotes thus far investigated for its presence [2], and appears to be an essential part of the RNA polymerase type II (RNAP II) machinery for gene transcription [3]. Mediator is composed of up to thirty protein subunits [3, 4], some of which interact with cellular transcriptional activators (such as Sp1, p53, the vitamin D receptor, the adenovirus E1A protein) [4, 5], as well as herpes simplex type 1 virus (HSV-1) and varicella-zoster virus (VZV) transcriptional activators [6–9]. These interactions result in a structural change of Mediator and subsequent RNAP II binding resulting in assembly of the transcription pre-initiation complex [10].

Subunit 25 of the human mediator (MED25) comprises 747 residues and was identified as the target of the acidic transactivation domains (TADs) of the HSV-1 protein VP16 [6, 7], and the VZV major transactivator protein IE62 [8, 9]. Although these two TADs do not exhibit any significant sequence similarity, they are both largely unstructured in solution [9, 11] and contain a high content of acidic and aliphatic amino acid residues [9, 11, 12]. Furthermore, both TADs bind to the polypeptide segment of MED25 comprising approximately residues 390 to 540, which was named activator-interacting domain (ACID), VP16-binding domain (VBD) or prostate tumor overexpressed (PTOV) protein domain [6, 7, 13]. Given the high content of basic amino residues in ACID and the fact that mutation of certain Phe residues in the TADs significantly decreases transactivation [9, 12, 14], one would expect that the formation of a TAD-ACID complex involves both electrostatic as well as hydrophobic interactions [15, 16]. Since the VP16 TAD folds upon binding to other cofactors [12, 17], it might very well be that the TADs fold upon binding to the ACID domain. Considering that ACID domain also interacts with the histone transacetylase CBP, which is involved in chromatin remodeling [13, 18], the structure of MED25 ACID domain is of key importance for understanding the structural biology of MED25 and its role in transcriptional activation.

MED25(391–543) (UniProt accession number Q71SY5) comprises the MED25 ACID domain and belongs to the Pfam [19] domain family PF11232 currently containing 50 members. These include the two ACID domains of protein PTOV1 which is overexpressed in prostate cancer tumors [20]. MED25(391–543) was selected as a community nominated target by the Protein Structure Initiative and assigned to the Northeast Structural Genomics Consortium (NESG; <http://www.nesg.org>; target ID HR6188A) for structure determination.

Materials and methods

MED25(391–543) was cloned, expressed, and purified following standard protocols developed by the NESG for production of uniformly (U) ^{13}C , ^{15}N -labeled protein samples [21, 22]. Briefly, the 391–543 fragment of Q71SY5 from *Homo sapiens* was cloned into pET15_NESG, a derivative of pET15 (Novagen), yielding the plasmid HR6188A-391–543-14.15. The resulting construct contains ten nonnative residues at the N-terminus (MGHHHHHSH) to facilitate protein purification. *Escherichia coli* BL21 (DE3) pMGK cells, a rare codon enhanced strain, were transformed with HR6188A-391–543-14.15, and cultured in MJ9 minimal medium containing ($^{15}\text{NH}_4$) $_2\text{SO}_4$ and U - ^{13}C -glucose as sole nitrogen and carbon sources. U - ^{13}C , ^{15}N MED25(391–543) was purified using an AKTExpress (GE Healthcare) based two step protocol consisting of IMAC (HisTrap HP)

and gel filtration (HiLoad 26/60 Superdex 75) chromatography. The final yield of purified $U\text{-}^{13}\text{C},^{15}\text{N}$ -MED25(391–543) (>98% homogenous by SDS-PAGE; 19.7 kDa by MALDI-TOF mass spectrometry) was ~23 mg/L. In addition, a uniformly ^{15}N - and 5% biosynthetically directed fractionally ^{13}C -labeled sample 5% $^{13}\text{C}; U\text{-}^{15}\text{N}$ -MED25(391–543) was produced using a mixture of 95% unlabeled and 5% $U\text{-}^{13}\text{C}$ -glucose as the sole carbon source [23]. The NMR samples of $U\text{-}^{13}\text{C},^{15}\text{N}$ -MED25(391–543) and 5% $^{13}\text{C}, U\text{-}^{15}\text{N}$ -MED25(391–543) were prepared at respective concentrations of ~0.7 and ~0.4 mM in 90% $\text{H}_2\text{O}/10\%$ D_2O solution containing 20 mM MES (pH 6.5), 100 mM NaCl, 10 mM DTT, 5 mM CaCl_2 , and 0.02% NaN_3 . An isotropic overall rotational correlation time of about 9 ns was inferred from ^{15}N spin relaxation times, indicating that MED25(391–543) is monomeric in solution. This was confirmed by analytical gel-filtration (Agilent Technologies) and static light scattering (Wyatt Technology Co.), using protocols described previously [22].

The following spectra were recorded for $U\text{-}^{13}\text{C};^{15}\text{N}$ -MED25(391–543) at 25 °C on (i) a Varian INOVA 750 spectrometer (total measurement time 4 days) equipped with a conventional $^1\text{H}\{^{13}\text{C},^{15}\text{N}\}$ probe: [$^{15}\text{N},^1\text{H}$]-HSQC, HNCQ, CBCA(CO)NH, HBHA(CO)NH, HN(CA)CO, aliphatic and aromatic (H)CCH, (H)CCH-TOCSY [24], and (ii) a Bruker AVANCE 900 spectrometer (total measurement time 6 days) equipped with a cryogenic $^1\text{H}\{^{13}\text{C},^{15}\text{N}\}$ probe: 2D [$^{15}\text{N},^1\text{H}$]-HSQC, aliphatic and aromatic 2D constant-time [$^{13}\text{C},^1\text{H}$]-HSQC, 3D HNCACB, 3D HNCA, simultaneous 3D $^{15}\text{N}/^{13}\text{C}_{\text{aliphatic}}/^{13}\text{C}_{\text{aromatic}}$ -resolved [$^1\text{H},^1\text{H}$]-NOESY [25] (mixing time 70 ms), 2D long-range [$^{15}\text{N},^1\text{H}$]-HSQC for determining the tautomeric states of His residues, and 2D (HB)CB(CGCDCE)HE [26]. To identify fast internal motional modes, a 2D NMR experiment to measure $^{15}\text{N}\{-^1\text{H}\}$ heteronuclear NOEs [27] was performed with 4 s pre-saturation of the amide protons using a Varian INOVA 750 spectrometer (measurement time 4 days). 2D constant-time [$^{13}\text{C},^1\text{H}$]-HSQC spectra were recorded as described [28] for 5% $^{13}\text{C}; U\text{-}^{15}\text{N}$ -MED25(391–543) on the Varian INOVA 750 spectrometer to obtain stereo-specific assignments for Val and Leu isopropyl groups [23]. All NMR spectra were processed using the programs PROSA v6.4 [29] and TopSpin v1.4 (Bruker Biospin), and analyzed using programs CARRA [30] and XEASY [31]. Sequence-specific backbone (H^{N} , H^{α} , N , C' , C^{α}) and $\text{H}^{\beta}/\text{C}^{\beta}$ resonance assignments were obtained by using the program AutoAssign [32, 33]. Resonance assignment of side-chains was accomplished using aliphatic and aromatic 3D (H)CCH, 3D (H)CCH-TOCSY, 2D (HB)CB(CGCDCE)HE and 3D $^{15}\text{N}/^{13}\text{C}_{\text{aliphatic}}/^{13}\text{C}_{\text{aromatic}}$ -resolved NOESY. Side chains assignments and α -protonated neutral tautomeric state of His 435 and His 502 were obtained using 2D long-range [$^{15}\text{N},^1\text{H}$]-HSQC [34]. Overall, for residues 391–543 sequence-specific resonance assignments were obtained for 99.3% of backbone (Fig. S1) and 100.0% of side chain resonances assignable with the NMR experiments listed above (i.e., excluding Pro ^{15}N , Lys and Arg side chain amino groups, hydroxyl protons of Ser, Thr and Tyr, thiol protons of Cys, carboxyl atoms of Asp and Glu, $\text{C}^{\epsilon 1}$, $\text{H}^{\epsilon 1}$ and imino groups of His, and nonprotonated aromatic carbons). Chemical shifts were deposited in the BioMagResBank on 11/24/2010 (BMRB ID: 17323). $^1\text{H}\text{-}^1\text{H}$ upper distance limit constraints for structure calculation were obtained from 3D $^{15}\text{N}/^{13}\text{C}_{\text{aliphatic}}/^{13}\text{C}_{\text{aromatic}}$ -resolved [$^1\text{H},^1\text{H}$]-NOESY, and backbone dihedral angle constraints for residues located in well-defined regular secondary structure elements were derived from chemical shifts using the program TALOS+ [35].

Automated NOE assignment was performed iteratively with CYANA v3.0 [36, 37], and the results were verified by interactive spectral analysis. Stereospecific assignments of methylene protons were performed with the GLOMSA module and the final structure calculation was performed with CYANA followed by refinement of selected conformers in an ‘explicit water bath’ [38] using the program CNS v1.2 [39]. Validation of the 20 refined conformers was performed with the Protein Structure Validation Software (PSVS) server [40]. Homology models of the PTOV1 ACID domains were generated using the SWISS-

MODEL server [41], starting with the lowest-energy MED25(391–543) conformer as a template.

Results and discussion

A high-quality structure of the 152-residue domain construct MED25(391–543) was obtained (Table 1; Fig. 1a, b) and the coordinates were deposited in the Protein Data Bank [42] (<http://www.rcsb.org>) on 11/24/2010 (accession code 2L6U). MED25(391–543) exhibits a mixed α/β fold resembling the shape of a stein: seven β -strands form a barrel of topology A($\uparrow$)B($\downarrow$)D($\uparrow$)G($\downarrow$)F($\uparrow$)E($\downarrow$)C($\uparrow$) with strands C, E, F and G flanked by α -helices I and III, and helix II connecting strands D and E at one end of the barrel. The locations of the regular secondary structure elements are: β -strands A (residues 399–409), B (424–433), C (447–454), D (468–473), E (494–499), F (510–516) and G (521–527), and α -helices I (455–466; with a kink between Gly 462 and Pro 463), II (481–491) and III (531–540). β -strands A and B are connected by a long flexibly disordered loop comprising residues 410–423, and residues Ser 517, Lys 518, Lys 519 and Lys 520 form a type I β -turn connecting β -strands F and G.

In parallel with the structure determination of MED25(391–543) presented here, the NMR structures of MED25(391–548) (PDB accession code: 2L23) [43], MED25(391–553) (PDB accession code: 2KY6) [44], and MED25(394–543) (PDB accession code: 2XNF) [45] were solved. These four structures are the first structural representatives for protein family PF11232. Comparison with our MED25(391–543) structure (Fig. 1c–e) reveals that the spatial arrangement of the regular structure elements is very similar: the root mean square deviation (RMSD) calculated between the mean coordinates of the backbone heavy atoms N, C $^{\alpha}$ and C $^{\prime}$ is only 0.97, 1.03 and 1.00 Å, respectively. Moreover, the long loop connecting β -strands A and B is highly disordered in all structures. Differences are observed, however, for the conformation of the N- and C-terminal segments which is likely due to variations in construct lengths. Notably, the structure presented here, as well as the structures of MED25(391–553) [44] and MED25(394–543) [45], exhibit better defined conformations of Trp 408, His 499 and Phe 500 side chains when compared with MED25(391–548) [43]. However, in MED25(394–543) [45] the indole moiety of Trp 408 is flipped around χ_2 when compared with the other structures. Furthermore, the side-chain conformations of Trp 402 and Trp 444 are distinctly different in the MED25(391–548) [43] when compared with the other structures. Since the chemical shifts of MED25(391–548) [43] and MED25(394–543) [45] are not available in the BMRB, it remains to be seen if differences in resonance assignments and/or the completeness of resonance assignment account for the observed structural differences.

Inspection of heteronuclear $^{15}\text{N}\{-^1\text{H}\}$ NOEs (Fig. S2a) shows that polypeptide backbone segments exhibiting increased global displacements (Figs. 1, S2b), that is, a reduced precision of the structural description, are affected by the presence of fast internal motional modes. Most prominently, the long loop connecting β -strands A and B is highly flexibly disordered and exhibits the largest displacements. Increased disorder manifested in lower $^{15}\text{N}\{-^1\text{H}\}$ NOEs and increased displacements is likewise observed for the loops connecting β -strands B and C, E and F, the loop connecting β -strand D and helix II, and the β -turn between strands F and G. In contrast, the $^{15}\text{N}\{-^1\text{H}\}$ NOE measured for Gly 493, which is located between α -helix II and β -strand E, is comparably low while the displacements of Gly 493 and its neighboring residues are hardly increased. This finding suggests that the internal motion registered by this NOE primarily affects the N-H moiety of Gly 493. Furthermore, reduced $^{15}\text{N}\{-^1\text{H}\}$ NOEs are also observed for the kinked α -helix I (average: 0.73 ± 0.06) while the corresponding global displacements are only slightly higher than in the other regular structure elements. In fact, except for the terminal α -helix III

(average 0.76 ± 0.07), the average $^{15}\text{N}\{-^1\text{H}\}$ NOEs are significantly higher for the other regular secondary structure elements: 0.81 ± 0.03 (β -strand A), 0.79 ± 0.09 (B), 0.82 ± 0.04 (C), 0.82 ± 0.05 (D), 0.81 ± 0.04 (α -helix II), 0.80 ± 0.04 (E), 0.84 ± 0.01 (F), and 0.81 ± 0.06 (G). Since the binding site for VP16 C-terminal domain, as judged from chemical shift perturbations [43], includes residues Gln 456, Leu 458, Thr 459, Phe 465 and Arg 466 located in helix I, as well as Asn 438 in the loop connecting β -strands B and C, and Lys519 in the β -turn connecting β -strands F and G, it may very well be that the increased flexibility of these polypeptide segments are important for MED25-TAD complex formation.

The electrostatic potential of MED25(391–543) (Fig. 1f) exhibits two distinct positively charged regions on opposite sides of the protein molecule which are likely important for TAD binding. Furthermore, there is an extended groove on the surface which is ‘wrapped around’ the protein and passes through the two positively charged regions. A total of 42 residues, most of which are positively charged or hydrophobic (Ser 396, Lys 398, Trp 408, Gln 409, Tyr 432, Glu 437, Asn 438, Leu 439, Gln 455, Arg 469, Met 470, Gln 472, Phe 473, His 474, Phe 475, Lys 478, Lys 484, Tyr 487, Arg 488, Met 490, Gly 491, Gly 493, Phe 494, Pro 500, His 502, Thr 503, Ala 504, Cys 506, Glu 507, Val 508, Arg 509, Leu 514, Tyr 515, Ser 517, Lys 519, Lys 520, Ile 521, Phe 522, Met 523, Tyr 528, Gln 530) participate in forming the groove. Consistent with the hypothesis that the charged patches and the groove are important for TAD binding, substitution of amino acid residues 447–450 or 484–488 to Ala resulted in a decreased interaction with the IE62 TAD [9] and VP16 TAD (Yamamoto and Ruyechan, unpublished results).

A search of the PDB for structurally similar proteins using the DALI server [46] resulted in only two significant hits (Fig. 2a, b) other than MED25(391–548): (i) the β -barrel domains of the Ku70/Ku80 heterodimer (PDB accession codes 1JEY, 1JEQ; highest Z-score 8.5; 108 residues with 10% sequence identity aligned with an RMSD value of 3.1 Å for C $^{\alpha}$ atoms) and (ii) the Spen paralog and ortholog C-terminal (SPOC) domain of SMRT/HDAC1-associated repressor protein (SHARP) (PDB accession code 1OW1; Z-score 7.2; 107 residues; 19%; 2.9 Å). Ku70/Ku80 is implicated in a wide range of functions, including DNA helicase activity, transcriptional activation, but mainly in double-stranded DNA break repair. In these crystal structures the β -barrel domains of Ku make extensive contacts with each other, as well as with other regions of the opposite subunit and the phosphate backbone of the DNA. SHARP, on the other hand, binds to nuclear receptors and represses transcription by interacting with histone deacetylase complexes (HDAC) [47, 48], and the acidic co-repressor SMRT/NCOR [47, 49] *via* the C-terminal SPOC domain.

MED25(391–543) exhibits 81 and 73% sequence identity with the N- and C-terminal ACID domains of human PTOV1 protein, respectively (Fig. 2c). The high sequence identity allowed us to generate high-quality homology models of the PTOV1 ACID domains (Fig. 2d, e). Inspection of the models shows that residues which are different in MED25(391–543) and the PTOV1 ACID domains are grouped peripherally around the highly conserved charged surface regions. This finding indicates that the differential biological activity of ACID domains in MED25 and PTOV1 [50] arises from a modulation of quite similar protein-protein interaction patterns by these peripheral residues.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ACID	Activator-interacting domain
CBP	CREB-binding protein
HDAC	Histone deacetylase complex
HSV-1	Herpes simplex virus type 1
NESG	Northeast Structural Genomics Consortium
MED25	Subunit 25 of the human mediator complex
NOE	Nuclear Overhauser effect
PDB	Protein Data Bank
PTOV	Prostate tumor overexpressed
RMSD	Root mean square deviation
SHARP	SMRT/HDAC1-associated repressor protein
SPOC	Spen paralog and ortholog C-terminal domain
TAD	Transactivation domain
VBD	VP16-binding domain
VZV	Varicella-zoster virus

Fig. 1.

Fig. 2.

Table 1

Statistics for MED25(391–543) structure

Completeness of resonance assignments (%) ^a	
Backbone/side-chain	99.3/100.0
Completeness of stereospecific assignments (%)	
Val and Leu isopropyl/ β CH ₂ / α CH ₂ of Gly	97/26/20
Conformation-restricting distance constraints	
Intraresidue [$i = j$]	596
Sequential [$ i - j = 1$]	622
Medium range [$1 < i - j < 5$]	428
Long range [$ i - j \geq 5$]	1,016
Total	2,662
Dihedral angle constraints (ϕ/ψ)	60/60
Average number of constraints per residue	18.3
Average number of long-range distance constraints per residue	6.7
CYANA target function ($\text{\AA}^2$)	0.19 ± 0.04
Average number of distance constraint violations per conformer	
0.2–0.5 $\text{\AA}$	8.2
>0.5 $\text{\AA}$	0.0
Average number of dihedral angle constraint violations per conformer	
>10°	0.0
Average RMSD from mean coordinates ($\text{\AA}$)	
Regular secondary structure elements ^b , backbone heavy atoms	0.6
Regular secondary structure elements ^b , all heavy atoms	1.1
Ordered residues ^c , backbone heavy atoms	0.7
Ordered residues ^c , all heavy atoms	1.2
Global quality scores ^c (raw/Z-score ^d)	
PROCHECK G-factor (ϕ/ψ)	−0.38/−1.18
PROCHECK G-factor (all dihedral angles)	−0.24/−1.42
MOLPROBITY clash score	12.25/−0.58
Verify3D	0.39/−1.12
ProsaII	0.47/−0.74
RPF scores ^e	
Recall/precision/F-measure	0.993/0.876/0.931
DP-score	0.841
Ramachandran plot summary (%) ^c	
Most favored regions	94.9
Additionally allowed regions	5.3
Generously allowed regions	0.2
Disallowed regions	0.0

^aResidues 391–543, excluding Pro ¹⁵N, Lys and Arg side chain amino groups, hydroxyl protons of Ser, Thr and Tyr, thiol protons of Cys, carboxyl atoms of Asp and Glu, C^ε1, H^ε1 and imino groups of His, and non-protonated aromatic carbons

^bResidues in regular secondary structure elements: 399–409, 424–433, 468–473, 521–527, 510–516, 494–499, 447–454, 455–461, 463–465, 481–491, 531–540

^cOrdered residues: 399–409, 424–432, 443–465, 468–473, 480–500, 509–515, 518–528, 531–541

^dCalibrated relative to a set of high-resolution X-ray crystal structures for which the corresponding mean structure-quality score corresponds to Z score = 0.0 [40]

^eAs described in [52]