The Six Zinc Fingers of Metal-responsive Element Binding Transcription Factor-1 Form Stable and Quasi-ordered Structures with Relatively Small Differences in Zinc Affinities*

Received for publication, May 31, 2005
Published, JBC Papers in Press, May 31, 2005, DOI 10.1074/jbc.M505217200

Belinda M. Potter‡, Linda S. Feng‡, Priya Parasuram‡, Viktor A. Matskevich‡, Jed A. Wilson‡, Glen K. Andrews§, and John H. Laity‡‡

From the ‡Division of Cell Biology and Biophysics, School of Biological Sciences, University of Missouri-Kansas City, Kansas City, Missouri 64110 and the §Department of Biochemistry and Molecular Biology, University of Kansas Medical Center, Kansas City, Kansas 66160

Six Cys2His2 zinc fingers (F1–6) comprise the DNA binding domain of metal-responsive element binding transcription factor-1 (MTF-1). F1–6 is necessary for basal and zinc-induced expression of metallothionein genes. Analysis of NMR structural and dynamic data for an F1–6 protein construct demonstrates that each zinc finger adopts a stable βαβα fold in the presence of stoichiometric Zn(II), provided that all cysteine ligands are in a reduced state. Parallel studies of protein constructs spanning the four N-terminal core DNA binding fingers (F1–4) and two C-terminal low DNA affinity fingers (F5–6) reveal similar stable zinc finger structures. In both the F1–6 and F5–6 proteins, the finger 5 cysteines were found to readily oxidize at neutral pH. Detailed spectral density and hydrodynamic analysis of 15N relaxation data revealed quasi-ordered anisotropic rotational diffusion properties of the six F1–6 zinc fingers that could influence MTF-1 DNA binding function. A more general effect on the rotational diffusion properties of Cys2His2 zinc fingers was also uncovered that is dependent upon the position of each finger within multifinger domains. Analysis of NMR 1H,15N-heteronuclear single quantum coherence spectral peak intensities measured as a function of added Zn(II) in conjunction with Zn(II) binding modeling studies indicated that the Zn(II) affinities of all MTF-1 zinc fingers are within ~10–50-fold. These analyses further suggested that metal sensing by MTF-1 in eukaryotic cells involves multiple zinc fingers and occurs over a 100-fold or less range of accessible Zn(II) concentration.

Organisms ranging from bacteria to mammals maintain intracellular Zn(II) levels within a functional range through homeostatic mechanisms that include metal-dependent regulation of gene expression. It has recently been proposed that the high Zn(II) binding affinities reported for the bacterial Zur and ZntR proteins suggest that Zn(II) homeostasis in Escherichia coli is tightly regulated, such that essentially no labile or “accessible” cytoplasmic Zn(II) is available (1). In this model, Zn(II) substitution reactions between Zur and ZntR and as yet unidentified metal chaperone transporters could mediate the metal response. In higher eukaryotic organisms, multiple homeostatic mechanisms regulate intracellular Zn(II) levels (2, 3). However, the accessible concentration range over which Zn(II) is regulated in eukaryotic cells is unknown. In higher eukaryotic organisms, Zn(II)-inducible expression of genes encoding for the zinc transporter-1 and cysteine-rich metallothionein class of proteins is mediated through the metal-responsive element binding transcription factor-1 (MTF-1) shown schematically in Fig. 1. Metallothioneins are the most well studied target of MTF-1 and are important for Zn(II) homeostasis, protection against oxidative stress, and heavy metal detoxification.

The MTF-1 transcription factor was first cloned from mouse (4) and has since been identified in humans (5, 6), Drosophila melanogaster (7), Takifugu rubripes (8), zebraﬁsh (9, 10), and chicken.2 Mouse MTF-1 (mMTF-1) is a 75-kDa, 675-amino acid protein that contains a six-Cys2His2 zinc ﬁnger DNA binding domain (F1–6) (11) and three distinct transcriptional activation domains (Fig. 1). With the exception of the Drosophila protein, 92% sequence identity exists between all other known orthologs of MTF-1 in the zinc ﬁnger domain (8). The human and mouse MTF-1 zinc ﬁnger domains dier by a single amino acid. By contrast, considerable sequence divergence occurs in the remaining domains of MTF-1. The high sequence conservation of the MTF-1 DNA binding domain is consistent with a proposed Zn(II) regulatory role. Transcriptional speciﬁcity of MTF-1 is mediated through F1–6, which recognizes a metal-responsive DNA promoter element (MRE). The minimal MRE consensus sequence is TGCCNC on the 5′-end of a larger 12-base pair site that contains a loosely conserved GC-rich region on the 3′-end (12–15). Although variable in orientation,

* This work was supported in part by National Institutes of Health Grant ES05704 (to G. K. A.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed: UMKC, 103 BSB, 5007 Rockhill Rd., Kansas City, MO 64110. Tel.: 816-235-5345; Fax: 816-235-6584; E-mail: laity@umkc.edu.

‡ The abbreviations used are: MTF-1, metal-responsive element binding transcription factor-1; mMTF-1, mouse MTF-1; F1–6, DNA binding domain of MTF-1 containing six Cys2His2 zinc ﬁngers; F1–4, four N-terminal zinc ﬁngers of MTF-1; F5–6, two C-terminal zinc ﬁngers of MTF-1; MRE, metal-responsive DNA promoter element; 1H-NOE, steady-state 1H-heteronuclear nuclear Overhauser enhancement; BME, β-mercaptoethanol; U-, uniform isotopic enrichment (98%); HSQC, heteronuclear single quantum coherence; MOPS, 3-(4-morpholino)propanesulfonic acid; d4-MES, deuterated 2-(N-morpholinoethanesulfonic acid; Rl, longitudinal relaxation time constant; Rr, transverse relaxation time constant; Tr, rotational correlation time; TF1–3, the three N-terminal zinc ﬁngers of TPiha from Xenopus laevis; WT1–4, the four zinc ﬁngers from the human Wilms tumor suppressor protein.

‡‡ H. M. Jiang and G. K. Andrews, unpublished data.
MREs are often found in multiple copies within the proximal promoter sites of target genes.

Multiple lines of evidence point to zinc finger metal coordination as an essential mechanistic contributor to Zn(II)-dependent activation of MTF-1. Electrophoretic mobility shift assays on nuclear extracts from various higher eukaryotic cell lines demonstrated that MTF-1 binding to the MRE is reversibly activated by the presence of 30 μM Zn(II) at 30–37 °C (6, 16–18). These and other experimental observations led to an estimation of in vitro Zn(II)-responsive MRE binding by MTF-1 as being in the low micromolar range (6, 16–18). Because these in vitro systems contained numerous other Zn(II)-binding proteins in relatively high concentration, a quantitative estimation of the Zn(II) affinities of the MTF-1 zinc fingers from these studies was not possible. However, recent Co(II) affinity measurements of each MTF-1 zinc finger in the context of the full-length F1–6 DNA binding domain indicated that two or three of the fingers do not adopt the predicted canonical secondary structures and linker regions indicated above the sequence. Commonly occurring DNA base-contacting residues are also indicated above the sequence as numerical positions relative to the start of the predicted α-helices.

FIG. 1. Schematic representation of the mMTF-1 gene. A, proceeding from N to C terminus, mMTF-1 contains six Cys_{2}His_{2} zinc fingers that comprise the DNA binding domain, followed by acidic, proline-rich, and serine/threonine-rich regulatory domains. B, amino acid sequence of the mMTF-1 zinc finger region (residues 129–312) aligned by individual fingers (F) with predicted canonical secondary structures and linker regions indicated above the sequence. Complexes occurring DNA base-contacting residues are also indicated above the sequence as numerical positions relative to the start of the predicted α-helices.

To reconcile conflicting structural and metal binding observations and to gain insights into the higher eukaryotic cellular Zn(II)-responsive range, we have characterized the structure, dynamics, and Zn(II) binding properties of all six MTF-1 zinc fingers (F1–6). Chemical shift, 3JHN-Hα scalar coupling, and 15N-{1}H NOE dynamics NMR measurements clearly demonstrate that each Cys_{2}His_{2} domain of MTF-1 adopts a stable ββα structure at submillimolar protein concentrations in the presence of 5 mM β-mercaptoethanol (BME) and stoichiometric quantities of Zn(II). Parallel studies show that the four N-terminal (F1–4) and two C-terminal (F5–6) zinc fingers of MTF-1 also form stable structures that are similar to those of the same fingers in the context of the full-length F1–6 DNA binding domain. An unusual tendency of zinc finger 5 toward cysteine oxidation is also reported. 15N relaxation measurements and subsequent spectral density and hydrodynamic analyses reveal that the rotational diffusion properties of F1–6 are relatively well correlated (quasi-ordered) compared with several other multizinc finger proteins, which suggests interfinger interactions could play a functional role in MTF-1 DNA binding. Position-specific effects on the rotational diffusion motions of zinc fingers that are likely general phenomena of the Cys_{2}His_{2} class of zinc fingers are also reported. Titration experiments monitored by 1H-15N-HSQC spectra peak intensities combined with theoretical binding studies also suggest that the Zn(II) affinities of all six MTF-1 zinc fingers are within 10–50-fold. These results are also consistent with a eukaryotic MTF-1 metalloregulatory mechanism involving multiple zinc fingers that occurs over a 100-fold or less concentration range of accessible Zn(II).

MATERIALS AND METHODS

Plasmid Construction and Expression—cDNA encoding residues 136–312 that encompass F1–6 was amplified by polymerase chain reaction (PCR) from a murine expressed sequence tag clone 1528077 (American Type Culture Collection). The DNA primers used for PCR amplification also incorporated the XbaI-NdeI region of pET21a(+) (Novagen) and an EcoRI restriction site onto the 5′- and 3′-ends of the gene, respectively. Gel-purified PCR fragments were digested and sub-
Cells were harvested by centrifugation and resuspended in lysis buffer (0.05% NaCl, 1 mM MgCl₂ and 1 mM CaCl₂) containing 50 μg/ml ampicillin. After overnight growth, gently centrifuged cells were resuspended in argon-saturated d₁₃-MES, pH 6.9, with 5% 3-(trimethylsilyl)-1-propane-sulfonic acid, and 1 mM NaN₃.

Proteins were prepared using standard methods. Mass spectrometry confirmed essentially identical protein compositions for MTF-1 zinc finger proteins F₁–₆ or F₁–₄ except for F₅–₆, which was soluble to 40% acetonitrile.

Protein extinction coefficients and concentration determinations for MTF-1 protein samples as determined by atomic absorption spectroscopy (Varian AA400, 599.7 MHz for 1H, 150.8 MHz for 13C, and 60.8 MHz for 15N) were calculated as described elsewhere (40, 41) using values of δ and R₂. All exponential data curves were fitted with the program CurveFit 1.30 (

Reduced spectral densities J(0), J(t), and J(0, t) were calculated for each finger from the R₁, R₂, and R₃ data sets. Theoretical J(t) values were calculated using empirical J(t) values for each finger.

An approximate isotropic chemical shift for each amino acid residue was calculated from the chemical shifts of the proton resonances (42). The chemical shifts were calculated as described elsewhere (31) using values of δ and R₂. All exponential data curves were fitted with the program CurveFit 1.30 (A. G. Palmer, III; Columbia University, New York, NY).

**Spectral Density Mapping**—Reduced spectral densities Σ(0), Σ(ω), and an average J(0.57Δω) in place of J(ω=0), J(ω + Δω), and J(ω=0) were calculated as described elsewhere (40, 41) using values of δ and R₂. All exponential data curves were fitted with the program CurveFit 1.30 (A. G. Palmer, III; Columbia University, New York, NY).
average ratio of R_2/R_1 values measured for rigid amide nitrogens according to Ref. 43 as shown in Equation 1

\[ \tau_{90} = (\gamma_{90} \sigma_{90}) (R_2/R_1) - 7 \]  
(Eq. 1)

using the program mapsdf generously provided by P. E. Wright (Scripps Research Institute, La Jolla, CA). Criteria used to identify rigid nitrogens with no significant contributions from subnanosecond-to-nanosecond internal (\( \tau_i \)) or microsecond-to-millisecond segmental motions due to chemical exchange (R_\( \tau_e \)) were those of Barbato et al. (44).

Rotational Diffusion Calculations—Both isotropic and anisotropic models were used to characterize the overall rotation diffusion properties of the F1–6, F1–4, and F5–6 MTF-1 zinc finger constructs using the TENSOR2 program (45). Because three-dimensional structural models were needed for each MTF-1 zinc finger as input for TENSOR2, a homology modeling approach using the MODELLER program version 8v2 was used to generate these structures (46). Suitable zinc finger structural templates for MODELLER were chosen from a recently compiled list of zinc finger proteins (47) for which structural models were available in the RCSB Protein Data Bank (www.rcsb.org/pdb/). Single or multiple templates used for final modeling calculations had at least 35% sequence identity to the respective MTF-1 zinc finger target and a z-score of at least 3.5 and produced homology models with a backbone root mean square deviation between template(s) and target of ≤1 Å (Table 1).

In addition to the selection criteria for rigid amides already described, residues with calculated differences in backbone \( \phi \) and/or \( \psi \) dihedral angles of >35° between the zinc finger models and the corresponding dihedral angles measured for F1–6 using the TALOS method (48) were also excluded. Confidence in the isotropic and anisotropic diffusion models was assessed in TENSOR2 using Monte Carlo sampling methods to compare experimental \( \chi^2 \) values (\( \chi^2_{\text{exp}} \)) with those Monte Carlo simulated values (\( \chi^2_{\text{MC}} \)) determined at the 90% confidence limit from the optimal fitted diffusion parameters (45). Because of the inherent deviations in individual residue chemical shift anisotropy, R_2 and R_1 uncertainties were set at 5% of the time constant value for residues with lower fitted uncertainties (generally 1–3%) (49).

Zinc Titrations—Zn(II)-coupled binding/folding of each apo F1–6 finger was monitored by \(^{1}H\)-\(^{15}N\)-HSQC fractional peak intensities, which were calculated from the ratio of each peak intensity corresponding to a given residue \(^{1}H\)-\(^{15}N\) correlation to the maximum peak intensity value measured from all of the \(^{1}H\)-\(^{15}N\)-HSQC experiments for that residue.

Theoretical Zn(II) Occupancy—Calculations of Zn(II) occupancies for each MTF-1 zinc finger within F1–6 were carried out using the Berkeley Madonna software package (version 8.0.1, ©1997–2000, Robert I. Macey and George F. Oster, Kagi Shareware, Berkeley CA). Solutions to differential equations using the Runge-Kutta 4 integration method provided steady-state metal-bound concentrations for each finger. Six non-cooperative simultaneous coupled metal binding-folding equilibrium described in Equation 2 were assumed (50)

\[ \text{Zn}(II) + F_i \rightleftharpoons \text{Zn}(II)F_i \ (i = 1 - 6) \]  
(Eq. 2)

where F_i and Zn(II)F_i are the apo and metal bound forms for each zinc finger of MTF-1, respectively. Equilibrium Zn(II) occupancies were calculated for each finger using the full range of \( K_{i} \) values reported previously for zinc finger binding to Co(II) or Zn(II) (10 \(^{10} – 10^6 \) M\(^{-1}\)) (19, 51). An estimation of the minimum high/low Zn(II) occupancy ratio from all six F1–6 fingers for a given range of affinities was sought. Therefore, within each \( K_{i} \) range modeled, one finger had the highest Zn(II) affinity and the remaining five fingers had the lowest affinity because this scenario always produced this minimum high/low Zn(II) occupancy ratio. Experimental concentrations of 180 \( \mu \)M for each F1–6 zinc finger (F_i) and 270 \( \mu \)M Zn(II) were used for modeling calculations. These concentrations of 1.5 molar equivalents Zn(II)/mole F1–6 protein were chosen because they represent the minimum Zn(II)/protein ratio in which resonances from all six MTF-1 fingers in the 180-\( \mu \)M experimental protein sample could be observed.

RESULTS

MTF-1 F5 Cysteines Are Hypersensitive to Thiol Oxidation—MTF-1 zinc finger constructs encompassing F1–6, F1–4, and F5–6 were isolated as lyophilized apo proteins in the free thiol (reduced) state. Despite exhaustive efforts to maintain anaerobic samples, analysis of preliminary NMR triple resonance spectra recorded for the F1–6 and F5–6 proteins in the presence of stoichiometric Zn(II) produced evidence of progressive cysteine thiol oxidation. Although well dispersed peaks corresponding to residues within folded zinc finger domains were evident in these early spectra, a second set of poorly dispersed resonances with increasing intensity over the course of data collection was also observed. Peaks with the greatest loss of intensity by far were determined to correspond to finger 5 in the folded state. Nearly complete assignments of the poorly dispersed resonances from the F5–6 spectra revealed a sequential run of finger 5 residues with essentially random coil chemical shifts and downfield-shifted (40–42 ppm) cystine \(^{13}C\beta \) values (data not shown). Because Zn(II) coordination to the two cysteine ligands is a necessary stabilizing force for canonical \( \beta \) structure formation within each finger (52), these observed random coil shifts are consistent with a disulfide-bonded zinc finger 5 polypeptide. To prevent thiol oxidation, 5 mM BME was added to zinc finger protein samples used to record all subsequent NMR spectra in these studies. BME was chosen for its relatively low Zn(II) binding affinity (53). \(^{1}H\)-\(^{15}N\)-HSQC spectra recorded over a 2–3-week period for F1–6, F1–4, and F5–6 protein samples treated with BME had stable intensities and monodispersed resonances including those corresponding to the finger 5 polypeptide. Addition of 5 mM BME to a partially oxidized F1–6 sample was sufficient to regenerate a stable NMR sample with the same spectral properties. An \(^{1}H\)-\(^{15}N\)-HSQC spectrum for F1–6 annotated with residue assignments for most resonances is shown in Fig. 2. Corresponding \(^{1}H\)-\(^{15}N\)-HSQC spectra of similar quality were recorded for F1–4 and F5–6 at 20 °C under otherwise identical solution conditions (not shown).

All Six MTF-1 Zinc Fingers Form Stable \( \beta \) Folds in the Presence of Stoichiometric Zn(II)—A total 161 of 169 possible backbone \(^{1}H\)-\(^{15}N\) correlations from residues 139–312 comprising the zinc finger domain of MTF-1 (Fig. 1) were assigned from...
a U^{15}N,^{13}C-labeled F1–6 protein sample in the presence of 5 mM BME and 1.05 molar equivalents of added Zn(II)/finger at indicated with H_{9252}. Predicted secondary structure schematic drawings for each finger are indicated above the plot. Bars and secondary structure schematics corresponding to residues in canonical β-sheet and α-helical structures in upper and lower panels are indicated with grey and black bars, respectively.

Comparison of the chemical shifts obtained for the MTF-1 F1–6 protein to those of random coil showed in Fig. 3 is a good indicator of secondary structure (35, 55). The most prominent repeating feature within each finger is the C-terminal α-helix that is strongly predicted by the presence of positive Δδ^{13}Ca and ^{13}CO values in these regions. The only deviation from predicted structure of any F1–6 zinc finger indicated from the data in Fig. 3 is the sequence position of the first finger 5 α-helical residue at His-274. For a canonical zinc finger, the first α-helical residue occurs 2 amino acids C-terminal to the second β-strand (Ser-273 for finger 5). Another prominent feature of the chemical shift data descriptive of canonical zinc finger structure is the significantly upfield-shifted ^{13}Ca and downfield-shifted ^{13}Cβ resonances for the residues in the −1 position relative to the start of each predicted α-helix attributed to an N-cap (56). By first approximation, negative Δδ^{13}Ca and ^{13}CO values are expected for the 6 residues within each finger predicted to comprise the small anti-parallel β-sheet. Although the ^{13}Ca and ^{13}CO trends in these regions are only partially obeyed, chemical shift comparisons to random coil throughout each finger have a very similar pattern to those reported for the three N-terminal fingers of TFIIA (TFI–3) and the four zinc fingers from the human Wilms tumor suppressor protein (WT1–4) (57, 58). Moreover, the backbone ^{13}Ca shift differences from random coil for each F1–6 cysteine at residue position 3 of β-strand 1 are positive (except finger 6), likely because of the effects of thiolate coordination to Zn(II) (58). The topology of F1–6 was further analyzed using the TALOS method (48), which produced dihedral angle restraints consistent with βα secondary structures for all fingers (Fig. 3).

Comparison of weighted HN chemical shift differences from random coil values for each F1–6 residue (139–312) (35). Predicted secondary structure schematic drawings for each finger are indicated above the plot. Bars and respective spectral resolutions (0.02 ppm for 1H, 0.15 ppm for ^{13}N, 0.3 ppm for ^{13}Ca, and 0.1 ppm for ^{13}CO) and backbone ^{13}C chemical shift differences between residues from F1–6 with those of F1–4 (A) and F5–6 (B). Predicted secondary structure schematic drawings for each finger are indicated above the plot.

FIG. 3. The six MTF-1 zinc fingers (F) comprising the DNA binding domain form canonical βα folds. Upper panel, bar plot of backbone ^{1}H, ^{15}N, ^{13}Cα, and side chain ^{13}Cβ chemical shift differences from random coil values for each F1–6 residue (139–312) (35). Predicted secondary structure schematic drawings for each finger are indicated above the plot. Lower panel, scalar ΔJ_{HN-Hα}, coupling constants for F1–6 residues obtained from three-dimensional HNHA (31) with secondary structure features predicted from backbone ϕ, ϑ angles using the TALOS method (48) indicated schematically above the plot. Bars and secondary structure schematics corresponding to residues in canonical β-sheet and α-helical structures are indicated with grey and black bars, respectively.

FIG. 4. Folded structures of individual zinc fingers (F) in smaller N- (F1–4) and C-terminal (F5–6) protein constructs are similar to those in the F1–6 context. Bar plots of HN ([δH^2+(δN[swN/swH])^2]^{1/2}, ^{13}CO, and ^{13}Ca chemical shift differences relative to the start of each predicted α-helix attributed to an N-cap (56). By first approximation, negative Δδ^{13}Ca and ^{13}CO values are expected for the 6 residues within each finger predicted to comprise the small anti-parallel β-sheet. Although the ^{13}Ca and ^{13}CO trends in these regions are only partially obeyed, chemical shift comparisons to random coil throughout each finger have a very similar pattern to those reported for the three N-terminal fingers of TFIIA (TFI–3) and the four zinc fingers from the human Wilms tumor suppressor protein (WT1–4) (57, 58). Moreover, the backbone ^{13}Ca shift differences from random coil for each F1–6 cysteine at residue position 3 of β-strand 1 are positive (except finger 6), likely because of the effects of thiolate coordination to Zn(II) (58). The topology of F1–6 was further analyzed using the TALOS method (48), which produced dihedral angle restraints consistent with βα secondary structures for all fingers (Fig. 3). Backbone ΔJ_{HN-Hα}, scalar coupling constants measured for residues in canonical α-helical and β-sheet regions were generally <5 and >7 Hz, respectively, which are qualitatively consistent with the respective secondary structure predictions (Fig. 3).
also worth noting that the linker region between fingers 5 and 6 and β-strand 2 of finger 6 in the F5–6 protein may have some subtle structural or dynamic difference manifested by changes in HN and 13Cα or 13CO shifts (Fig. 4).

MTF-1 Zinc Fingers Have Similar Fast Internal Dynamics with Quasi-correlated Position-specific Anisotropic Rotational Diffusion Properties—A summary of 15N relaxation data recorded at 60 MHz 15N frequency for F1–6, F1–4, and F5–6 is provided in Fig. 5. Only those residues with well resolved 1H-15N resonances corresponding to a single amino acid were used in the subsequent analyses. The two-dimensional 15N relaxation spectra of the repeating zinc finger domains contained a rather large number of overlapped resonances because numerous residue positions in different fingers contain identical or chemically similar residues that are likewise in similar chemical environments within each domain (see Fig. 1). As the largest multi-Cys2His2 zinc finger protein used for NMR studies to date with six repeating domains, it is not surprising that resonance overlap was most severe for the F1–6 protein. In addition, a small number of resonances with insufficient intensity for accurate measurement in one or more of the three 15N-1H-NOE, R2, or R1 data sets were also excluded. Residues for which 15N relaxation parameters were determined based on the above criteria are indicated in Fig. 5.

15N-1H-NOE measurements provide information about protein fast internal dynamics (backbone flexibility) that occur on the nanosecond-picosecond timescale such as librational or internal motions for linker residues of F1–6 (0.45 ± 0.07, 3) 0.67 ± 0.06, 5) 0.69 ± 0.11, and 6) 0.65 ± 0.09; and F1–4, 1) 0.66 ± 0.07, 2) 0.67 ± 0.05, 3) 0.65 ± 0.08, and 4) 0.61 ± 0.07; F5–6, 5) 0.64 ± 0.05, and 6) 0.65 ± 0.06 were calculated. Corresponding values for linker residues of F1–6 (0.45 ± 0.12), F1–4 (0.45 ± 0.08), and F5–6 (0.60 ± 0.01) suggest that residues within the typically disordered linkers connecting adjacent fingers are more flexible than those residues from the finger regions, although the single F5–6 linker appears to be decidedly less so.

R2/R1 ratio is directly affected by rotational diffusion motions that occur on the one to tens of nanoseconds timescale. Both the F1–6 and F1–4 zinc finger proteins have uncharacteristically large and heterogeneous R2/R1 ratios when compared with other zinc finger proteins studied, including ADR1 (transcription factor containing two zinc fingers that regulates alcohol dehydrogenase and other peroxisomal enzymes) (61) and WT1–4 (5) (Fig. 5). By contrast, R2/R1 ratios measured for F5–6 are much smaller and more homogeneous. The generally larger R2/R1 ratios observed in helical residues within each internal MTF-1 finger of F1–6 and F1–4 (Fig. 5) are consistent with a predicted greater alignment of these N-H vectors with the long diffusion axis of anisotropic multifinger domains (62) (finger-finger alignment and rotational diffusion is analyzed in detail later in this section). A systematic trend of increasing average R2/R1 ratios starting from the N- and C-terminal fingers of F1–6 and F1–4 that progresses to the internal fingers is also evident in Fig. 5. Localized effects of R2 chemical exchange processes on the microsecond-millisecond timescale also affects the T2 or transverse relaxation times (1/R2) for some of the residues in the MTF-1 zinc fingers. Most notably, Thr-142 and Gly-145 in zinc finger 1 from both F1–4 and F1–6 have R2 values (22–24 s−1) essentially twice the per finger averages calculated for the respective proteins. Interestingly, these two residues within the putative metalloregulatory finger 1 are adjacent to the two metal binding cysteines (20). A possible correlation between the microsecond-millisecond dynamics of Thr-142 and Gly-145 and the putative metal-sensing mechanism of finger 1 is currently under investigation. 15N relaxation data recorded for F1–6 at an ~50% lower protein concentration (~400 μM) produced very similar R2 and R1 time constants (data not shown), diminishing the possibility that aggregation could account for the heterogeneous and generally larger R2/R1 ratios observed for the MTF-1 F1–4 and F1–6 proteins.

Reduced spectral density maps of J(0), J(ωN), and J(0.87ωH) presented in Fig. 6 were calculated to qualitatively characterize the complex dynamic properties of the tethered multifinger MTF-1 proteins. The progressively larger average J(0) and lower average J(ωN) and J(0.87ωH) spectral densities for the internal fingers in F1–6 and F1–4 suggest correspondingly more restricted rotational diffusion for these interior domains, which are tethered to one or more adjacent zinc fingers on both ends. Overall, the Fig. 6 spectral density maps and Fig. 7 plots

3 J. H. Laity, H. J. Dyson, and P. E. Wright, unpublished data.
structure schematic drawings for each finger are indicated above plot.

FIG. 6. MTF-1 zinc fingers have quasi-ordered rotational dynamics. Reduced spectral density plots as a function of residue number for F1–6 (A), F1–4 (B), and F5–6 (C). Spectral densities were calculated at J(0) (upper panel), J(wN) (middle panel), and J(0.87wH) (lower panel) frequencies. Predicted secondary structure schematic drawings for each finger are indicated above plot.

are dependent on T², they alone are affected by Rₓₓ and are also very sensitive to anisotropy. J(wN) spectral densities are dependent on T¹ (1/R₁) and therefore are also affected by anisotropic rotational diffusion, albeit to a lesser extent. By contrast the timescales of the two F5–6 zinc finger rotational diffusion motions appear to be very similar and faster than those of even the most rapidly tumbling domains from the larger MTF-1 zinc finger proteins.

Apparent rotational correlation times (τₐ) of: F1–6, 1) 8.2 ± 0.4, 2) 11.0 ± 1.1, 3) 11.8 ± 0.9, 4) 10.2 ± 1.1, 5) 9.2 ± 1.0, and 6) 8.0 ± 0.5; F1–4, 1) 7.9 ± 0.3, 2) 10.6 ± 0.9, 3) 10.1 ± 0.8, and 4) 7.1 ± 0.3; F5–6, 5) 4.5 ± 0.4 and 6) 4.2 ± 0.3 ns were calculated using residues with rigid amides (44) (no significant Rₓₓ or Rᵧᵧ) from each zinc finger of the different MTF-1 constructs. A total of 12 F1–6, 11 F1–4, and 2 F5–6 non-rigid amides were excluded from these τₐ calculations. Given the heterogeneous Rₓₓ/Rᵧᵧ ratios measured for the remaining residue amides that are consistent with significant anisotropic rotational diffusion motions (Fig. 5), τₐ only represents an approximate weighted average of the inverse diffusion constants from each of the three principal axes of diffusion ([Dₓₓ]⁻¹, [Dᵧᵧ]⁻¹, [Dzz]⁻¹) (62). Despite the isotropic approximation inherent to τₐ, the rotational diffusion properties of zinc fingers from MTF-1 F1–6 and F1–4 appear to be more restricted and thus more correlated than those of the WT1–4 protein, which has τₐ values of 1) 5.2 ± 0.8, 2) 6.6 ± 0.8, 3) 6.3 ± 1.0, and 4) 5.4 ± 0.9 ns (60). It should be noted that WT1–4 also appears to have similar but more subdued position-specific rotational diffusion properties (internal fingers are more restricted).

To probe the MTF-1 zinc finger hydrodynamics in more depth, calculations of rotational diffusion properties incorporating both isotropic and anisotropic tumbling models were performed using the TENSOR2 program (45). Homology-based structural models of each MTF-1 zinc finger were constructed as described under “Materials and Methods” and in Table I. With the exception of residues 1) Arg-148, 2) Gln-173, 2) Gly-177, 3) Asp-202, and 5) Lys-267 from F1–6, all TALOS-derived backbone ϕ and ψ dihedral angles from residues with non-excluded amides were within 35° of those same residues in the homology models (most ϕ and ψ angles of residues in the α-helices were within 1–10°). However, because of the large number of overlapped resonances and other non-rigid residue exclusions, of 25 possible amino acids per finger, a total of F1–6, 1) 14, 2) 11, 3) 12, 4) 11, 5)15 and 6) 8; F1–4, 1) 12, 2) 14, 3) 14, and 4) 12; F5–6, 5) 18 and 6) 18 residues provided useful ¹⁵N relaxation parameters for hydrodynamic calculations.

A summary of these calculations is given in Table II. With the exception of finger 6 from F5–6, all of the MTF-1 zinc fingers from F1–6, F1–4, and F5–6 were fit successfully to the anisotropic diffusion model at the 90% confidence interval. By
much of the experimental R2 and R1 heterogeneity. One possibility more pronounced in the internal fingers, clearly accounts for the domain orientation relative to the axes of diffusion for each an anisotropic model (compare isotropic models, albeit with mostly lower quality fits than those obtained with 6 from F1–6 and F5–6 produced accepted isotropic models, contrast, only data from fingers 1 and 4 from F1–4 and finger 6 from F1–6 and F5–6 produced accepted isotropic models, albeit with mostly lower quality fits than those obtained with an anisotropic model (compare x^2_{MC} with x^2_{MC} for both models in Table II). A plot of experimental R2/R1 ratios bracketed with 5% uncertainties (49) is superimposed with back-calculated R2/R1 ratios in Fig. 8. The rotational anisotropy in F1–6, which is more pronounced in the internal fingers, clearly accounts for much of the experimental R2 and R1 heterogeneity. One possible domain orientation relative to the axes of diffusion for each Finger (F) | Protein Data Bank template | Identity | Root mean square deviation | Z-score
|---|---|---|---|---|
| F1 | 1UBD (F1) | 56 | 0.7 | 3.7 |
| F2 | 1UBD (F2) | 40 | 0.6 | 3.7 |
| F3 | 1UBD (F1) | 40 | 0.6 | 3.7 |
| F4 | 1UBD (F1) | 40 | 1.0 | 3.7 |
| F5 | 1UBD (F1) | 40 | 0.6 | 3.7 |
| F6 | 1UBD (F1) | 40 | 0.8 | 3.7 |

Table II Hydrodynamic parameters from MTF-1 zinc finger domains

| Protein finger (F) | Isotropic model | Anisotropic model |
|---|---|---|
| | Rotational correlation time, τ_0 | Diffusion tensor elements \( \times 10^{-7} \text{ s}^{-1} \) | Rotational correlation time elements (ns) |
| | \( \tau_0 \) (ns) | \( x^2_{exp} \) | \( x^2_{MC} \) | \( (6D_{xx})^{-1} \) | \( (6D_{yy})^{-1} \) | \( (6D_{zz})^{-1} \) |
| F1-6 | F1 | 2.4 ± 1.0 | 1.7 ± 0.3 | 1.9 ± 0.3 | 7.8 | 12.9 | 6.8 |
| F2 | 1.4 ± 0.2 | 1.1 ± 0.2 | 2.3 ± 0.4 | 4.6 | 9.0 | 11.8 |
| F3 | 0.9 ± 0.3 | 1.6 ± 0.3 | 2.3 ± 0.6 | 6.0 | 10.5 | 18.6 |
| F4 | 1.2 ± 0.2 | 1.6 ± 0.2 | 2.7 ± 0.4 | 6.8 | 9.0 | 14.3 |
| F5 | 0.9 ± 0.1 | 2.3 ± 0.1 | 2.1 ± 0.1 | 7.6 | 14.4 | 18.2 |
| F6 | 1.7 ± 0.5 | 2.2 ± 0.7 | 2.5 ± 0.2 | 3.2 | 3.7 | 9.7 |

Table I MTF-1 F1–6 zinc finger modeling parameters

Homology models calculated using MODELLER version 6v2 (46).

| Finger (F) | Protein Data Bank template | Template/target | Identity | Root mean square deviation | Z-score |
|---|---|---|---|---|---|
| F1 | 1UBD (F1) | | 56 | 0.7 | 3.7 |
| F2 | 1UBD (F2) | | 40 | 0.6 | 3.7 |
| F3 | 1UBD (F1) | | 36 | 0.8 | 3.7 |
| F4 | 1UBD (F1) | | 40 | 1.0 | 3.7 |
| F5 | 1UBD (F1) | | 40 | 0.6 | 3.7 |
| F6 | 1UBD (F1) | | 40 | 0.8 | 3.7 |

* Structural templates for modeling corresponding to RCSB Protein Data Bank codes given.
* Percent sequence identity of template and model.
* Backbone root mean square deviation between template and calculated modeled structures.
* Template was Finger F if multizinc finger protein.

Hydrodynamic parameters were calculated using the TENSOR2 program (45).

Hydrodynamic parameters were calculated using the TENSOR2 program (45).
protein fragment, estimations of the order parameter $S_2$ calculated for each residue directly from $J(0)$ and $J(\rho)$ using the model free approach (64, 65) with isotropic and anisotropic constraints in TENSOR2 (46) as a function of residue number for each MTF-1 finger (F).

Efforts to fit the MTF-1 F1–6 and F5–6 $^{15}$N relaxation data using the model free approach (64, 65) with isotropic and anisotropic models in TENSOR2 were unsuccessful. Similar problems have been encountered with other proteins, such as an 200-amino acid fragment of the prion protein that does not exhibit a single global correlation time (66). As with the prion protein fragment, estimations of the order parameter $S_2$ calculated for each residue directly from $J(0)$ and $J(\rho)$ often exceeded unity, and as such were not meaningful. A model free analysis was successful for the F5–6 protein in that most residues (except Lys-267) were fit to model 1 or 2 (67) with no $R_s$ and $t$ less than 100–200 ps. However, the interpretation of these data alone did not seem to add any new insights into the structural and functional properties of the MTF-1 metalloregulatory DNA binding domain.

**MTF-1 Zinc Fingers Have Similar Zn(II) Binding Affinities**—The relative Zn(II) binding affinity of each MTF-1 finger within F1–6 was investigated using $^1$H-$^{15}$N-HSQC-based Zn(II) titrations starting from the apo form of the protein. Representative spectra and results of the titration are presented in Fig. 9. Analyzed $^1$H-$^{15}$N-HSQC peaks corresponded to residues in the folded protein that were in slow exchange on the NMR timescale (the vast majority of all resonances). A fractional peak intensity corresponding to a given residue and Zn(II) concentration, which is defined as measured intensity divided by maximum peak intensity corresponding to the same residue measured from spectra at all Zn(II) concentrations, was averaged over all observable residues from each finger (Fig. 9, C and D). Moreover, because zinc finger folded structure is fully coupled to Zn(II) binding (52), this average fractional intensity was used to estimate the relative extent of folding for each finger as a function of added Zn(II). Data from a minimum of 9 residues/finger show that each finger has a measurable Zn(II) occupancy by 0.75 equivalents except for finger 6, which is observable at 1.5 equivalents (Fig. 9B).

Based on relative Zn(II) occupancies of each finger at substoichiometric Zn(II) levels (Fig. 9D), the relative affinities for the MTF-1 fingers appear to be in qualitative agreement with those reported recently for Co(II): (finger, F, highest to lowest) $F_4 > F_2 = F_5 > F_1 = F_3 = F_6$ (19). However, given the overlapping uncertainties of some Zn(II) titration points between 0.75–2.2 equivalents, the ranking of closely spaced fingers is approximate. Finally, a surprising heterogeneity is evident in the finger 2 binding data in which residues from the loop connecting adjacent $\beta$-strands and the C-terminal region of the $\alpha$-helix (light blue data points) appear to have less ensemble structural order at substoichiometric quantities of Zn(II). The cause of this structural heterogeneity is unknown and is currently under investigation.

**DISCUSSION**

The results of the NMR studies presented here clearly show that all six MTF-1 zinc fingers are capable of forming stable canonical $\beta\beta\alpha$ secondary structures at submillimolar protein concentrations in the presence of stoichiometric quantities of Zn(II) and millimolar BME (Fig. 3). Chemical shift comparisons of smaller N- and C-terminal zinc finger proteins shown in Fig. 4 also suggest that context-dependent effects on the structure and stability of each MTF-1 finger are not likely to be significant. However, a hypersensitivity of zinc finger 5 to cysteine thiol oxidation indicates that the metal binding properties of this finger may be somewhat unusual. A 50-fold greater thiolate reactivity for a subset of approximately three MTF-1 fingers has been reported previously (23). One possible explanation for hypersensitivity to thiol oxidation is labile Zn(II) binding, which is consistent with previous studies in which unusually high susceptibility to thiol alkylation by finger 5 was also observed (68). However, the observations reported here of a relatively stable $\beta\beta\alpha$ fold for finger 5 appear on the surface to contradict a Zn(II) lability theory. Although the cause of the increased susceptibility of cysteine oxidation in finger 5 is still under investigation, elevation of both “on” and “off” rates for Zn(II) binding to the metal site could be consistent with all experimental observations to date (i.e. stability and
contribution to the metalloregulatory function of MTF-1 (via protein inactivation) from a more labile zinc finger could also be envisaged whereby a drop in cellular Zn(II) levels would produce a rapid and potentially irreversible (thiol oxidation) loss of ββββ structure. It is tempting to speculate that the reported instability of finger 5 in the absence of any reducing agents from an earlier NMR study could be because of hyper-reactive cysteine thiol oxidation (25). However, a spectrophotometric assay from that study indicated that all cysteines were in the free thiol state, suggesting that another explanation may be needed to reconcile the conflicting structural observations for finger 5.

The two stably folded zinc fingers (finger 4 and finger 6) from an earlier study of MTF-1 F4–6 had $^{15}$N-$^1$H-NOE values of 0.61 (finger 4) and 0.64 (finger 6), which is in the range of the values reported here. The MTF-1 F1–6, F1–4, and F5–6 $^{15}$N-$^1$H-NOE values are also consistent with those reported previously for the TF1–3, WT1–4, and ADR1 multizinc finger proteins (59–61), suggesting that the backbone flexibility of the metal-responsive MTF-1 zinc fingers is similar to those of other non-metalloregulatory DNA binding domains.

Rotational diffusion motions of proteins are influenced by molecular weight, hydration, and shape. It seems highly unlikely that large hydration differences exist between different DNA binding zinc finger proteins that have similar surface charge potentials. The effective shape of a multizinc finger protein is defined by the ensemble average of finger-finger orientations that reside somewhere between two extremes: (i) individual tumbling domains restricted only by tethering to adjacent zinc fingers via flexible peptide linkers, and (ii) a single more anisotropic rigid body of connected modules. The significantly greater isotropically approximated rotational diffusion values calculated for MTF-1 F1–4 (13.7 kDa) compared with WT1–4 (14.1 kDa) indicate that the two zinc finger proteins, each with four canonical domain structures, have different ensemble average solution conformations. F1–6, F1–4, and F5–6 are characterized by significant anisotropic rotational diffusion properties (Table II) with partial alignment of the principal axes of diffusion for each internal finger (Fig. 8, data not shown for F1–4 and F5–6). Rotational diffusion properties based on an anisotropic model were not reported for WT1–4. However, the more homogeneous $R_2/R_1$ ratios calculated for WT1–4 suggest less anisotropy compared with the MTF-1 F1–4 and F1–6 fingers. Overall, the most plausible model suggested from the $^{15}$N relaxation data from all three F1–6, F1–4, and F5–6 proteins is one in which each F1–6 zinc finger is characterized by significant interdomain interactions that produce an elongated ensemble-averaged multifinger conformation. Indeed, an ensemble extended three-zinc-finger domain from TF1–3 has been reported previously (62).

The clear increasing trend of $R_2/R_1$ and $J(0)$ shown in Figs. 5 and 6, respectively, that starts from N- and C-terminal fingers and progresses inward to internal fingers demonstrates that finger tethering plays a predictable and more general role in Cys2His2 zinc finger rotational diffusion properties. Specifically, individual domain motions become increasingly more restricted for internal fingers that are tethered to adjacent single or multiple domains on both ends. The rotational diffusion motions of the MTF-1 zinc fingers are complex, because greater finger-finger interactions also increase the effective molecular weight experienced by the residues within each affected finger. This complexity more than likely contributed to the lack of success fitting model free parameters (64, 65) to the MTF-1 F1–6 $^{15}$N relaxation data.

An elongated ensemble conformation resulting from more restricted domain motion compared with other previously stud-
ied zinc finger proteins would have functional implications for MTF-1 DNA binding properties (60, 61), although a direct link to MTF-1 metal sensing is not obvious. Because the linkers connecting adjacent zinc fingers are flexible in the DNA-free state (lower \(^{15}\)N-,\(^{1}H\)-NOE values), the structural determinants of the quasi-ordered MTF-1 zinc fingers suggest as yet unidentified interdomain contacts may be present within each finger. By contrast, the relative domain orientations of DNA-bound zinc finger structures along the major groove of DNA are typically at least partially determined by highly specific residue-DNA base contacts and DNA-induced canonical ordered and less flexible linker conformations (60, 70, 71). Therefore, potential enthalpic DNA binding penalties could result from the disruption of interdomain contacts within the free F1–6 protein concomitant with DNA-induced changes in finger orientations. Even if the free F1–6 interdomain interactions orient the fingers in such a way that is optimal for DNA binding, a loss of at least some of the DNA-induced finger-finger packing stabilization is likely if the free fingers are already ordered (62). However, a stabilizing entropic DNA binding effect for F1–6 stemming from reduced finger-finger mobility in the free state could compensate the destabilizing enthalpic contributions, although the extent of this compensation is unknown.

The narrow 25-fold range in Co(II) affinities reported for the MTF-1 zinc fingers by Berg and coworkers (19) could suggest that multiple fingers are involved in metalloregulation if Zn(II) binding properties follow a similar trend. However, the Zn(II) affinities of individual MTF-1 zinc fingers are unknown, and parallel studies of Zn(II) and Co(II) binding by other Cys4His2 zinc fingers have reported variable Zn(II)/Co(II) affinity ratios in the range of \(10^2-10^5\) (52, 72). Therefore, we sought to estimate an upper limit for the range of MTF-1 F1–6 Zn(II) affinities that would be consistent with our titration results. Theoretical Zn(II) occupancies of the MTF-1 zinc fingers were calculated from a general binding model of six metal sites, each at an experimental protein concentration of 180 \(\mu\)M and a total Zn(II) concentration of 1.5 equivalents (270 \(\mu\)M) using Ka, spanning the maximum reported for any zinc finger (11.1 \(M^{-1}\)) to the lowest measured MTF-1 Co(II) affinity (10.6 \(M^{-1}\)) (19).

The Zn(II) concentration used for modeling is the minimum concentration at which resonances from all six MTF-1 fingers could be observed (Fig. 9D). For all solutions to this model, any of the six zinc fingers one order of magnitude higher in \(K_a\) compared with the lowest affinity finger would have at least 4.0-fold higher Zn(II) occupancy at 1.5 equivalents. Zinc fingers two orders of magnitude higher in \(K_a\) would be at least 8.1-fold higher. Actual high/low Zn(II) occupancy ratios would probably be substantially greater because this is the predicted outcome of having several higher affinity fingers, and fingers 1–5 appear to be higher affinity than finger 6 (Fig. 9, C and D). Close inspection of the NMR data recorded at 180 \(\mu\)M protein indicates that the average peak intensity at 1.5 equivalents of Zn(II) for all observable resonances from the highest affinity MTF-1 zinc finger (finger 4) was 0.063 ± 0.014, whereas the absolute threshold for peak detection was ≥0.010. By comparison, average peak intensities for the lowest affinity finger at 1.5 equivalents of Zn(II) was 0.012 ± 0.002. Given the strong correlation of peak intensity with Zn(II) occupancy for each finger shown clearly in Fig. 9B, this 5-fold finger 4/finger 6 peak intensity ratio provides strong evidence that the relative affinities for Zn(II) of all MTF-1 zinc fingers are remarkably similar (within ±50-fold).

Overall, the Zn(II) binding studies presented here strongly support the role of multiple similar affinity zinc fingers in governing metal sensing by MTF-1 in eukaryotic cells. Moreover, because two of the medium Zn(II) affinity fingers (finger 1 and finger 3) are also needed for high affinity DNA binding and concomitant MTF-1 function (20, 22), an upper limit for the concentration range over which accessible Zn(II) is sensed and responded to by MTF-1 can be estimated using the binding model described by Equation 2. Assuming similar affinities for all F1–6 fingers indicated from Fig. 9D (within 50-fold), F1–6 occupancies ranging from <10 to >90% of the maximum attainable will always occur within a Zn(II) concentration range of 100-fold or less for all finger \(K_a\) values within the range of 10^6–10^11 \(M^{-1}\) regardless of MTF-1 or accessible Zn(II) concentrations. In this model, no explicit consideration is given for potential finger-finger or DNA binding cooperative effects on Zn(II) binding affinities, although these effects would be expected to decrease the Zn(II)-responsive range for metal sensing. Indeed, evidence from Co(II) binding and thiol alkylation studies suggest modest cooperativity from both finger-finger and DNA binding (19, 68). The absolute affinity and \(K_a\) copy number range for the regulatory fingers needed to establish the intracellular accessible Zn(II) “set point” concentration and actual concentration range that is sensed by MTF-1 is currently under investigation.

REFERENCES
and Wright, P. E. (1992) Biochemistry 31, 4394–4406
40. Farrow, N. A., Zhang, O., Forman-Kay, J. D., and Kay, L. E. (1995) Biochemistry 34, 868–878
41. Peng, J. W., and Wagner, G. (1995) Biochemistry 34, 868–878
42. Palmer, A. G., III (2004) Chem. Rev. 104, 3623–3640
43. Fushman, D., Ohlenshlager, O., and Ruterjans, H. (1994) J. Biomol. NMR 4, 61–78
44. Barbato, G., Ikura, M., Kay, L. E., Pastor, R. W., and Bax, A. (1992) Biochemistry 31, 5269–5278
45. Dosset, P., Hus, J. C., Blackledge, M., and Marion, D. (2000) J. Biomol. NMR 16, 23–28
46. Sali, A., and Blundell, T. L. (1993) J. Mol. Biol. 234, 779–815
47. Luty, J. H. (2004) in Handbook of Metalloproteins (Messerchmidt, A., ed) pp. 307–323, John Wiley & Sons Press, West Sussex, UK
48. Cornilescu, G., Delaglio, F., and Bax, A. (1999) J. Biomol. NMR 13, 289–302
49. Volkman, B. F., Alam, S. L., Satterlee, J. D., and Markley, J. L. (1998) Biochemistry 37, 10906–10919
50. Krizek, B. A., Zawadzke, L. E., and Berg, J. M. (1993) Protein Sci. 2, 1313–1319
51. Krizek, B. A., Amann, B. T., Kilfoil, V. J., Merkle, D. L., and Berg, J. M. (1991) J. Am. Chem. Soc. 113, 4518–4523
52. Berg, J. M., and Merkle, D. L. (1989) J. Am. Chem. Soc. 111, 3759–3761
53. Cornell, N. W., and Crivaro, K. E. (1972) Anal. Biochem. 47, 203–208
54. Potter, B. M., Kuusinen, N. A., Feng, L. S., Matskevich, V., Wilson, J. A., Andrews, G. K., and Laity, J. H. (2005) J. Biomol. NMR, in press
55. Wishart, D. S., Sykes, B. D., and Richards, F. M. (1991) J. Mol. Biol. 222, 311–333
56. Gronenborn, A. M., and Clore, G. M. (1994) J. Biomol. NMR 4, 455–458
57. Foster, M. P., Wuttke, D. S., Clemens, K. R., Jahnke, W., Radhakrishnan, I., Tennant, L., Reymond, M., Chung, J., and Wright, P. E. (1998) J. Biomol. NMR 12, 51–71
58. Laity, J. H., Dyson, H. J., and Wright, P. E. (2000) J. Mol. Biol. 295, 719–727
59. Foster, M. P., Wuttke, D. S., Radhakrishnan, I., Case, D. A., Gottesfeld, J. M., and Wright, P. E. (1997) Nat. Struct. Biol. 4, 605–608
60. Laity, J. H., Dyson, H. J., and Wright, P. E. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 11932–11935
61. Hye, D. E., and Kleev, H. E. (1998) J. Mol. Biol. 270, 929–943
62. Bruschweiler, R., Liao, X., and Wright, P. E. (1995) Science 268, 866–889
63. Palmer, A. G., Rance, M., and Wright, P. E. (1991) J. Am. Chem. Soc. 113, 4371–4380
64. Lipari, G., and Szabo, A. (1984) J. Am. Chem. Soc. 104, 4546–4549
65. Lipari, G., and Szabo, A. (1984) J. Am. Chem. Soc. 104, 4559–4570
66. Viles, J. H., Dunne, D., Kroom, G., Prusiner, S. B., Cohen, F. E., Dyson, H. J., and Wright, P. E. (2001) Biochemistry 40, 2743–2753
67. Palmer, A. G., III (2001) Annu. Rev. Biophys. Biomol Struct. 30, 129–155
68. Apuy, J. L., Chen, X. H., Russell, D. H., Baldwin, T. O., and Giedroc, D. P. (2001) Biochemistry 40, 15164–15175
69. Bird, A. J., McCall, K., Kramer, M., Blankman, E., Winge, D. R., and Eide, D. J. (2003) EMBO J. 22, 5137–5146
70. Pavletich, N. P., and Pabo, C. O. (1991) Science 252, 809–817
71. Wuttke, D. S., Foster, M. P., Case, D. A., Gottesfeld, J. M., and Wright, P. E. (1997) J. Mol. Biol. 273, 183–206
72. Blasie, C. A., and Berg, J. M. (2002) Biochemistry 41, 15068–15073
The Six Zinc Fingers of Metal-responsive Element Binding Transcription Factor-1 Form Stable and Quasi-ordered Structures with Relatively Small Differences in Zinc Affinities

Belinda M. Potter, Linda S. Feng, Priya Parasuram, Viktor A. Matskevich, Jed A. Wilson, Glen K. Andrews and John H. Laity

J. Biol. Chem. 2005, 280:28529-28540.
doi: 10.1074/jbc.M505217200 originally published online May 31, 2005

Access the most updated version of this article at doi: 10.1074/jbc.M505217200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 70 references, 15 of which can be accessed free at http://www.jbc.org/content/280/31/28529.full.html#ref-list-1