Yeast heat shock transcription factor N-terminal activation domains are unstructured as probed by heteronuclear NMR spectroscopy

HO S. CHO,^{*1} COREY W. LIU,^{*1} FRED F. DAMBERGER,² JEFFREY G. PELTON,³
HILLARY C. M. NELSON,^{3,4} AND DAVID E. WEMMER^{1,3}

¹ Department of Chemistry, University of California, Berkeley, California 94720

² Biophysics Graduate Group, University of California, Berkeley, California 94720

³ Structural Biology Division, Lawrence Berkeley Laboratory, 1 Cyclotron Road, Berkeley, California 94720

⁴ Department of Molecular and Cell Biology, University of California, Berkeley, California 94720

(RECEIVED September 19, 1995; ACCEPTED November 21, 1995)

Abstract

The structure and dynamics of the N-terminal activation domains of the yeast heat shock transcription factors of *Kluyveromyces lactis* and *Saccharomyces cerevisiae* were probed by heteronuclear ¹⁵N{¹H} correlation and ¹⁵N{¹H} NOE NMR studies. Using the DNA-binding domain as a structural reference, we show that the protein backbone of the N-terminal activation domain undergoes rapid, large-amplitude motions and is therefore unstructured. Difference CD data also show that the N-terminal activation domain remains random-coil, even in the presence of DNA. Implications for a “polypeptide lasso” model of transcriptional activation are discussed.

Keywords: activation domains; dynamics; heat shock factor; NMR; unstructured

Transcription factors orchestrate the regulated production of key proteins in eukaryotic cells during development and in response to extracellular stimuli. Typical transcription factors consist of a DNA-binding domain, an oligomerization domain, and one or more activation domains (Tjian & Maniatis, 1994). The structural basis for the function of the DNA-binding and oligomerization domains have been well characterized (Nelson, 1995); however, similar information for the activation domains remains scarce (Triezenberg, 1995). Many transcriptional activation domains fall into one of three categories on the basis of their predominant amino acid compositions: acidic, proline-rich, or glutamine-rich (Mitchell & Tjian, 1989). Fusion proteins produced by joining these domains with heterologous DNA-binding domains have exhibited transcriptional activation (Tjian & Maniatis, 1994). Several recent studies conducted on the “acidic” activation domains of Vmw65 protein of herpes simplex virus (Donaldson & Capone, 1992; O’Hare & Williams, 1992), NF- κ B p65 (Schmitz et al., 1994), and the τ 1 core of the human glucocorticoid receptor (Dahlman-Wright et al., 1995) concluded that these domains lacked well-defined structure. These results were based essentially on negative information: a lack of long-range

NOEs and poor proton chemical shift dispersion in ¹H NMR spectra.

In this report, we use information on the dynamics of the protein backbone obtained from ¹⁵N{¹H} heteronuclear NOE, along with CD experiments, to characterize the structures of the N-terminal transcriptional activation domains of the heat shock transcription factor (HSF) from two species of yeast. HSF is an inducible transcriptional activator that regulates the expression of the heat shock proteins when eukaryotic cells are exposed to elevated temperatures or other environmental insults (Lis & Wu, 1993; Morimoto, 1993). HSF contains a DNA-binding domain, a trimerization domain, and one or two transcriptional activation domains. In yeast, HSF is constitutively bound at heat shock elements containing the sequence 5'-nGAAn-3' (Amin et al., 1988; Xiao & Lis, 1988) and acts as a transcription factor even under nonstressed conditions (Jakobsen & Pelham, 1988; Sorger & Pelham, 1988; Wiederrecht et al., 1988; Gross et al., 1990; Jakobsen & Pelham, 1991; Chen et al., 1993). Functional HSF is required for yeast viability at all temperatures (Sorger & Pelham, 1988; Wiederrecht et al., 1988; Gallo et al., 1993). Heat shock or stress conditions effect a higher level of transcriptional activation, which is mediated through mechanisms that are currently being investigated (Nieto-Sotelo et al., 1990; Sorger, 1990; Gallo et al., 1993).

Biochemical and genetic experiments have been used to map the transcriptional activity of yeast HSF to distinct regions of

Reprint requests to: David E. Wemmer, Department of Chemistry, University of California, Berkeley, California 94720; e-mail: dewemmer@lbl.gov.

*The first two authors contributed equally to this work.

the N- and C-termini (Nieto-Sotelo et al., 1990; Sorger, 1990; Jakobsen & Pelham, 1991) (Fig. 1). These activation domains could not be grouped into any known classes by their amino acid content, nor could they be categorized into any known folding motifs by structure prediction programs. The C-terminal activation domains from *Kluyveromyces lactis* and *Saccharomyces cerevisiae* exhibit high levels of constitutive activity when fused to a heterologous DNA-binding domain (Nieto-Sotelo et al., 1990; Sorger, 1990; Jakobsen & Pelham, 1991). The intact C-terminal activation domains are too large (300+ residues) for practical NMR studies. Although the residues most involved in activation in the C-terminal domain have been mapped to a much smaller segment (amino acids 592–623), its activity is clearly modulated by other regions of the protein (Chen et al., 1993), making this segment a questionable target for structural studies.

Deletions of the entire C-terminal region result in a functional HSF at physiological temperatures (Nieto-Sotelo et al., 1990; Sorger, 1990); this implies that the N-terminal activation domain is sufficient for the required constitutive level of transcriptional activity. In fact, the N-terminal activation domains from both yeast strains also show a low level of transcriptional activity when fused to a heterologous DNA-binding domain (Nieto-Sotelo et al., 1990; Sorger, 1990). The smaller size of the N-terminal activation domain (170–190 residues) makes it suitable for study by NMR. In addition, the N-terminal region is flanked by the well-studied and stable DNA-binding domain (Damberger et al., 1994; Harrison et al., 1994).

Using two constructs that contained both the N-terminal activation and the DNA-binding domains from *K. lactis* and *S. cerevisiae*, we were able to compare the structure and dynamics of the N-terminal activation domain against an internal structural control, the DNA-binding domain. $^{15}\text{N}\{^1\text{H}\}$ heteronuclear single quantum coherence (HSQC) spectra showed that the chemical shift dispersion of the ^1H resonances of the activation domains was poor, typical of that observed in denatured proteins (Neri et al., 1992; Shortle & Abeygunawardana, 1993). In addition, we used two-dimensional heteronuclear $^{15}\text{N}\{^1\text{H}\}$ NOE NMR and measurements of ^{15}N relaxation parameters to show that the N-terminal activation domains from both yeast strains have a high degree of flexibility, which is consistent with an unstructured state in solution. The results are particularly compelling because they offer the first positive evidence for a dynamically disordered transcriptional activation domain.

Results

Dynamics probed by heteronuclear relaxation

Heteronuclear relaxation studies provide a residue-specific probe of the conformational dynamics of proteins (Kay et al., 1989; Palmer, 1993). Current NMR approaches couple dynamic measurements with structural information to gain insight into pro-

tein function. Typical heteronuclear relaxation studies result in the determination of order parameters for backbone amide N-Hs (Clare et al., 1990; Barbato et al., 1992; Redfield et al., 1992; Cheng et al., 1993; Farrow et al., 1994; Zink et al., 1994). Order parameters, which define the amplitude and range of local molecular motions, are obtained using data acquired from a suite of three heteronuclear relaxation NMR experiments: ^{15}N T1 longitudinal relaxation, ^{15}N T2 transverse relaxation, and heteronuclear $^{15}\text{N}\{^1\text{H}\}$ NOE. In a recent example, Bax and coworkers applied such studies to support the "flexible tether" model for calmodulin function (Barbato et al., 1992).

The heteronuclear $^{15}\text{N}\{^1\text{H}\}$ NOE provides a qualitative assessment of the mobility of N-H bond vectors for individual residues. It is sensitive to both the overall tumbling time of the protein (τ_m) and fast internal motions. Maximal NOE values ($+0.83$) occur in the slow-tumbling limit ($\omega_N \tau_m \gg 1$), indicating the N-H bond vectors reorient with the overall tumbling of the molecule. Minimal NOE values (-3.5 ; assuming isotropic rotation, ^{15}N resonance frequency of 60.8 MHz, and 1.02 Å ^{15}N - ^1H bond length) occur in the fast-tumbling limit ($\omega_N \tau_m \ll 1$) and are indicative of rapid, large-amplitude motions with respect to the overall tumbling of the molecule (Palmer, 1993).

Although heteronuclear NOE data alone are not sufficient for fully quantifying the dynamic behavior of molecules, they can be used as a probe for assessing the structural state of a protein. In one recent example, heteronuclear NOE data were particularly enlightening when comparing the backbone flexibilities between the folded and unfolded states of the drkN SH3 domain (Farrow et al., 1995). The data showed dramatic differences in dynamic behavior between the folded and unfolded states: the structured, folded state exhibited positive NOEs, and the unfolded state showed primarily negative NOEs.

Dynamic variations within the *K. lactis* HSF DNA-binding domain

Most of the cross peaks in the $^{15}\text{N}\{^1\text{H}\}$ HSQC spectra of the *K. lactis* HSF DNA-binding domain are well resolved. The spectrum for the heteronuclear NOE version of this experiment is shown in Figure 2A. Assignments were based on the previous NMR study (Damberger et al., 1994). Calculated NOE values, shown in Figure 3A, have an average of 0.67 (0.81 average in regions of secondary structure). The heteronuclear NOEs for the structured regions of the DNA-binding domain are positive and near the slow tumbling limit, as opposed to the C-terminal residues His-91 and Ala-92, which show negative NOEs indicative of rapid, large-amplitude motions. Areas of intermediate NOEs reflect an increase in dynamic flexibility. The best example of this can be seen for the L1 loop (residues 69–83). Previous structural work had suggested that there was higher mobility in this loop region, based on the lack of long-range contacts in the NMR structure (Damberger et al., 1994) and the lack of electron density for residues 76–79 in the crystal structure (Harrison et al., 1994). The heteronuclear NOE gives direct evidence that this region has a higher degree of dynamic flexibility with respect to the overall tumbling of the protein.

Structural assessment of the *S. cerevisiae* HSF DNA-binding domain

There are no published structures of the DNA-binding domain of *S. cerevisiae*; however, its high sequence homology (73%

Fig. 1. Schematic representations of *K. lactis* and *S. cerevisiae* HSF.

N-terminal activation domain of yeast HSF is unstructured

The remaining peaks in Figure 2B, all negative in intensity, can be attributed to the N-terminal activation domain. These ad-

Fig. 3. Representations of the NOE data for the DNA-binding domains of the *K. lactis* HSF constructs. **A:** Assigned residues for the DNA-binding domain construct (1–92). **B:** Assigned DNA-binding domain residues (193–284) for the construct of the N-terminal activation domain plus the DNA-binding domain.

ditional peaks have poor shift dispersion in the proton dimension, as is the case for unstructured proteins. Integrating the volumes of the signals in the spectra could account for approximately 183 of the possible 192 residues; however, only 103 peaks were sufficiently resolved to calculate NOEs. These NOE values (Fig. 4) are sorted by proton chemical shift because there are no sequence-specific assignments currently available for this domain. Negative values indicate that all of the residues in this region exhibit a high degree of rapid internal motion, typical for an unstructured protein. Rapid motions of the activation domain also affect the dynamics of the N-terminus of the DNA-binding

domain. Arg 2 in the DNA-binding domain alone showed a small positive NOE (Fig. 3A), but the presence of the highly dynamic activation domain in the fusion construct of the N-terminal activation domain plus the DNA-binding domain seems to drive the corresponding residue (Arg 194) into a rapid motion regime (Fig. 3B).

Identical experiments were performed on the comparable construct for *S. cerevisiae* HSF. Despite the lack of residue-specific assignments for the DNA-binding domain, we were still able to identify the corresponding peaks in the full-length construct by comparison with spectra of the DNA-binding domain alone.

Fig. 4. NOE data for the activation domain peaks of the construct of the N-terminal activation domain plus the DNA-binding domain. Histogram sorted by proton chemical shift.

Again, the calculated NOEs fell predominately in the slow tumbling regime. The remaining signals, attributable to the N-terminal activation domain, all had negative NOEs indicative of rapid, large-amplitude motions. This shows that the N-terminal activation domain of *S. cerevisiae* also behaves as an unstructured protein.

To confirm that the N-terminal activation domains were not unstructured because of denaturation at low pH, further NMR spectroscopic studies were performed at pH 5.75 (data not shown). Comparison of the chemical shift distribution in spectra acquired at higher pH with those at lower pH indicated no change in the structural state of the constructs. The DNA-binding domains remained folded, whereas the activation domains persisted in an unstructured state.

Far-UV CD spectroscopy, performed at pH 7.0, also indicate that the N-terminal activation domains of yeast HSF are unstructured. Figure 5A shows CD spectra for the DNA-binding domain, and for the N-terminal activation domain plus the DNA-binding domain, as well as the difference between the two. The difference spectrum is characteristic of a random coil. We also examined the possibility that the N-terminal activation domain might behave differently when the DNA-binding domain is bound to a DNA-binding site (Fig. 5B). An examination of this CD difference spectrum indicates that binding of the DNA-binding domain to the DNA does not induce any structural changes in the N-terminal activation domain. Identical results were obtained for the *K. lactis* constructs (not shown).

Discussion

Progress is being made in the effort to understand the mechanism of transcriptional activation. Structural studies of proteins often reveal important aspects of their function and are a powerful complement to biochemical and genetic studies. Heteronuclear relaxation NMR spectroscopy can be used to characterize the behavior of unstructured or partially structured protein states in solution. Using heteronuclear NOE experiments, we have been able to show that the N-terminal activation domain of yeast HSF undergoes rapid local motions and is unstructured in solution. It is possible that the structural state of the N-terminal activation domain might be affected by the presence of the deleted C-terminal regions. However, this seems unlikely because the N-terminal activation domain is functional when fused to heterologous DNA-binding domains (Nieto-Sotelo et al., 1990), and C-terminal deletion constructs are sufficient for cell viability (Nieto-Sotelo et al., 1990; Sorger, 1990).

There have been several previous studies of functional fragments of acidic activation domains, varying in both size and source. The consensus of these studies is that these peptides alone in solution have little secondary structure, although data suggest that either α helices (Donaldson & Capone, 1992; Schmitz et al., 1994; Dahlman-Wright et al., 1995) or β sheets (Leuther et al., 1993; Vanhoy et al., 1993) can be induced under specific solvent conditions. It has then been concluded that these conformations might be important in the function of the domains, although there is no direct evidence for this. Models have also been presented that suggest that activation domains interact through amphipathic helices (Giniger & Ptashne, 1987), but these models have limited support from either genetic or biochemical data. It has been clearly shown, however, that specific patterns of acidic and hydrophobic residues are required for ac-

Fig. 5. Far-UV CD spectra of *S. cerevisiae* HSF constructs. **A:** Protein only. **B:** Protein and DNA. DBD, DNA-binding domain construct; AD + DBD, construct of the N-terminal activation domain plus the DNA-binding domain; AD (diff.), activation domain difference spectra.

tivity of acidic activation domains (Cress & Triezenberg, 1991; Regier et al., 1993), although this does not seem to hold for glutamine- or proline-rich domains (Gerber et al., 1994). The studies presented here are the first on a general sequence activation domain (not falling into acidic, proline-rich, or glutamine-rich classes), and also the first on a complete activation domain. The NMR data clearly show that this domain is highly disordered, with internal motions on a very rapid time scale. We have reached somewhat similar conclusions about an activation domain fragment from c-jun (unpubl. data), which again does not fall into any of the classes noted above. These data, taken together with previous studies of acidic activation domains, argue strongly that the disorder observed is in fact a general property of activation domains of all classes.

It is not obvious how an unstructured activation domain might interact with the components of the general transcriptional machinery. An "induced fit" model proposes that some proteins

may function by acquiring structure in the presence of target factors (Koshland, 1958). Induced fit structural changes have been found in systems such as b-ZIP binding to DNA (Talanian et al., 1990), calmodulin binding to target proteins (Ikura et al., 1992), and staphylococcal nuclease binding to inhibitor (Hynes & Fox, 1991). However, these examples are of proteins that already possess some degree of structure in their "apo" forms. The structural changes induced in these proteins by their interacting partners are relatively small and are localized around the substrates. No conclusions can be drawn about the structure of activation domains in their activated complexes until further data is gathered, but it would be surprising to find that systems as large and dynamically disordered as activation domains could become completely folded via an induced fit mechanism.

With respect to this, we suggest that activation domains only serve as "polypeptide lassos" that rein in and increase the local concentrations of the factors needed for activated transcription. Evidence for this type of role can be drawn from reports indicating that activation domains interact with several different factors of the general transcriptional machinery (Tjian & Maniatis, 1994). The localization of these factors could stimulate the formation of the transcriptional complex and thereby initiate transcription. The exact nature of this recruiting process is still not clear, but some insight can be gained from examining the biochemical data on the interaction of acidic domains with the general transcription factor TFIIB. Recently published results show that acidic activators interact with the C-terminal region of TFIIB to recruit this factor into the preinitiation complex (Roberts & Green, 1994). However, the protein-protein interaction that mediates this and similar events are unlikely to be highly specific in nature. Experiments using heterologous transcription factors formed by a fusion of GAL4 or GCN4 DNA-binding domains to random polypeptides coded by fragments of genomic *Escherichia coli* DNA found the polypeptides that provided the highest levels of enhanced transcription to be rich in acidic side chains but to possess no other obvious sequence similarity (Ma & Ptashne, 1987). This reinforces the notion that nonspecific interactions can play a large role in the process of activated transcription, a generalization of the "acid blobs and negative noodles" idea of Sigler (1988). It would then not be too surprising if other activation domains recruited their factors in a similar, nonspecific fashion. Once needed factors are gathered, their close proximity to each other could stimulate the assembly of the active transcription complex. Similar proposals have been outlined by Frankel and Kim (1991).

Thus, it may not be necessary for activation domains to form any well-defined and unique structure. The idea of activation domains functioning as "polypeptide lassos" better supports the impression that a small number of transcription factors are able to regulate the production of thousands of proteins (Tjian & Maniatis, 1994). Their lack of a well-defined and unique structure could make activation domains more versatile in their abilities to recruit the various factors necessary for formation of the transcriptional complex.

Materials and methods

Cloning

The HSF activation domain plus DNA-binding domain constructs were cloned from plasmids developed previously (Flick

et al., 1994; Harrison et al., 1994). The plasmid pHN200, which contains the coding sequence for the *K. lactis* HSF, was modified by site-directed mutagenesis with two oligomers to insert an *Nde*I site at the N-terminus and an *Sph*I site at amino acid 284. This restriction fragment was subcloned into *Nde*I/*Sph*I digested pHN104, a T7-driven expression vector. The *S. cerevisiae* construct was made in a similar manner from plasmid pHF153, which codes for the first 259 amino acids of HSF. It already contained the correct *Sph*I site at residue 259. An *Nde*I site was introduced at the N-terminus with site-directed mutagenesis, and again the *Nde*I/*Sph*I fragment was subcloned into pHN104 for expression.

Sample preparation

Plasmid constructs were transformed into *E. coli* (strain BL21 (DE3)/pACYC) (Flick et al., 1994; Harrison et al., 1994). Uniform ^{15}N -labeled protein was obtained by growing the bacteria on M9 minimal medium with $[^{15}\text{N}]\text{NH}_4\text{Cl}$ (CIL) as the sole nitrogen source. Induction occurred at $\text{OD}_{594} = \sim 0.6$ with 1.2 g IPTG/1L, and the cells were harvested 4 h later by centrifugation at 4,000 rpm, 4 °C, in a Sorvall GS3 rotor. Protein purification was accomplished by a simplified version of a previously reported method (Harrison, 1994). Cells were resuspended in 10 mL/g cells isotonic wash buffer, spun down, resuspended in 1 M NaCl lysis buffer, and lysed by sonication for 6 min in a dry ice/ethanol bath. A high-speed spin was used to get rid of cellular debris, and the resultant supernatant was diluted to 100 mM NaCl and loaded onto a pre-equilibrated heparin column. Prior to elution, the column was washed with a low-salt buffer. The desired protein was eluted with 500 mM NaCl. PAGE (15%) was used to assess the purity of fractions collected. Electrospray-ionization mass spectroscopy confirmed >98% ^{15}N incorporation. Fractions containing >95% of the desired protein were dialyzed into NMR buffer to yield final 0.5-mL samples of $\sim 3\text{--}5$ mM protein in 10 mM KH_2PO_4 , 90% $\text{H}_2\text{O}/10\%$ D_2O , at pH 3.4 or pH 5.75.

NMR spectroscopy

$^{15}\text{N}\{^1\text{H}\}$ heteronuclear NOE spectra were measured on a Bruker AMX-600 instrument as described (Barbato et al., 1992). Spectra were initially acquired at pH 3.4 to minimize proton exchange and maximize the signal-to-noise ratio. The 8-k data points were collected in the t_2 dimension to increase resolution. Data were processed with the NMRPipe suite of programs (Delaglio, 1993), and peaks picked with the CAPP/PIPP suite of analysis programs (Garrett et al., 1991). Spectra at pH 5.75 were collected with the PEP-Z-HSQC experiment (Akke et al., 1994). Data were processed with the Felix processing package (Biosym Technologies).

CD spectroscopy

Far-UV CD spectroscopy was performed on an AVIV Model 62DS equipped with a temperature-controlled cell holder and connected to an IBM-compatible workstation for data analysis. Samples were prepared as 1 mg/mL solutions in 25 mM sodium phosphate buffer, pH 7.0, at 25 °C. Concentrations (2:1) of DNA(5'-CCGGTGAATTTTCTTGAATGGCC-3'):protein were used for the protein/DNA experiments. Difference spectra were

obtained using Microsoft Excel. A three-point moving average was used for data smoothing.

Acknowledgments

We gratefully thank Karen Flick for assistance with the CD measurements and HPLC, Dr. David King for performing ESI mass spectral analysis, and Prof. Susan Marqusee for use of her CD spectrometer. This work has been supported by an award from the PEW Scholars Program in Biomedical Sciences (to H.C.M.N.), the Director, Office of Energy Research, Office of Biological & Environmental Research, General Life Science Division of the U.S. Department of Energy under Contract No. DE-AC03-76F00098 (to D.E.W.), and NIH Traineeship GM08295 (to C.W.L.). Instrumentation grants were provided by the U.S. Department of Energy, DE-FG05-86ER75281 and the NSF, DMB86-09305 and BBS 87-20134 (to D.E.W.).

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