Accelerated Publications

Alternating Zinc-Finger Motifs in the Human Male-Associated Protein ZFY†

Michael A. Weiss,*,‡,§ Kathleen A. Mason,§ Charles E. Dahl,‡ and Henry T. Keutmann§

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts 02115, and Department of Medicine, Massachusetts General Hospital, Boston, Massachusetts 02114

Received March 28, 1990; Revised Manuscript Received May 1, 1990

ABSTRACT: ZFY, a putative transcription factor encoded by the human Y chromosome, contains a distinctive two-finger repeat: odd-numbered and even-numbered CC/HH metal-binding motifs exhibit systematic alternation in sequence pattern. Such alternation, which is not generally observed in zinc-finger proteins, has also been described in an extensive family of Kruppel-like genes in *Xenopus laevis* and in the AIDS-associated human DNA-binding protein HIV-EP1. The strict conservation of a two-finger repeat among ZFY-, Kruppel- and HIV-related zinc-finger proteins suggests distinct mechanisms of protein–nucleic acid recognition. To test whether this sequence pattern reflects an underlying alternation in domain structure, we have synthesized and characterized single-finger peptides from the human ZFY gene. Remarkably, systematic differences in metal-dependent folding are observed in the circular dichroism spectra of even- and odd-numbered domains. Our results suggest the existence of distinct CC/HH finger submotifs, which may play different roles in nucleic acid recognition.

The Zn-finger motif defines a conserved class of eukaryotic nucleic acid binding proteins involved in the regulation of gene expression and the specification of cell fate (Klug & Rhodes, 1987). Originally described as a metal-binding site in *Xenopus* transcription factor TFIIIA (Brown et al., 1985; Miller et al., 1985), the Zn finger contains two cysteine and two histidine residues with characteristic spacing (Berg, 1987). An isolated Zn finger forms an independent folding unit (Frankel et al., 1987), and 2D NMR[1] studies of single-finger peptides demonstrate a compact globular domain in which the divalent metal is encaged (Parraga et al., 1988; Lee et al., 1989; Parraga et al., 1990). The N-terminal portion, containing the conserved cysteines, forms a β-sheet and -turn; the C-terminal portion, containing the conserved histidines, forms an α-helix. The NMR data confirm major features of structural models proposed earlier on the basis of an analysis of known metal-binding sites (Berg, 1988) and independently by computer-based molecular modeling methods (Gibson et al., 1988).

Although the mechanism of DNA recognition by Zn fingers is not presently understood, Klug and colleagues have proposed two general models (Fairall et al., 1986). In model I the protein follows the helical path of the major groove; consecutive fingers are equivalently oriented with respect to the DNA, and so the structural repeat is one finger. In model II the protein lies along one face of the double helix; alternating fingers make inequivalent DNA contacts in accord with a two-finger repeat. A majority of Zn-finger sequences exhibit a single-finger repeat, consistent with model I (Gibson et al., 1988). However, families of sequences exhibiting second-order repeats are also observed, as defined by systematic differences between odd- and even-numbered domains. Examples include the ZFY-related gene family (Page et al., 1987b; Schneider-Gadicke et al., 1989), Kruppel-related sequences in *Xenopus laevis* (Nietfeld et al., 1989), and a recently described human DNA-binding protein that recognizes the HIV-1 enhancer (Maekawa et al., 1989) and β-interferon promoter (Fan & Maniatis, 1990). The structural implications of these sequence repeats are not presently understood.

ZFY-related sequences define a highly conserved vertebrate gene family (Page et al., 1987b; Bull et al., 1988; Sinclair et al., 1988; Mardon & Page, 1989; Mardon et al., 1989, 1990; Mitchell et al., 1989; Schneider-Gadicke et al., 1989; Nagamine et al., 1990; DiLella et al., 1990) and thus provide a model for structural studies of second-order repeats. Originally identified from studies of apparent sex reversal in man (de la Chapelle, 1972; Page et al., 1987a,b), ZFY is presently thought to be involved in spermatogenesis (Palmer et al., 1989; Koopman et al., 1989). Like previously characterized eukaryotic transcription factors (Hope & Struhl, 1986; Ma & Ptashne, 1987), ZFY encodes on separate exons an acidic domain (presumed to activate transcription) and a putative DNA-binding domain. The latter contains 13 Zn fingers, shown in Figure 1. Whereas the odd-numbered domains are similar to the general Zn-finger consensus (Klug & Rhodes, 1987; Gibson et al., 1988), the even-numbered domains exhibit systematic differences. To explore the structural meaning of these sequence differences, we have synthesized six single-finger peptides from the human ZFY sequence. Three correspond to odd-numbered fingers (domains 3, 5, and 7), and three correspond to even-numbered fingers (domains 6, 8, and 12). Remarkably, odd and even domains are observed to exhibit an alternation in domain architecture.

Materials and Methods

Peptide Synthesis. Odd-numbered domains were synthesized as peptides corresponding to domain positions 74–101 (designated ZFY-3; 28 residues), 134–162 (designated ZFY-5;

† This work was supported in part by grants from the NIH and the Whitaker Foundation to M.A.W. and by the Markey Charitable Trust at Harvard Medical School. M.A.W. was supported in part by the Pfizer Scholars Program for New Faculty and by an American Cancer Society Junior Faculty Research Award.

* Address correspondence to this author at the Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School.

‡ Harvard Medical School.

§ Massachusetts General Hospital.

[1] Abbreviations: CD, circular dichroism; HPLC, high-performance liquid chromatography; 2D NMR, two-dimensional nuclear magnetic resonance.

0006-2960/90/0429-5660$02.50/0

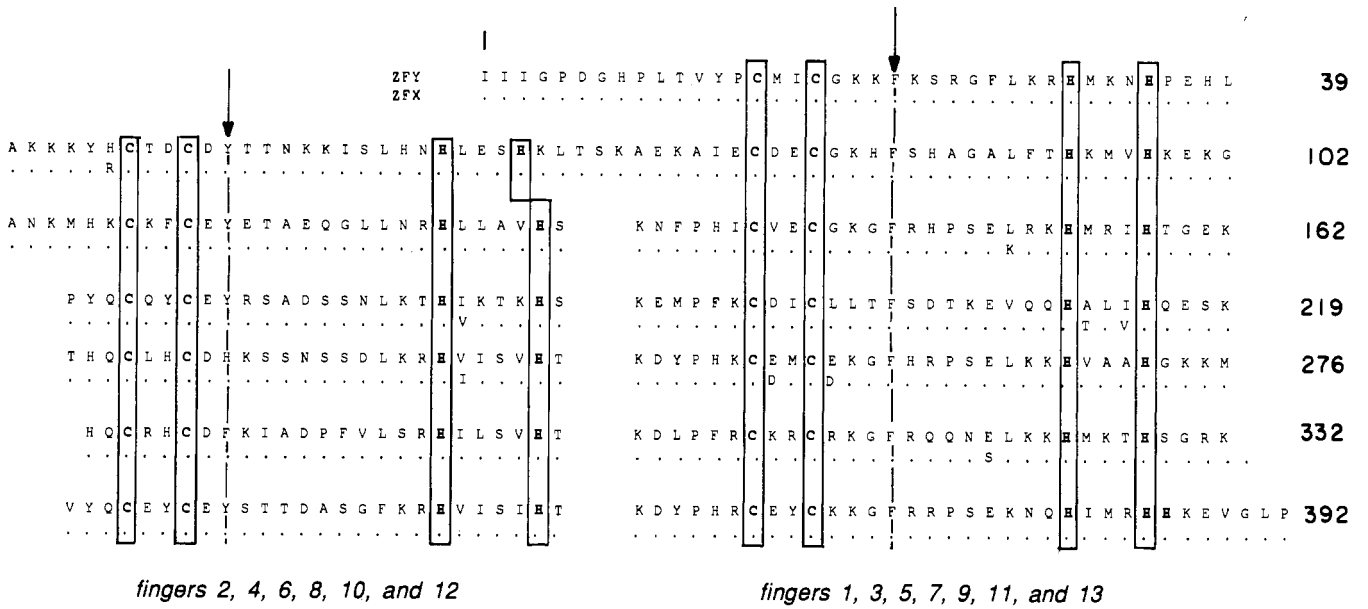

FIGURE 1: Sequences of ZFY and ZFX exhibiting an alternation between odd and even sequence patterns. The cysteine and histidine residues presumed to be involved in metal coordination are boxed, and the alternating position of the central aromatic residue is indicated by arrows. Odd-numbered peptides have the following sequences: ZFY-3, KAIECDECGKHFSHAGALFTHKMVHKEK; ZFY-5, KPHICVECGKGFRHPSELRKHMRIHTGEK; ZFY-7, KPFKCDICLLTFSDTKEVQQHALIHQESK. Even-numbered peptides have the following sequences: ZFY-6 (N), KPYQCQYCEYRSADSSNLKTHIKTKHSKEK; ZFY-8, KTHQCLHCDHKSSNSSDLKRHVISVHTKDK; ZFY-12, KVYQCEYCEYSTTDASGFKRHVISIHTKDK.

29 residues), and 191–219 (designated ZFY-7; 29 residues) (Figure 1). The even-numbered domains were synthesized as 30-residue peptides corresponding to residues 162–191 (ZFY 6), 219–248 (ZFY 8), and 332–361 (ZFY 12). To enhance the solubility of the peptide and avoid methionine oxidation, C- or N-terminal hydrophobic or methionine residues in the native sequence were replaced by lysine (residues F74, M191, Y248, and Y361; Figure 1). Domains 5, 6, and 7 were synthesized by the solid-phase procedure (Barany & Merrifield, 1979; Stewart & Young, 1984) using the Applied Biosystems synthesizer and *t*-BOC amino acids. Peptides commmenced with 0.5 mmol of initial residue on PAM-resin, providing a C-terminal carboxyl group. Double symmetric anhydride couplings were used throughout, monitored by quantitative ninhydrin tests (Stewart & Young, 1984) to assure 99.1%–99.3% coupling at each step. Domains 3, 8, and 12 were synthesized by using fluorenylmethoxycarbonyl (Fmoc) amino acids (Chang & Meienhofer, 1978; Atherton et al., 1978) on a Biosearch/Milligen synthesizer. The initial residue was linked to a *p*-alkoxybenzyl alcohol resin, also providing a C-terminal carboxyl group.

Peptide Purification. A total of 75 mg of lyophilized, cleaved peptide was dissolved in 0.1% acetic acid, passed over a Sephadex G-50 column, and eluted with 50 mM ammonium acetate (pH 5.0). A single predominant peptide peak was eluted with a position consistent with that of a peptide monomer. The gel-filtered material was further purified following reduction (below) by reversed-phase HPLC using a C-18 semipreparative column.

Reduction of cystine to cysteine was accomplished by reaction with 0.5 M dithiothreitol in 100 mM Tris-HCl (pH 7.7 at 20 °C) at 60 °C for 1–3 h. Reduced and oxidized peptides were separated by reversed-phase HPLC using an acetonitrile gradient. The reduced peptide was lyophilized and stored in vacuo.

Peptide Characterization. The amino acid composition and sequence of each peptide were determined to verify the predicted structure. Preview analysis, performed as described by Tregear et al. (1977), showed all purified peptides to be a single sequence free from any deleted residues or truncated fragments. The reduction status of cysteines following DTT treatment was confirmed by reaction with iodoacetate followed by sequencing.

The aggregation state of representative odd and even fingers (domains 5 and 6, respectively) was determined by gel-filtration chromatography using Sephadex G-50 (fine) and found to be monomeric at the concentration and conditions of study. The elution position expected for a monomeric peptide was calibrated in reference to a fragment of parathyroid hormone (residues 1–34). The column buffer contained 1 mM $ZnCl_2$ in 50 mM Tris-HCl (pH 7.5).

Absorption Spectroscopy. Metal binding of the cobalt complex was evaluated by absorption from 220 to 800 nm (Frankel et al., 1987). A total of 0.5 mg of reduced peptide was dissolved in 100 μL of 0.1 N acetic acid containing 15 mM cobalt chloride. The solution was brought to 1 mL by addition of 100 mM Tris-HCl (pH 8.0), providing a final concentration of 0.15 mM peptide and 0.15 mM cobalt chloride. A control solution, prepared similarly but without peptide, was used as a reference.

Circular Dichroism. CD spectra were obtained with an Aviv spectropolarimeter. Samples were dissolved in Tris-HCl buffer (see below) at a peptide concentration of 0.1 mM and placed in a 1-mm path-length cuvette. Peptide concentrations were measured in CD samples by quantitative amino acid analysis following acid hydrolysis.

Buffers. Absorption measurements and CD were performed in 50 mM Tris-HCl (pH 7.5) containing successive aliquots of $CoCl_2$ or $ZnCl_2$ as described in the appropriate figure legends. For pH titrations this buffer was mixed with aliquots of 0.1% acetic acid (containing the same concentration of $CoCl_2$ or $ZnCl_2$) to achieve intermediate pH conditions. To delay oxidation of the peptide, buffers and solvents were flushed with Ar or N_2 immediately prior to use.

RESULTS

Metal Binding. The participation of cysteine in tetrahedral metal coordination may be inferred from the absorption

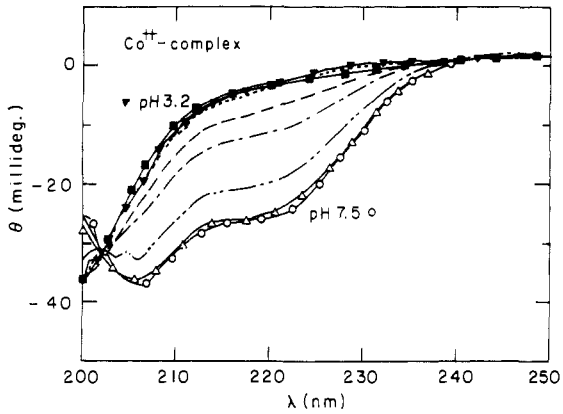

FIGURE 2: Metal-dependent folding and helix formation as demonstrated by CD spectra in the presence (○) and absence (■) of Co^{2+} at pH 7.5. The stability of the ZFY-6/Co^{2+} complex may be monitored by the pH dependence of the ellipticity at 222 nm; a parallel pH dependence is observed by absorbance spectroscopy (Figure 3). (△) pH 6.8; (- - -) pH 6.0; (- -) pH 5.4; (--) pH 5.2; (- - -) pH 4.3; (▼) pH 3.2.

spectrum of the Co^{2+}–peptide complex (Frankel et al., 1987). The Co^{2+} spectra of the even and odd ZFY domains exhibit identical d–d and charge-transfer bands. In addition, both odd- and even-numbered complexes exhibit displacement of Co^{2+} by equimolar Zn^{2+} (data not shown).

Metal-Dependent Folding. Following reduction, both odd and even domains exhibit metal-dependent folding, as indicated by CD studies of 1:1 Zn^{2+}– and Co^{2+}–peptide complexes. The changes observed in the CD spectrum upon addition of equimolar $ZnCl_2$ or $CoCl_2$ (negative ellipticity with inflections near 208 and 222 nm; Chang et al., 1978) are in accord with the formation of an α-helix. Such changes are not observed with oxidized peptides (data not shown). Metal-dependent helix formation is illustrated in the case of ZFY-6 in Figure 2: the CD spectrum at pH 7.5 in the presence of Co^{2+} is indicated by open circles and in the absence of metal by solid squares. No further spectroscopic changes at pH 7.5 are observed with additional $ZnCl_2$ or $CoCl_2$, consistent with a 1:1 complex (data not shown). Progressive loss of ordered structure is observed under acidic conditions and correlates with loss of metal binding (see below). The CD spectrum of the acid-dissociated form (pH 3.2; solid triangles) is similar to that of the reduced peptide in the absence of metal at pH 7.5 (solid squares).

Structural Stability. To evaluate the relative stabilities of odd and even fingers, metal-dependent folding may be observed as a function of pH. Titration of cysteine and/or histidine side chains results in release of the bound metal, as monitored by Co^{2+} absorbance (not shown), and attendant loss of ordered structure (Figure 2). The relative pH stabilities of odd and even domains are shown in Figure 3. No consistent distinction between odd- and even-numbered domains is observed.

General Differences between Odd and Even Fingers. Despite overall similarities in metal coordination and range of stabilities, odd and even Zn fingers exhibit systematic structural differences. The CD spectra of the six Zn^{2+} peptide complexes are shown in Figure 4. The even fingers (ZFY-6, -8, and -12; panel A) consistently exhibit more extensive helix formation than the odd fingers (ZFY-3, -5, and -7; panel B). These differences are not due to confounding changes in oligomeric state, to altered equilibria between folded and unfolded forms, or to kinetic barriers arising from conditions of initial dissolution. Odd- and even-numbered domains thus form distinguishable subclasses, providing evidence for a conserved structural repeat.

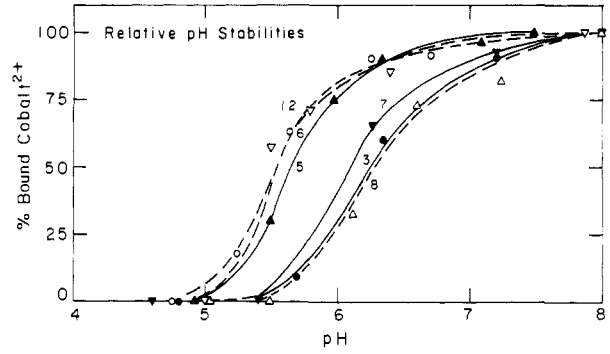

FIGURE 3: Relative pH stabilities of odd and even Zn fingers as measured by amplitude of Co^{2+} d–d absorbance bands. Three fingers are more stable [ZFY-5 (▲), ZFY-6 (▽), and ZFY-12 (○)], and three are less stable [ZFY-8 (△), ZFY-3 (●), and ZFY-7 (▼)]. No consistent difference between odd and even subclasses is observed.

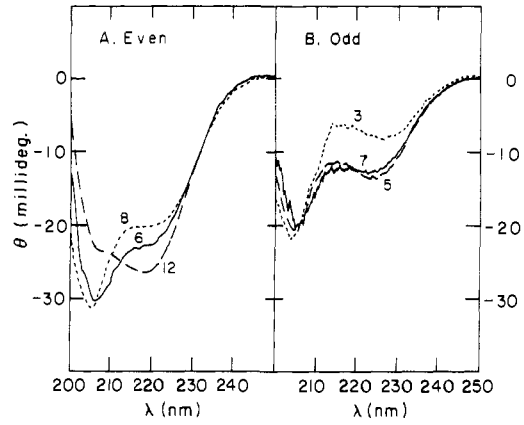

FIGURE 4: (A) CD spectra of even-numbered peptide–Zn^{2+} complexes: ZFY-6 (—), ZFY-8 (- - -), and ZFY-12 (--). (B) CD spectra of odd-numbered peptide-Zn^{2+} complexes: ZFY-3 (- - -), ZFY-5 (--), and ZFY-7 (—).

ZFY-3 contains substantially less α-helix than the other peptides of its class (dotted line in Figure 4B). Indeed, little ordered secondary structure is observed upon formation of the reduced Zn^{2+} complex relative to the oxidized complex (not shown). A tetrahedral binding site is nevertheless maintained by stable tertiary interactions, as indicated by guanidine denaturation studies of the Co^{2+} complex (not shown). Interestingly, the third domain is the only Zn-finger sequence that is not conserved as a functional metal-binding site among the ZFY gene family (Figure 5): Zfb (chicken) contains a Cys → Arg substitution in the first ligand site (DiLella et al., 1990), Zfa (*Mus musculus* autosomal sequence) contains a Cys → Tyr substitution in the second ligand site (Mitchell et al., 1989; Mardon et al., 1990), and Zfy-2 (*M. musculus*) contains a deletion of six residues spanning the third ligand site (Nagamine et al., 1990; R. Lovell-Badge, personal communication).

Discussion

2D NMR studies of ADR1 and Xfin fingers have revealed a globular minidomain in which Zn^{2+} is rigidly encaged (Parraga et al., 1987, 1990; Lee et al., 1988). These structures exhibit overall similarities in accord with the previous predictions of Berg (1987) and Gibson et al. (1987). However, the structures of ADR1a and Xfin-31 peptides differ in important respects, including the extent of metal-dependent helix formation. The origins and generality of such differences are of major interest, since the existence of distinct finger subtypes would have implications for the mechanism(s) of DNA recognition. The present study of ZFY-derived peptides demonstrates that significant variations exist in the structures of

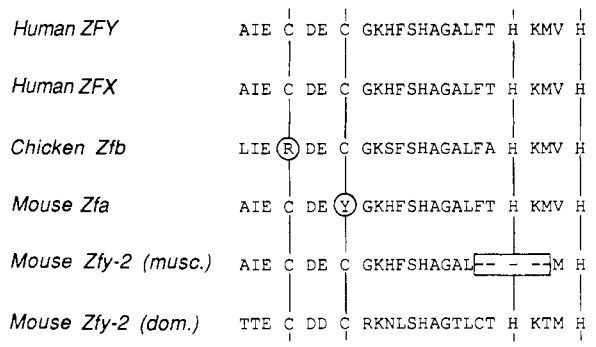

FIGURE 5: Sequences of the third Zn finger from various species demonstrating nonconservation of the functional metal-binding site. Sequences were obtained as follows: ZFY (Page et al., 1987); ZFX (Schneider-Gadicke et al., 1989); Zfb (DiLella et al., 1990); Zfa (Mardon et al., 1990); Zfy-2 *musc.* (Nagamine et al., 1990; R. Lovell-Badge, personal communication); Zfy-2 *dom.* (Mardon & Page, 1989; Mardon et al., 1989). Among these sequences the remaining 12 fingers are strictly conserved.

individual Zn fingers. Remarkably, variations in this system can be classified as odd and even in accord with general differences in CD features.

The structural origins of the differences between odd and even Zn fingers are not clear. Analysis of ZFY-related sequences suggests two major features that distinguish these subclasses: the position of the central aromatic residue (arrows in Figure 1) and the spacing between conserved histidines (HX_3H in odd domains and HX_4H in even domains). Odd-specific features are more generally observed among Zn-finger proteins (Gibson et al., 1988) and are seen in the NMR structure of the Xfin peptide (Lee et al., 1989). The HX_4H spacing is observed in ADR1a (Parraga et al., 1988). In this structure the C-terminal helix appears to break between the two histidines, resulting in a shorter metal-dependent helix than in the HX_3H Xfin structure. This is opposite to the trends observed among ZFY peptides. Understanding the differences in HX_4H-associated helix length will require detailed comparison of 2D NMR structures of ZFY, ADR1a, and related peptide models.

Within the general odd and even subclasses, particular features are observed in the spectra of individual domains. What are the implications of variations in local finger architecture? ZFY-8, for example, exhibits a less extensive α-helical transition (dotted line in Figure 4A) than ZFY-6 or ZFY-12 (solid and dashed lines, respectively). Interestingly, in this sequence the aromatic residues at positions 3, 7, and 10 are each His (rather than Tyr or Phe; Figure 1). This pattern is conserved in the ZFY-related gene family (DiLella et al., 1990) and thus reflects a feature of the ancestral protein. Since these residues are not part of the C-terminal α-helix (Parraga et al., 1988; Lee et al., 1989), a change in packing is presumably transmitted via the hydrophobic core of the domain; such a change would in turn be expected to alter the configuration of the putative DNA-binding surface. The conservation of these histidines in domain 8 is likely to reflect functional constraints on the details of local structure, such as the relative positioning of functional groups involved in DNA recognition. The importance of such positioning has been demonstrated by cocrystal studies of helix–turn–helix DNA-binding proteins (Pabo & Sauer, 1984; Jordan & Pabo, 1988; Aggarwal et al., 1988). Similar constraints may underlie the variation in helix content of ZFY-6 and ZFY-12, suggesting that any "recognition code" for Zn-finger–DNA interactions may involve nonlocal effects mediated by changes in overall domain architecture.

ZFY-3 exhibits an essentially nonhelical metal-dependent structure. The absence of significant secondary structure in the human ZFY-3 peptide presumably reflects the absence of selection pressure constraining the third domain as a "cannonical" finger. We propose that the third domain is not directly involved in protein–DNA contacts but has diverged as, for example, a spacer between conserved recognition elements. This proposal is in general accord with detailed studies of the interaction between *Xenopus* transcription factor TFIIIA and its cognate DNA and RNA target sites (Vrana et al., 1988). Promoter mutagenesis studies demonstrate that DNA contact points in the 5S internal control region are not regularly spaced (Pieler et al., 1987). In this system certain Zn fingers appear to contribute more to the overall free energy of binding than do others, and finger 1 is proposed to contact the transcriptional machinery rather than to bind DNA directly (Nietfeld et al., 1987).

DNA-binding proteins containing two-finger repeats may arise in vertebrate evolution through the duplication of an ancestral two-finger unit. Such sequence patterns may simply reflect corresponding duplications in target DNA sites (Page et al., 1987b). Alternatively, the observed sequence repeats may reflect the existence of distinct mechanisms of Zn-finger-mediated DNA recognition. Klug and colleagues have proposed two general models of the protein–DNA complex, which differ in their symmetry (Fairall et al., 1986). In model I the protein follows the helical path of the major groove; the structural repeat is one finger. In model II the protein lies along one face of the double helix, and the structural repeat is a two-finger unit (Berg, 1988). The second model predicts the existence of distinct odd- and even-specific architectures and is consistent with the conservation of tandem repeats in the ZFY- and Kruppel-related gene families (Page et al., 1987b; Nietfeld et al., 1989). It is important to note, however, that distinct architectures may recognize DNA by a common mechanism (conversely, similar architectures may be used in different ways). Accordingly, distinguishing between models I and II will require structural studies of representative protein–DNA complexes.

In summary, ZFY provides a model system in which to explore the relationship between sequence repeats and underlying structural features. We have shown that odd and even Zn-finger peptides from ZFY exhibit alternating patterns of metal-dependent folding. Further characterization of representative odd and even ZFY domains by 2D NMR is in progress and may provide a foundation for analyzing their respective modes of DNA binding.

ACKNOWLEDGMENTS

We thank D. C. Page and P. A. Sharp for helpful discussion and communication of results prior to publication, R. Lovell-Badge for communication of results prior to publication, C. T. Walsh and C. O. Pabo for critical reading of the manuscript, and S. Lehrer for use of his CD spectropolarimeter.

REFERENCES

Aggarwal, A. K., Rodgers, D. W., Drottar, M., Ptashne, M., & Harrison, M. (1988) *Science 242*, 899–907.

Ashworth, A., Swift, S., & Affara, N. (1989) *Nucleic Acids Res. 17*, 2864–2874.

Atherton, E., Fox, H., Harkiss, D., Logan, C., Sheppard, R. C., & Williams, B. J. (1978) *J. Chem. Soc., Chem. Commun.*, 537–539.

Barany, G., & Merrifield, R. B. (1979) in *The Peptides* (Gross, E., & Meienhofer, J., Eds.) Vol. 2, pp 1–284, Academic Press, New York, NY.

Berg, J. (1987) *Science 232*, 485–488.

Berg, J. M. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 99–487.
Brown, R. S., Sander, C., & Argos, P. (1985) *FEBS Lett. 186*, 271–274.
Bull, J. J., Hillis, D. M., & O'Steen, S. (1988) *Science 242*, 567–569.
Chang, C. D., & Meinhofer, J. (1978) *Int. J. Pept. Protein Res. 11*, 246–249.
Chang, C.-T., Wu, C.-S. C., & Yang, J. T. (1978) *Anal. Biochem. 91*, 13–31.
de la Chapelle, A. (1972) *Am. J. Human Genet. 24*, 71–105.
Diakun, G. P., Fairall, L., & Klug, A. (1986) *Nature 324*, 698–699.
DiLella, A. G., Page, D. C., & Smith, R. G. (1990) *New Biologist 2*, 49–55.
Evans, R. M., & Hollenberg, S. M. (1988) *Cell 52*, 1–3.
Fairall, L., Rhodes, D., & Klug, A. (1986) *J. Mol. Biol. 192*, 577–591.
Fan, C.-M., & Maniatis, T. (1990) *Genes Dev. 4*, 29–42.
Frankel, A. D., Berg, J. M., & Pabo, C. O. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 4841–4845.
Gibson, T. J., Postma, J. P. M., Brown, R. S., & Argos, P. (1988) *Protein Eng. 2*, 209–218.
Jordan, A., & Pabo, C. O. (1988) *Science 242*, 895–899.
Harshorne, T. A., Blumberg, H., & Young, E. T. (1986) *Nature 320*, 172–173.
Hope, I., & Struhl, K. (1986) *Cell 46*, 885–894.
Klug, A., & Rhodes, D. (1987) *Trends Biochem. Sci. 12*, 464.
Koopman, P., Gubbay, J., Colignon, J., & Lovell-Badge, R. (1989) *Nature 342*, 940–942.
Lee, M. S., Gippert, G. P., Soman, K. V., Case, D. A., & Wright, P. E. (1989) *Science 245*, 635–637.
Ma, J., & Ptashne, M. (1987) *Cell 51*, 113–119.
Maekawa, T., Sakura, H., Sudo, T., & Ishii, S. (1989) *J. Biol. Chem. 264*, 14591–14593.
Mardon, G., & Page, D. C. (1989) *Cell 56*, 765–770.
Mardon, G., Mosher, R., Disteche, C. M., Nishioka, Y., McLaren, A., & Page, D. C. (1989) *Science 243*, 78–80.
Mardon, G., Luoh, S.-W., Simpson, E. M., Gill, G., Brown, L. G., & Page, D. C. (1990) *Mol. Cell. Biol. 10*, 681–688.
Miller, J., McLachlan, A. D., & Klug, A. (1985) *EMBO J. 4*, 1609–1614.
Mitchell, M., Simon, D., Affara, N., Ferguson-Smith, M., Avner, P., & Bishop, C. (1989) *Genetics 121*, 803–809.
Nagai, K., Nakaseko, Y., Nasmyth, K., & Rhodes, D. (1988) *Nature 332*, 284.
Nagamine, C. M., Chan, K., Hake, L. E., & Lau, Y.-F. C. (1990) *Genes Dev. 4*, 63–74.
Nietfeld, W., El-Baradi, T., Mentzel, H., Pieler, T., Koster, M., Poting, A., & Knochel, W. (1989) *J. Mol. Biol. 208*, 639–659.
Pabo, C. O., & Sauer, R. T. (1984) *Annu. Rev. Biochem. 53*, 293–321.
Page, D. C., Brown, L. G., & de la Chapelle, A. (1987a) *Nature 328*, 437–440.
Page, D. C., Mosher, R., Simpson, E., Fisher, E. M. C., Mardon, G., Pollack, J., McGillivray, B., de la Chapelle, A., & Brown, L. G. (1987b) *Cell 51*, 1091–1104.
Palmer, M. S., Sinclair, A. H., Berta, P., Ellis, N. A., Goodfellow, P. N., Abbas, N. E., & Fellous, M. (1989) *Nature 342*, 937–939.
Parraga, G., Horvath, S. J., Eisen, A., Taylor, W. E., Hood, L., Young, E. T., & Klevit, R. E. (1988) *Science 241*, 1489–1492.
Parraga, G., Horvath, S., Hood, L., Young, E. T., & Klevit, R. E. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 137–141.
Pieler, T., Hamm, J., & Roeder, R. G. (1987) *Cell 48*, 91–100.
Schneider-Gadicke, A., Beer-Romero, P., Brown, L. G., Nussbaum, R., & Page, D. C. (1989) *Cell 57*, 1247–1258.
Sinclair, A. H., Foster, J. W., Spencer, J. A., Page, D. C., Palmer, M., Goodfellow, P. N., & Graves, J. A. (1988) *Nature 336*, 780–783.
Stewart, J. M., & Young, J. D. (1984) *Solid-Phase Synthesis*, Raven Press, New York, NY.
Tregear, G. W., van Reitschoten, J., Sauer, R. T., Niall, H. D., Keutmann, H. T., & Potts, J. T. (1977) *Biochemistry 16*, 2817–2823.
Vrana, K. E., Churchill, M. E. A., Tullius, T. D., & Brown, D. D. (1988) *Mol. Cell. Biol. 8*, 1684–1696.