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Resonance assignments of the Mrf-2 DNA-binding domain

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Biological context

A new DNA-binding sequence motif, which does not resemble any previously identified DNA-binding motif, is defined by at least 13 proteins. This motif was described in the mouse protein *bright* and shown to bind a specific AT-rich DNA sequence found in the matrix attachment regions (MARs) flanking the immunoglobulin heavy chain enhancer (Herrscher et al., 1995). This motif has also been characterized in the *Drosophila* protein dead ringer (*dri*) which binds the ATTA sequence (Gregory et al., 1996). The *dri* gene was found to be developmentally regulated and is essential for the development of *Drosophila* embryos.

One of the proteins in this family is Mrf-2. This is a human protein that recognizes the modulator region of the major immediate-early gene of human cytomegalovirus (HCMV) (Whitson et al., 1998). HCMV, a member of the herpes virus family, is an infectious agent of world-wide occurrence. The modulator region controls cell-type specific expression of the major immediate-early gene of HCMV (Lubon et al., 1989). This region represses transcription in some cell lines, but enhances transcription in others.

Recent studies indicate that this DNA-binding motif appears to have contacts with the minor groove of DNA (Herrscher et al., 1995; Quinn et al., 1996; Whitson et al., 1998). It is our goal to understand how the new DNA-binding motif recognizes specific DNA sequences. This information will increase our knowledge of protein–DNA recognition in transcription regulation. To date, conformational studies of the new DNA-binding motif and its complex with DNA are not available.

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Methods and Results

In order to analyze the three-dimensional structure of this novel DNA-binding motif, we have cloned the Mrf-2 DNA-binding domain (residues 14–121) into the vector PQE30 (from Qiagen, Inc.). The modified plasmid has an open reading frame that encodes the sequence Met-Arg-Gly-Ser-$(His)_6$-Gly-Ser, followed by the residues 14-121 of Mrf-2. The modified plasmid was used to transform competent *Escherichia coli* strain DH5a. The protein was enriched with ^{13}C and ^{15}N using standard labelling methods. The protein was purified on a Ni-NTA column (Qiagen) under denaturing conditions and was renatured by dialyzing out urea.

The NMR samples contained approximately 1 mM of protein in the NMR buffer (100 mM sodium phosphate buffer, pH 6.0, 100 mM KCl, 10 mM $Na_2S_2O_4$ as the reducing agent, and 0.015% NaN_3 as preservative) containing 90% H_2O/10% D_2O or 99% D_2O. NMR experiments were performed on a Varian Unityplus 500 NMR spectrometer equipped with four radiofrequency channels, pulse shaping and pulsed-field-gradient accessories. All NMR experiments were conducted at 25 °C or 26 °C with a Varian 5 mm triple resonance probe with an actively shielded Z gradient coil. The following NMR experiments were performed: 2D HSQC, 3D ^1H-^{15}N NOESY-HSQC, 3D ^1H-^{15}N TOCSY-HSQC with 35 ms and 50 ms isotropic mixing periods, HNCA, HNCACB, HBHA(CBCACO)NH, CBCA(CO)NH, HCCH-TOCSY, HCCH-COSY, and 3D HNHA (for reviews, see Bax et al., 1994; Clore and Gronenborn, 1994). The frequencies of proton resonances were referenced to DSS at 0.00 ppm. The chemical shifts of ^{13}C and ^{15}N resonances were referenced as described by Wishart et al. (1995). All spectra were processed and analyzed using FELIX 95.0 soft-

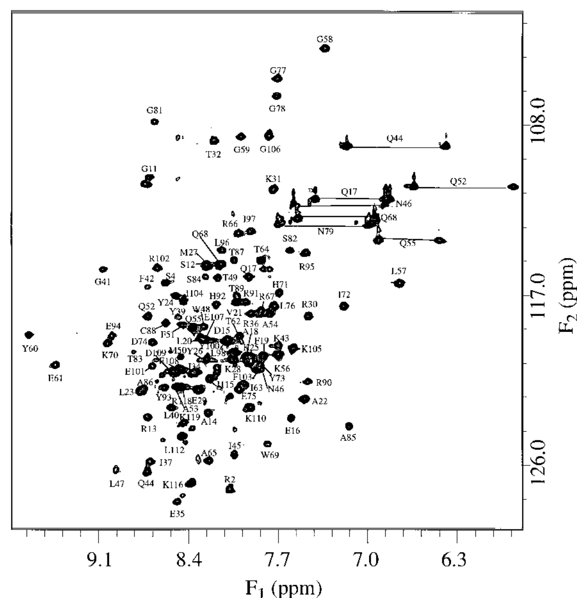

Figure 1. Gradient-enhanced 2D ^{1}H-^{15}N-HSQC 500 MHz spectrum of the Mrf-2 domain. The protein is enriched with ^{15}N. The sample (90% H_2O/10% D_2O) contained 1 mM protein, 100 mM KCl, 10 mM $Na_2S_2O_4$, 0.015% NaN_3 in 100 mM phosphate buffer, PH 6.0. The experiment was conducted at 26 °C. Bars connect side-chain NH_2 resonances.

ware from Molecular Simulations Inc. on SGI Indigo II workstations.

Sequence-specific assignments were obtained by triple resonance experiments. A combination of HNCA, HNCACB and CBCA(CO)NH experiments was used to obtain sequential connections. The ^{15}N-edited TOCSY spectrum in combination with the HBHA(CBCACO)NH experiment also provided sequential connectivities and was useful in resolving ambiguities. In most cases, C^{α} and H^{α} were used to establish the sequential connectivity. C^{β} and H^{β} resonances were also used when they were observed. These assignments were further confirmed by sequential NOEs between the backbone protons. Side chain resonances were assigned using HCCH-COSY and HCCH-TOCSY experiments.

Extent of assignments and data deposition

Assignments were obtained for all backbone $^{13}C^{\alpha}$, H^{α}, H^{N}, and ^{15}N resonances. Figure 1 shows the ^{1}H-^{15}N HSQC spectrum of the Mrf-2 domain. Resonance assignments are labeled in this spectrum. More than 95% of the side chain resonances have also been assigned. A table of chemical shifts has been deposited in the BioMagResBank Database (accession code 4100). Deviations of H^{α}, C^{α} and C^{β} chemical shifts from random coil values, characteristic sequential and medium range NOEs and $^{3}J_{HN\alpha}$ coupling constants indicate that the secondary structure of this domain contains mainly helices and no β-sheets (for reviews, see Wishart and Sykes, 1994; Wüthrich, 1986).

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