The N-terminal Methionine Is a Major Determinant of the DNA Binding Specificity of MEF-2C*

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Members of the MEF-2 family of transcriptional regulators positively modulate the activity of basic helix-loop-helix proteins in both myogenic and neurogenic cell lineages. Previous work had shown that MEF-2C(2–117), a protein fragment comprising the dimerization domain of MEF-2C but lacking the N-terminal methionine, bound to AT-rich DNA sequences with high affinity. MEF-2C(2–117) did not discriminate between different AT-rich sequences. We now report the in vitro DNA binding properties of a MEF-2C fragment containing the N-terminal methionine. Measurements of the apparent dissociation constants of the complexes of GG-MEF-2C(1–117) revealed that each AT-rich sequence is bound by different MEF-2C fragments. In particular, MEF-2C site containing DNA (CTATAAATTAG) is bound preferentially to DNA containing a SRF site (CTATAAATG). Strikingly, when the shorter AT run consisted of six alternating thymines and adenines, almost wild-type affinity was observed. Irrespective of the particular DNA sequence, all circular dichroism spectra of the DNA complexes of GG-MEF-2C(1–117) revealed that different AT-rich sequences are bound by different MEF-2C fragments. In particular, MEF-2C site containing DNA (CTATAAATG) is bound preferentially to DNA containing a SRF site (CTATAAATAG). Strikingly, when the shorter AT run consisted of six alternating thymines and adenines, almost wild-type affinity was observed. Irrespective of the particular DNA sequence, all circular dichroism spectra of the DNA complexes of GG-MEF-2C(1–117) revealed that different AT-rich sequences are bound by different MEF-2C fragments. In particular, MEF-2C site containing DNA (CTATAAATG) is bound preferentially to DNA containing a SRF site (CTATAAATAG). Strikingly, when the shorter AT run consisted of six alternating thymines and adenines, almost wild-type affinity was observed. Irrespective of the particular DNA sequence, all circular dichroism spectra of the DNA complexes of GG-MEF-2C(1–117) revealed that different AT-rich sequences are bound by different MEF-2C fragments. In particular, MEF-2C site containing DNA (CTATAAATG) is bound preferentially to DNA containing a SRF site (CTATAAATAG). Strikingly, when the shorter AT run consisted of six alternating thymines and adenines, almost wild-type affinity was observed. Irrespective of the particular DNA sequence, all circular dichroism spectra of the DNA complexes of GG-MEF-2C(1–117) revealed that different AT-rich sequences are bound by different MEF-2C fragments. In particular, MEF-2C site containing DNA (CTATAAATG) is bound preferentially to DNA containing a SRF site (CTATAAATAG). Strikingly, when the shorter AT run consisted of six alternating thymines and adenines, almost wild-type affinity was observed. Irrespective of the particular DNA sequence, all circular dichroism spectra of the DNA complexes of GG-MEF-2C(1–117) revealed that different AT-rich sequences are bound by different MEF-2C fragments. In particular, MEF-2C site containing DNA (CTATAAATG) is bound preferentially to DNA containing a SRF site (CTATAAATAG). Strikingly, when the shorter AT run consisted of six alternating thymines and adenines, almost wild-type affinity was observed. Irrespective of the particular DNA sequence, all circular dichroism spectra of the DNA complexes of GG-MEF-2C(1–117) revealed that different AT-rich sequences are bound by different MEF-2C fragments. In particular, MEF-2C site containing DNA (CTATAAATG) is bound preferentially to DNA containing a SRF site (CTATAAATAG). Strikingly, when the shorter AT run consisted of six alternating thymines and adenines, almost wild-type affinity was observed. Irrespective of the particular DNA sequence, all circular dichroism spectra of the DNA complexes of GG-MEF-2C(1–117) revealed that different AT-rich sequences are bound by different MEF-2C fragments. In particular, MEF-2C site containing DNA (CTATAAATG) is bound preferentially to DNA containing a SRF site (CTATAAATAG).
Production and Purification of MEF-2C(2–117), GG-MEF-2C(1–117), and GG-MEF-2C(1–117)/R(3)K—An N-terminal fragment of the murine MEF-2C protein comprising amino acids 2 to 117 was produced in BL21(DE3)pLys cells (32) from the T7 promoter in the plasmid pGJetita as described previously (21, 33). To construct the expression plasmid for the production of GG-MEF-2C(1–117), two synthetic oligonucleotides 5′-TATGGTGGG and 5′-TACCAATCA were annealed and ligated with the expression plasmid for MEF-2C(2–117), which had been digested with the restriction enzyme NdeI which is located at the initiator methionine codon. The cDNA insert in the expression plasmid pGJetGGG-MEF-2C(1–117) therefore coded for MEF-2C(1–117) with the additional tripeptide MGG at the N terminus. To construct the expression plasmid for the recombinant proteins lacking the N-terminal methionines, MALDI-TOF mass spectroscopy revealed molecular masses of 13,386 units for MEF-2C(2–117), 13,628 units for GG-MEF-2C(1–117), and 13,605 units for GG-MEF-2C(1–117)/R(3)K, which corresponded well with the calculated masses of 13,377 units, 13,622 units, and 13,594 units for the recombinant proteins lacking the N-terminal methionines. Protein concentrations were determined by measuring the UV absorption at 210, 215, and 220 nm (37). The protein yields were approximately 5 mg of protein.

Experimental Procedures

Production and Purification of MEF-2C(2–117), GG-MEF-2C(1–117), and GG-MEF-2C(1–117)/R(3)K—An N-terminal fragment of the murine MEF-2C protein comprising amino acids 2 to 117 was produced in BL21(DE3)pLys cells (32) from the T7 promoter in the plasmid pGJetita as described previously (21, 33). To construct the expression plasmid for the production of GG-MEF-2C(1–117), two synthetic oligonucleotides 5′-TATGGTGGG and 5′-TACCAATCA were annealed and ligated with the expression plasmid for MEF-2C(2–117), which had been digested with the restriction enzyme NdeI which is located at the initiator methionine codon. The cDNA insert in the expression plasmid pGJetGGG-MEF-2C(1–117) therefore coded for MEF-2C(1–117) with the additional tripeptide MGG at the N terminus. To construct the expression plasmid for the recombinant proteins lacking the N-terminal methionines, MALDI-TOF mass spectroscopy revealed molecular masses of 13,386 units for MEF-2C(2–117), 13,628 units for GG-MEF-2C(1–117), and 13,605 units for GG-MEF-2C(1–117)/R(3)K, which corresponded well with the calculated masses of 13,377 units, 13,622 units, and 13,594 units for the recombinant proteins lacking the N-terminal methionines. Protein concentrations were determined by measuring the UV absorption at 210, 215, and 220 nm (37). The protein yields were approximately 5 mg of protein.

Circular Dichroism Spectroscopy—CD spectra were measured on a Jasco J600 circular dichroism spectrometer at 25 °C using strain free quartz cuvettes with a path length of 0.5 cm. For every measurement, the CD spectra were freshly diluted from a stock solution into 1 mm Tris, pH 8.0, 0.25 mM dithiothreitol, which had been filtered and degassed prior to use. Spectra were measured for protein concentrations of 2 μM. The concentrations of the double-stranded oligonucleotides were 1 μM. CD spectra of DNA complexes are reported as difference spectra. In Fig. 3 the spectrum of the free DNA was subtracted from the spectra of the complexes, while the contribution from the free protein was subtracted from the spectra of the complexes in Fig. 4.

Electrophoretic Mobility Shift Assays—Electrophoretic mobility shift assays (Fig. 5) were performed as described previously (36, 38). In essence, bacterially expressed proteins were serially diluted into EMSA buffer (50 mM Tris, pH 7.9, 6 mM MgCl₂, 40 mM ammonium sulfate, 0.2 mM EDTA, 1 mM dithiothreitol, 100 mM KCl, and 5% glycerol). This solution was incubated in the presence of 10 μM labeled oligonucleotide for 10 min at ambient temperature. Samples were applied to 5% polyacrylamide gels in 0.5 M Tris, pH 8.9, 1 mM MgCl₂, and 0.5 mM EDTA, and electrophoresis was performed at 1000 V for 1 h at 20 °C. The bands were visualized by autoradiography, and the mobility of the retarded band was measured. The mobility of the retarded band was measured as the activity (F) divided by the activity (F) of the free DNA (F). The concentrations of the double-stranded oligonucleotides were 1 μM. CD spectra of DNA complexes are reported as difference spectra. In Fig. 3 the spectrum of the free DNA was subtracted from the spectra of the complexes, while the contribution from the free protein was subtracted from the spectra of the complexes in Fig. 4.

DNA Binding Specificity of MEF-2C

2C(1–117) bends all DNA sequences examined by 70°, a value which is comparable to the bending observed in the DNA complex of SRF. In agreement with the crystal structures of the DNA complex of SRF and of the MCM1-MATα2-DNA complex (20, 31) our results support the notion that the N-terminal methionine of MEF-2C is central to anchoring MEF-2C to the ends of the AT run, thereby properly orienting the protein on the DNA most likely through stabilization of the conformation of Arg-3.
DNA Binding Specificity of MEF-2C

RESULTS

CD Spectroscopic Characterization of MEF-2C(2–117) and GG-MEF-2C(1–117)—Starting from a cDNA encoding the first 117 amino acids of MEF-2C (21, 33), a gene was constructed which at the 5′-end contained three additional base triplets coding for the tripeptide MGG (Fig. 1). This gene was expressed in E. coli and the gene product purified to apparent homogeneity. N-terminal Edman sequencing and MALDI-TOF mass spectroscopy revealed that the protein GG-MEF-2C(1–117) lacked the N-terminal methionine but contained two additional glycine residues just N-terminal to methionine 1 of MEF-2C (Fig. 1).

Circular dichroism spectroscopy of recombinant GG-MEF-2C(1–117) showed that the protein was at least partially folded for all concentrations examined (100 nM to 1 μM) even in the absence of DNA (Fig. 3A). Analysis of the shape of the CD spectrum indicated that the α-helical content of GG-MEF-2C(1–117) was approximately 23% (41). These observations mirrored the results obtained for MEF-2C(2–117) which lacks the N-terminal methionine (Fig. 3B) (21). The addition of a double-stranded oligonucleotide containing a binding site for MEF-2C did not induce a change in the conformation of GG-MEF-2C(1–117) or MEF-2C(2–117) (Fig. 3, A and B) (21). Similarly no conformational changes were observed when oligonucleotides of heterologous sequence were added (data not shown).

DNA Binding Specificity of GG-MEF-2C(1–117) Determined by EMSA—The DNA binding affinities of GG-MEF-2C(1–117) with various double-stranded oligonucleotides (Fig. 2) were measured in electrophoretic mobility shift assays (Fig. 5A). A constant amount of DNA was titrated with increasing amounts of the protein. The fraction Φ of DNA bound was determined from such experiments as the activity of the retarded band (PD) divided by the sum of the retarded and the unretarded band (D) (Fig. 4, A-C). In agreement with the observations made for the DNA-binding reaction of MEF-2C(2–117) (21), the best fit for the binding isotherm (Equation 1) was found under the assumption of one preformed MEF-2C dimer binding to one double-stranded oligonucleotide (n = 1).

\[ \Phi_i = \left\{ \frac{(P/K_D)_i}{(1 + (P/K_D)_i)} \right\} \]

(Eq. 1)

103 nM GG-MEF-2C(1–117) bound 50% of an oligonucleotide containing a MEF site (Table I), while we had previously measured a \( K_D \) of 110 nM for the binding of MEF-2C(2–117) to MEF site DNA (Table I) (21). The presence of the tripeptide GGM at the N terminus did not therefore alter the affinity for MEF site containing DNA. However, the measurement of the dissociation constants of GG-MEF-2C(1–117) and MEF-2C(2–117) with mutant DNA sites revealed that this tripeptide greatly enhanced the specificity of DNA binding (Table I and Fig. 5, A-C). While the affinities of MEF-2C(2–117) for the oligonucleotides MEF site, MEF-1T, MEF(-3T), and MEF(-2A),(-1T) (Fig. 1) were indistinguishable within experimental error, the presence of the N-terminal tripeptide GGM allowed the protein to discriminate between these binding sites which differ in the sequence of the AT core (Table I). The highest affinity binding (\( K_D = 82 \) nM) was observed with a core region of four TA base steps. The inherently rigid A-tract DNA was bound by MEF-2C(2–117) and GG-MEF-2C(1–117) with, respectively, 7- and 10-fold reduced affinity. The interruption of the AT-rich core
region through a GC step increased the amount of MEF-2C(2–117) needed to bind to DNA half-maximally by 10-fold. On the other hand, almost 20 times more GG-MEF-2C(1–117) was needed to bind 50% of MEF(-1G),1C when compared with MEF site DNA.

Strikingly, the discrimination against DNA-binding sites containing AT runs of reduced length is also increased significantly by the presence of the N-terminal methionine (Figs. 5, B and C). While MEF-2C(2–117) could not discriminate between AT-rich cores of length 7 and 8, GG-MEF-2C(1–117) showed a slight preference for the longer sequence (Fig. 2 and Table I). Reducing the length of the core region to 6 by removing the first and the last base pairs of the AT core of the MEF site DNA (Fig. 2) reduced the affinity for MEF-2C(2–117) approximately 3-fold, while an almost 20 times higher concentration of GG-MEF-2C(1–117) was needed to bind 50% of MEF-D(1–117). However, the replacement of adenine with purine in positions −3 or −4 reduced the amount of GG-MEF-2C(1–117) needed for half-maximal DNA binding by almost a factor of two, while a purine in position 1 or −1 only slightly increased the affinity for GG-MEF-2C(1–117). Replacing adenes in positions −1 or 1 of MEF site containing DNA with 2,6-diaminopurine reduced the affinity for both MEF-2C(2–117) and GG-MEF-2C(1–117) by 4–5-fold (Table II).

DNA Binding Specificity of GG-MEF-2C(1–117)R(3)K—The crystal structure of the DNA complex of SRF revealed that Arg-143 lies in an extended conformation along the floor of the minor groove (20). One guanidinium N(η) is hydrogen bonded to N-3 of adenine (1) and to O-2 of thymine (2) thereby stabilizing the high propeller twists observed in these positions. The sequence alignment in Fig. 1 indicates that R-3 of MEF-2C corresponds to R-143 of SRF. Replacing R-3 in GG-MEF-2C(1–117) with lysine slightly reduced the affinity for DNA sequences containing runs of 8 adenines and thymines (Table I). Interestingly, the DNA binding specificity of GG-MEF-2C(1–117)R(3)K was similar to the specificiry displayed by MEF-2C(2–117). As had been observed for the protein lacking the

![CD spectra of DNA in the complexes with GG-MEF-2C(1–117) are independent of the nucleotide sequence. CD spectroscopy of double-stranded oligonucleotides MEF site (panel A), MEF-D(-1),1 (panel B), MEF-2A, (-1T) (panel C), and MEF(-1G),1C (panel D) in the presence and absence of MEF-2C(2–117) (left row) or GG-MEF-2C(1–117) (right row). The specific core sequence is given and the position of the maximum ellipticity in the spectra of the complexes is indicated by a vertical line. The spectra of the complexes are difference spectra in that the contribution from the CD spectrum of the free protein is subtracted from the spectrum of the complex. [MEF-2C(2–117)] = [GG-MEF-2C(1–117)] = 2 μM; [DNA] = 1 μM.](image)
DNA Binding Specificity of MEF-2C

played by GG-MEF-2C(1–117) suggesting that arginine 3 and methionine 1 cooperate in the wild-type protein to define its binding specificity (see below).

Conformational Properties of DNA in Complexes of MEF-2C(2–117) and GG-MEF-2C(1–117) Determined by CD Spectroscopy—The CD spectra of all double-stranded oligonucleotides used in this study exhibited the positive maximum ellipticity between 270 and 275 nm characteristic of B-DNA (Fig. 4). Previous work has shown that on binding to MEF-2C(2–117) the maximum ellipticity in the CD spectrum of the MEF site oligonucleotide was shifted to higher wavelength by approximately 4 nm and its intensity reduced significantly, while a slight blue shift of less than 1 nm was observed for the mutant DNA MEF(-1G),1C and the intensity of the signal was only slightly reduced (Fig. 4) (21). We now show that for DNA complexes of MEF-2C(2–117) both the shape of the CD signal and the position of its maximum are dependent on the specific DNA sequence (Fig. 4). The maximum ellipticity for the complexes of MEF-2C(2–117) with MEF site, MEF-D(-1),1, MEF(-2A),(-1T), and MEF(-1G),1C were found to be 278, 272, 275, and 271 nm, respectively (Fig. 4).

When 1 equivalent of GG-MEF-2C(1–117) dimer was added to double-stranded oligonucleotides, the CD signal around 275 nm was also affected. However, in this case the CD spectrum of the DNA in the complexes was independent of the particular DNA sequences. Indeed, the spectra of the complexes are superimposable between 250 and 300 nm and the maximum ellipticity is always at 269.5 nm (Fig. 4). These results suggested that the DNA conformation in the complexes with GG-MEF-2C(1–117) were similar irrespective of the exact DNA sequence, while the DNA adopted different conformations in the various MEF-2C(2–117) complexes.

DNA Binding in MEF-2C(2–117), GG-MEF-2C(1–117), and GG-MEF-2C(1–117)R(3)K Complexes—In order to further characterize the conformational behavior of the DNA in the protein complexes, circular permutation assays were performed. To this end, several MEF-binding sites were cloned into pTK401 (39). Digestion of the resulting plasmids with restriction enzymes gave a set of probes of identical length and base composition, but with the binding site at different positions along the length of the probes (Fig. 6A). The electrophoretic mobility of bent DNA is dependent on the location of the bend and the reduction in migratory speed is greatest when the DNA is bent at the center, and least, when near its end (40). It had been shown previously that MEF site DNA is bent by 49° in the complex with MEF-2C(2–117) (Fig. 6, B and C) (21).

The relative mobilities of the complexes of GG-MEF-2C(1–117) with probes containing a MEF site were even more strongly dependent on the position of the binding site along the long axis of the probes when compared with the complexes with MEF-2C(2–117) (Fig. 6B). Analysis of the relative mobilities of the complexes as a function of the flexure displacement indicated that the DNA bending induced by GG-MEF-2C(1–117) mapped to the center of the MEF site (Fig. 6B). Comparing the relative electrophoretic mobilities of the GG-MEF-2C(1–117) complexes to the relative mobility of A-tract DNA indicated that the bend angle was approximately 70° (42), significantly larger than that observed in the corresponding MEF-2C(2–117) complex.

In good agreement with the CD experiments the bend angle of the DNA in the GG-MEF-2C(1–117) complexes was independent of the specific DNA sequence even for the MEF(-1G),1C site in which two GC-base pairs interrupt the AT run (Fig. 6C). In the complex of MEF-2C(2–117) with MEF(-1G),1C the DNA remained largely unbent (21).

The DNA bending properties of the GG-MEF-2C(1–
117)R(3)K were similar to those observed for MEF-2C(2–117). Replacing arginine 3 with lysine led to a 23° reduction in the bend angle observed in the complex with MEF site containing DNA. Like MEF-2C(2–117), the mutant protein induced a DNA bend of 49° which mapped to the center of the MEF site (Fig. 6).

**DISCUSSION**

The x-ray structure of the DNA complex of SRF revealed that for the interaction with its AT-rich target site this MADS box protein employs an α-helix which contacts the phosphodiester in the major groove and a N-terminal extension which reaches over the DNA backbone and penetrates into the minor groove (20). The high sequence similarity between MEF-2C and SRF suggests that MEF-2C interacts with its DNA target through a similar mechanism. In the SRF complex the DNA is bent by 72°. Surprisingly, the bending analysis of the DNA complexes of MEF-2C(2–117) by circular permutation assays revealed a bend angle of only 49° (Fig. 6) (21). However, MEF-2C(2–117)
Table I

DNA binding parameters for binding of MEF-2C(2–117), GG-MEF-2C(1–117), and GG-MEF-2C-R3/K to various DNA sites

| DNA sequence | MEF-2C(2–117) | GG-MEF-2C(1–117) | GG-MEF-2C-R3/K |
|--------------|---------------|-----------------|---------------|
| TATATAA | 110 (±20)
TATATAA | 103 (±20) | 82 (±5) | 82 (±2) |
| TTATAA | 116 (±27) | 88 (±27) | 163 (±15) |
| TGCTGC | 123 (±30) | 128 (±32) | 193 (±27) |
| ACAGGC | 102 (±21) | 61 (±15) | 147 (±19) |
| ACAGGC | 165 (±39) | 50 (±13) | 172 (±24) |
| TGCTGC | 498 (±98) | 586 (±169) | 446 (±51) |
| TGCTGC | 390 (±79) | 418 (±67) | 383 (±44) |

*Protein concentration, [P]1/2 (%), for which 50% of the DNA-binding sites are filled. Values for [P]1/2 were determined by EMSA as described under “Experimental Procedures.”

*For DNA sequences see Table II.

*The sequence of the proteins is given in Fig. 1.

*From Ref. 21.

*ND, not determined.

Table II

Effect of purine and DAP substitutions on the DNA binding parameters of MEF-2C(2–117), GG-MEF-2C(1–117), and GG-MEF-2C-R3/K

| DNA sequence | MEF-2C(2–117) | GG-MEF-2C(1–117) | GG-MEF-2C-R3/K |
|--------------|---------------|-----------------|---------------|
| TATATAA | 110 (±20)
TATATAA | 103 (±20) | 82 (±5) | 82 (±2) |
| TTATAA | 116 (±27) | 88 (±27) | 163 (±15) |
| TGCTGC | 123 (±30) | 128 (±32) | 193 (±27) |
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*Protein concentration, [P]1/2 (%), for which 50% of the DNA-binding sites are filled. Values for [P]1/2 were determined by EMSA as described under “Experimental Procedures.”

*The AT-rich core region is indicated in bold. P: purine; D, 2,6-diamino-purine (DAP). Mutations are underlined.

*From Ref. 21.

Lacks the N-terminal methionine. Since the inspection of the crystal structure of SRF had suggested an important function for this residue for DNA recognition, we have now measured the DNA binding properties of GG-MEF-2C(1–117), a protein which contains the N-terminal methionine and two additional glycine residues.

Circular permutation experiments revealed that in the presence of the N-terminal methionine MEF-2C bends its DNA target sequences by approximately 70° (Fig. 6). This result was independent of the specific DNA sequence used since all oligonucleotides studied were bent to the same extent upon binding to GG-MEF-2C(1–117). This result is in sharp contrast to the behavior of MEF-2C(2–117), which bends MEF site containing DNA by 49°, while no bending was observed with the mutant oligonucleotide MEF(1-1),(1C) (21).

The binding specificity of a protein is determined by the difference in binding affinities of the protein for the specific and the nonspecific sites. DNA bending is an energy costing process even for sequences of enhanced bendability (Fig. 7). Some of the binding free energy must be used to bend a DNA molecule which, in the absence of protein, adopts either an un bent or an only slightly bent conformation. Therefore, protein induced DNA bending can contribute to the overall specificity of a DNA binding protein (43–45).

MEF-2C for instance must use some of the binding free energy from specific interactions to induce bending at the specific site. However, in the case of a MEF-2C mutant lacking the N-terminal methionine no binding free energy appears to be used for the bending of the nonspecific complex (21). Consequently, the difference between the free energies of the specific and the nonspecific complexes is reduced because of the unfavorable contribution from bending the specific DNA. On the other hand, GG-MEF-2C(1–117) bends both specific and nonspecific DNA to the same extent, thereby increasing the difference in the free energy between the specific and the nonspecific complexes. The increased DNA binding specificity of GG-MEF-2C(1–117) as compared with MEF-2C(2–117) relies therefore in part on the fact that GG-MEF-2C(1–117) bends DNA irrespective of the particular sequence. GG-MEF-2C(1–117) appears to select DNA-binding sites which are characterized by increased bendability since for these DNA sequences less binding free energy must be expended to force them into the bent conformation necessary for the optimal shape complementarity observed in the complexes (Fig. 7).

CD spectroscopy provided further information about the structure of the DNA in the complexes with MEF-2C proteins. While the CD spectra of the DNA in the complexes with MEF-2C(2–117) were strongly dependent upon the specific nucleotide sequence, the spectra of the DNA complexes of GG-MEF-2C(1–117) were superimposable and characterized by an identical maximal ellipticity at 269.5 nm (Fig. 4). These results suggested that in the MEF-2C(2–117) complexes the conformation of the DNA was sequence dependent. On the other hand, the presence of the N-terminal methionine appears to allow GG-MEF-2C(1–117) to force all oligonucleotides into a similar conformation.

Even in the absence of a protein, sequence dependent bending of double-stranded DNA often occurs at the junctions between regions of G-C and A-T base pairs. DNA bending of 10° to 20° has been observed in the crystal structures of oligonucleotides containing an AT core (Refs. 23, 25, and 46, and references cited therein). The transition from G-C to A-T base pairs renders this region of the DNA flexible and capable of potentially undergoing a bend. The molecular mechanism of
such facultative bending has been reviewed in detail elsewhere (21, 30). The x-ray structure of the DNA complex of SRF has shown that this protein takes advantage of the inherent bendability of its DNA target (20). The helical bend is thereby mainly generated by large positive roll angles at the junctions between GC and AT base pairs. The AT-rich core is characterized by a small average negative roll which is made possible through increased propeller twists in this region which in turn are facilitated because A-T pairs are held together by only two hydrogen bonds rather than the three formed between guanine and cytosine.

The affinities of MEF-2C(2–117) for AT-rich DNA sequences did not depend on the exact nucleotide sequence (Table I). However, due to its poor stacking, the deformability of a TA step is inherently greater than that of an AT or an AA step (22, 47). Similarly, a simple mechanical model would predict that a run of alternating thymines and adenines should be most easily be deformed due to the absence of steric hinderance preventing the DNA from bending by a roll mechanism (29, 30, 48). As a consequence, MEF-2C should preferentially bind to MEF-1T as was indeed observed with GG-MEF-2C(1–117) but not with MEF-2C(2–117) (Table I). Approximately 20% less GG-MEF-2C(1–117) was needed to bind half-maximally to MEF-1T than to MEF site DNA. Unlike MEF-2C(2–117), GG-MEF-2C(1–117) was generally able to discriminate between different AT-rich sequences. In addition, the discrimination of GG-MEF-2C(1–117) against the mutant MEF-(-1G),1C site, in which the AT step was present.

The DNA bending specificity and the extent of DNA bending depend significantly on the presence of the N-terminal methionine. However, methionine 1 exerts its function in cooperation with Arg-3. The mutant GG-MEF-2C(1–117)R(3)K displays the relaxed DNA binding specificity of MEF-2C(2–117). Like MEF-2C(2–117), GG-MEF-2C(1–117)R(3)K bends DNA by only 49° upon complex formation (Fig. 6C).

The crystal structure of the DNA complex of SRF revealed that the N-terminal arm penetrates the minor groove (20). Arg-143, which corresponds to Arg-3 of MEF-2C, lies in an extended conformation buried in this narrow minor groove and makes hydrogen bonds to N-3 of adenine (1) and O-2 of thymine (2). This interaction allows a discrimination against a G-C base pair because the third hydrogen bond would block this orientation of the arginine side chain, which in turn is necessary to stabilize the increased propeller twists observed in this region. Increased propeller twists are important to allow proper bending of the DNA to occur. The conformation of Arg-143 in the minor groove is stabilized through extensive hydrophobic interaction with Val-144 and Ile-146 and the sugar rings. While Ile-146 is conserved in MEF-2C, the position which corresponds to Val-144 in SRF is occupied by a lysine in MEF-2C (Fig. 1). All the results described above suggest that Met-1 could substitute for valine in stabilizing the embedded structure of the N-terminal extension. When both valine and methionine are absent, the contacts between the DNA and the guanidinium group of arginine might no longer be strong enough to prevent the N-terminal extension from swinging away from the DNA. This explanation is supported by the structure of the DNA complex of MCM1 (31). This MADS box protein contains neither a methionine nor a valine in the relevant part of the N-terminal extension (Fig. 1). Val-144 of SRF is replaced by arginine, while methionine 1 of MEF-2C is substituted by a lysine residue. Generally this region of MCM1 is very hydrophilic and indeed no contacts between Arg-18 of MCM1, which corresponds to Arg-3 of MEF-2C and Arg-143 of SRF, and the bases in the minor groove of the DNA were observed in the crystal structure (31). In one of the subunits of the dimeric MCM1, the N-terminal extension is guided into the major groove of the DNA through hydrogen bonds with phosphate oxygens and through van der Waals interactions between Arg-18 and the methyl group of a thymine and C-8 of an adenine. No minor groove contacts are made by this subunit. The second subunit does make a hydrogen bond to O-2 of thymine as was observed in the structure of the DNA complex of SRF. However, it is not Arg-18 but Arg-19 which mediates this contact in what appears to be the consequence of crystal packing forces as has been pointed out earlier (31).
In summary, the specificity of DNA recognition by MEF-2C is governed by both intrinsic and inducible properties of the DNA target site. MEF-2C exploits the intrinsically high deformability of AT-rich sequences and on binding induces the DNA to bend by 70°. This bending and the DNA binding specificity of MEF-2C are to a significant extent controlled by amino acids in the N-terminal region of the protein, especially methionine (1), which most likely restricts the conformational freedom of arginine (3) in the minor groove.

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