Homodimeric MyoD Preferentially Binds Tetraplex Structures of Regulatory Sequences of Muscle-specific Genes*

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Myogenic transcription is activated by the binding of heterodimers of the basic helix-loop-helix proteins MyoD and E12 or E47 to a consensus E-box sequence, d(CANNTG), in promoter or enhancer regions of muscle-specific genes. Homodimers of MyoD bind E-box less tightly and are less efficient activators of transcription. Recent results from our laboratory (Yafe, A., Etzioni, S., Weisman-Shomer, P., and Fry, M. (2005) Nucleic Acids Res. 33, 2887–2900) indicate that regulatory sequences of several muscle-specific genes contain a disproportionate high content of guanine clusters that readily form hairpin and parallel-stranded unimolecular and bimolecular tetraplex structures. Here we have shown that homodimers of full-length recombinant MyoD formed complexes with bimolecular tetraplex structures of muscle-specific regulatory sequences but not with their double-stranded, hairpin, or unimolecular tetraplex forms. Preferential binding of homodimeric MyoD to bimolecular tetraplex DNA structures over E-box DNA was reflected by the 18.7–39.9-fold lower dissociation constants, $K_d$, of the MyoD-tetraplex DNA complexes. Conversely, MyoD-E47 heterodimers formed tighter complexes with E-box as indicated by their 6.8–19.0-fold lower $K_d$ relative to complexes with bimolecular tetraplex DNA structures. Similarly, homodimers of the 60-amino acid basic helix-loop-helix domain of MyoD bound E-box more efficiently and tetraplex DNA less efficiently than homodimers of full-length MyoD. It might be that the favored binding of MyoD homodimers to tetraplex DNA structures lowers their ability to activate muscle-specific gene transcription, whereas the formation of MyoD-E47 heterodimers and their preferential binding to E-box DNA enhance transcription.

The development of skeletal muscle from pluripotent mesodermal stem cells involves multiple consecutive steps. At first, cells commit to myogenic precursor and proliferate as myoblasts. In a following differentiation step, myoblasts cease to divide, begin to express muscle-specific genes, and finally fuse to form fully differentiated syncytial myotubes (1, 2). Four myogenic regulatory factors (MRFs), 3 MyoD, Myf-5, myogenin, and MRF4, regulate the coordinated activation of multiple muscle-specific genes during myogenesis. These transcription factors comprise a subgroup within the superfamily of basic helix-loop-helix (bHLH) proteins (for review see Ref. 3). Targeted inactivation of the various MRFs in the germ line of mice indicated that the commitment of proliferating somitic cells to the myogenic lineage is controlled by MyoD and Myf-5 (4–6), whereas the subsequent differentiation of committed myoblasts into myocytes and myotubes requires the action of myogenin and MRF4 (7–10). The HLH section of the bHLH domain is responsible for oligomerization of MRF proteins, whereas its basic region is required for their specific binding to DNA (11). Heterodimers of MyoD with the bHLH proteins E12, E47, and ITF1 were found to be formed at a greater efficiency than homodimers of MyoD (Refs. 11,12, reviewed in Ref. 13). Studies of differentiation in vitro revealed that heterodimers of MyoD with E12 or E47 proteins activated transcription by their binding to a conserved E-box motif, d(CANNTG), in the promoter or enhancer regions of muscle-specific genes. Also, the affinity of MyoD-E12 heterodimers for E-box was significantly higher than that of their respective homodimers (12, 14). Homodimers of the 60 amino-acid-long protein fragment that spans just the bHLH domain of MyoD were shown to be sufficient for specific DNA binding in vitro (12), and similar to full-length MyoD, they were capable of inducing myogenesis in stably transfected mouse fibroblasts (15).

As originally proposed by Larsen and Weintraub (16), some regulatory proteins may affect transcription by specifically recognizing altered DNA conformation rather than a nucleotide sequence in B-DNA. In line with this idea, the ability of myogenic proteins to recognize DNA conformations other than double strands was first inferred from the sequence-specific binding of MyoD and of a muscle protein designated MP3 to single-stranded E-box motif (17). More significantly, recombinant MyoD was reported to form complexes with tetrahedral structures of a guanine-rich mouse creatine kinase enhancer sequence or of Tetrahymena telomeric DNA (18). Measurements of dissociation constants indicated that the binding of MyoD to tetraplex DNA structures is 4–5-fold tighter than to E-box DNA (18).

We reported recently that promoter and enhancer regions of several muscle-specific genes contained segments with a disproportional high frequency of clusters of contiguous guanine residues (19). Interestingly, guanine-rich DNA tracts in the regulatory regions of the muscle-specific genes readily folded into a variety of secondary structures, hairpin and parallel-stranded unimolecular and bimolecular tetraplexes (19). To examine whether these secondary structures may be of potential regulatory significance, we examined in this work the association of MyoD homodimers and MyoD-E47 heterodimers...
with E-box DNA and with the various secondary structures of guanine-rich muscle gene regulatory sequences. We report that MyoD homodimers specifically bound bimolecular DNA tetraplexes but not single or double strands or hairpin or monomolecular tetraplex structures of the guanine-rich muscle gene DNA sequences. Further, measurements of dissociation constants of complexes of MyoD homodimers with DNA indicated that complexes of this protein with the bimolecular tetraplexes were more stable than its complex with E-box DNA. Conversely, MyoD-E47 heterodimers bound E-box DNA more tightly than bimolecular tetraplex DNA structures. We speculate that the preferential binding of the relatively inactive MyoD homodimers or the transcriptionally active MyoD-E47 heterodimers to tetraplex or E-box DNA, respectively, may contribute to the timed regulation of muscle-specific gene expression during myogenesis.

**Experimental Procedures**

Preparation of Hairpin, Double-stranded, and Monomolecular and Bimolecular Tetraplex DNA Structures—The nucleotide sequences of the synthetic DNA oligomers (Genosys) that are listed in Table I were derived from guanine-rich promoter regions of the genes sarcomeric mitochondriald kinase ( MtCK oligomer) or a7 integrin integrin 26 and integrin 29 oligomers (see Ref. 19) or contained the core E-box sequence. The oligomers were purified by denaturing gel electrophoresis in 8.0 M urea, 12% polyacrylamide (acyrlis/bisacrylamide, 19:1) (20). The single-stranded oligonucleotides were labeled at their 5′-ends by 32P in bacteriophage T4 polynucleotide kinase-catalyzed reaction. Hairpin and monomolecular and bimolecular tetraplex structures of the DNA oligomers were formed as we described (19). Double-stranded DNA was prepared by annealing under previously described conditions (21) equimolar amounts of two complementary single-stranded oligomers.

Expression and Purification of Recombinant Proteins—Expression and purification of recombinant E47N, an N-terminal-truncated version of E47 protein, were conducted as described (22). Sequences of cDNA that encode full-length MyoD protein or its bHLH domain (residues 102–162 of MyoD) in pRK171 cDNA that encode full-length MyoD protein or its bHLH domain (residues 102–162 of MyoD) in pRK171 vector (23) were PCR amplified using primers that contained at their respective 5′- or 3′-ends EcoRI and XhoI restriction sequences. The product cDNA molecules were purified (QiAquick; Qiagen) and ligated to XhoI- and EcoR1-digested pGEX-6P vector. Recombinant plasmids were electroporated into Escherichia coli DH5α, and the presence of intact cDNA inserts was verified by determination of their nucleotide sequences. To isolate recombinant MyoD protein and its bHLH domain, pGEX-6P plasmids harboring MyoD or bHLH cDNA were electroporated into competent E.coli BL21(DE3)/pLyS85 cells, the bacteria were grown to an of ~0.6 in Luria Bertani medium containing ampicillin and chloramphenicol, and synthesized glutathione S-transferase fused proteins was induced by exposure to 100 μM isopropyl α-thio-β-D-galactopyranoside for 3 h. The recombinant proteins were purified to ~95% homogeneity from the bacterial cell extracts by glutathione-agarose (Sigma) affinity column chromatography. The glutathione S-transferase residue was cleaved by incubating 100 μg of fusion protein for 4 h at 4 °C with 20 μL of preScission protease (Amersham Biosciences).

Electrophoretic Mobility Shift Assay of Protein Binding to DNA and Determination of Dissociation Constants of the Protein-DNA Complexes—Homodimers of MyoD or its bHLH domain or MyoD-E47N heterodimers were formed prior to their binding to the various DNA probes by incubating for 10 min at 37 °C specified amounts of purified recombinant MyoD or bHLH alone or an equimolar mixture of MyoD and E47N in reaction mixtures that contained, in a final volume of 10 μL 45 mM KCl, 4.5 mM MgCl2, 0.5 mM EDTA, 1 mM dithiothreitol, 20% glycerol, 20 mM Tris-HCl buffer, pH 8.0, and 0.5 μg of HeLa whole cell extract protein. Reaction mixtures for protein-DNA binding contained, in a final volume of 10 μL, specified amounts of MyoD or bHLH homodimers or MyoD-E47N heterodimers and 32P-labeled DNA probe, 14.5 mM KCl, 0.45 mM MgCl2, 0.5 mM EDTA, 1 mM dithiothreitol, 20% glycerol in 20 mM Tris-HCl buffer, pH 8.0, and 0.05 μg of HeLa whole cell extract protein. Reaction mixtures for the binding of end-labeled double-stranded E-box DNA or double-stranded forms of the examined guanine-rich myogenic sequences also contained 100-fold (w/w) excess of unlabeled poly d(I-C) (Sigma). Mixtures for the binding of end-labeled bimolecular tetraplex DNA structures of muscle-specific regulatory sequences contained 100-fold (w/w) excess of unlabeled single-stranded oligomer of the same sequence. The mixtures were incubated for 20 min at 30 °C. Protein-DNA complexes of E-box DNA and bimolecular tetraplex integrin 26 DNA are bound by homodimeric and heterodimeric forms of MyoD and E47N proteins. Oligomerization of recombinant MyoD or E47N proteins was promoted by incubating each protein separately or their equimolar mixture with HeLa whole cell extract protein (see “Experimental Procedures”). The oligomeric forms of MyoD (12 pmol), E47N (6 pmol), or their equimolar mixture (12 pmol) were incubated under DNA binding conditions with 65 fmol 32P-labeled double-stranded E-box DNA or bimolecular tetraplex integrin 26 DNA. DNA-protein complexes were resolved from free DNA by non-denaturing electrophoresis in 4% polyacrylamide gel, 0.25× TBE buffer, 10 mM KCl as described under "Experimental Procedures." To determine the binding of the released DNA-protein complexes, electrophoresis was conducted in a long gel that allowed migration of a bromphenol blue marker dye to 15 cm into the gel. Shown are phosphorimages of the dried gels.

To determine values of dissociation constants, Kd, of complexes of MyoD or MyoD-E47 dimers with E-box DNA or with bimolecular tetraplex structures of guanine-rich muscle-specific DNA sequences, increasing amounts of 32P-labeled DNA were incubated with a constant amount of protein as described above. Following electrophoretic mobility shift resolution of the protein-DNA complexes from free DNA, their relative amounts were determined by phosphorimaging quantification of the dried gel. Kd values were derived from the negative reciprocal of the slope of a Scatchard plot of the results as detailed elsewhere (24).

**Results**

MyoD and E47N Proteins Bind E-box and Bimolecular Tetraplex DNA in Their Homodimeric and Heterodimeric Forms—The HLH domains of MyoD or E proteins mediate formation of homodimers of each protein or their association to form heterodimers. We first inquired whether the binding of double-stranded E-box DNA and bimolecular tetraplex structure of integrin 26 DNA is mediated by the monomeric or oligomeric forms of these proteins. Oligomerization of MyoD and E47N was promoted by incubating each protein separately or their equimolar mixture thereof in the presence of a HeLa whole cell extract protein (see “Experimental Procedures”). The proteins were subsequently incubated under DNA binding conditions with 5μl 32P-labeled E-box DNA or bimolecular tetraplex integrin 26 DNA. Formed protein-DNA complexes were resolved by gel electrophoresis at 4 °C and 200–250 V in non-denaturing 4% polyacrylamide gel (acyrlis/bisacrylamide, 19:1) in 10 mM KCl, 0.25× TBE buffer (1.2 mM EDTA in 0.5 mM Trisborate buffer, pH 8.3). Electrophoresis of the DNA was conducted until a bromphenol blue marker dye migrated 7.5 cm into the gel. In a case that required greater resolution of the protein-DNA complexes, (see Fig. 1), electrophoresis was conducted until the marker dye migrated 15 cm into a longer gel. The gels were dried on DE81 filter paper, and the relative proportions of bands of free and protein-bound DNA structures were quantified by phosphorimaging analysis.

FIG. 1.
from free DNA by non-denaturing polyacrylamide gel electrophoresis. Representative results of this experiment are shown in Fig. 1. These data indicated that both MyoD and E47 formed complexes with the two types of end-labeled DNA. The MyoD-DNA complexes migrated in the gel more slowly than the E47N-DNA complexes, as expected for the 318-amino acid-long MyoD as compared with the 157-amino acid-long E47N (22). Most importantly, incubation of each type of DNA with the mixture of MyoD and E47N yielded protein-DNA complexes that migrated midway between the slower and the more rapid complexes of MyoD and E47N, respectively (Fig. 1). The intermediate electrophoretic mobilities of DNA complexes with the mixture of MyoD and E47N strongly suggested that heterodimers of the larger and smaller sized MyoD and E47N, respectively, were responsible for the binding of DNA. Likewise, when compared with the midway migration of these complexes, the slower or more rapid migration of either MyoD or E47N alone, respectively, was consistent with a homodimeric structure of each protein.

**Homodimeric MyoD Selectively Binds Bimolecular Tetraplex Structures of Muscle-specific Regulatory Sequences—**Recently gathered data show that short guanine-rich tracts derived from enhancer or promoter sequences of muscle-specific genes readily folded into hairpin forms or parallel-stranded unimolecular or bimolecular tetraplex structures (19). Also, as was previously demonstrated, MyoD associated with tetraplex forms of sequences of mouse creatine kinase enhancer (18) or sMtCK promoter (19) more efficiently than with E-box DNA. Because MyoD regulates the transcription of muscle-specific genes whose regulatory sequences formed secondary structures, we compared the binding of homodimeric MyoD to single-stranded, double-stranded, hairpin, or tetraplex structures of these guanine-rich tracts. Increasing amounts of MyoD homodimers were incubated with a constant amount of end-labeled bimolecular tetraplex structures of the integrin 26 or sMtCK DNA oligomers or with the double-strands that these oligomers formed with their respective complementary sequences (see Table I for oligomer sequences). As seen in Fig. 2, MyoD associated avidly with the bimolecular tetraplex structures of both sequences, whereas its binding to the double-stranded forms of both sequences was negligible. As was shown (19), the integrin 26 sequence formed a mixture of unimolecular and bimolecular parallel-stranded tetraplexes. Results presented in the upper electrophoretogram of Fig. 2 indicated that, whereas the MyoD homodimers bound bimolecular tetraplex integrin 26 DNA efficiently, they did not detectably associate with the unimolecular tetraplex form of this sequence. In other experiments we found that in contrast to the efficient association of MyoD with bimolecular tetraplex sMtCK DNA (Fig. 2), it failed to form complexes with hairpin structures of the same sequence (19). Thus, MyoD in its homodimeric state appeared to bind preferentially bimolecular tetraplex structures of guanine-rich tracts of promoter sequences of the muscle-specific integrin and sMtCK genes.

**Binding of MyoD Stabilizes Tetraplex sMtCK DNA—**We next inquired whether the stability of the bimolecular tetraplex structure of a promoter-derived sequence of the muscle-specific sMtCK gene was affected by its association with MyoD. Bimolecular tetraplex structures of the sMtCK DNA oligomer were bound to homodimeric recombinant MyoD to form protein-DNA complexes of muscle-specific gene promoters. Homodimers of full-length recombinant MyoD protein at amounts ranging between 0 and 30 pmol were incubated for 20 min at 30 °C under DNA binding conditions and in the presence of an appropriate unlabeled DNA competitor (see “Experimental Procedures”) with 65 fmol each 5'-32P-labeled bimolecular tetraplex integrin 26 DNA, bimolecular tetraplex sMtCK DNA, double-stranded integrin 26 DNA-anti-integrin 26 DNA or double-stranded sMtCK DNA-anti-sMtCK DNA (Table I). Protein-bound and free DNA were resolved from one another by non-denaturing gel electrophoresis in 4% polyacrylamide, 0.25 × TBE buffer, 10 mM KCl. Shown are respective phosphorimages of the dried gels and plots of the results as quantified by phosphorimaging analysis. The notation G'2 refers to bimolecular tetraplex forms of specific DNA sequences.
complexes, whereas control mixtures did not contain MyoD. Aliquots of the binding or control mixtures were either incubated for 10 min each at increasing temperatures or were heated at 50 °C for various periods of time. DNA denaturation was terminated by rapid cooling of the mixtures to 4 °C, the DNA was stripped of bound protein by 0.5% sodium dodecyl sulfate, and bimolecular tetraplex sMtCK DNA was resolved from denatured single-stranded sMtCK DNA by non-denaturing gel electrophoresis in 10% polyacrylamide, 0.5× TBE buffer, 10 mM KCl. The relative amounts of tetraplex and single-stranded DNA were quantified by phosphorimaging analysis. Shown are plots of percent tetraplex DNA remaining as a function of temperature. B, denaturation at 50 °C of free or MyoD-bound tetraplex sMtCK DNA. Protein-bound or free 5-32P-labeled bimolecular tetraplex sMtCK DNA was incubated at 50 °C for various periods of time. Termination of the denaturation reaction, gel electrophoresis resolution of the DNA, and quantification of the denatured single-stranded and remaining tetraplex sMtCK DNA were conducted as in panel A.

**Fig. 3.** Homodimeric MyoD increases the heat stability of bound bimolecular tetraplex sMtCK DNA. A, denaturation of free or MyoD-bound bimolecular tetraplex sMtCK DNA at increasing temperatures. 5-32P-labeled bimolecular tetraplex sMtCK DNA was incubated under complex formation conditions either in the presence of a saturating amount of MyoD homodimers or in the absence of protein as described under “Experimental Procedures” and in Fig. 1. Aliquots of mixtures containing protein-bound or free DNA were incubated for 10 min each at increasing temperatures and rapidly cooled on ice. SDS was added to a final concentration of 0.5%. Denatured single-stranded sMtCK DNA was resolved from the remaining intact bimolecular tetraplex sMtCK DNA by non-denaturing gel electrophoresis in 10% polyacrylamide, 0.5× TBE buffer, 10 mM KCl. The relative amounts of tetraplex and single-stranded DNA were quantified by phosphorimaging analysis. Shown are plots of percent tetraplex DNA remaining as a function of temperature. B, denaturation at 50 °C of free or MyoD-bound tetraplex sMtCK DNA. Protein-bound or free 5-32P-labeled bimolecular tetraplex sMtCK DNA was incubated at 50 °C for various periods of time. Termination of the denaturation reaction, gel electrophoresis resolution of the DNA, and quantification of the denatured single-stranded and remaining tetraplex sMtCK DNA were conducted as in panel A.

**Fig. 4.** Full-length MyoD, but not its bHLH domain, binds bimolecular tetraplex integrin 26 DNA more efficiently than E-box DNA. Increasing amounts of homodimers of recombinant full-length MyoD or its bHLH domain were incubated with 5-32P-labeled bimolecular tetraplex integrin 26 DNA or E-box DNA under binding conditions as described in Fig. 2. Protein-DNA complexes were resolved from free tetraplex or double-stranded DNA by non-denaturing gel electrophoresis in 4% polyacrylamide, 0.25× TBE buffer, 10 mM KCl, and their relative amounts were determined by phosphorimaging analysis. Shown are typical phosphorimages of the dried gels and plots of the quantified results.
isolated bHLH domain is also capable of activating the muscle differentiation program by associating in a sequence-specific manner with an E-box DNA motif, d(CANNTG) (11, 15). In view of the capacity of MyoD to bind tetraplex structures of muscle-specific DNA sequences, we compared the relative binding of homodimers of full-length MyoD or its bHLH domain to bimolecular integrin 26 DNA and E-box DNA. Representative results shown in Fig. 4, upper panel, indicated that the full-length MyoD protein formed complexes with tetraplex integrin 26 DNA in preference over E-box DNA. For example, employing the same binding conditions, 30 pmol full-length MyoD bound 22 fmol bimolecular tetraplex integrin 26 DNA, but only about 2 fmol E-box DNA. The isolated bHLH domain, however, associated with E-box DNA more avidly than with the tetraplex DNA. As seen in Fig. 4, 30 pmol bHLH bound 22 fmol E-box DNA and 13 fmol bimolecular tetraplex integrin 26 DNA. Thus, relative to full-length MyoD, bHLH bound E-box DNA at an ~10-fold greater efficiency, whereas its capacity to bind the bimolecular tetraplex DNA structure was decreased by close to 2-fold. The preferential avidity to bimolecular tetraplex DNA that marked full-length MyoD was lost therefore by the isolated bHLH domain, mainly because of its greatly increased affinity for E-box DNA.

Unlike MyoD homodimers, MyoD-E47 Heterodimers Favor E-box DNA over Bimolecular Tetraplex DNA—Homodimers of MyoD are formed at a lower efficiency than its heterodimers with the bHLH proteins E12 or E47 (12). Further, transcription of muscle-specific genes is enhanced more effectively by MyoD-E47 heterodimers than by MyoD homodimers (14, 23). We therefore next compared the affinities of MyoD homodimers or MyoD-E47N heterodimers for E-box DNA and for bimolecular tetraplex structures of promoter sequences of two muscle-specific genes. To this end, we determined values of dissociation constants, $K_d$, of complexes of MyoD homodimers or heterodimers with the various DNA structures. Representative results shown in Fig. 5 illustrate the distinct affinities of MyoD homodimers or heterodimers for a representative bimolecular tetraplex DNA structure. The homodimeric MyoD possessed a significantly higher affinity for bimolecular tetraplex integrin 26 DNA than the MyoD-E47N heterodimer. This was reflected by a 10-fold higher $K_d$ value of the complex of the tetraplex DNA with MyoD-E47N heterodimer relative to MyoD homodimer (Fig. 5, D and B, respectively). In contrast to the preferential binding of MyoD homodimers to tetraplex DNA (Figs. 2 and 4), representative results shown in Fig. 6 indicated that MyoD-E47N heterodimers had a significantly higher affinity for E-box DNA than for bimolecular tetraplex DNA. As observed in this experiment, the $K_d$ value of the MyoD-E47N heterodimer complex with E-box DNA was >20-fold lower than the $K_d$ of its complex with the bimolecular tetraplex structure of integrin 26-integrin 29 DNA. The disparate preference of MyoD homodimers and heterodimers for tetraplex DNA structures and E-box DNA was established in repeated measurements of $K_d$ values of the respective protein-DNA complexes. Results of these experiments summarized in Table II showed that the average dissociation constant of the complex of E-box DNA with MyoD homodimers was 55-fold higher than the $K_d$ value of its complex with MyoD-E47N heterodimer. Con-
versely, MyoD homodimers bound bimolecular tetraplex DNA structures more tightly than E-box DNA. Thus, the $K_d$ values of complexes of MyoD homodimers with bimolecular tetraplex structures of the sMtCK, integrin 26, and integrin 26/integrin 29 DNA sequences were 4.9–7.8-fold lower than dissociation constants of the respective complexes with MyoD-E47N heterodimers (Table II). In sum, these results clearly indicated that MyoD homodimers associated preferentially with bimolecular tetraplex DNA structures of muscle-specific regulatory sequences, whereas E-box DNA was a preferred target for the binding of MyoD-E47N heterodimers.

**DISCUSSION**

Cellular proteins from diverse organisms interact with tetraplex structures of DNA or RNA. Some proteins bind tetraplex DNA preferentially and at high affinity (26–30). Others act as nucleases that specifically hydrolyze DNA and RNA next to quadruplex structures (31–33). Proteins of yet other types either enhance the formation of tetraplex DNA forms (34–37) or, conversely, unwind or destabilize such structures in DNA or RNA (20, 38–41). A starting point to this work was the reported tight binding of the master transcription factor MyoD to tetrahelical structures of guanine-rich tracts in the muscle creatine kinase enhancer sequence or telomeric DNA (18). In fact, the higher affinity of MyoD for tetrahelical DNA than for its E-box target sequence (18) raised the possibility that tetraplex structures in DNA may contribute to the regulation of the transcription of muscle-specific genes. Involvement of tetraplex DNA structures in gene transcription is not without precedents. Evidence indicates that a tetrahelical DNA domain

![Image](http://www.jbc.org/DownloadedFrom.png)
formed upstream to the P1 promoter of c-myc suppressed its expression (42–46). Conversely, tetraplex structures in the human insulin-linked polymorphic region acted to enhance transcription of this gene (47, 48).

Recent results from our laboratory showed that promoter or enhancer regions of several muscle-specific genes included tracts rich in guanine clusters that readily folded in vitro into hairpin and parallel-stranded unimolecular and bimolecular tetraplex structures (19). Examination of the binding of homodimers of recombinant MyoD to the different secondary structures of these guanine-rich sequences revealed its clear preference for association with bimolecular tetraplex DNA structures. Thus, for instance, homodimeric MyoD efficiently bound bimolecular tetraplex formations of the integrin 26 or sMtCK DNA sequences, whereas their double-stranded structures did not form detectable complexes with this protein (Fig. 2). Similarly, neither unimolecular tetraplex integrin 26 DNA (Fig. 2) nor hairpin sMtCK DNA (19) formed detectable complexes with the MyoD homodimers. The tight association of MyoD with bimolecular integrin 26 DNA was manifested by the significant increase in the thermal stability of the protein-bound tetrahelix (Fig. 3). Most interestingly, however, the binding of MyoD homodimers to bimolecular tetraplex DNA was tighter than to the E-box DNA motif. Results illustrated in Fig. 5 and summarized in Table II indicated that the dissociation constants of complexes of homodimeric MyoD with bimolecular tetraplex structures of the integrin and sMtCK DNA sequences were 18.7–39.9-fold lower than the $K_d$ value of its complex with E-box DNA. However, heterodimers of MyoD with E47N displayed a converse relative affinity to tetraplex complex with E-box DNA. We speculate that the release of homodimeric MyoD from its complex with the heterodimer could also lead to the release of associated histone acetylases to the heterodimer-bound myogenic promoters. It is tempting to speculate that the release of homodimeric MyoD from its complex with tetraplex DNA might be affected by specialized DNA helicases (20, 39) or by DNA destabilizing proteins (41, 50) that melt tetrahedral DNA.

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*Preferential Binding of Tetraplex DNA by MyoD*
Homodimeric MyoD Preferentially Binds Tetraplex Structures of Regulatory Sequences of Muscle-specific Genes
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