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Accelerated Publications

Generation and Statistical Mechanical Modeling of Z-DNA in the Mouse Metallothionein I Promoter†

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Received July 9, 1987; Revised Manuscript Received August 12, 1987

ABSTRACT: The mouse metallothionein I promoter has a potential Z-DNA sequence 86–102 bases upstream from the transcription start. The alternating purine/pyrimidine nature of this sequence, d-(GCGCGTG*AC*TATGCGTG), is interrupted by a central d(AC) dinucleotide. We reversed the d(AC) to d(CA) by site-directed mutagenesis and subcloned the wild-type and mutated 218 base pair (bp) *Sst*I–*Bgl*II fragment containing this region into pUC12. By two-dimensional chloroquine gel analysis, the mutant promoter clearly underwent a B–Z transition with a resulting loss of at least five twists. Purines within this sequence exhibited diethyl pyrocarbonate (DEP) sensitivity, which extended in both the 5′ and 3′ direction encompassing a region of approximately 32 base pairs. When subjected to sufficient torsional stress, the wild-type sequence showed weak evidence of a transition on chloroquine gels and clear DEP sensitivity with a similar, yet distinct pattern. Statistical mechanical modeling of the chloroquine gel analysis demonstrated that the average free energy of propagation for the mutant sequence (0.7–0.9 kcal mol^{-1} bp^{-1}) was approximately twice that for $d(CG)_n$ sequences and that the sequence d(GACGCGGGGCGCGTGCA-TATGCGTGG) forms the core Z-DNA region.

Left-handed Z-DNA and its dynamics are well characterized for synthetic oligonucleotides in vitro [reviewed in Rich et al. (1984)]. Alternating purine/pyrimidine (pur/pyr)[1] sequences most readily form Z-DNA in a syn/anti conformation, respectively (Haschemeyer & Rich, 1967; Wang et al., 1979, 1984), but only two of the three redundant alternating pur/pyr sequences [$d(CG)_n$ and $d(CA)_n$] adopt Z-conformation when subjected to the torsional stress of negative superhelicity (Singleton et al., 1982; Peck et al., 1982; Peck & Wang, 1983; Haniford & Pulleyblank, 1983; Wang, 1984). $d(AT)_n$ forms Z-DNA under certain conditions (Patel & Kozlowski, 1985; Adam et al., 1986; McLean et al., 1986) but preferentially adopts a cruciform structure under superhelical stress (Greaves et al., 1985), as does $d(CATG)_{10}$ (Naylor et al., 1986). Although Z-DNA formation is enhanced by solvent conditions, base modification, and protein binding (Pohl & Jovin, 1972; Wang et al., 1984; Nordheim et al., 1982; Azorin & Rich, 1985), it is clearly sequence dependent. The presence of d(A/T) base pairs or interruptions of pur/pyr alternation may impose a substantial energetic cost to Z-DNA formation (Wang et al., 1984; Patel & Kozlowski, 1985; McLean et al., 1986; Ellison et al., 1986).

Indirect evidence suggests that Z-DNA occurs naturally (Nordheim & Rich, 1983; Rich et al., 1984) and may be involved in recombination (Slightom et al., 1980; Kmiec & Holloman, 1984), DNA packaging (Miller et al., 1985), and gene regulation (Nordheim & Rich, 1983; Hipskind & Clarkson, 1983). However, sequence constraints on the B–Z transition of natural DNA are poorly understood. The mouse metallothionein I (mMT-I) promoter has potential Z-DNA sequences adjacent to regulatory regions. Similar observations were made for the human metallothionein IIA promoter (Karin et al., 1984). We investigated this region in the

† This research was supported in part by the Medical Research Council of Canada and the Natural Sciences and Engineering Research Council of Canada during the tenure of an MRC Scholarship (C.B.H.) and a Gerontological Research Council of Ontario Advanced Student Bursary held within the Medical Sciences Growth and Development Program (D.V.).

[1] Abbreviations: pur/pyr, purine/pyrimidine; mMT-I, mouse metallothionein I; 2-D, two dimensional; bp, base pair(s); TBE, Tris–borate–EDTA; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; DEP, diethyl pyrocarbonate.

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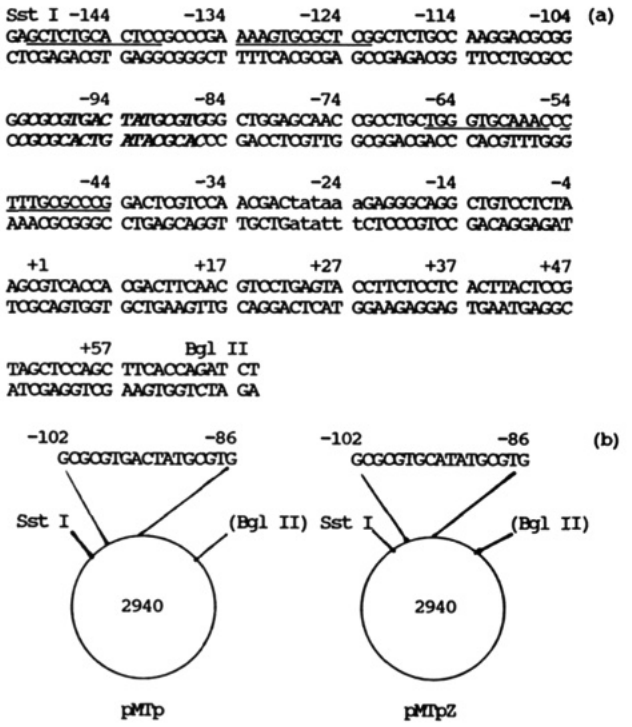

FIGURE 1: (a) DNA sequence of the mMT-I promoter region from the *Sst*I to *Bgl*II site. The potential Z region (italics) is located 102–86 bp upstream from the cap site (+1). Metal regulatory elements (underlined) and the TATAA box (lower case) are indicated. (b) Promoter plasmids. The wild-type mMT-I promoter *Sst*I/*Bgl*II fragment was cloned into *Sst*I/*Bam*HI-digested pUC12, yielding plasmid pMTp. A mutant promoter plasmid (pMTpZ) was generated by reversing bases −94 and −95 (see Materials and Methods).

wild-type mMT-I promoter and a mutant mMT-I promoter generated to improve its Z-DNA potential. Two-dimensional (2-D) chloroquine gel analysis (Peck & Wang, 1983; Haniford & Pulleyblank, 1983) and chemical mapping (Johnston & Rich, 1985; Herr, 1985) provided independent measures of structural changes in this region. These data, together with statistical mechanical modeling of the transition (Peck & Wang, 1983; Ellison et al., 1986), were used to quantitatively estimate the energetics of the B–Z transition. This analysis provides new information on the energetics of this transition and the possible involvement of Z-DNA in natural sequences.

Materials and Methods

Mutagenesis. The 218-bp *Sst*I–*Bgl*II fragment of mMT-I (Figure 1a) obtained from pMK (Brinster et al., 1982) was cloned into *Sst*I/*Bam*HI-digested bacteriophage M13mp10. A synthetic mutagenic primer 5′-d(CACGCATATGCACGCGCC)-3′ was synthesized and verified for hybridization specificity by its ability to act as a primer in sequencing the M13 recombinant. This primer and an M13 sequencing primer (BRL) were used to generate in one step an AC to CA dinucleotide mutation at positions −94 and −95 (Zoller & Smith, 1983; Messing, 1983). Mutants were identified by high stringency hybridization of the 18-base primer to phage DNA (Zoller & Smith, 1983) and were verified by dideoxy sequencing (Sanger et al., 1977).

Plasmids and Plasmid Topoisomers. The *Sst*I–*Pst*I fragments from wild-type and mutant mMT-I/mp10 replicative forms were cloned into *Sst*I/*Pst*I-digested pUC12, yielding plasmids pMTp and pMTpZ (Figure 1b). The promoter region of purified plasmids (Pulleyblank et al., 1983; Radloff et al., 1967) was verified by double-stranded, dideoxy sequencing using reverse transcriptase (Zagursky et al., 1985)

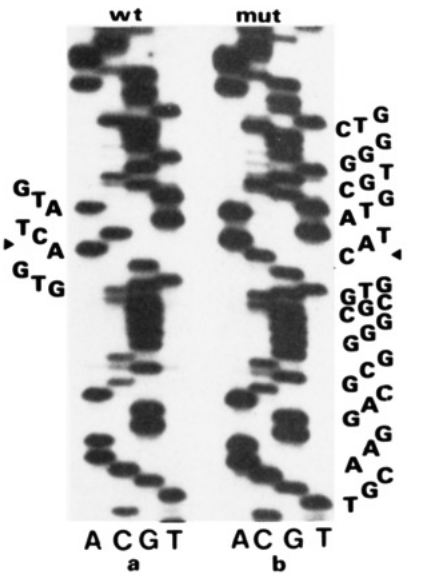

FIGURE 2: Autoradiogram of a sequencing gel showing the relevant promoter region of plasmids pMTp (a) and pMTpZ (b). The AC to CA mutation is indicated by arrows.

(Figure 2). Topoisomers differing in linking number were generated by topoisomerase I in the presence of 0–7 μM ethidium bromide (Peck & Wang, 1981).

Two-Dimensional Chloroquine Gel Electrophoresis. Agarose (1%) in TBE buffer (1.5 L, 90 mM Tris–borate–2.5 mM EDTA, pH 8.3) was poured into a 50 cm × 50 cm horizontal slab gel apparatus. DNA (2 μg) was loaded into wells formed at the top corner by using glass rods 0.5 cm in diameter and 12 cm apart. Gels were run at 150 V for 48–54 h at room temperature in the first dimension with buffer circulation, then soaked in 4.5 L of TBE buffer and 1.3 μM chloroquine for 16–24 h with circulation, rotated 90°, and run in the second dimension at 150 V for 36–40 h in TBE–chloroquine buffer. Buffer chambers were 27 ± 2 °C during electrophoresis. Gels were stained in 1 μg/mL ethidium bromide for 30 min, illuminated with ultraviolet light, and photographed.

"Mixed" gels obtained by running equal amounts of mutant and wild-type DNA loaded in the same well were also analyzed. These gels were stained, photographed, and transferred onto nitrocellulose (Southern, 1975). Blots were hybridized with the mutagenic primer at low and then high stringency to detect total and mutant DNA, respectively (Zoller & Smith, 1983).

ΔTw and $\alpha - \alpha_0°$ Measurements and Statistical Mechanical Modeling. Measurements of ΔTw (change in twist) and $\alpha - \alpha_0°$ (linking difference) and statistical mechanical modeling of the B–Z transition were done as described by Peck and Wang (1983) and Ellison et al. (1986). When a topoisomer did not fall directly on the apex ($\alpha - \alpha_0° = 0$) of the 2-D gel, values of $\alpha - \alpha_0°$ were based on symmetry about the apex. In Figure 3, for example, the topoisomers to the left and right of the apex were assigned $\alpha - \alpha_0° = -0.8$ and +0.2, respectively. Thus, $\alpha - \alpha_0°$ for topoisomers on either side of the discontinuity (arrow, Figure 3b) were −11.8 and −12.8. ΔTw for topoisomers near the discontinuity were calculated by determining their apparent $\alpha - \alpha_0°$ from a curve of $\alpha - \alpha_0°$ vs mobility. In Figure 3b, ΔTw for the topoisomer immediately to the right of the arrow was −3.5 since actual and apparent values of $\alpha - \alpha_0°$ were −12.8 and −9.3, respectively. This represents a retardation in mobility in the first dimension due to a loss of 3.5 negative supercoils.

DEP Sensitivity Assays. DEP sensitivity assays were done as described by Herr (1985) with the exception of the pi-

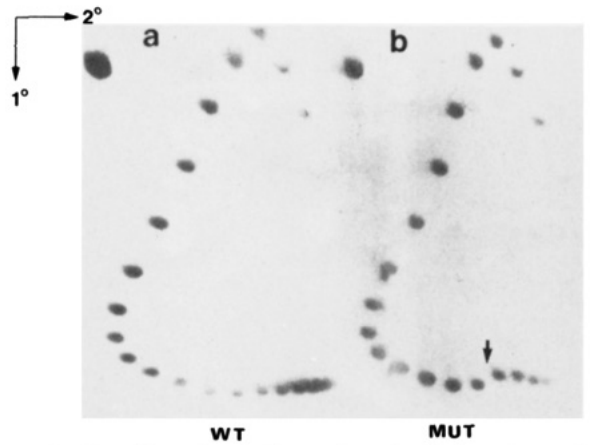

FIGURE 3: Two-dimensional chloroquine gel of (a) pMTp and (b) pMTpZ. Pooled topoisomers from ethidium bromide/topoisomerase I reactions were resolved as described under Materials and Methods. A typical ethidium bromide stained gel of (a) wild-type pMTp (WT) and (b) mutant pMTpZ (MUT) plasmids is shown. Arrows indicate the direction of the first and second dimensions for electrophoresis and, in (b), the discontinuity indicating the B–Z transition.

peridine cleavage reaction. On the basis of time course experiments (Figure 4b), piperidine cleavage reactions were typically carried out at 95 °C for 5 min to minimize background. For strand-specific labeling, plasmids were cut with *Eco*RI and end labeled with T4 kinase ("top" strand labeled) or Klenow fragment ("bottom" strand labeled) (Johnson & Rich, 1985; Herr, 1985). A second cut with *Hin*dIII liberated a 254-bp fragment that was isolated by polyacrylamide gel electrophoresis. G + A sequencing reactions were carried out as described by Maxam and Gilbert (1980).

Results and Discussion

Mutagenesis, Cloning, and Sequencing. The mMT-I promoter (Figure 1a) has two groups of metal regulatory elements responsible for heavy metal induction of transcription (Stuart et al., 1984). The sequence d(GCGCGTGACTATGCGTG) (−102 to −86) which falls between these two groups contains a central d(AC) which interrupts an otherwise fully alternating pur/pyr pattern. This region and flanking bases form potential Z-DNA sequences. We reversed the central d(AC) dinucleotide to d(CA) by site-directed mutagenesis, generating a mutant promoter with a greater expected propensity to flip to Z-DNA. Wild-type and mutant promoters were subcloned into a small plasmid to facilitate analysis of the B–Z transition (Figure 1b). The sequence of the promoter region of these plasmids verified that they differed only at the mutated dinucleotide (Figure 2).

Mutant and Wild-Type mMT-I Promoters Undergo Structural Transitions. Chloroquine gel analysis was used to examine the Z-forming ability of pMTp and pMTpZ (Figure 3). The discontinuity in electrophoretic pattern in pMTpZ (Figure 3b) represents a B–Z transition in the region around −102 to −86 since the mutant promoter differs from wild type only in this region. There was no obvious transition in the wild-type sequence although a slight reduction in mobility was apparent within the subpopulation of highly negatively supercoiled plasmids (Figure 3a). We cannot discount a substantial loss of twist in this subpopulation since these topoisomers were not resolvable in the first dimension.

DEP reacts preferentially with purines in the syn conformation (Herr, 1985) and has been used as a chemical probe to identify bases in Z-DNA. However, it is not known with certainty that DEP sensitivity reflects Z-conformation only. We used this chemical probe to map the structural alteration

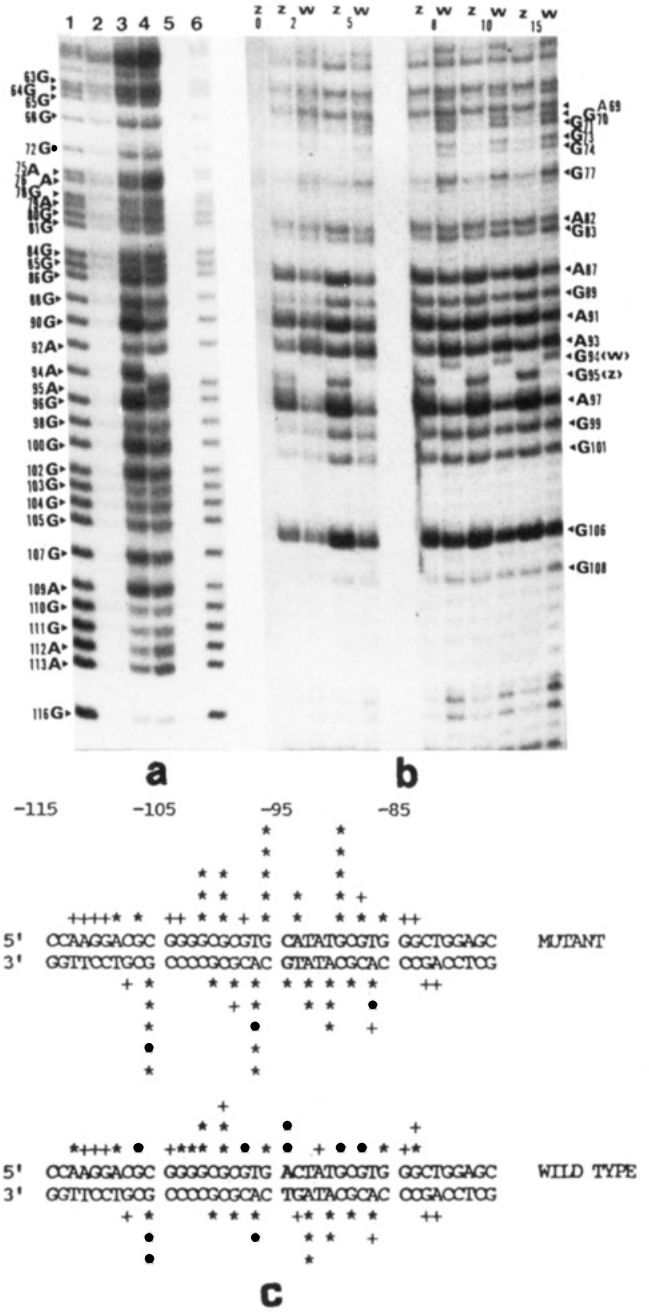

FIGURE 4: DEP sensitivities in wild-type pMTp (W) and mutated pMTpZ (Z) plasmids. Plasmids were treated as described under Materials and Methods. Equal counts were loaded in each lane of a 10% polyacrylamide sequencing gel with the exception of the G + A sequencing lanes. (a) Autoradiogram of the "top" strand (Figure 1): pMTpZ G + A (lane 1); pMTpZ relaxed closed circular plasmid (lane 2); pMTpZ supercoiled plasmid ($\alpha - \alpha_0°$) = −10 to −16 (lane 3); pMTp supercoiled plasmid ($\alpha - \alpha_0°$) = −10 to −16 (lane 4); pMTp relaxed closed circular plasmid (lane 5); pMTp G + A (lane 6). (b) Autoradiogram of the "bottom" strand (Figure 1). A time course for 1 M piperidine cleavage at 95 °C for 0, 2, 5, 8, 10, and 15 min for pMTpZ (Z) and pMTp (W) is shown. (c) Schematic of DEP sensitivity in the mutant and wild-type mMT-I promoters. Relative band intensities were determined by densitometric scanning of autoradiograms in (a) and (b). Increasing sensitivity is represented by +, *, *+, **, etc.

in pMTp and pMTpZ (Figure 4). Negatively supercoiled pMTpZ was clearly sensitive to DEP in the region defined by the alternating pur/pyr stretch. Interestingly, pMTp also displayed sensitivity with a similar but distinct pattern. Presumably, only highly supercoiled species of the wild-type plasmid are modified by DEP since chloroquine gels did not show evidence of a B–Z transition at moderate levels of su-

perhelicity (Figure 3a). This is supported by the fact that although equal amounts of radiolabeled pMTp and pMTpZ were loaded from plasmid samples of identical superhelical ranges ($\alpha - \alpha_0° = -10$ to -16), autoradiographic bands from pMTp were less intense than those from pMTpZ.

In both mutant and wild-type sequences, DEP reacted with purines in the flanking sequences beyond the central stretch of 17 alternating pur/pyr bases. In addition, pMTp had sensitive bases well beyond the central region that were not sensitive in pMTpZ (e.g., Figure 4b, G-70 and G-71). These sensitive regions were often purine rich and may represent altered DNA structures in the wild-type plasmid resulting from incomplete transition of the Z-DNA region.

DEP sensitivity delimits the region involved in the B–Z transition to the 32-bp sequence from −82 to −113 (Figure 4c). Substantial variation in sensitivity exists within this region, with the boundary purines being only marginally sensitive. Therefore, it was not possible to define an exact junction between the B and Z regions in these promoters. Herr (1985) observed that dG residues 5′ of dT were relatively insensitive to DEP compared to the hypersensitive dA residue opposite the 3′ dT. Our data support this observation: in all seven cases where d(GT/AC) is present within the sensitive region, dA is more reactive than dG.

Statistical Mechanical Modeling of the B–Z Transition. Free energy stored in a supercoiled closed circular plasmid is a function of the linking difference between supercoiled and relaxed states

$$\Delta G_\tau = K(\alpha - \alpha_0°)^2 \quad (1)$$

where $K = 1100RT/N$, R = gas constant, T = temperature, and N = number of base pairs in the plasmid ($N > 2000$) (Pulleyblank et al., 1975; Depew & Wang, 1975). This free energy drives supercoil formation and, if sufficiently large, structural changes such as local melting, cruciforms, and Z-DNA. Data from 2-D chloroquine gels of ΔTw versus $\alpha - \alpha_0°$ and statistical mechanical analysis provide information on the energetics of these transitions.

Peck and Wang (1983) modeled $d(CG)_n$ transitions in terms of the free energy required to form a B–Z junction (ΔG_j), the energy required to flip one dinucleotide unit ($2\Delta G_{bz}$), and the loss of twist at the B–Z junction (b). The partition function for the B–Z transition in a closed circular supercoiled plasmid is given by

$$Ps = \exp[-K(\alpha - \alpha_0°)^2/RT] + \sum_{i=1}^{n}(n - i + 1)\sigma s^i \exp[-K(\alpha - \alpha_0° - ai - 2b)^2/RT] \quad (2)$$

where $\sigma = \exp(-2\Delta G_j/RT)$, $s = \exp(-2\Delta G_{bz}/RT)$, n = number of dinucleotide pairs in Z-DNA, and $a = -2[1/(10.5 + 1/12)]$. Thus, the average change in twist is

$$\langle \Delta Tw \rangle = \{\sum_{i=1}^{n}(ai + 2b)(n - i + 1)\sigma s^i \times \exp[-K(\alpha - \alpha_0° - ai - 2b)^2/RT]\}/Ps \quad (3)$$

$$= \sum_{i=1}^{n}(ai + 2b)\beta/(\sigma^{-1} + \sum_{i=1}^{n}\beta) \quad (4)$$

where $\beta = (n - i + 1)s^i \exp[-K(ai + 2b)[ai + 2b - 2(\alpha - \alpha_0°)]/RT]$. Simultaneous nonlinear least-squares minimization of data from plasmids containing $d(CG)_n$ inserts yields values of $\Delta G_j = 5$ kcal mol^{-1} junction^{-1}, $\Delta G_{bz} = 0.33$ kcal mol^{-1} bp^{-1}, and $b = -0.4$ turn per junction (Peck & Wang, 1983).

To estimate mean energetic parameters of the heterogeneous Z-DNA sequence within the mutant mMT-I promoter, a similar analysis was applied to nine independent 2-D chloroquine gels of pMTpZ (Figure 5). Technical variations and uncertainty in the accuracy of ΔTw measurements contribute to scatter in the data. Maximal unwinding (ΔTw ≥5, Figure 5) and chemical sensitivity data (Figure 4) indicated that 22–32 bp were involved in the B–Z transition. Therefore, the analysis was carried out over the range n = 11–16 dinucleotides. For all values of n tested, the average energetic requirement for dinucleotides in this region to propagate through the B–Z transition was 0.80–0.82 kcal mol^{-1} bp^{-1} (Table I). The data did not permit an accurate determination of b and ΔG_j but suggest that the parameters for n = 13 or 14 best represent the transition that includes regions of nonalternating pur/pyr character at both junctions.

To estimate the confidence level for the B–Z propagation energy of this sequence, curves were generated by varying ΔG_{bz} while keeping n, b, and ΔG_j fixed at intermediate values. Approximately 70% of the data points were enveloped by curves generated with $\Delta G_{bz} = 0.7$ and 0.9 (Figure 5, dotted lines). This range of ΔG_{bz} was insensitive to changes in the fixed parameters and is more than twice that required to propagate d(CG) dinucleotides in Z-DNA (0.33 kcal mol^{-1} bp^{-1}).

The above analysis assumes a homogeneous population of transition intermediates and thus generates an "average" ΔG_{bz}

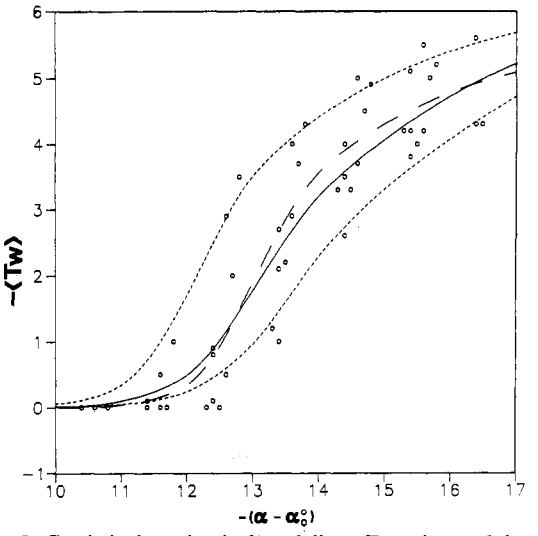

FIGURE 5: Statistical mechanical modeling. Experimental data (O) from nine independent 2-D chloroquine gels were subjected to nonlinear least-squares analysis as described under Materials and Methods. The best fit curve for n = 14 based on the values for b, ΔG_j, and ΔG_{bz} presented in Table I is shown (solid line). Theoretical curves for ΔG_{bz} = 0.7 (dotted, uppermost), ΔG_{bz} = 0.9 (dotted, lowermost), and the sequence d(GACGCGGGGCGCGTGCATATGCGTGGGC) generated with the Ellison model (Ellison et al., 1986) (long dashed line) are also shown. All 52 data points were included in each analysis. The estimated errors in $-(\alpha - \alpha_0°)$ and −ΔTw measurements were ±0.2 and ±0.3, respectively.

Table I: Simultaneous Least-Squares Minimization for b, ΔG_j, and ΔG_{bz}

no. of dinucleotides	b	ΔG_j (kcal mol^{-1} jn^{-1})	ΔG_{bz} (kcal mol^{-1} bp^{-1})	SS[a]
11	−0.99	6.4	0.81	40.5
12	−0.90	6.0	0.82	40.2
13	−0.80	5.6	0.82	40.1
14	−0.65	4.9	0.82	40.1
15	−0.38	3.7	0.80	40.2
16	−0.15	2.7	0.80	40.3

[a] Denotes sum of squares of differences between theoretical and observed values of ΔTw for the 52 data points in Figure 5. jn = junction.

for the heterogeneous sequence. Recent work defining ΔG_{bz} for 4 of the 20 possible dinucleotide pairs shows that these values vary significantly (Vologodskii & Frank-Kamenetskii, 1984; Ellison et al., 1985, 1986). Therefore, we attempted to analyze the transition in Figure 5 with a model developed by Ellison et al. (1986) for heterogeneous sequences, which takes into account estimated propagation energies for each dinucleotide. Using their parameters, we generated theoretical curves for all mutant sequences with n = 13–16 in the region defined by DEP sensitivity. Curves including the core sequence d(GACGCGGGGCGCGTGCATATGCGTGG) (n = 13) best represented the data (not shown). Expanding this sequence one full dinucleotide in either direction or in both the 5′ and 3′ directions yielded similar curves. The curve for this core sequence extended by GC at the 3′ end is presented in Figure 5 (long dashes). The average ΔG_{bz} for these sequences determined from theoretical contributions of each dinucleotide was 0.7–0.9 kcal mol^{-1} bp^{-1}, which agrees with the Peck and Wang minimization (Table I). These results suggest that the sequential dinucleotide context defined by the core sequence above forms the central Z region of pMTpZ. Although modeling this sequence fits the data well and provides support for the Ellison model, further work involving mutations in this region and their effects on the transition is required.

The wild-type sequence could not be modeled experimentally since its transition was not resolvable by chloroquine gels. Although there was DEP sensitivity in the Z-region, it was generally weaker, and there were also hypersensitive sites in relatively distant regions. However, theoretical predictions for the pMTp transition using the analytical model (Ellison et al., 1986) suggest that it should undergo a B–Z transition at superhelical densities roughly one linking number greater than pMTpZ (not shown). Our chloroquine gels do not allow us to resolve this question.

It is noteworthy that the energetics of the mutant transition (approximately 5 kcal mol^{-1} junction^{-1}, 0.8 kcal mol^{-1} bp^{-1}) could reasonably be met in vivo, and thus it is feasible that the wild-type sequence might also flip to Z-DNA or alter its conformation under physiological conditions. We are attempting to characterize further these transitions within the mMT-I promoter and examine the possible biological effects of the mutation on transcription in vivo.

Acknowledgments

We thank Carol Greider, Bruce Futcher, Lawrence Peck, and Michael Ellison for helpful discussion. We also thank Michael Ellison for the program TREZ2, which defines theoretical B–Z transitions in heterogeneous sequences, and Richard Palmiter for plasmid pMK.

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The 90-Kilodalton Peptide of the Heme-Regulated eIF-2α Kinase Has Sequence Similarity with the 90-Kilodalton Heat Shock Protein†

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Received July 21, 1987

ABSTRACT: Highly purified preparations of the heme-controlled eIF-2α (eukaryotic peptide initiation factor 2 α subunit) kinase of rabbit reticulocytes contain an abundant 90-kilodalton (kDa) peptide that is immunologically cross-reactive with spectrin and that modulates the activity of the enzyme [Kudlicki, W., Fullilove, S., Read, R., Kramer, G., & Hardesty, B. (1987) *J. Biol. Chem. 262*, 9695–9701]. The amino-terminal sequence of the 90-kDa protein has a high degree of similarity with the known amino-terminal sequences of the *Drosophila* 83-kDa heat shock protein (20 out of 22 residues) and with other related heat shock proteins. The amino acid sequence of a tryptic phosphopeptide isolated by high-performance liquid chromatography from the eIF-2α kinase associated 90-kDa protein after phosphorylation by casein kinase II is shown to be identical with a 14 amino acid segment of the known sequence of the *Drosophila* 83-kDa heat shock protein. Results of hydrodynamic studies indicate a highly elongated structure for the reticulocyte protein, characteristic of a structural protein. Additional structural similarities between the eukaryotic heat shock proteins, the reticulocyte eIF-2α kinase associated 90-kDa peptide, and spectrin are discussed.

Protein synthesis in rabbit reticulocytes is controlled by the availability of heme. In the absence of heme, a translational inhibitor, called the heme-controlled repressor (HCR)[1] (Gross & Rabinovitz, 1972), is activated in reticulocytes or their cell-free lysates. An enzyme system consisting of several constituent peptides has been shown to be responsible for the observed inhibition and possesses protein kinase activity toward the smallest or α subunit of eukaryotic peptide initiation factor 2, eIF-2 [reviewed in Ochoa (1983) and Hardesty et al. (1985)]. Highly purified preparations of the heme-controlled kinase contain a relatively abundant 90-kDa peptide as judged by SDS/PAGE. This peptide does not phosphorylate eIF-2α and does not inhibit the eIF-2-mediated binding of Met-tRNA$_f$ to 40S ribosomal subunits (Wallis et al., 1980). Rather, it appears to effectively increase the activity of the kinase that mediates these effects (Kudlicki et al., 1987).

Recently, a similarity between the 90-kDa peptide associated with eIF-2α kinase and the β subunit of spectrin, the most abundant component of the erythroid membrane skeleton (Bennett, 1985), was demonstrated (Kudlicki et al., 1985). The evidence for relatedness of the two proteins involves immunological cross-reactivity of antibodies from several distinct monoclonal hybridomas derived from different fusions and apparent similarities as substrates for two different protein kinases. Spectrin α or β subunits as well as the authentic 90-kDa peptide increase the enzymatic activity of the eIF-2α kinase, and some of the antibodies raised against spectrin affect the biological activity of the kinase. The association of the eIF-2α kinase with spectrin-related peptides has led to the development of a model in which this enzyme system essential for the modulation of protein synthesis interacts with (and possibly is regulated by) structural components of the cell (Hardesty et al., 1985).

Heme deprivation appears not to be the only stimulus responsible for the activation of HCR. It has been shown in intact reticulocytes as well as their cell-free lysates (Ernst et al., 1982), and more recently in HeLa cells (Duncan & Hershey, 1984; de Benedetti & Baglioni, 1986), that the inhibition of protein synthesis associated with the heat shock response correlates with the phosphorylation of eIF-2α. Furthermore, the kinase responsible for this phosphorylation appears to be sensitive to heme and in one case (de Benedetti & Baglioni, 1986) was shown to be inhibited by polyclonal anti-HCR antibodies. It seems likely that eIF-2α phosphorylation by an enzyme analogous to HCR has at least a contributory effect on translational inhibition subsequent to heat shock (Burdon, 1986).

† This project was supported by National Institutes of Health Grant CA 16608 (to B.H.) and by a grant from the National Health and Medical Research Council of Australia (to R.E.H.W.). D.W.R. is a recipient of a fellowship from the U.S. Public Health Service (Grant CA 09182).
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[1] Abbreviations: HCR, heme-controlled repressor; NBRF, National Biomedical Research Foundation; HPLC, high-performance liquid chromatography; TSTA, tumor-specific transplantation antigen; eIF-2, eukaryotic peptide initiation factor 2; eIF-2α, smallest subunit of eIF-2; SDS/PAGE, polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate; RP, reversed phase; kDa, kilodalton(s); Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid.

0006-2960/87/0426-6583$01.50/0