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ORIGINAL PAPER

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2-Chloro-2′-deoxyadenosine: alteration of DNA:TATA element binding protein (TBP) interactions

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Abstract 2-Chloro-2′-deoxyadenosine (CldAdo, cladribine) is used therapeutically for hairy cell and other leukemias. The nucleoside is converted in leukemia cells to the 2-Cl-analog of dATP and incorporated into DNA, where it may alter binding of transcription factors to gene regulatory AT-rich sequences. Here we model the effects of CldATP incorporation into the adenovirus major late promoter TATA element recognized by TATA binding protein (TBP). The modeling results are consistent with experimental studies of DNA:TBP binding and indicate which positions in the canonical TATA sequence are most severely affected by CldATP incorporation.

Keywords 2-Chloro-2′-deoxyadenosine · TATA binding protein · Adenovirus major late promoter · Transcriptional process · Hairy cell leukemia

Introduction

2-Chloro-2′-deoxyadenosine (CldAdo, cladribine) is used therapeutically for hairy cell leukemia and various myeloid malignancies, for immunosuppression, and in the treatment of multiple sclerosis [1, 2, 3, 4]. CldAdo is taken up by cells, converted to 2-chloro-2′-deoxyadenosine triphosphate (CldATP), and incorporated into DNA; it exerts its cytotoxic effects in part by down-regulating cellular ribonucleotide reductase and by inhibiting DNA synthesis [5, 6, 7, 8]. CldATP is also an effective substrate for human DNA polymerases and is incorporated into DNA in place of dATP. However, CldATP is not an absolute chain terminator for DNA polymerase-alpha [9, 10, 11]. Using a polymerase chain reaction (PCR)-based method, we reported that one CldATP is incorporated into DNA every 1,000 bases when cultured leukemia cells were incubated for four hours with therapeutic doses of CldAdo [11]. Therefore, it is likely that CldATP is inserted into gene regulatory regions, particularly AT-rich sequences such as the canonical TATA element.

We have shown, using gel shift assays, that substitution of 2-CldAMP for one or more of the dAMP residues in the TATA box region of DNA alters the affinity and stability of TATA binding protein (TBP), a subunit of transcription factor IID (TFIID) [12] for promoter-containing DNA. Initial binding is decreased 30–55%, depending on the site of substitution. Once the complex is formed, however, the protein is more difficult to dissociate from substituted DNA than from control sequences. Further, once bound to a CldAMP-substituted TATA element, the conformation of TBP is altered, as demonstrated by proteolytic digestion. Here we report a molecular modeling investigation of the effects of CldATP incorporation on the DNA:TBP complex. When CldATP is inserted into DNA, the 2-chloro-substituent extends into the minor groove (Fig. 1). In the DNA:TBP complex, the DNA is bent in a "U" shape, opening the minor groove. This exposed minor groove forms an extensive interface with specific hydrophobic and hydrogen bonding amino acid side chains of TBP, as described by Nikolov et al. (Fig. 2) [13]. We have examined the effect of CldAMP substitution at seven different dAMP positions along the TATA box element (Fig. 3) to determine which positions are most likely to affect the affinity of DNA for TBP and which positions are most likely to alter TBP conformation.

Materials and methods

Modeling was carried out using Quanta 98 [14] with the CHARMM force field (Version 22) [15]. All figures except 2–4 were created using Molecular Operating Environment, Version 2003.02 [16].

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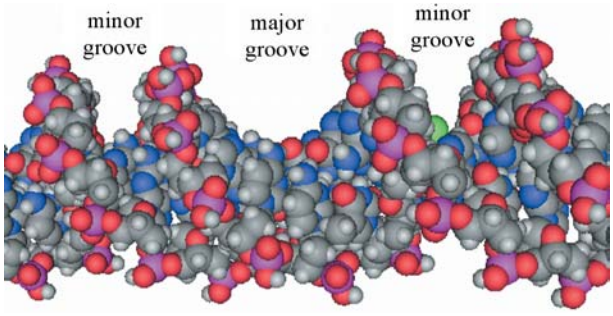

Fig. 1 A segment of double-stranded DNA with a chloroadenine residue modeled in place of an adenine. The DNA was modeled as a B-DNA helix, and the view is along the major and minor grooves. Note the chlorine atom (*green*) of the chloroadenine in the minor groove on the right

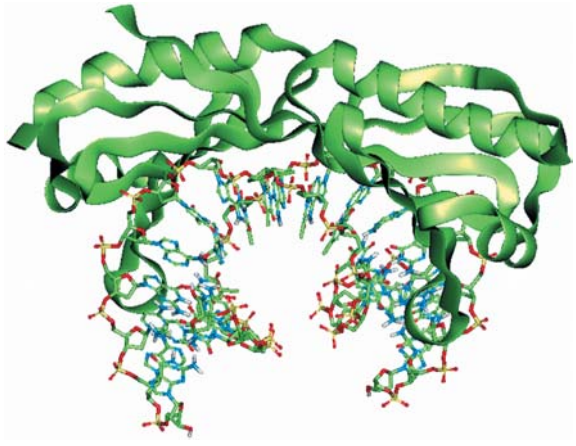

Fig. 2 Structure of the DNA:TBP complex, based on the X-ray crystal structure of Nikolov et al. [13]. The protein is shown as a ribbon structure, and the DNA as a stick model. Note that the DNA is bent into a horseshoe conformation, with the minor groove opened and exposed to the protein interface

DNA conformations were analyzed using the 3DNA program (Version 1.2) [17].

Our starting point for modeling studies was the X-ray crystallographic structure of a human TATA box-binding protein complexed with a TATA element, determined by Nikolov et al. to 1.9 Å resolution [13, 18]. Polar hydrogen atoms were added, and the crystal structure was minimized to convergence (1,200 steps). One position at a time, adenines within the canonical consensus sequence from positions 3 through 13 and its complementary region (adenines 6, 8, 9, 10, 11, 110, and 112, shown in Fig. 3),

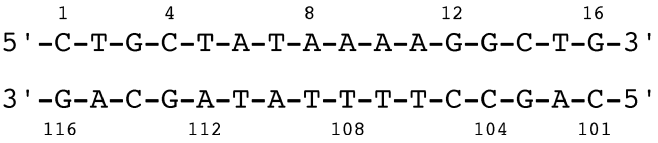

Fig. 3 Sequence and numbering of the DNA that was modeled

were converted to 2-chloroadenine, using a C–Cl bond length of 1.73 Å, and polar hydrogens were again added. Each of the listed structures was also minimized for 1,200 steps; this did not always lead to convergence of the minimization, since in some cases the chloro-substituent produced considerable steric interactions, but 1,200 steps were sufficient to show which structures were most severely affected. Obviously, simple minimization is not likely to produce the correct final structures of the chloro-substituted complexes, but it provides an excellent indication of the extent to which the chlorine atom will interfere with *normal* binding at different positions. The final CHARMM energies listed in Table 1 can only be considered as approximate indicators of relative stability, due to the limitations of the minimization methodology.

Each chloro-substituted structure was compared to the minimized native structure to determine the extent of conformational change in both the DNA and the protein. Modeled structures (each minimized for 1,200 steps) were superimposed using a rigid body fit algorithm. Root mean square deviations (RMSD) from the starting conformation to the final minimized conformation were determined for TBP and for DNA as measures of the extent of conformational change induced by chloro-substitution.

Results

Figure 4 is a schematic representation of the protein residues near the minor groove of the DNA. Table 1 summarizes basic information about the minimization results. The chlorine atom movement ranged from 1.2 Å (for $ClAMP_{11}$) to 3.0 Å (for $ClAMP_{10}$). RMSD for TBP ranged from 1.63 Å (for $ClAMP_9$) to 1.79 Å (for $ClAMP_8$); RMSD for the DNA ranged from 1.34 Å (for $ClAMP_9$) to 1.47 Å (for $ClAMP_8$). The starting crystal structure has 42 DNA base-base hydrogen bonds. Each individual substitution, regardless of site, disrupted 50% or more of the normal H-bonds upon minimization; in some cases, new (mispaired) H-bonds were formed. The table lists the number of DNA base-base hydrogen bonds remaining at the end of the minimization.

Table 1 Summary of effects induced by ClAMP substitution

ClAMP position	Cl atom movement (Å)	RMSD for TBP (Å)	RMSD for DNA (Å)	DNA base–base H-bonds remaining	Final CHARMM energy (kcal mol^{-1})
A6	1.3	1.75	1.39	20	−13212.5
A8	2.7	1.79	1.47	18	−13341.3
A9	1.6	1.63	1.34	23	−13180.4
A10	3.0	1.70	1.44	17	−13191.3
A11	1.2	1.76	1.44	16	−13310.2
A110	1.7	1.66	1.45	17	−13206.2
A112	2.3	1.70	1.36	16	−13054.3

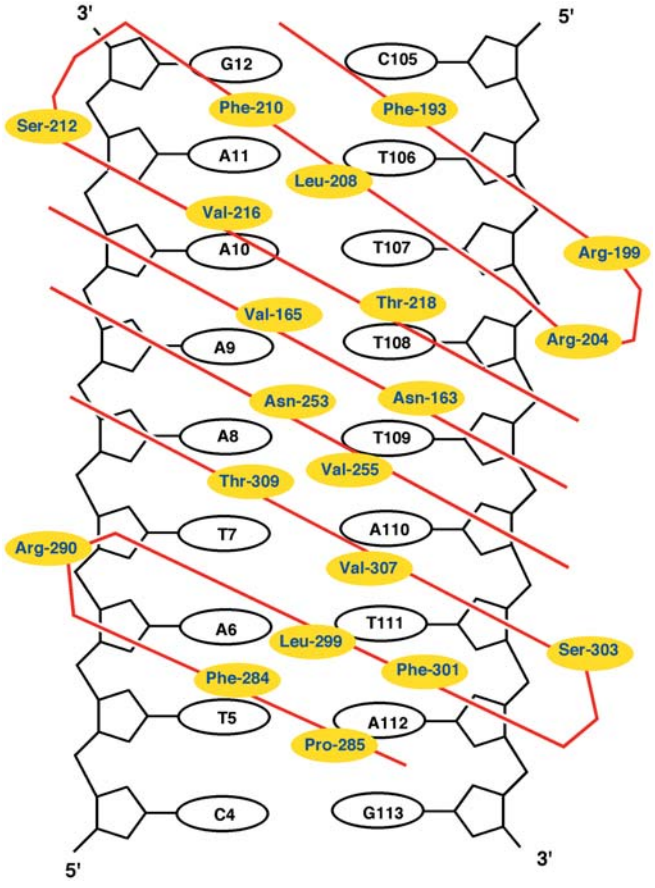

Fig. 4 Schematic representation of the protein residues of interest in the region of the minor groove of the DNA

Incorporation at A_6

The chlorine atom at this position is initially only 2.1 Å from a side-chain methyl group of Leu-299, presenting a serious steric overlap. On minimization (Fig. 5), the chlorine atom moves 1.3 Å and the leucine side chain rotates 120° around the C-beta/C-gamma bond. The chloroadenine and attached ribose move ~1.3 Å, and the phosphodiester bond in this region undergoes conformational changes, which move the adjacent protein residues Leu-283 by 2.9 Å and Ile-288 by 1.9 Å. These movements cause the neighboring Phe-284 and Phe-301 to move ~1.5 Å.

Incorporation at A_8

At this position, the chlorine atom is initially 2.4 Å from a methyl group of Val-255 and is almost close enough to hydrogen-bond to the side-chain NH of Asn-253. On minimization (Fig. 6), both the protein and DNA undergo the largest overall movements, as evidenced by the RMSDs. The chlorine atom moves 2.7 Å, alleviating the steric contact with Val-255 and forming favorable H-bonding/electrostatic interactions with the side chain of Asn-253. The protein backbone around Val-255 changes its conformation substantially; the backbone NH of Val-255 moves 2.6 Å in order to form a favorable H-bond/electrostatic interaction with the chlorine atom. These movements cause Val-165 to move 0.9 Å. The plane of the CldAMP ring rotates 25°, and the ribose shifts ~1.7 Å. Flanking DNA bases also rotate 15°–20°. On the opposing DNA strand, T-109 moves 0.7 Å and rotates 22° out of its original plane.

Incorporation at A_9

Initially the chlorine atom at this position has steric overlap with a methyl group of Val-165, and it is close to the side chain NH_2 groups of Asn-163 (2.8 Å) and Asn-253 (3.2 Å). The RMSD movements on minimization are the smallest observed for both the protein and the DNA. The chlorine moves only 1.6 Å on minimization (Fig. 7), alleviating the steric interaction with Val-165 and making a favorable interaction with the side chain NH_2 group of Asn-163. The CldAMP base moves less than 1 Å and rotates ~10°, as do the flanking DNA bases. The T-108 on the opposing strand remains in its original plane and shifts 1.3 Å. Asn-253 moves 1.1 Å in order to strengthen the

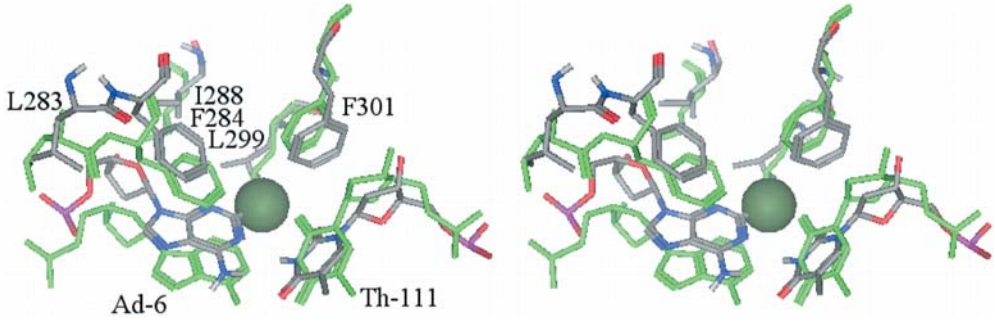

Fig. 5 Stereo view of CldAde-6 and neighboring residues which were affected by chlorine substitution. The chlorine atom is shown as a *dark green* sphere; other atoms are shown as sticks with the following color scheme: carbon=*gray*, nitrogen=*blue*, oxygen=*red*, phosphorus=*pink*, polar hydrogens=*white*; other hydrogens were omitted for clarity. Native structure is shown for comparison, as *green* stick models

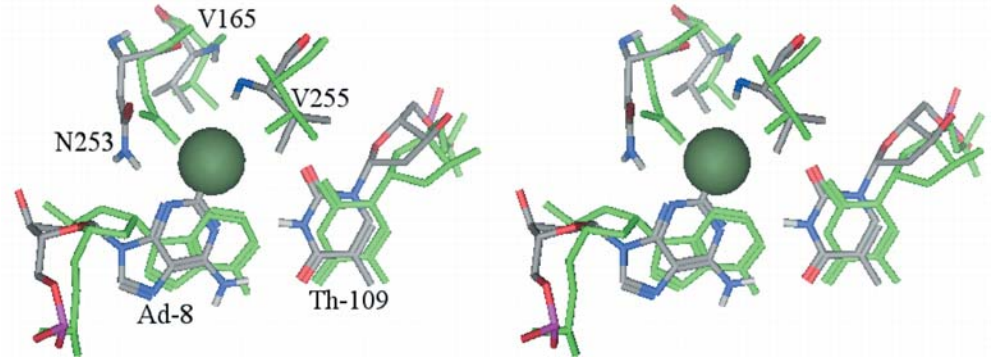

Fig. 6 Stereo view of CldAde-8 and neighboring residues which were affected by chlorine substitution. Scheme is as described for Fig. 4

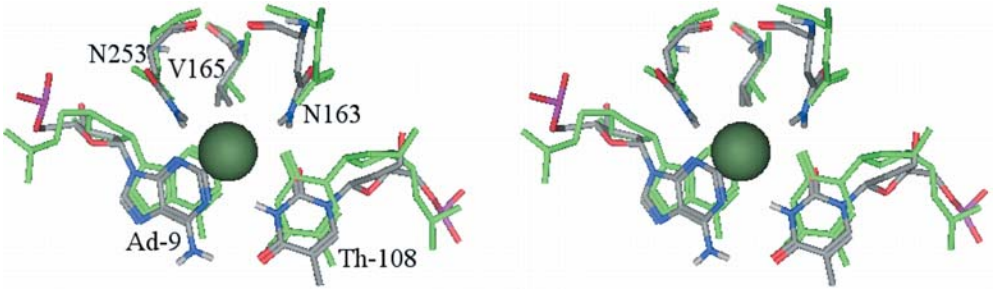

Fig. 7 Stereo view of CldAde-9 and neighboring residues which were affected by chlorine substitution. Scheme is as described for Fig. 4

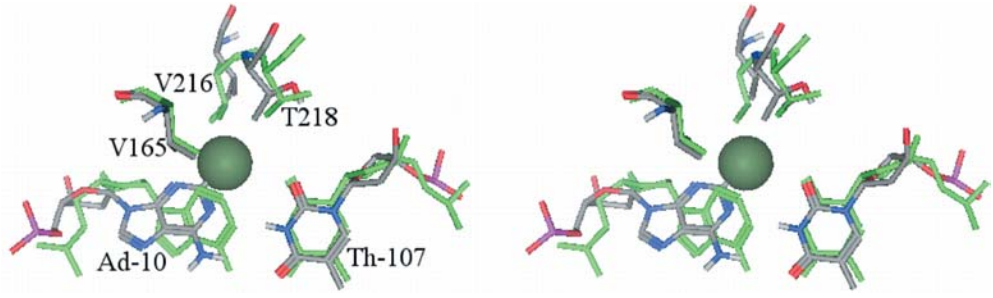

Fig. 8 Stereo view of CldAde-10 and neighboring residues which were affected by chlorine substitution. Scheme is as described for Fig. 4

interaction of Cl with the side chain NH_2 group of Asn-253.

Incorporation at A_{10}

The chlorine atom at this position is initially in steric conflict with Thr-218 (3.2 Å from a side chain methyl) and is in steric contact with methyl groups of Val-165 and Val-216. On minimization (Fig. 8), the CldAMP base undergoes extensive movement and the ring rotates ~40° out of its original plane, moving the chlorine atom 3.0 Å from its starting point (largest movement observed). This causes movement of several flanking bases on the DNA: A-9 moves 1.9 Å and rotates 30°; A-8 rotates 22°; A-7 shifts 0.6 Å; A-11 moves 0.6 Å and rotates 8°. There is also considerable local movement of the protein. Thr-218 moves 1.1 Å away from the chlorine atom to alleviate steric conflict; Val-165 moves 0.7 Å away; Val-216 moves 1.5 Å but maintains steric contact between the chlorine atom and the side chain methyl groups.

Incorporation at A_{11}

At this position, the chlorine atom is in steric conflict with a side chain methyl of Leu-208. On minimization (Fig. 9), the CldAMP base moves 0.6 Å and rotates out of its original plane 16°, resulting in a chlorine atom movement of 1.2 Å, the smallest chlorine movement observed. Despite the limited movement of this base, there is considerable movement of neighboring bases: G-12 moves 1.2 Å, G-13 moves 1.4 Å, C-14 moves 1.9 Å, and A-10 moves 0.6 Å, while T-106 moves 0.6 Å and T-107 moves 1.9 Å. Leu-208 moves 1.1 Å away from CldAMP to relieve steric strain; the neighboring Val-216 moves 1.5 Å, and Phe-193 moves 1.6 Å and rotates 30° out of its original plane.

Incorporation at A_{110}

Initially the chlorine atom at this position has no significant steric conflicts, although it lies close to Thr-

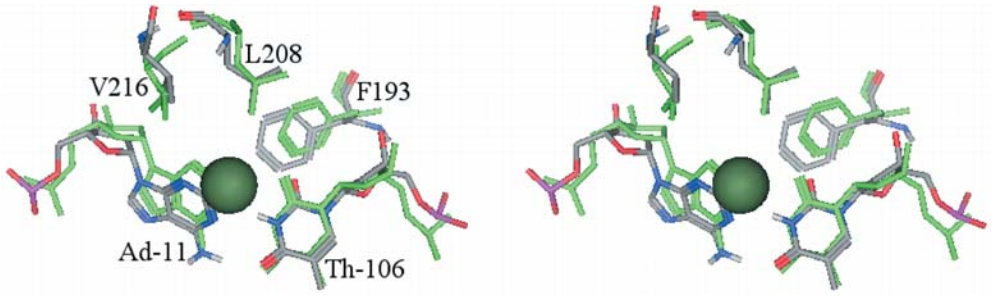

Fig. 9 Stereo view of CldAde-11 and neighboring residues which were affected by chlorine substitution. Scheme is as described for Fig. 4

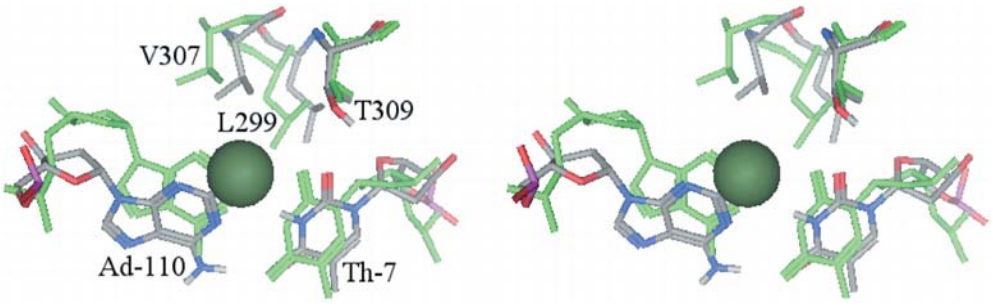

Fig. 10 Stereo view of CldAde-110 and neighboring residues which were affected by chlorine substitution. Scheme is as described for Fig. 4

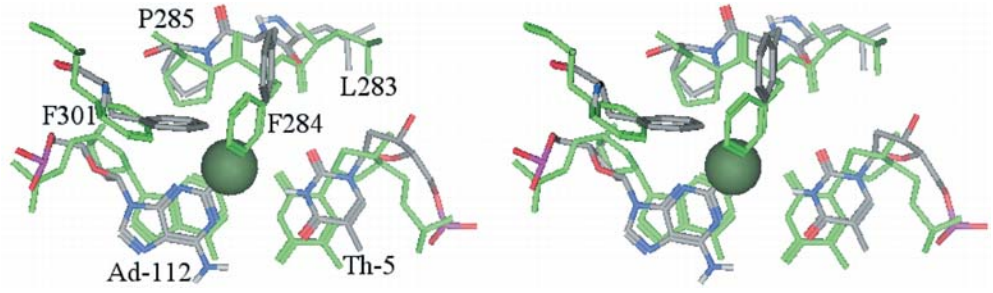

Fig. 11 Stereo view of CldAde-112 and neighboring residues which were affected by chlorine substitution. Scheme is as described for Fig. 4

309 and Val-307. However, the base stacking is quite tight in this region, and the chlorine atom appears to disrupt stacking. On minimization (Fig. 10), the chlorine atom moves 1.7 Å as the CldAMP residue shifts and rotates 27° out of its original plane. Flanking residues are strongly affected: T-109 rotates 21°, T-111 rotates 20°, A-112 moves 0.6 Å, and T-108 moves 0.6 Å and rotates 20°. On the opposite chain, T-7 and A-8 move 0.6 Å and 0.7 Å, respectively. Among the protein residues, Val-307 moves 1.4 Å, while Leu-299 moves 1.1 Å and the side chain rotates 35°.

Incorporation at A_{112}

When the chlorine atom is incorporated at A-112, there is steric conflict with the side chains of Phe-284 and Pro-285. Minimization (Fig. 11) causes the CldAMP ring to move 1.3 Å and to rotate 25° out of its original plane; the chlorine atom moves 2.3 Å. The flanking base T-111 rotates 16° out of its original plane, and G-113 moves 1.0 Å. On the opposing strand, T-5, A-6, C-4, and G-3 move 1.5 Å, 1.4 Å, 1.5 Å, and 1.2 Å, respectively. Phe-284 and Leu-283 each move 3.0 Å to alleviate steric repulsion, and these movements cause Phe-301 to move 2.7 Å.

Discussion

As described above, a 2-chloro-substitution on adenine in the TATA region may have many effects on binding. In some positions, considerable conformational change would be required to accommodate the chlorine atom. This could result in the decreased affinity initially observed by *in vitro* binding assays. In some positions, additional electrostatic or hydrogen bonding interactions may take place involving the chlorine atom and could account for the tighter affinity observed experimentally.

Comparison to mutation studies

It is also useful to compare our results to experimental studies in which dAMP residues of the consensus TATA sequence were systematically mutated to other bases by

Wobbe and Struhl. [19] They found that mutation of positions 8 and 110 had the greatest negative effect on transcription. In our studies, chlorine substitution at A-8 produced the largest RMSD for both the protein and the DNA; substitution at A-110 produced the second-largest RMSD for the DNA and one of the most serious disruptions of DNA base-base hydrogen bonding. In the mutation studies, mutation of positions 9, 10, and 11 were least detrimental to activity. In our studies, substitution at position 9 produced the smallest RMSD for both the DNA and the protein, and preserved the largest number of hydrogen bonds intact. Substitution at position 11 produced the smallest chlorine atom movement.

CldAdo exerts many effects following insertion into DNA. When it is incorporated into the TATA element, it alters the affinity of the DNA:TBP complex, and it modifies TBP conformation. Our modeling results are consistent with these experimental data; there are substantial conformational changes in both the DNA and the protein, and the final CHARMM energies are very different for different substitution positions. Our results suggest that the most severe conformational changes may occur on substitution at A-8, and the least severe at A-9; these results are consistent with experimental studies on mutation of the TATA sequence. We expect further studies employing molecular dynamics simulations to produce a clearer picture of the binding modes of CldAMP-substituted sequences.

Supplementary material

The minimized chloro-substituted structures are available in PDB format.

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