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Isolated Oligopurine Tracts Do Not Significantly Affect the Binding of DNA to Nucleosomes†

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ABSTRACT: Nucleosomal-length DNA was constructed to contain one of two 10 bp oligopurine·oligopyrimidine sequences, either $d(A_{10}·T_{10})$ or $d(G_{10}·C_{10})$. The 146 base pair (bp) sequences were then each tandemly cloned. This allowed for the production of circularly-permuted sequence variants in which the oligopurine tract was located at eight different positions. The permuted sequences were then assayed for their ability to reconstitute into nucleosomes by competitive reconstitution. The results of the assay indicate that the free energy of nucleosome formation differs only by several tenths of a kilocalorie per mole for an oligopurine tract at any position along the DNA, including the central dyad region.

Since the great majority of DNA in a eukaryotic cell is organized into nucleosomes, the DNA sequence requirements for forming such a complex are necessarily quite nonspecific. Past experiments have shown that the nucleosome does indeed have the ability to accommodate a wide variety of DNA sequences, including prokaryotic DNA (Bryan et al., 1979), the glucosylated DNA of bacteriophage T7 (McGhee & Felsenfeld, 1982), synthetic polymers such as poly[d(A-T)]·poly[d(A-T)] (Bryan et al., 1979), poly[d(G-C)]·poly[d(G-C)] (Simpson & Künzler, 1979), poly(dG·dC) (Jayasena & Behe, 1989a,b), and poly(dA·dT) (Puhl et al., 1991), and even an RNA-DNA copolymer (Jayasena & Behe, 1989a).

There do, however, seem to be exceptional sequences which appear to deviate in either a positive or a negative direction from bulk DNA in their abilities to form nucleosomes. On the positive side, Satchwell et al. (1986) showed that there was a preference for having AA and GC dinucleotides occur with a phasing of ~10 bp, offset from each other by ~5 bp, in nucleosomal DNAs that they cloned and sequenced. Shrader and Crothers (1989, 1990), using the "rules" established by Satchwell et al. (1986), then designed synthetic polymers of the general sequence $(A/T)_3N_2(G/C)_3N_2$ and showed that they formed nucleosomes very well, with a differential free energy ($\Delta\Delta G$) of nucleosome formation significantly more favorable than bulk DNA. On the negative side, polymers that

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are in the Z conformation have been shown to resist nucleosome formation (Nickol et al., 1982; Ausio et al., 1987).

In this paper, we attempt to determine if oligopurine tracts 10 base pairs in length can significantly affect the free energy of nucleosome binding. There are several reasons to suppose they might do so: (1) tracts of contiguous oligopurines are considerably overrepresented in eukaryotic, but not prokaryotic, DNA and are present in sufficient number to potentially act as structural elements (Behe, 1987); (2) although poly(dA·dT) can form nucleosomes as well as heterogeneous-sequence DNA (Puhl et al., 1991), it was reported earlier that $d(A_n \cdot T_n)$ tracts were found mainly at the ends of nucleosomal DNA, away from the central dyad region (Satchwell et al., 1986; Kunkel & Martinson, 1981), implying that isolated $d(A_n \cdot T_n)$ tracts bind in nucleosomes less strongly than other sequences. In this paper, we report, however, that the results of competitive nucleosome reconstitution experiments show that cloned $d(A_{10} \cdot T_{10})$ and $d(G_{10} \cdot C_{10})$ tracts bind almost equally well at many locations along the nucleosome. It therefore seems unlikely that such sequences exert a significant influence on the strength of nucleosome binding in vivo.

Experimental Procedures

Materials. Micrococcal nuclease and the oligonucleotides pdA_{10}, pdT_{10}, pdG_{10}, pdC_{10}, pd(CGGATCCG) (*Bam*HI linker), and pd(GGAATTCC) (*Eco*RI linker) were purchased from Pharmacia. T4 polynucleotide kinase, T4 polynucleotide ligase, calf intestine alkaline phosphatase (CIP), Klenow fragment, and Sequenase 2.0 sequencing kit were obtained from United States Biochemical. All restriction enzymes were purchased from New England Biolabs. [γ-^{32}P]ATP was from DuPont. Chicken blood was obtained in Alsever's solution from Lampire Biologicals and generally arrived in the laboratory within 4 h of bleeding.

Construction of Nucleosomal-Length DNA. An 8 bp *Bam*HI linker was cut with its cognate restriction enzyme, and a 10-fold excess of the oligonucleotide was ligated with pUC18 that had also been cut with the enzyme and dephosphorylated. This results in the production of some plasmids with linkers on both ends which then blunt-end-ligate to yield a closed circular plasmid. The ligation mixture was used to transform *Escherichia coli* JM109 cells, and transformants were screened for the presence of the *Bam*HI linker by restriction with *Pst*I and *Eco*RI, followed by electrophoresis in 8% acrylamide. The *Pst*I/*Eco*RI restriction fragment of this plasmid has a length 8 bp greater than that for pUC18 itself. The plasmid was then cut with *Hin*cII, and the oligonucleotides $pd(A_{10} \cdot T_{10})$ and $pd(G_{10} \cdot C_{10})$ were separately ligated into the blunt-ended site. The ligation mixture was again used to transform JM109 cells. Transformants were assayed for the presence of a 10 bp insert by restriction with *Pst*I/*Eco*RI, followed by electrophoresis.

The recombinant plasmids were separately digested with *Fnu*4HI, and the fragment containing the $d(A_{10} \cdot T_{10})$ or $d(G_{10} \cdot C_{10})$ insert ($\sim$320 bp), or the same region but without an oligopurine insert, was isolated by electrophoresis through 6% polyacrylamide, elution into 3 M ammonium acetate, and ethanol precipitation. The sticky ends of the fragments were filled in using Klenow fragment, and an 8 bp *Eco*RI linker was then ligated to the ends. *Eco*RI digestion of this DNA produced two different length fragments, 146 and 180 bp (136 and 180 bp for no oligopurine insert). The 146 bp fragment, containing the oligopurine insert, and the 136 bp fragment were isolated by electrophoresis as described above, purified, and briefly self-ligated. The mixture was subsequently ligated into the *Eco*RI site of another pUC18 plasmid and transformed into JM109 cells. Cell lysates were screened by restriction analysis for the presence of plasmids containing two tandem inserts. Finally, the sequences of the plasmid constructs were confirmed by dideoxy sequencing using a Sequenase 2.0 kit according to the manufacturer's instructions.

Circularly-permuted, nucleosomal-length DNAs were produced by digesting the plasmids with eight restrictions enzymes: *Pst*I, *Sph*I, *Hin*dIII, *Eae*I, *Bst*NI, *Mse*I, *Sac*I, and *Xba*I (Figure 1). The 146 bp fragments (136 bp for no-insert DNA) were then isolated by electrophoresis through 10% polyacrylamide, elution into 3 M ammonium acetate, and ethanol precipitation. The DNAs were subsequently dephosphorylated with CIP and labeled with $^{32}PO_4$ using T4 polynucleotide kinase.

Bulk Nucleosome Monomers. Nucleosome monomers were prepared from chicken chromatin by limited digestion of erythrocyte nuclei with micrococcal nuclease (Simpson & Whitlock, 1976). The monomers were purified by ultracentrifugation at 20000 rpm in a Beckman SW-27 rotor at 4 °C for 40 h on a 5–20% linear sucrose gradient containing 10 mM NaCl, 10 mM Tris·HCl, pH 8.0, and 0.1 mM EDTA.

Competitive Nucleosome Reconstitution Assay. In order to quantitatively evaluate the ability of each permuted fragment to form nucleosomes, a competitive reconstitution assay was used (Shrader & Crothers, 1989, 1990; Jayasena & Behe, 1989b). Briefly, 0.5 μg of radioactively-labeled permuted fragment was incubated with 18 μg of nucleosome monomers and 9 μg of bulk unlabeled DNA (1:1 mole ratio of monomers of free bulk DNA) in 20 μL of 800 mM NaCl, 5 mM Tris·HCl, pH 8.0, 0.1 and mM EDTA for 20 min at 37 °C. The mixture was slowly diluted to 80 mM NaCl by the addition of aliquots of 5 mM Tris·HCl, pH 8.0, and 0.1% Nonidet P-40 once every 20 min. The nucleosome-bound DNA was separated from unbound DNA by electrophoresis at 10 mA through 6% polyacrylamide. The gel was autoradiographed, the free and nucleosomal DNA bands were excised, and the radioactivity in the bands was quantitated by liquid scintillation. The differential free energy of nucleosome formation was then calculated as follows

$$\Delta\Delta G = -RT \ln (K_p/K_s)$$

where $\Delta\Delta G$ is the differential free energy of reconstitution, K_p is the ratio of counts in the nucleosome band to counts in the free DNA band for a permuted DNA, and K_s is the same ratio for the standard DNA (heterogeneous chicken DNA).

Hydroxyl Radical Cleavage. For hydroxyl radical cleavage reactions, permuted fragments, labeled at their 5′ ends, were exchanged onto chicken nucleosomes. The exchange procedure was similar to that described above for competitive reconstitution, but no free chicken DNA was added. This results in virtually all of the labeled DNA being incorporated into nucleosomes. The hydroxyl radical cleavage reaction was then performed as described (Tullius & Dombroski, 1986).

Following the cleavage reaction, labeled material was electrophoresed, and the band corresponding to intact nucleosomes was excised and eluted. This step ensures that only DNA from intact nucleosomes, uncontaminated by free or dissociated DNA, is processed further. The eluted material was then extracted 3 times with phenol and chloroform, precipitated with ethanol, dissolved in 97% formamide, and electrophoresed through 8% polyacrylamide/7 M urea. The gel was then autoradiographed and the film scanned with an LKB XL Ultrascan densitometer.

Results

Construction of Circularly-Permuted Fragments. In order to synthesize nucleosomal-length DNAs containing a 10 bp

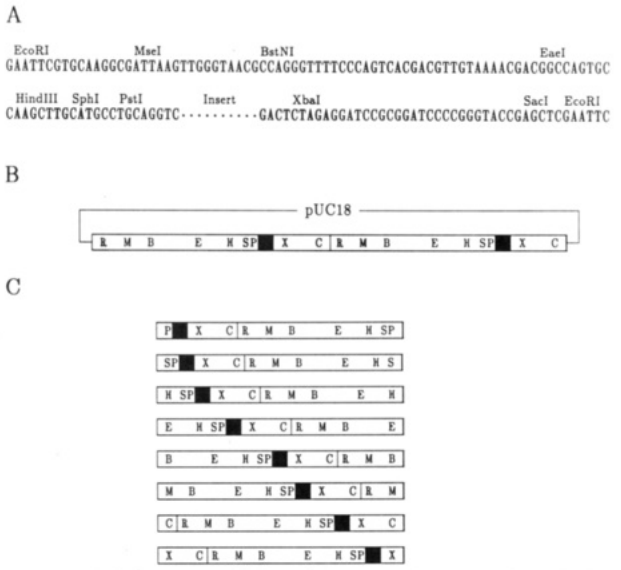

FIGURE 1: (A) Sequence of DNA fragment prior to tandem cloning. The fragment was subsequently cut with *Eco*RI and tandemly cloned into pUC18. The region marked "insert" contains either $d(A_{10}\cdot T_{10})$ or $d(G_{10}\cdot C_{10})$. Restriction sites are listed above the sequence. (B) Schematic drawing of plasmid containing tandemly cloned copies of the sequence listed in (A). Restriction sites are abbreviated as follows: R, *Eco*RI; M, *Mse*I; B, *Bst*NI; E, *Eae*I; H, *Hin*dIII; S, *Sph*I; P, *Pst*I; X, *Xba*I; C, *Sac*I. The oligopurine insert is shown as a black box. (C) Permuted nucleosomal-length fragments produced by digesting the plasmid shown in (B) with various restriction enzymes. The locations of the restriction sites insert are designated by one-letter abbreviations; the location of the oligopurinone insert is shown as a black box. The fragments are designated in the text by a letter, either A (oligoadenosine), G (oligoguanosine), or N (no-insert), and a subscript showing the restriction enzyme used to produce them.

homopurine tract at different positions along the length of the DNA, we have tandemly cloned 146 bp (and 136 bp) DNA fragments into pUC18. Figure 1A shows the sequence of the DNA fragment before tandem cloning. The locations of the restriction sites are indicated above the sequence. Six of the restriction enzymes used to generate the permuted fragments used here leave four-base-overhanging ends. Thus, although it is 146 bp between the centers of identical restriction sites in the tandemly cloned fragment, when cut the resulting DNA is a 142 bp double-strand fragment with a four-base single-strand overhang on both ends. *Mse*I leaves a two-base overhang, and *Bst*NI leaves a one-base overhang. The fragments contain either a $d(A_{10}\cdot T_{10})$ or a $d(G_{10}\cdot C_{10})$ insert or no insert at the *Hinc*II restriction site. A plasmid containing the tandemly cloned fragments is represented in Figure 1B. The location of the oligopurine inserts is marked, along with the locations of relevant restriction sites. Figure 1C shows the circularly-permuted fragments generated by cutting the tandem clone with one of the eight restriction enzymes used here. The location of the oligopurine insert varies, from the ends of permuted fragments to the middle. Although the protein component of a nucleosome has 2-fold symmetry, the DNA component usually does not. That is the case here; therefore, the occurrence of the insert near one end of the fragment is not necessarily energetically equivalent to its occurrence at the other end.

Sequence analysis of the permuted fragments constructed here shows that the DNA is not featureless. Figure 2 presents the base composition of a five-base window sliding along the DNA fragment. There are five AT-rich peaks that each contain three or more contiguous AT base pairs, occurring within a 70 bp region of the fragments. Four of the peaks, numbered 2–5, are phased to occur at integral multiples of

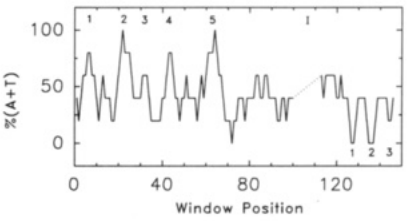

FIGURE 2: Base composition of a sliding, 5 bp window of a *Sac*I fragment. The region of the oligopurine insert in shown as a dotted line marked "I". AT-rich and GC-rich peaks are numbered. Similar figures for other permuted fragments can be generated simply by permuting this figure.

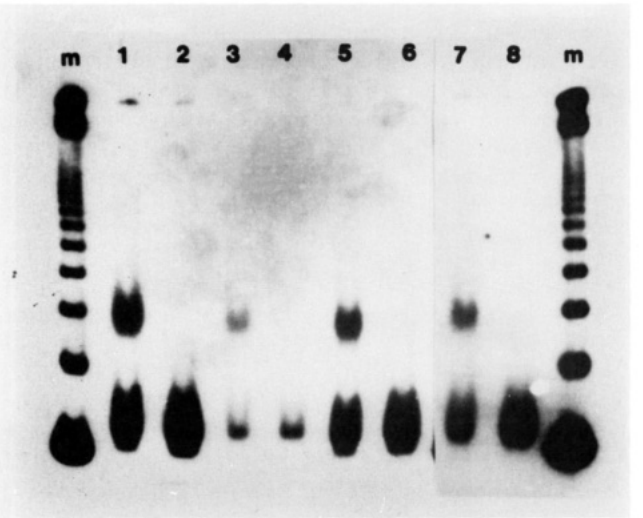

FIGURE 3: Electrophoresis of competitively-reconstituted no-insert DNA. Lanes 1, 3, 5, and 7 show reconstituted mixtures of N_{BstNI}, N_{EaeI}, N_{XbaI}, and N_{MseI}. Lanes 2, 4, 6, and 8 are the corresponding unreconstituted fragments. The lanes marked "m" contain 123 bp ladder as a size standard.

10 bp from each other; this region is from the parent plasmid. It has been shown that such phased, AT-rich sequences promote nucleosome formation, probably due to sequence-dependent, anisotropic DNA flexibility (Satchwell et al., 1986; Shrader & Crothers, 1989, 1990). The other AT-rich peak, number 1, has its center about 5 bp out of phase with the other four. This peak, however, which is due to an *Eco*RI restriction site, was actually introduced into the plasmid sequence by the cloning methods we used. Additionally, the region between the *Xba*I and *Sac*I restriction sites is rather GC-rich, and three GC peaks, phased by ~10 bp from each other, can be seen. However, these peaks arise from manipulation of the parent plasmid: from the polylinker region and from the *Bam*HI linker we inserted.

Competitive Nucleosome Reconstitution. The results from the electrophoresis of a typical competitive nucleosome reconstitution experiment are shown in Figure 3. Here permuted fractions of the no-insert DNA compete with chicken DNA for incorporation into nucleosomes, and the labeled DNA distributes itself between the bound and free DNA bands. After excision of the bands and quantitation of radioactivity, the $\Delta\Delta G$ of the fragment, relative to chicken DNA, is calculated according to eq 1. Each fragment was reconstituted 5 times. The reproducibility of the reconstitution is quite good, with standard deviations usually of ~0.05 kcal/mol (Figure 4). This is comparable to the experimental error experienced by other workers for this type of experiment (Shrader & Crothers, 1989, 1990).

Figure 4 shows $\Delta\Delta G$ for $d(A_{10}\cdot T_{10})$ (top), $d(G_{10}\cdot C_{10})$ (middle), and no-insert (bottom) fragments relative to chicken

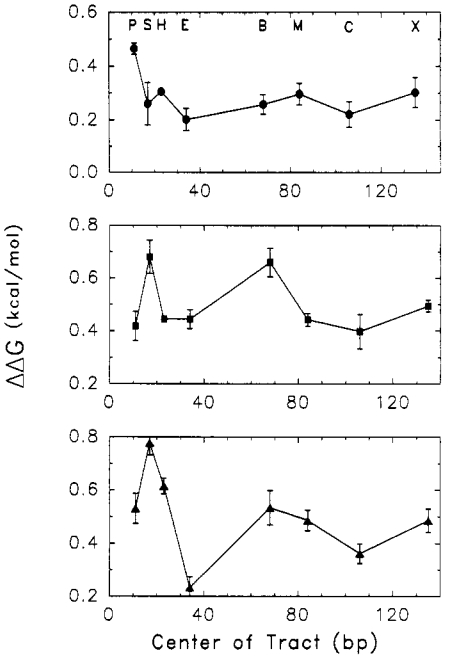

FIGURE 4: Differential free energy, $\Delta\Delta G$, of reconstitution vs the distance of the center of the oligopurine tract from the end of the DNA. Also shown for each point is the standard deviation for each set of measurements. The restriction enzymes used to generate the fragments are listed at the top of the figure. (Top panel) $d(A_{10}\cdot T_{10})$ fragments; (middle panel) $d(G_{10}\cdot C_{10})$ fragments; (bottom panel) no-insert fragments.

DNA as a function of the position of the oligopurine tract along the permuted fragment, along with the standard deviation for the set of five independent measurements represented by each point. (All $\Delta\Delta G$ values seen here for the homogeneous, prokaryotic, and plasmid-derived DNAs are somewhat more positive than those for the heterogeneous-sequence, eukaryotic DNA. This does not affect the interpretation of our results, however, since all permuted DNAs are compared to the same standard). Distance is measured from the middle of the oligopurine insert, through the *Pst*I site, to the end of the permuted fragment. (A four-base overhang at the end is counted as 2 base pairs.) The same distance is assigned to the analogous permuted fragments of the no-insert DNA, even though they do not contain an oligopurine tract. This is done to facilitate comparison in Figure 4.

The value of $\Delta\Delta G$ is rather stable for permuted fragments containing $d(A_{10}\cdot T_{10})$ (top); most have values of ~0.2–0.3 kcal/mol. Only the *Pst*I fragment, in which the $d(A_{10}\cdot T_{10})$ tract is located close to one end, is noticeably different, with a $\Delta\Delta G$ of 0.45 kcal/mol. The value of $\Delta\Delta G$ is also quite constant for permuted fragments containing $d(G_{10}\cdot C_{10})$ (middle); in this case, most values are between ~0.4 and 0.5 kcal/mol. Two permuted fragments containing $d(G_{10}\cdot C_{10})$ do not fall within this range: the fragments formed by restriction with *Sph*I and *Bst*NI. In both cases, the efficiency of nucleosome binding is decreased, with values of $\Delta\Delta G$ of ~0.6–0.7 kcal/mol.

The no-insert permuted fragments, which are only 136 bp in length, have the widest range of $\Delta\Delta G$ values, but the variation is restricted to two fragments. The six fragments generated by restriction with *Pst*I, *Hind*III, *Bst*NI, *Mse*I, *Sac*I, and *Xba*I all have values of $\Delta\Delta G$ in the range ~0.4–0.6 kcal/mol, which is the same range in which most fragments containing $d(G_{10}\cdot C_{10})$ occur. However, the fragments generated by restriction with *Sph*I and *Eae*I fall outside of this range. These two fragments differ from each other in binding energy by ~0.6 kcal/mol, even though their sequences differ only by the permutation of 17 bp.

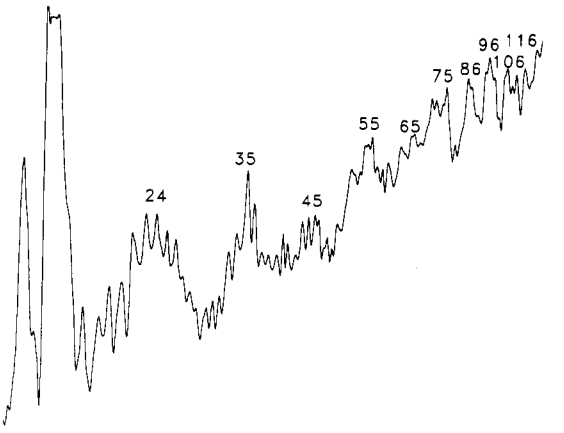

FIGURE 5: Densitometer trace of an autoradiograph of $A_{HindIII}$ that was cut with hydroxyl radical while reconstituted and then electrophoresed.

Hydroxyl Radical Cleavage Studies. Hydroxyl radical cleavage studies were performed in order to determine the rotational phasing of the permuted fragments when incorporated into a nucleosome. Figure 5 shows a typical densitometer trace of an autoradiogram from such an experiment. The lengths of fragments were determined by running in a neighboring lane the same fragment which had been partially cleaved at guanosine sites by the Maxam–Gilbert sequencing reaction.

Peak cutting sites for each of the permuted fragments are shown in Table I. Several points can be made. (1) For fragments produced by restricting with a given enzyme, the peaks, and thus the rotational phasing, are very similar across the length of the DNA regardless of the sequence of the insert. (2) All fragments with four-base overhanging ends have similar rotational phases. Fragments produced by cutting with *Bst*NI or *Mse*I, which leave one- and two-base overhanging ends, respectively, have somewhat shifted rotational phases.

Discussion

Variations in $\Delta\Delta G$ with Permutation of the DNA Sequence. The 136 bp DNA fragment, which lacks an oligopurine insert, shows the most variation in binding affinity for the N_{SphI}, $N_{HindIII}$, and N_{EaeI} permutations (Figure 4, bottom). In these fragments, AT-rich peak 1 (Figure 2), which is out of phase by 5 bp with the other four AT-rich peaks, is 50, 56, and 67 bp, respectively, from an end of the fragment. Thus, the out-of-phase, AT-rich peak is coming near to the dyad axis. It has been shown that nucleosomal DNA near the dyad undergoes several sharp turns (Richmond et al., 1984) so that requirements for bending in this region differ from the rest of the nucleosome. It seems likely that the fluctuation in $\Delta\Delta G$ in this region results from the AT-rich peaks, four in-phase and one out-of-phase, being accommodated more or less well to the dyad region.

The fragments containing oligoguanosine tracts show behavior that is somewhat analogous to the no-insert DNA (Figure 4, middle) in that the G_{SphI} permutation also forms nucleosomes that are 0.2–0.3 kcal/mol less stable than the majority of permuted fragments. In this fragment, AT-rich peak 1 is 60 bp from an end, so again, as with the analogous no-insert fragment, the out-of-phase, AT-rich peak is coming near to the dyad axis. The other oligoguanosine permutation whose $\Delta\Delta G$ value is measurably different from the remaining fragments is G_{BstNI}. In this permutation, the oligoguanosine

Table I: Hydroxyl Radical Peak Cutting Sites for Reconstituted, Permuted Fragments[a]

*Bst*NI			*Eae*I			*Hind*III			*Mse*I		
A	G	N	A	G	N	A	G	N	A	G	N
25	28	29	23	24	25	24	24	25	25	22	22
35	38	39	34	35	34	35	34	35	33	34	33
46	48	48	44	45	45	45	43	45	44	44	43
57	59	59	55	55	55	55	54	55	54	54	54
69	69	68	65	65	65	65	64	65	64	64	64
79	79	78	75	75	76	75	75	75	74	74	74
91	89	89	86	85	85	86	86	85	84	84	85
101	100	99	96	95	96	96	96	96	94	94	95
112	110	109	106	106	106	106	106	106	104	104	106
122			117	116	116	116	117	115	115	114	117
			127	126		124	126		123	126	126
*Pst*I			*Sac*I			*Sph*I			*Xba*I		
A	G	N	A	G	N	A	G	N	A	G	N
23	24	25	28	24	25	25	26	25	24		25
35	35	35	37	35	35	35	36	35	33	34	33
45	44	45	45	46	45	45	46	46	44	45	44
56	55	56	56	56	56	56	56	56	54	55	55
67	66	66	66	66	66	66	66	66	64	65	65
76	76	76	76	76	76	77	76	76	75	75	76
86	87	86	86	87	86	87	87	86	86	86	86
97	98	97	97	97	97	97	97	97	96	96	96
107	108	108	106	107	107	107	107	107	106	106	107
119	118	115	115	118	118	117	116		117	116	115
									127		

[a] The enzyme used to generate the permuted fragments is listed above a set of three columns. In each set, the column marked "A" shows data for fragments containing oligoadenosine, the column marked "B" shows data for fragments containing oligoguanosine, and the column marked "N" shows data for no-insert fragments.

tract itself is located very near the dyad axis (center at 69 bp from an end) and may be accommodated there only with some slight difficulty.

The permutations containing oligoadenosine tracts actually show the most regularity in forming nucleosomes (Figure 4, top). All fragments except A_{PstI}, which is several tenths of a kilocalorie per mole higher in energy and lies very close to the values for the analogous no-insert and oligoguanosine permutations, lie within ~0.1 kcal/mol of each other.

It is interesting to note that, while most of the fragments which contain oligoguanosine tracts and most of the no-insert fragments lie in the same range of binding energies, with $\Delta\Delta G$ values of ~0.4–0.6 kcal/mol, most fragments containing oligoadenosine tracts consistently bind several tenths of a kilocalorie per mole more favorably, with $\Delta\Delta G$ values of ~0.2–0.3 kcal/mol. Although this value is small, it apparently reflects the ability of the oligoadenosine tract to be more easily accommodated in a nucleosome than either an oligoguanosine tract or the plasmid DNA. This may be due to the structure of the oligoadenosine tract. Hayes et al. (1990) have shown that the helical repeat is not uniform over the length of nucleosomal DNA. Flanking DNA has a repeat of 10.04 bp/turn while the central 32 bp has a periodicity of 10.68 bp/turn. Most free DNA has a helical repeat of ~10.5 bp/turn (Wang, 1979). Since oligoadenosine tracts have a helical repeat of ~10.0 bp/turn (Peck & Wang, 1981; Rhodes & Klug, 1981), essentially identical to that found in nucleosomal flanking sequences, such tracts would have to undergo less torsional stress to be accommodated in the flanking sequences of the nucleosome.

Oligopurine Tracts and Nucleosome Structures. The synthetic homopurines poly(dA)·poly(dT) and poly(dG)·poly(dC) were first reported to resist nucleosome formation over a decade ago (Simpson & Künzler, 1979; Rhodes, 1979). The reason for their refractory behavior was speculated to be the altered conformations seen in the polymers. Several years ago, our laboratory examined a large number of sequences in the GenBank database for the occurrence of tracts of oligopurine·oligopyrimidines, and we showed that, while prokaryotic sequences contained approximately the number of tracts predicted from statistical considerations, eukaryotic sequences were strongly biased in favor of long tracts of oligopurine·oligopyrimidines (Behe, 1987; Beasty & Behe, 1988). These results led us to think it was plausible that the sequence of eukaryotic DNA was biased in this manner in order to modulate interactions with nucleosomes, and we set out to test the possibility.

In the course of examining the hypothesis, however, we demonstrated that the homopurines poly(dG)·poly(dC) and poly(dA)·poly(dT) could indeed form nucleosomes and that the differential free energy of nucleosome formation using the homopolymers was essentially equivalent to that using heterogeneous sequence chicken DNA (Jayasena & Behe, 1989a,b; Puhl et al., 1991). Additionally, we showed that the alternating polypurine poly[d(A-G)]·poly[d(T-C)] could also form nucleosomes (Jayasena & Behe, 1989a). However, a possibility that was not addressed by our previous experiments was that both favorable and unfavorable interactions occurred with homopurine over the length of the nucleosome and that such interactions balance out. To examine this possibility, it was necessary to create a set of nucleosomal-length DNA fragments containing oligopurine tracts in different relative positions and to examine the energy of nucleosome binding as a function of the position of the tract.

Oligoadenosine tracts in particular have special structural characteristics which could affect their ability to bind along the length of a nucleosome. Tracts of as few as three adenosines contain a bifurcated hydrogen bond not seen in normal B-form DNA (Nelson et al., 1987), the helical repeat of adenosine tracts has been shown to be ~10.0 bp/turn (Peck & Wang, 1981; Rhodes & Klug, 1981) instead of the 10.5 bp/turn found for heterogeneous-sequence DNA (Wang, 1979), short tracts of three to seven contiguous adenosines, phased to occur at integral multiples of 10 bp, lead to the

occurrence of macroscopically bent DNA (Koo et al., 1986), and polymers with such phased tracts do form nucleosomes more readily than bulk DNA (Shrader & Crothers, 1989, 1990). Longer, unphased tracts of adenosines, however, were thought to be detrimental to nucleosome formation. This idea is based on unsuccessful attempts to reconstitute poly(dA)·poly(dT) (Simpson & Künzler, 1979; Rhodes, 1979), the appearance of a long cloned dA·dT tract in dinucleosomes but not mononucleosomes (Kunkel & Martinson, 1981), and the preference of longer dA_n·dT_n ($n \geq 6$) tracts in cloned nucleosomal DNAs isolated from high-salt buffers to appear toward the ends of the nucleosome cores versus their appearance near the dyad axis (Satchwell et al., 1986). However, in none of these experiments was the actual or relative free energy of nucleosome formation measured. Satchwell and Travers (1989) also showed that nucleosomal DNAs isolated at low salt had apparently randomly distributed dA_n·dT_n tracts and speculated that the presence or absence of H5, which is lost in high-salt washes, may act as a molecular switch to make adenosine tracts occupy different positions.

We have shown in this paper, however, that a 146 bp length of DNA containing a dA_{10}·dT_{10} tract forms nucleosomes with a free energy ~0.2 kcal/mol more favorable than the identical sequence containing a dG_{10}·dC_{10} tract or containing no oligopurine tract and that, for any given set of permuted fragments, most of them lie within ±0.1 kcal/mol of each other. (Figure 4). It is useful to compare the magnitude of the values of $\Delta\Delta G$ seen in Figure 4 to those obtained for other polynucleotides, both synthetic and natural, that have been previously examined. Our laboratory has investigated double-stranded polydeoxynucleotides of the general formula poly-[$d(G_n$-$C_n)$]·poly[$d(G_n$-$C_n)$] and poly[$d(G_n$-C)]·poly[d(G-C_n)] and showed that the differential free energy of nucleosome formation varies over a range of ~1.5 kcal/mol as a function of n (Jayasena & Behe, 1989b). Shrader and Crothers (1989, 1990), in examining 5S RNA gene from several sources and synthetic polymers containing $(A/T)_3N_2(G/C)_3N_2$-repeating sequences, saw differences in binding energy approaching 3 kcal/mol. Thus, the occurrence or position of the oligopurine tracts examined here results in a much more restricted range of $\Delta\Delta G$ values than are seen for other sets of polymers.

Recently Hayes et al. (1991) examined the energetics of reconstitution of a length of DNA containing several long dA_n·dT_n tracts, with n ranging from 10 to 16, and showed that nucleosomes formed with a $\Delta\Delta G$ 1.1 kcal/mol less favorable than heterogeneous-sequence DNA. In the same paper, it was demonstrated by hydroxyl radical cleavage that the dA_n·dT_n tracts are constrained to adopt the same conformation in the nucleosome as other sequences. Thus, the weight of the evidence now shows that long tracts of either dG_n·dC_n (Jayasena & Behe, 1989a,b) or dA_n·dT_n (this work; Puhl et al., 1991; Hayes et al., 1991) can be accommodated in nucleosomes without difficulty.

We conclude, then, that despite their overabundance in eukaryotic DNA and their special structural characteristics isolated, unphased oligopurine tracts do not significantly affect the binding of DNA to nucleosomes. Whether such tracts play a different role in the cell is not yet known.

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