Contribution of Histones H2A and H2B to the Folding of Nucleosomal DNA†

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ABSTRACT: We have studied the structural properties of nucleosomal particles deficient in histones H2A and H2B produced by modification of histone amino groups with dimethylmaleic anhydride [Jordano, J., Montero, F., & Palacián, E. (1984) *Biochemistry* (preceding paper in this issue)]. Digestion with DNase I of residual particles containing only 15% of the original H2A·H2B complement produces only discrete DNA fragments no longer than 70 nucleotides. As compared with the original nucleosomes, thermal denaturation of the residual particles shows a decrease from 140 to about 90 in the number of nucleotide base pairs per particle that melt at the highest temperature transition as well as a drop in the temperature of this transition. Circular dichroism spectra of the residual particles give ellipticity values around 275 nm, much higher than those corresponding to the control nucleosomes, which appears to indicate a loss in the compact DNA tertiary structure. When regeneration of the modified amino groups of the residual particles takes place in the presence of the complementary fraction containing histones H2A and H2B, but not in its absence, nucleosomal particles with the structural properties of the original nucleosomes are reconstituted. Therefore, the structural change observed in the residual particles can be assigned to the lack of histones H2A and H2B and not to the modified amino groups of the histones present in the residual particles. The results are consistent with the stabilization by histones H2A and H2B of a DNA length of 50–70 base pairs per nucleosome.

Arginine-rich histones H3 and H4 play a fundamental role in the folding of DNA into nucleosomal particles (Camerini-Otero et al., 1976; Sollner-Webb et al., 1976). These proteins, in the absence of H2A and H2B, interact with double-stranded DNA to form particles with many of the structural properties of nucleosomal cores (Camerini-Otero et al., 1976; Sollner-Webb et al., 1976; Boseley et al., 1976; Camerini-Otero & Felsenfeld, 1977; Moss et al., 1977; Bina-Stein & Simpson, 1977; Bradbury et al., 1978; Oudet et al., 1978). In contrast, histones H2A and H2B are unable by themselves to fold DNA into the ordered condensed structure found in nucleosomes (Camerini-Otero et al., 1976; Sollner-Webb et al., 1976). Moreover, during in vitro assembly of nucleosomal core particles from core histones and DNA, a tetramer of H3 and H4 binds first to DNA to form a nucleosome-like structure, being followed by the incorporation of H2A and H2B to complete the nucleosomal particle (Wilhelm et al., 1978; Jorcano & Ruiz-Carrillo, 1979). However, in spite of the preponderant role played by histones H3 and H4, histones H2A and H2B are required to obtain particles with all the structural characteristics of native nucleosomes (Camerini-Otero et al., 1976; Sollner-Webb et al., 1976; Boseley et al., 1976; Oudet et al., 1978; Klevan et al., 1978; Bina-Stein, 1978). Therefore, it is of interest to evaluate the nucleosomal DNA structuring properties of histones H2A and H2B.

In the preceding paper (Jordano et al., 1984), we have studied the rearrangement of nucleosomal components brought about by modification of lysine residues with carboxylic acid anhydrides (Jordano et al., 1984). During disassembly of nucleosomal particles with DMMA,[1] residual particles are produced which are deficient in histones H2A and H2B and at the same time have an excess of H3 and H4. Since regeneration of the modified groups as well as reconstitution of nucleosomes from the residual particles and the complementary released fraction can be easily obtained, this experimental system could be useful to investigate the contribution of histones H2A and H2B to the correct folding of DNA into nucleosomes.

In this paper, we use DNase I digestion, circular dichroism, and thermal denaturation to study the structural properties of the nucleosomal particles deficient in histones H2A and H2B, obtained after DMMA treatment, in an attempt to evaluate the contribution of these histones to the nucleosomal structure. The lysine residues of these histones appear to play an important role in the folding of a substantial length of nucleosomal DNA.

Materials and Methods

Preparation of Different Nucleosomal Particles. Native nucleosomal particles with an average DNA length of 160 ± 10 base pairs were obtained from chicken erythrocytes as described in the preceding paper (Jordano et al., 1984). The concentration of nucleosomal particles was determined spectrophotometrically, taking A_{260nm} = 20.0 for a solution containing 1.0 mg of DNA/mL.

The residual nucleosomal particles were obtained from native nucleosomes by treatment with different amounts of DMMA, followed by dialysis to eliminate the hydrolyzed reagent, and separation of the residual particles from the released fraction (free DNA and proteins) by differential centrifugation in linear 5–20% sucrose gradients (Jordano et al., 1984).

When required, the modified amino groups of the residual particles were regenerated by dialysis against a buffer solution at pH 6.0 (Jordano et al., 1984). Reconstituted nucleosomes were obtained by applying the regeneration procedure to modified preparations containing all the nucleosomal com-

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[1] Abbreviations: CD, circular dichroism; DMMA, dimethylmaleic anhydride; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; EDTA, ethylenediaminetetraacetic acid; bp, base pairs.

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ponents and isolating the particles by centrifugation in 5–20% sucrose gradients.

Composition of Nucleosomal Particles. Histone composition of nucleosomal particles was evaluated by densitometry of the patterns of electrophoresis obtained on 15% polyacrylamide gels containing 0.1% sodium dodecyl sulfate (Laemmli, 1970), as previously described (Jordano et al., 1984). DNA concentration was determined by measuring the absorbance at 254 nm, a wavelength at which the relative absorbance of histones is negligible.

Digestion of Nucleosomal Particles with DNase I. Samples (30–100 μg of DNA in 1–2 mL), in 10 mM Tris-HCl (pH 8.0), 8 mM $MgCl_2$, and 8 mM $CaCl_2$, were digested at 37 °C for 1–10 min with 20–170 units of pancreatic deoxyribonuclease (DNase I) (type I, Sigma). The reaction was stopped by addition of EDTA to a concentration of 10 mM and by cooling the mixture to 2 °C. The DNA fragments were separated from the protein essentially as described by Marmur (1961) and subjected to electrophoresis in gels containing 15% polyacrylamide and 7 M urea, following the procedure of Maniatis et al. (1975). Gels were stained with ethidium bromide and photographed under ultraviolet light. Densitometric profiles were obtained from the patterns on Polaroid type 55 film with an Optronic digital microdensitometer connected to a PDP 11/45 computer.

Circular Dichroism. Spectra were obtained with a Mark III dicrograph (Jobin-Yvon) using quartz cells with 1.0-cm optical path. Ellipticity is expressed on the basis of DNA nucleotide residue concentration. Ellipticity was calculated and the CD spectra were plotted with the use of a minicomputer HP9815A connected to a HP7225A plotter through a HP-IB interphase. The nucleosomal particles (25–75 μg of DNA/mL) were studied in 0.25 mM EDTA (pH 8.2) at room temperature, and a sensitivity of (2–5) × 10^{-6} ΔA/mm was used.

Thermal Denaturation. After dialysis of the samples against 0.25 mM EDTA (pH 8.2), denaturation profiles were obtained with a Beckman DU-8 spectrophotometer equipped with a TM·S module by using a heating rate of 0.5 °C/min. Absorbance at 260 nm was registered at 1 °C intervals between 30 and 102 °C by using a slit of 0.5 nm. Reported denaturation profiles are averages of five determinations. Hyperchromicity values and the derivative dH/dt were obtained from the registered data by using an HP-85 minicomputer as previously described (Mârquez et al., 1982). The derivative denaturation curves were resolved into component thermal transitions by Gaussian curve fitting with a BMDP-3R nonlinear regression program run on an IBM 360-65I computer provided with an operating system OS/360. T_m, the transition midpoint, is the temperature of maximum dH/dt for each transition.

Results

Nucleosomal Particles Studied. Modification of nucleosomes with DMMA produces, in addition to free DNA and proteins, residual nucleosomal particles with an excess of histones H3 and H4 but deficient in H2A and H2B (Jordano et al., 1984). Table I shows the composition of the residual nucleosomal particles used in this work. The four preparations were obtained after treatment with different amounts of DMMA. Since the presence in the nucleosomal particle of additional molecules of H3 and H4 does not seem to affect the superhelical structure of DNA (Klevan et al., 1978; Eisenberg & Felsenfeld, 1981), these residual particles can be used to study the effects on nucleosomal DNA structure produced by the lack of histones H2A and H2B.

Table I: Composition of the Residual Nucleosomal Particles Studied[a]

	native nucleosome	residual particles			
		P1	P2	P3	P4
DNA (%)	100.0	90.7	83.7	45.7	67.6
H2A·H_2B (%)	100.0	82.6	43.0	15.8	10.0
H2A·H_2B:DNA (relative values)	1.00	0.91	0.51	0.35	0.15
H3·H4:DNA (relative values)	1.00	1.10	1.19	2.19	1.48

[a] The residual particles were obtained after treatment of nucleosomal particles with the following amounts of DMMA (mg/mL): 0.29, P1; 0.58, P2; 1.80, P3; 1.12, P4. The percentages of DNA and of histones H_2A·H2B shown are relative to the same components present in the nucleosomal sample from which each preparation of residual particles is obtained. The relations between DNA loss, histone H2A·H2B deficiency of the residual particles, and amount of DMMA used in the treatment are discussed in the preceding paper (Jordano et al., 1984).

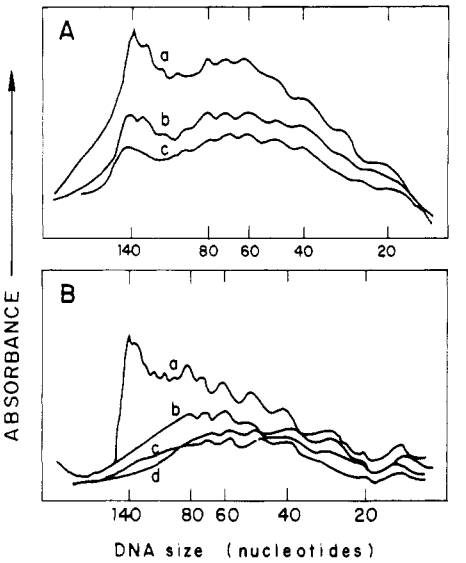

FIGURE 1: DNase I digestion patterns of residual nucleosomal particles. Densitometric tracings of the digestion DNA fragments after electrophoretic separation by size. (A) Original nucleosomes (a) and residual particles P1 (b) and P2 (c). In all three cases the nucleosomal particles (30 μg of DNA) were digested with 100 units of DNase I for 10 min. (B) Original nucleosomes (a) and residual particles P4 (b–d). Nucleosomal particles (29 μg of DNA) were digested under the following conditions: 100 units of DNase I and 5 min (a and d); 20 units of DNase I and 1 min (b) ; 50 units of DNase I and 2 min (c).

Digestion of Nucleosomal Particles with DNase I. Digestion of the DNA of these residual particles with DNase I takes place at a higher rate than that of native nucleosomes. The digestion rate increases with the extent of H2A·H2B deficiency (Figure 1). This effect is indicative of a relaxation of nucleosomal structure. Figures 1 and 2 show the digestion profiles of different residual particles obtained with DMMA as compared with that of the original nucleosomes. Treatment of the control nucleosomes with DNase I is accompanied by the formation of DNA fragments which are multiple of 10 nucleotides in length and range from 10 to 140 nucleotides, this digestion pattern being the result of the different accessibility of the potential sites of attack of double-stranded DNA in the particle (McGhee & Felsenfeld, 1980); Mirzabekov, 1980). As compared with the control, the residual particles show relatively lower amounts of fragments longer than about 70 nucleotides. The proportion of these longer fragments decreases with increasing loss of histones H2A and H2B. The residual particles with the lowest H2A·H2B content do not show discrete fragments longer than 70 nucleotides, even when

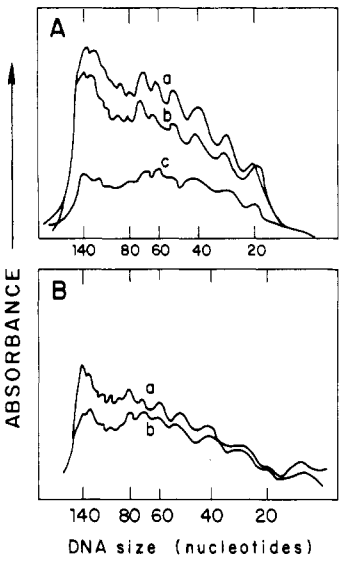

FIGURE 2: DNase I digestion patterns of regenerated residual particles and reconstituted nucleosomes. Densitometric tracings of the digestion DNA fragments after electrophoretic separation by size. (A) Original nucleosomes (a), reconstituted nucleosomes from residual particles P3 and the corresponding released fraction (b), and residual particles P3 with or without regeneration of their modified amino groups (c). Nucleosomal particles (50 μg of DNA) were digested with 170 units of DNase I for 5 min. (B) Original nucleosomes (a) and nucleosomes reconstituted from residual particles P4 and the corresponding released fraction (b). Nucleosomal particles (29 μg of DNA) were digested with 100 units of DNase I for 5 min.

digestion takes place under conditions milder than those of the control (Figure 1B), indicating that a DNA segment of this length is the longest that is efficiently protected from digestion in these particles.

Regeneration of the modified amino groups of the residual particles is accompanied by little or no change in rate or pattern of digestion (Figure 2A). However, when regeneration takes place in the presence of the complementary fraction released by the DMMA treatment, which contains histones H2A and H2B, the reconstituted particles produced give digestion patterns very similar to those of the control nucleosomes, with a high proportion of discrete DNA fragments longer than 70 nucleotides.

Circular Dichroism of the Nucleosomal Particles. The circular dichroism spectra of the residual particles are also different from the spectrum of the original nucleosomes (Figure 3). The ellipticity at 275 nm is increased in the residual nucleosomes, while the wavelength at the crossover point is lowered. The increase in ellipticity depends on the H2A·H2B content of the residual particles, being larger for those containing lower amounts of H2A·H2B. While regeneration of the modified amino groups produces only a small decrease in ellipticity, the nucleosomes reconstituted from the residual particles show a substantial recovery of the characteristics of the original spectrum (Figure 4).

According to the analysis made by Cowman & Fasman (1978, 1980), the CD spectrum between 250 and 320 nm of nucleosomes can be resolved in two components. One corresponds to the secondary structure of free DNA (B-DNA), and the other is a negative band, centered at 275 nm, which is attributed to the condensation of B-DNA into an asymmetric tertiary structure. The assumptions made by Cowman and Fasman allow a tentative interpretation of the CD spectrum of nucleosomes in quantitative structural terms. The CD contribution of a nucleotide residue in the altered condensed structure would be equal to the observed contribution in core particle DNA. This assumes 100% participation of core

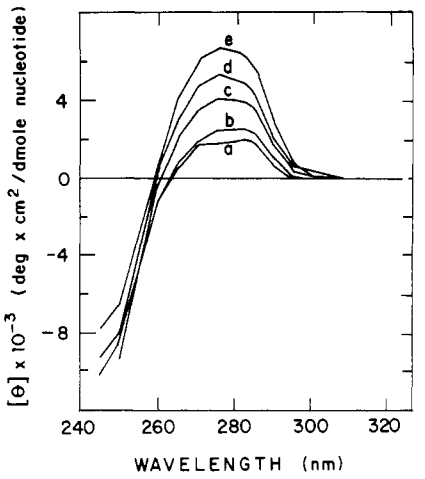

FIGURE 3: Circular dichroism spectra of residual nucleosomal particles. Original nucleosomes (a) and residual particles P1 (b), P2 (c), P3 (d), and P4 (e).

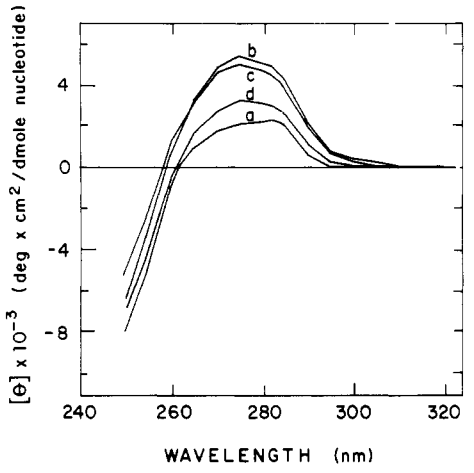

FIGURE 4: Circular dichroism spectra of regenerated residual particles and the corresponding reconstituted nucleosomes. Residual nucleosomal particles P3 (b); residual particles P3 after regeneration of the modified amino groups (c), nucleosomes reconstituted from residual particles P3 and the corresponding released fraction (d), and original nucleosomes subjected to the procedure used for reconstitution (a).

particle DNA (140 base pairs) in the altered structure. Only two states are considered for DNA nucleotide residues: altered, with contribution of both secondary and tertiary structures, and nonaltered, with contribution of only the secondary structure. If these considerations are used to calculate the number of nucleotide base pairs that maintain the nucleosomal DNA tertiary arrangement in the different nucleosomal particles studied, the values shown in Table II are obtained. The residual particles with the lowest H2A·H2B content have only 46 nucleotide base pairs in the condensed tertiary configuration, while the original nucleosomes have 141 and the reconstituted particles 115.

Thermal Denaturation of the Nucleosomal Particles. Thermal denaturation is another useful tool in the investigation of nucleosomal structure. Melting profiles of nucleosomal core particles are biphasic, with transition midpoints at 60 and 74 °C (Weischet et al., 1978). The thermal denaturation of core particles is interpreted as melting of about 40 base pairs in the first transition, corresponding to the ends of the DNA coil, followed by melting of the remaining 100 base pairs located in the central region of DNA, in the second transition. However, nucleosomal particles containing histone H1 give denaturation profiles with practically all the DNA melting in one thermal transition with a high midpoint temperature, indicating that histone H1 seems to produce a stabilization

Table II: Circular Dichroism Parameters for Different Nucleosomal Particles[a]

		nucleosomal particles							
	DNA[b]	core[b]	nucleosome	P1	P2	P3	P3r	RN3	P4
$[\theta]_{275}$	9300	800	1800	2500	4100	5400	5000	3200	6800
$[\theta]_{275} - [\theta]_{275,DNA}$	0	−8500	−7500	−6800	−5200	−3900	−4300	−6100	−2500
F		1.00	0.88	0.80	0.61	0.46	0.51	0.72	0.29
condensed DNA length/particle (bp)		140	141	128	98	74	82	115	46

[a] P1, P2, P3, and P4, residual nucleosomal particles; P3r, P3 after regeneration of amino groups; RN3, nucleosomes reconstituted from P3 and the corresponding released fraction. F, fraction of DNA contributing to CD difference band; equal to the ratio of sample difference band intensity ($[\theta]_{275} - [\theta]_{275,DNA}$) to core particle CD difference band intensity. Parameters were calculated according to Cowman & Fasman (1980). [b] Values taken from Cowman & Fasman (1980).

Table III: Transition Temperatures and Relative Areas of Thermal Transitions in Native and Residual Nucleosomal Particles

	thermal transitions								
	native nucleosomes			residual particles					
				P2		P3		P4	
	a	b	c	a	c	a	c	a	c
T_m (°C)	65	69	79	64	79	61	78	58	76
hyperchromicity (%)	7	5	88	30	70	46	54	41	59
DNA (bp)	11	8	141	48	112	74	86	66	94

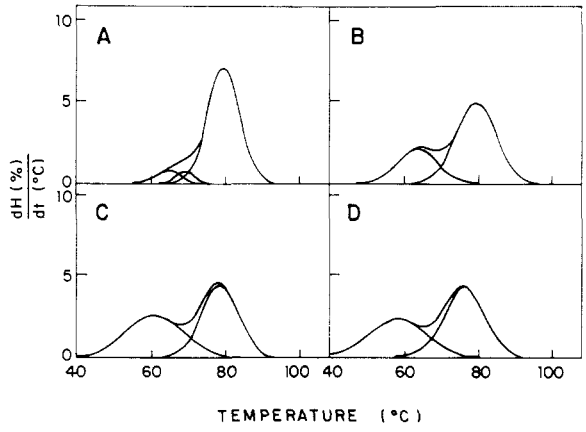

FIGURE 5: Thermal denaturation profiles of the residual particles and the reconstituted nucleosomes. Original nucleosomes or nucleosomes reconstituted from residual particles P4, both profiles identical (A), and residual particles P2 (B), P3 (C), and P4 (D). In addition to the experimental curves, the resolved Gaussian transitions are included.

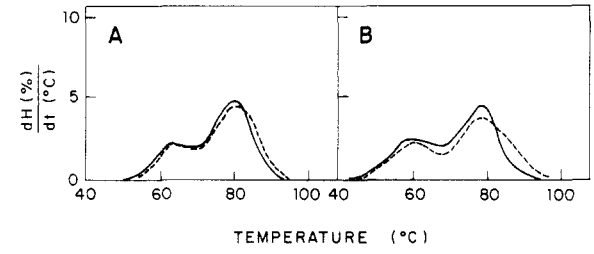

FIGURE 6: Effect of regeneration of the residual particles on the denaturation profile. Thermal denaturation profiles of the residual particles (—) and of the same particles after regeneration of their modified amino group (---). Residual particles P2 (A) and P3 (B).

of the DNA ends (Cowman & Fasman, 1980).

The denaturation profile of control nucleosomes is shown in Figure 5A. Most DNA denatures in a thermal transition with a T_m equal to 79 °C. The residual nucleosomal particles give different denaturation profiles according to their histone composition and degree of modification (Figure 5). With the loss of histones H2A·H2B the fraction of total DNA denaturing in the neighborhood of 79 °C decreases, whereas the DNA in a thermal transition with a T_m of 58–64 increases (Table III). At the same time, the T_m of both transitions decreases with increasing loss of H2A·H2B. All particles studied show a similar change in hyperchromicity which amounts to about 37%. The nucleosomes reconstituted from the residual particles give a denaturation profile identical with that of the original preparation (Figure 5A). In contrast, regeneration of the modified amino groups of the residual particles is accompanied by only small changes in denaturation (Figure 6).

Discussion

The residual nucleosomal particles obtained by treatment with DMMA are deficient in histones H2A and H2B and have extra copies of H3 and H4 (Jordano et al., 1984). Since additional molecules of H3 and H4 or even extra core histone octamers have been shown to bind on the surface of nucleosomal cores without affecting DNA structure (Klevan et al., 1978; Voordouw & Eisenberg, 1978; Stein, 1979; Eisenberg & Felsenfeld, 1981), the residual particles obtained with DMMA could be used to study the structural effects caused on the nucleosomal particle by the presence of modified lysine residues and by the lack of histones H2A and H2B.

Our results indicate that the residual particles have an altered structure as compared with the original nucleosomes and that the observed change increases with the extent of H2A·H2B deficiency. This structural change can be attributed to the lack of histones H2A·H2B, to the distortion produced by the modified lysine residues of the histones present in the residual particles, or to both causes. Since regeneration of the modified amino groups in the residual particles is accompanied by only a very small recovery of the original structure as compared with reconstitution in the presence of histones H2A and H2B, most of the structural change observed can be assigned to the lack of H2A and H2B. The structural results are consistent with the destabilization of a DNA length of 50–70 base pairs in the H2A·H2B-depleted residual particles as compared to the original nucleosomes.

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Kinetics of Incorporation of O^6-Methyldeoxyguanosine Monophosphate during in Vitro DNA Synthesis†

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ABSTRACT: O^6-Methyldeoxyguanosine triphosphate (m^6dGTP), known to be produced in vivo by methylation of deoxyguanosine triphosphate with simple methylating mutagens, is utilized by prokaryotic DNA polymerases during in vitro replication of synthetic and natural DNA template-primers. A study of the kinetic behavior of m^6dGTP during DNA replication in vitro and of its effect on DNA replication indicates that m^6dGTP acts as an analogue of dATP with K_m^{app} of about 6 μM for *Escherichia coli* DNA polymerase I (Klenow fragment) compared to the K_m^{app} of about 0.8 μM for dATP. m^6dGTP is not incorporated in the complete absence of dATP (a competitive inhibitor). m^6dGTP also inhibits in vitro DNA synthesis. Different DNA polymerases behave differently in utilization and turnover of m^6dGTP. T4 DNA polymerase shows stronger discrimination against m^6dGMP incorporation than either T5 DNA polymerase or *E. coli* DNA polymerase I. The possibility that m^6dGTP is unlikely to contribute significantly to in vivo mutation is discussed.

O^6-Methylguanine is an important promutagenic lesion produced by direct methylation of DNA by simple methylating carcinogens (Loveless, 1969; Pegg, 1977; Lawley, 1979; Singer, 1979). Following the proposal of Loveless (1969) that O^6-alkylguanine is promutagenic, indirect in vitro and in vivo experiments (Abbott & Saffhill, 1979; Lawley & Martin, 1975; Coulondre & Miller, 1977) supported the idea that the pairing of the alkylated base with thymine during DNA replication causes mutation. More recently, Nagata et al. (1982) and Klopman & Ray (1982) proposed, on theoretical grounds, that the m^6dG·dT[1] pair is similar to the dA·dT pair in terms of energy of formation, bond angles, and bond distances. Our previous studies (Snow et al., 1984) indicate that m^6dG in the DNA template has a strong preference for dT during in vitro DNA synthesis, but at the same time, the m^6dG·dT pair is not recognized as a normal substitute for dA·dT by prokaryotic DNA polymerases. In the present studies, we have investigated the recognition of the m^6dG·dT pair by DNA polymerases during utilization of m^6dGTP as a substrate in DNA synthesis. This is important because it has been shown recently that m^6dGTP can also be produced in vivo by direct alkylation of

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[1] Abbreviations: m^6dGTP, O^6-methyldeoxyguanosine triphosphate; m^6dG, O^6-methyldeoxyguanosine; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetate; HPLC, high-performance liquid chromatography.

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