DNA Condensation by the Rat Spermatidal Protein TP2 Shows GC-Rich Sequence Preference and Is Zinc Dependent†

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Received October 14, 1994; Revised Manuscript Received January 9, 1995⊗

ABSTRACT: Transition protein-2 (TP2), isolated from rat testes, was recently shown to be a zinc metalloprotein. We have now carried out a detailed analysis of the DNA condensing properties of TP2 with various polynucleotides using circular dichroism spectroscopy. The condensation of the alternating copolymers by TP2 (incubated with 10 μM $ZnSO_4$), namely, poly(dG−dC)•poly(dG−dC) and poly(dA−dT)•poly(dA−dT), was severalfold higher than condensation of either of the homoduplexes poly(dG)•poly(dC) and poly(dA)•poly(dT) or rat oligonucleosomal DNA. Between the two alternating copolymers, poly(dG−dC)•poly(dG−dC) was condensed 3.2-fold more effectively than poly(dA−dT)•poly(dA−dT). Preincubation of TP2 with 5 mM EDTA significantly reduced its DNA-condensing property. Interestingly, condensation of the alternating copolymer poly(dI−dC)•poly(dI−dC) by TP2 was much less as compared to that of poly(dG−dC)•poly(dG−dC). The V8 protease-derived N-terminal fragment (88 aa) condensed poly(dA−dT)•poly(dA−dT) to a very small extent but did not have any effect on poly(dG−dC)•poly(dG−dC). The C-terminal fragment (28 aa) was able to condense poly(dA−dT)•poly(dA−dT) more effectively than poly(dG−dC)•poly(dG−dC). These results suggest that TP2 in its zinc-coordinated form condenses GC-rich polynucleotides much more effectively than other types of polynucleotides. Neither the N-terminal two-thirds of TP2 which is the zinc-binding domain nor the C-terminal basic domain are as effective as intact TP2 in bringing about condensation of DNA.

The chromatin structure undergoes extensive modification during mammalian spermatogenesis resulting in the generation of highly compact and condensed spermatozoa (Meistrich, 1989). The process of spermiogenesis following the meiotic division is characterized by a transition stage (steps 12−15) wherein the nucleosomal histones (both somatic and testis specific variants) are replaced by a set of small basic proteins, TP1, TP2, and TP4 (Meistrich, 1989; Unni & Meistrich, 1992). Finally, these proteins are themselves replaced by protamine S1 during steps 16−19. The physiological significance of the appearance of the transition proteins, only in mammals, is not clearly defined yet. However, three important events occur during steps 12−15 of spermiogenesis. They are (1) transformation of nucleosomal type of chromatin into a smooth chromatin fiber, (2) initiation of chromatin condensation, and (3) cessation of transcription. Therefore, it is generally believed that the transition proteins might be involved in one or more of these events.

TP1 is a protein of 6000 Da (Kistler *et al.*, 1973) and has a DNA-destabilizing property manifested through its two tyrosine residues flanked by basic amino acids (Singh & Rao, 1987). Since addition of TP1 to isolated nucleosome core particles resulted in destabilization of the compact structure of the nucleosome core particle, Singh and Rao (1988) speculated that TP1 may be involved in the process of displacement of histones. The transition protein TP4 was recently purified to homogeneity and characterized (Unni & Meistrich, 1992). It has a molecular mass of approximately 16 kDa and is a basic protein with arginine and lysine constituting 24 mol %. The DNA-binding properties of this newly characterized protein, however, have not been studied.

The transition protein TP2 has a molecular mass of 13 000 Da (Grimes *et al.*, 1975), and in contrast to TP1 it was shown to have a DNA-condensing property (Baskaran & Rao, 1990). Recently TP2 has been shown to be a zinc metalloprotein by atomic absorption spectroscopy and to contain two atoms of zinc bound per molecule (Baskaran & Rao, 1991). Analyzing the cDNA-derived amino acid sequence, they observed that the protein could be folded to generate two zinc finger motifs and speculated that such a structure could have important implications in relation to the biochemical events described above through specific DNA interactions. In a recent communication, we have shown that both cysteine and histidine residues, located in the V8 protease-derived N-terminal two-thirds of TP2, are involved in coordination with zinc (Kundu & Rao, 1994). Thus, it becomes imperative that delineation of the structure of the zinc-coordinated motifs in TP2 and its interaction with DNA are very crucial to understanding the physiological significance of the transitory appearance during mammalian spermiogenesis. Earlier studies on the DNA-condensing properties of TP2 (Baskaran & Rao, 1990) were carried out with pBR322 DNA in the presence of EDTA before it was discovered that TP2 is a zinc metalloprotein. On the basis of our recent observations, we were prompted to answer two important questions: (1) Does zinc have any role in the DNA

† This work was financially supported by the Council of Scientific and Industrial Research. T. K. Kundu is a Senior Research Fellow of the University Grants Commission.

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⊗ Abstract published in *Advance ACS Abstracts,* April 1, 1995.

0006-2960/95/0434-5143$09.00/0

condensation process? (2) Is there a nucleotide sequence preference for condensation by TP2? We report here our studies on the DNA-condensing properties of TP2 with various synthetic polynucleotides as well as rat oligonucleosomal DNA in the presence of zinc and also after chelation of zinc with EDTA using circular dichroism spectroscopy. The results of these experiments have suggested that zinc has a significant role in the condensation of both rat DNA and synthetic polynucleotides brought about by TP2. Interestingly, alternating GC copolymer is condensed much more efficiently over other types of polynucleotides, and the condensation is zinc dependent. The significance of these observations with reference to the chromatin condensation process during spermiogenesis has been discussed.

MATERIALS AND METHODS

Male albino Wistar rats (60 days) were used for isolation of TP2. All the chemicals used were of analytical reagent grade. Synthetic polynucleotides poly(dG−dC)•poly(dG−dC), poly(dA−dT)•poly(dA−dT), poly(dG)•poly(dC), poly(dA)•poly(dT), and poly(dI−dC)•poly(dI−dC) were purchased from Pharmacia LKB, Biotechnology, Sweden. V8 protease (*Staphylococcus aureus*) and micrococcal nuclease were purchased from Sigma Chemical Co.

Purification of TP2 and Its V8 Protease-Digested Polypeptide Fragments. TP2 was purified from the sonication resistant spermatid (SRS) nuclei of rat testis as described recently using the Hg ligand-based affinity chromatography technique (Kundu & Rao, 1994). Briefly, the SRS nuclei prepared from rat testes according to the method of Platz *et al.* (1977) were extracted with 0.4 N HCl, the acid-soluble proteins were processed for organomercurial Sepharose column chromatography, and the bound TP2 was eluted with 0.2 M cysteine hydrochloride.

TP2 (500 μg) was digested with V8 protease (Houmard & Drapeau, 1972) to cleave at glutamate 88 in 50 mM ammonium bicarbonate, pH 7.8, at 37 °C for 10 h at an enzyme to protein ratio of 1:50. The N-terminal (88 aa) and C-terminal (28 aa) polypeptide fragments were purified by preparative polyacrylamide gel electrophoresis followed by gel filtration on Sephadex G-25. Purified TP2 and its N- and C-terminal fragments were checked for purity on a 15% polyacrylamide acid−urea gel (Panyim & Chalkley, 1969) and confirmed by amino acid composition analysis.

Isolation of Rat Oligonucleosomal DNA. Rat liver nuclei were isolated and digested with micrococcal nuclease as described by Rao *et al.* (1983) for either 1 min (to generate 0.8−4-kbp fragments) or 10 min (to generate 0.2−0.8-kbp fragments). Purity and the size range of the DNA fragments were checked by A_{260}/A_{280} ratio and electrophoresis on 1% agarose gel, respectively.

Circular Dichroism Spectroscopy. All the synthetic polynucleotides and rat oligonucleosomal DNA were dialyzed overnight at 4 °C against 10 mM Tris-HCl, pH 7.5, and 20 mM NaCl in water obtained from a Millipore Milli-Q system. The nucleoprotein complexes were prepared by sequentially adding DNA and TP2 or its N- and C-terminal fragments in a total volume of 300 μL of buffer containing 10 mM Tris-HCl, pH 7.5, 20 mM NaCl, and 10 mM $ZnSO_4$ or 5 mM Na_2 EDTA. After each addition of a fixed aliquot (1 μL) of protein or polypeptide, the sample was incubated at room temperature for 10 min before recording the spectrum. There

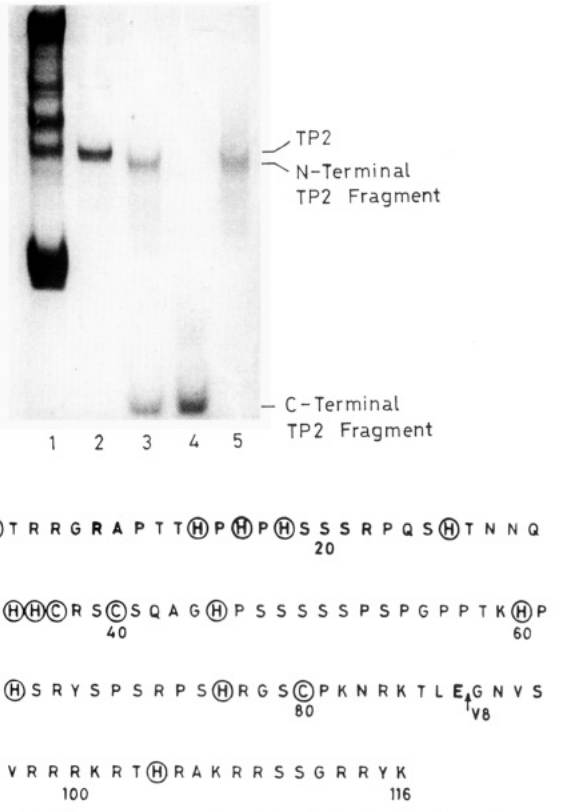

FIGURE 1: Acid−urea polyacrylamide gel electrophoretic patterns of TP2 and its V8 protease-derived N- and C-terminal fragments. (Top) Lane 1, 0.4 N HCl-soluble proteins of SRS nuclei; lane 2, purified TP2; lane 3, V8 protease-digested TP2; lane 4, purified C-terminal; lane 5, purified N-terminal. (Bottom) cDNA-derived amino acid sequence of rat TP2 [from Luerssen *et al.* (1989)].

was no further change in the spectrum beyond 10 min suggesting that the spectrum recorded represented the spectral property of the nucleoprotein complexes at equilibrium. The circular dichroism spectra of nucleic acids and nucleoprotein complexes were recorded at room temperature in a JASCO (J500A) spectropolarimeter from 300 to 200 nm. Each spectrum is an average of four recordings stored and normalized in a data processor. The data collection parameters were sensitivity, 1 mdeg/cm; time constant, 8 s; scan speed, 1 cm/min; and wavelength expansion, 10 nm/cm with a slit width of 2 mm. The protein TP2 or its N-terminal fragment was preincubated with either 10 μM Zn SO_4 or 5 mM EDTA for 6 h at 4 °C. The final concentrations of nucleic acids were calculated using the following molar extinction coefficients (per mole of phosphate at 260 nm and 25 °C) and expressed as mole concentration of bases: rat liver DNA, 6500; poly(dG−dC)•poly(dG−dC) and poly(dG)•poly(dC), 6350; poly(dA−dT)•poly(dA−dT) and poly(dA)•poly(dT), 9750; and poly(dI−dC)•poly(dI−dC), 6900. A mean residue weight of 330 was used to calculate the mean residue ellipticity of nucleic acids and their complexes with TP2. The concentrations of TP2 and its polypeptide fragments were determined by amino acid composition. The molecular weights of TP2 and its N- and C-terminal fragments were taken as 13 000, 10 120, and 3220, respectively. All the nucleoprotein complexes were monitored for absorbance at 400 nm routinely for any light scattering, and absorbances were in the range of 0.002−0.004, indicating that the nucleoprotein complexes did not form insoluble aggregates.

RESULTS

The electrophoretic pattern of purified TP2 which we have used in all our studies is shown in Figure 1A. Authenticity of the protein was confirmed by amino acid composition analysis which closely agreed with the composition based on the cDNA-derived amino acid sequence of rat TP2 (Luerssen *et al.*, 1989; Figure 1B) as well as the composition reported earlier (Cole & Kistler, 1987). The cDNA-derived amino acid sequence shown in Figure 1B has a glutamate residue at the 88 position which enabled us to digest with V8 protease and purify the N- and C-terminal polypeptide fragments. The purity of the isolated fragments are also shown in Figure 1A. A minor slow moving band in the TP2 lane is not a contaminant but actually an aggregate of TP2 (Cole & Kistler, 1987), as revealed by Western blot analysis using polyclonal anti-TP2 antibodies (data not shown), and constituted approximately 2% of the total purified TP2 preparation.

Interaction of TP2 with Synthetic Polynucleotides. The major emphasis of the present investigation was to compare the DNA-condensing properties of TP2 with defined synthetic nucleic acid sequences and also to examine the role of zinc in the condensation process. Circular dichroism spectroscopy is often used to study the condensation behavior of nucleic acids. For studying the circular dichroic spectra, nucleoprotein complexes were prepared in a buffer containing 10 mM Tris-HCl and 20 mM NaCl. Although this concentration of salt is not phyisological, we could not use NaCl above 20 mM NaCl concentration because the nucleoprotein complexes started precipitating out of solution (Baskaran & Rao, 1990).

Figure 2A,C shows the circular dichroic spectra of two alternating copolymers, poly(dG−dC)·poly(dG−dC) and poly(dA−dT)·poly(dA−dT), respectively, with increasing concentrations of TP2. Curve 1 in each panel of the figure represents the spectrum of the nucleic acid in the absence of added TP2. The shape of the curves obtained are typical of the B type spectrum and agree well with those reported in the literature (Gary *et al.*, 1992). There was no change in the spectrum of the nucleic acids recorded in the presence or absence of $ZnSO_4$. TP2 used for these studies was preincubated with 10 μM $ZnSO_4$ or 5 mM EDTA. The buffer for recording CD spectra also contained 10 μM $ZnSO_4$ or 5 mM EDTA. It can be seen from Figure 2A that addition of TP2 (zinc preincubated) at a mole ratio of 0.0034 (protein/nucleotide) resulted in a decrease in the positive ellipticity, θ_{max}, at 272 nm of poly(dG−dC)·poly(dG−dC). At higher input of TP2 (ratio of 0.017), there was a steep decrease in the ellipticity at 272 nm toward the negative side. It can also be seen that there was a red shift in the spectrum of poly(dG−dC)·poly(dG−dC) upon addition of TP2. Addition of TP2 (zinc preincubated) to poly(dA−dT)·poly(dA−dT) also resulted in a decrease in the positive ellipticity value at 260 nm. It can be noted, however, that at an equivalent ratio of TP2/DNA of 0.017, the ellipticity was not reversed to the negative side in the case of poly(dA−dT)·poly(dA−dT) in contrast to that observed with poly(dG−dC)·poly(dG−dC). The dotted line in panels A and B represents the CD spectrum of TP2 alone in the absence of nucleic acid. The protein does not contribute to the spectrum between 230 and 300 nm, and therefore any changes that are observed in the DNA spectrum upon complexing with TP2 represent

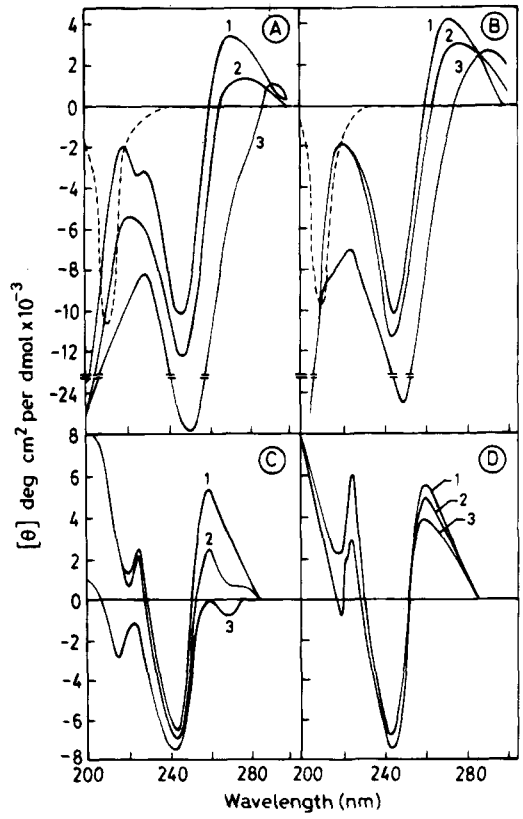

FIGURE 2: Effect of TP2 on the circular dichroic spectra of alternating copolymers poly(dG−dC)·poly(dG−dC) and poly(dA−dT)·poly(dA−dT). TP2 was directly mixed with the two copolymers in the presence of 10 mM Tris-HCl, pH 7.5, 20 mM NaCl, and 10 μM $ZnSO_4$ or 5 mM EDTA as described under methods, and the spectra were recorded in a JASCO (J500A) spectropolarimeter. Spectra of poly(dG−dC)·poly(dG−dC) in the presence of 10 μM $ZnSO_4$ (A) or 5 mM EDTA (B): 1, DNA alone; 2, DNA + TP2 (protein to nucleotide ratio of 0.0034); 3, DNA + TP2 (protein to nucleotide ratio of 0.017). Spectra of poly(dA−dT)·poly(dA−dT) in the presence of 10 μM $ZnSO_4$ (C) or 5 mM EDTA (D): 1, DNA alone; 2, DNA + TP2 (protein to nucleotide ratio of 0.016); 3, DNA + TP2 (protein to nucleotide ratio of 0.027). The dotted line in panels A and B represents the CD spectrum of TP2 alone in the respective buffers.

conformational changes of the nucleic acids. A decrease in the positive ellipticity has often been interpreted as condensation of the nucleic acids, while a steep negative ellipticity has been explained as due to formation of ordered aggregates in the form of liquid crystals. Such a Ψ type of spectrum has also been observed upon interaction of DNA with histone H1 (Liao & Cole, 1981). There was no increase in the absorbance at 400 nm with the nucleoprotein complexes studied here indicating that the decrease in the ellipticity observed is not due to precipitation of the nucleoprotein complexes. Figure 2B,D shows the circular dichroic spectra of the two alternating copolymers upon interaction with TP2 which had been preincubated with 5 mM EDTA. On comparison of panel B and A and panel D with C, it is clear that upon preincubation of TP2 with EDTA there was a significant decrease in the ability of TP2 to condense both poly(dG−dC)·poly(dG−dC) and poly(dA−dT)·poly(dA−dT). The DNA-condensing properties of TP2 reported here were reproducible with different batches of the protein purified from rat testes. A summary of the ellipticity values of the two alternating copolymers upon interaction with TP2 is given in Table 1.

Table 1: Summary of the Ellipticity Values Obtained from Circular Dichroic Spectra of Various Nucleic Acids upon Interaction with TP2

nucleic acids	protein/DNA ratio (mol/nucleotide)	mean residue ellipticity[a] +ZnII	+EDTA
Synthetic Polynucleotides			
poly(dG−dC)·poly(dG−dC)		3468	4335
	0.0034	1238	2972
	0.017	−6193	−2472
poly(dA−dT)·poly(dA−dT)		5437	5558
	0.0034	4641	5558
	0.016	2416	4833
	0.027	241	3866
Rat Oligonucleosomal DNA			
rat DNA, 0.8−4 kbp		6000	6000
	0.036	5750	6000
	0.018	5000	5750
	0.025	4364	5750
rat DNA, 0.2−0.8 kbp		5875	5750
	0.0036	5875	5750
	0.018	2937	4764
	0.025	750	3125

[a] The ellipticity values are from Figures 2 and 6. The CD spectra of the nucleoprotein complexes were recorded with two independent preparations of TP2. Each spectrum is an average of four repeated recordings. The variation between the two independent experiments is within an error of 5%.

We then investigated the ability of TP2 to condense homoduplexes poly(dG)·poly(dC) and poly(dA)·poly(dT). Figure 3 shows the circular dichroic spectra obtained upon addition of zinc-preincubated TP2 (Figure 3A,C) and EDTA-preincubated TP2 (Figure 3B,D) with poly(dG)·poly(dC) (Figure 3A,B) and poly(dA)·poly(dT) (Figure 3C,D), respectively. There was only a marginal decrease of positive ellipticity at 253 nm from 9661 to 7184 in the case of poly(dG)·poly(dC) upon interaction with zinc-preincubated TP2 at a protein/nucleotide ratio of 0.013. It can be noted that even this marginal condensation was partially sensitive to EDTA treatment. The marginal condensation of homoduplex poly(dG)·poly(dC) by TP2 is in contrast to the effect observed with alternating copolymer poly(dG−dC)·poly(dG−dC) in Figure 2. The circular dichroic spectrum of the synthetic homoduplex poly(dA)·poly(dT) shown in Figure 3C,D has two positive θ maxima, one at 256 nm and the other at 280 nm, which agree with the spectrum reported in the literature (Gary *et al.*, 1992). On the basis of the X-ray diffraction analysis of crystals of d($CGCA_3T_3GCG$), it has been proposed that poly(dA)·poly(dT) exists in a modified B conformation, Bp, which is associated with a large propeller twist of the bases with continuous bifurcated hydrogen bonds along the major groove (Coll *et al.*, 1987). TP2 (zinc preincubated) upon addition to poly(dA)·poly(dT) at a mole ratio of 0.021 did not alter the ellipticity at both 256 and 280 nm. EDTA pretreatment also did not have an appreciable effect on the positive ellipticity observed (Figure 3D).

For a better analysis and understanding of the relative ability of TP2 to condense various polynucleotides, we have calculated the decreases in the positive ellipticity observed upon addition of increasing concentrations of TP2 from those obtained with polynucleotides alone, termed as $\Delta\theta$, and plotted them against the protein/DNA ratio (mol/nucleotide). Such an analysis is shown in Figure 4A,B. Several important observations can be made from the curves presented in this

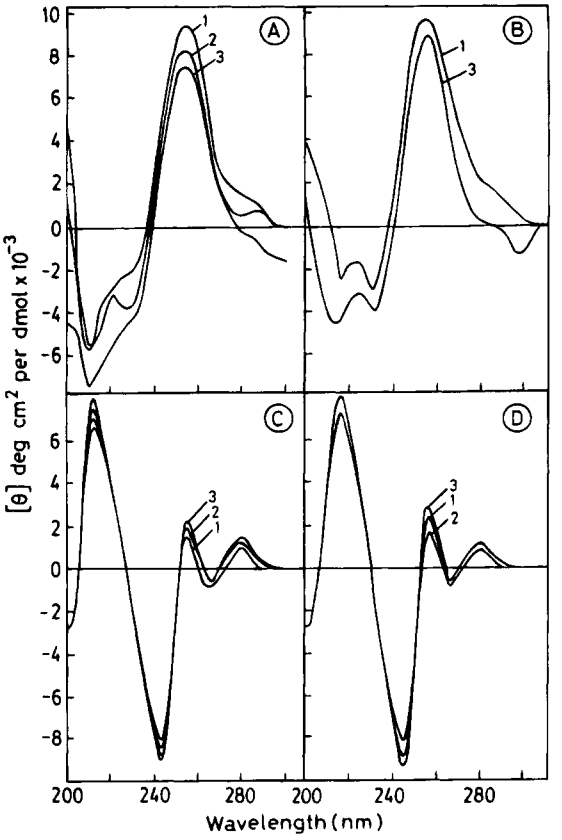

FIGURE 3: Effect of TP2 on the circular dichroic spectra of homoduplexes poly(dG)·poly(dC) and poly(dA)·poly(dT). Spectra were recorded as described in the legend to Figure 2. Spectra of poly(dG)·poly(dC) in the presence of 10 μM $ZnSO_4$ (A) or 5 mM EDTA (B): 1, DNA alone; 2, DNA + TP2 (protein to nucleotide ratio of 0.0034); 3, DNA + TP2 (protein to nucleotide ratio of 0.013). Spectra of poly(dA)·poly(dT) in the presence of 10 μM $ZnSO_4$ (C) or 5 mM EDTA (D): 1, DNA alone; 2, DNA + TP2 (protein to nucleotide ratio of 0.012); 3, DNA + TP2 (protein to nucleotide ratio of 0.021).

figure. Firstly, alternating copolymers are more efficiently condensed by TP2. Secondly, among the two copolymers, TP2 condenses poly(dG−dC)·poly(dG−dC) more effectively than poly(dA−dT)·poly(dA−dT). Thirdly, homoduplex poly(dG)·poly(dC) is condensed to a lesser extent than alternating copolymer poly(dG−dC)·poly(dG−dC). Fourthly, there is no effect of TP2 on the CD spectrum of homoduplex poly(dA)·poly(dT). More importantly it can be seen that removal of zinc from TP2 by preincubation with EDTA resulted in a significant decrease in the condensation of all the types of polynucleotides.

Since the results of the experiments described above indicated that the maximum condensation brought about TP2 was with alternating copolymer poly(dG−dC)·poly(dG−dC), we were curious to know the importance of the guanine base in the polynucleotide for the condensation to occur. We, therefore, studied the effect of TP2 on the circular dichroism spectrum of the alternating copolymer poly(dI−dC)·poly(dI−dC). These results are presented in Figure 5. The spectrum for the free polynucleotide is different from the classical B type spectrum in that it shows a negative band at 275 nm and a positive band at 258 nm. This unusual spectrum has been reported earlier by several groups of workers (Mirau & Kearus, 1984; Vorlickova & Sagi, 1991; Wang & Keiderling, 1993). Based on the unusual X-ray fiber diffraction pattern and the circular dichroism spectrum

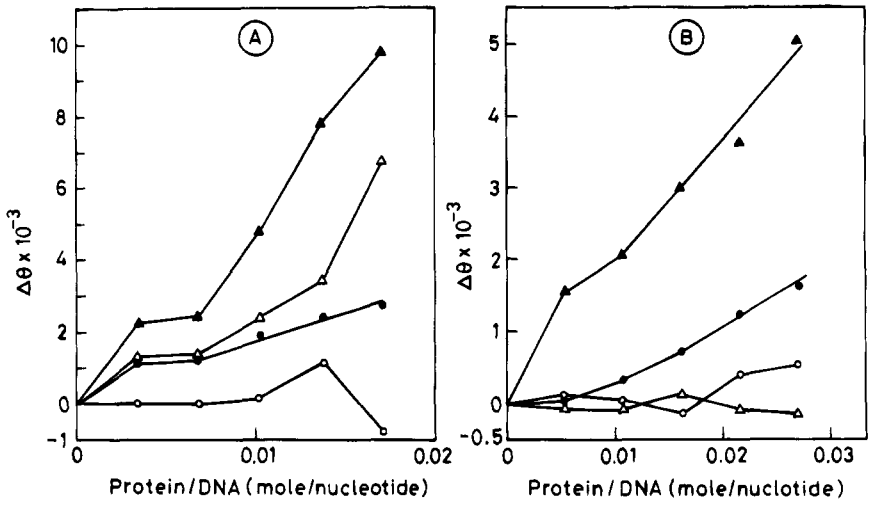

FIGURE 4: Effect of TP2 on the condensation of different nucleic acids. Ellipticity changes observed in Figures 2 and 3 with different nucleic acids upon binding to TP2, termed as $\Delta\theta$, are plotted as a function of the protein/DNA ratio (mol/nucleotide). (A) ▲−▲, poly(dG−dC)·poly(dG−dC) + TP2 (ZnII); △−△, poly(dG−dC)·poly(dG−dC) + TP2 (EDTA); ●−●, poly(dG)·poly(dC) + TP2 (ZnII); and ○−○, poly(dG)·poly(dC) + TP2 (EDTA). (B) ▲−▲, poly(dA−dT)·poly(dA−dT) + TP2 (ZnII); ●−●, poly(dA−dT)·poly(dA−dT) + TP2 (EDTA); ○−○, poly(dA)·poly(dT) + TP2 (ZnII); and △−△, poly(dA)·poly(dT) + TP2 (EDTA).

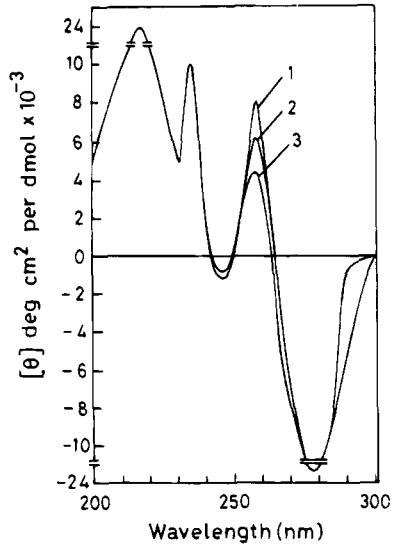

FIGURE 5: Effect of TP2 on the circular dichroic spectra of poly(dI−dC)·poly(dI−dC). Spectra were recorded as described in the legend to Figure 2. CD spectra with poly(dI−dC)·poly(dI−dC) in the presence of 10 μM $ZnSO_4$: 1, DNA alone; 2, DNA + TP2 (protein to nucleoside ratio of 0.0034); and 3, DNA + TP2 (protein to nucleotide ratio of 0.017).

(Mitsui *et al.*, 1970), models were initially proposed to show that this nucleic acid probably exists as a left-handed double helix (Mitsui *et al.*, 1970; Ramaswamy *et al.*, 1982). However, subsequent 2D NMR measurements (Mirau & Kearns, 1984), vacuum CD (Sutherland & Griffin, 1983), and vibrational CD (Wang & Keiderling, 1993) experiments have clearly ruled out the possibility of a left-handed helical nature of poly(dI−dC)·poly(dI−dC). These workers have suggested that it exists in a B or closely related B form. It is interesting to note that addition of TP2 did bring about a small but significant decrease in the ellipticity value of poly(dI−dC)·poly(dI−dC) from θ of 8000 to θ of 4400 at a mole ratio of protein/nucleotide of 0.017. However, this decrease is much less as compared to that observed with poly(dG−dC)·poly(dG−dC) at an equivalent protein/nucleotide ratio (Figure 2 and Table 1). Therefore, this experiment suggests that probably amino group(s) in the purine base plays an important role in recognition and subsequent condensation of the polynucleotide by TP2.

Interaction of TP2 with Rat Oligonucleosomal DNA. All the experiments described above are with respect to the condensation of various synthetic polynucleotides by TP2. We then studied the condensation effect of TP2 on rat oligonucleosomal DNA. For this purpose, we have used two size classes of oligonucleosomal DNA ranging from 0.8 to 4 kbp and 0.2 to 0.8 kbp isolated from rat liver nuclei following micrococcal nuclease digestion as described in the methods section. The dichroic spectra obtained are given in Figure 6, while the plot of $\Delta\theta$ versus protein/DNA ratio is shown in Figure 7. It is clear that the condensation of shorter DNA fragments is more efficient than that of the longer DNA fragments. Again it can be noted that pretreatment of TP2 with EDTA decreased significantly its condensation property (compare panels A and B and panels C and D of Figure 6). A comparison of $\Delta\theta$ observed at a protein/DNA ratio of 0.018 with respect to rat oligonucleosomal DNA (0.2−0.8 kb) and poly(dG−dC)·poly(dG−dC) (average size of 805 bp) again reveals that alternating GC copolymer is condensed at least 3-fold more efficiently than random sequence-containing DNA.

Interaction of V8 Protease-Derived Fragments of TP2 with Nucleic Acids. We have shown recently (Kundu & Rao, 1994) that the zinc-binding sites are localized to the N-terminal polypeptide fragment of TP2 obtained after V8 protease digestion. Therefore, we wanted to investigate the DNA-condensing properties of the N- and C-terminal fragments individually. For this purpose, we selected the two alternating copolymers poly(dG−dC)·poly(dG−dC) and poly(dA−dT)·poly(dA−dT) and studied the effect of the two polypeptide fragments in the presence of either 10 μM zinc sulfate or 5 mM EDTA. The purity of these polypeptide fragments used in our studies is demonstrated in Figure 1. The circular dichroic spectral data obtained are given in Figure 8. Addition of the N-terminal fragment to poly(dG−dC)·poly(dG−dC) did not alter the spectrum at an input ratio of 0.021 (Figure 8B). This is in contrast to the effect observed with intact TP2 shown in Figure 2. On the other hand, addition of the C-terminal fragment resulted in a small

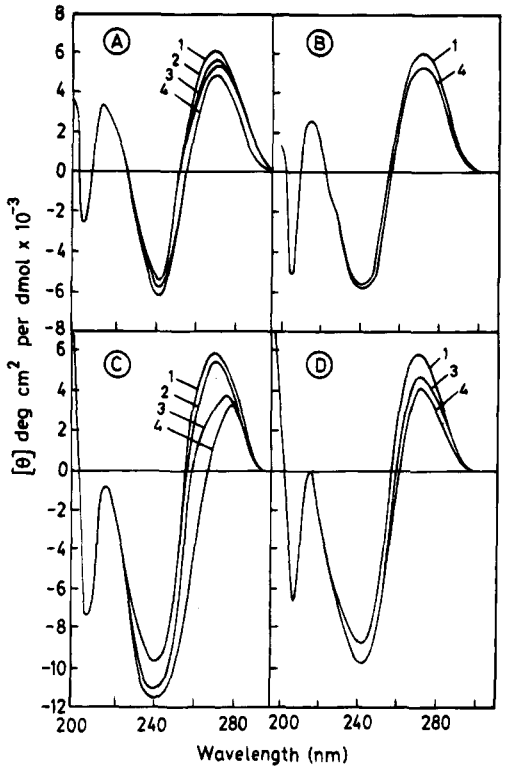

FIGURE 6: Effect of TP2 on the circular dichroic spectra of rat oligonucleosomal DNA. Spectra were recorded as described in the legend to Figure 2. Spectra of rat oligonucleosomal DNA (0.8−4 kbp) in the presence of 10 μM $ZnSO_4$ (A) or 5 mM EDTA (B): 1, DNA alone; 2, DNA + TP2 (protein to nucleotide ratio of 0.0036); 3, DNA + TP2 (protein to nucleotide ratio of 0.018); and 4, DNA + TP2 (protein to nucleotide ratio of 0.025). Spectra of rat oligonucleosomal DNA (0.2−0.8 kbp) in the presence of 10 μM $ZnSO_4$ (C) or 5 mM EDTA (D): 1, DNA alone; 2, DNA + TP2 (protein to nucleotide ratio of 0.0036); 3, DNA + TP2 (protein to nucleotide ratio of 0.025).

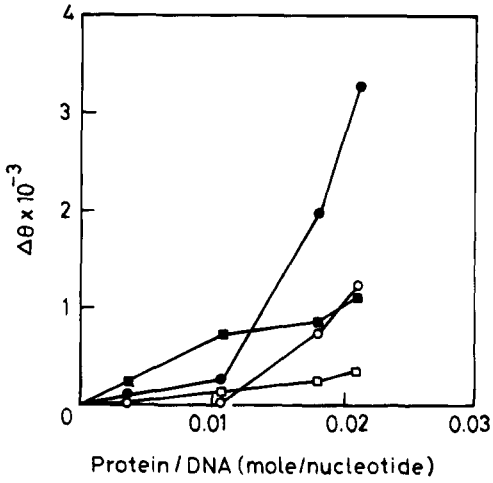

FIGURE 7: Effect of TP2 on the condensation of rat oligonucleosomal DNA. CD spectra of rat oligonucleosomal DNA were recorded after complexing with different concentrations of TP2 (CD spectra not shown. The changes in the ellipticity observed at 270 nm, $\Delta\theta$, are plotted against the protein/DNA ratio (mol/nucleotide): ●−●, rat oligonucleosomal DNA (0.2−0.8 kbp) + TP2 (ZnII); ○−○, rat oligonucleosomal DNA (0.2−0.8 kbp) + TP2 (EDTA); ■−■, rat oligonucleosomal DNA (0.8−4 kbp) + TP2 (ZnII); and □−□, rat oligonucleosomal DNA (0.8−4 kbp) + TP2 (EDTA).

but a significant decrease in the molar ellipticity at 270 nm (Figure 8A). However, this decrease in θ was severalfold

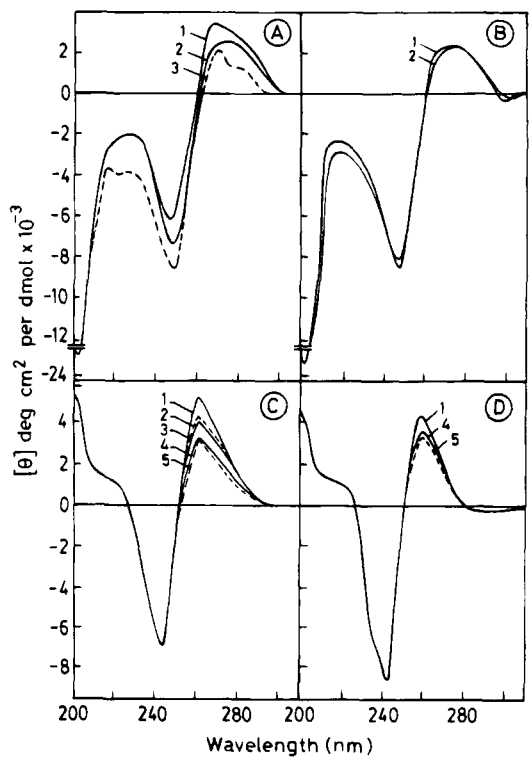

FIGURE 8: Effect of V8 protease-derived N- and C-terminal fragments of TP2 on the circular dichroic spectra of different copolymers. Peptides were directly mixed with different copolymers in the presence of 10 mM Tris-HCl, pH 7.5, 20 mM NaCl, and 10 μM $ZnSO_4$ or 5 mM EDTA as described under methods, and the spectra were recorded. (A) Spectra with poly(dG−dC)·poly(dG−dC) and poly(dG−dC)·poly(dG−dC)−C-terminal complex: 1, DNA alone; 2, DNA + C-terminal fragment incubated with ZnII (peptide to nucleotide ratio of 0.017); and 3, DNA + C-terminal fragment incubated with EDTA (peptide to nucleotide ratio of 0.017). (B) Spectra with poly(dG−dC)·poly(dG−dC) and poly(dG−dC)·poly(dG−dC)−N-terminal fragment complex: 1, DNA alone; 2, DNA + N-terminal fragment incubated with ZnII (peptide to nucleotide ratio of 0.021). (C) Spectra with poly(dA−dT)·poly(dA−dT) and poly(dA−dT)·poly(dA−dT)−C-terminal fragment complex: 1, DNA alone; 2, DNA + C-terminal fragment incubated with EDTA (peptide to nucleotide ratio of 0.017); 3, DNA + C-terminal fragment incubated with ZnII (peptide to nucleotide ratio of 0.017); 4, DNA + C-terminal fragment incubated with ZnII (peptide to nucleotide ratio of 0.021); and 5, DNA + C-terminal fragment incubated with EDTA (peptide to nucleotide ratio of 0.021). (D) Spectra with poly(dA−dT)·poly(dA−dT) and poly(dA−dT)·poly(dA−dT)−N-terminal fragment complex: 1, DNA alone; 2, DNA + N-terminal fragment incubated with ZnII (peptide to nucleotide ratio of 0.021); and 5, DNA + N-terminal fragment incubated with EDTA (peptide to nucleotide ratio of 0.021).

lower than that observed with intact TP2. Addition of EDTA did not affect this condensation brought about by the C-terminal fragment. In the case of poly(dA−dT)·poly(dA−dT), addition of the N-terminal fragment resulted in a small decrease in the ellipticity which was, however, EDTA insensitive (Figure 7D). On the other hand, addition of the C-terminal fragment to poly(dA−dT)·poly(dA−dT) at a similar mol/nucleotide ratio of that of the N-terminal fragment resulted in a substantial decrease in the ellipticity. But this decrease was also severalfold lower than that observed with intact TP2. Again it is interesting to note that this condensation is also EDTA insensitive. A summary of the ellipticity values obtained with N- and C-terminal fragments is given in Table 2.

Table 2: Summary of the Ellipticity Values Obtained from Circular Dichroic Spectra of Various Nucleic Acids upon Interaction with V8 Protease-Derived N- and C-Terminal Fragments of TP2

nucleic acids and polypeptides	polypeptide/DNA ratio (mol/nucleotide)	mean residue ellipticity[a] +ZnII	+EDTA
poly(dG−dC)·poly(dG−dC)		2477	2477
+N-terminal fragment	0.021	2477	2477
poly(dG−dC)·poly(dG−dC)		3468	3468
+C-terminal fragment	0.017	3468	3468
	0.021	2477	1981
poly(dA−dT)·poly(dA−dT)		4459	4459
+N-terminal fragment	0.017	4459	4459
	0.021	3468	3368
poly(dA−dT)·poly(dA−dT)		4458	4458
+C-terminal fragment	0.017	3591	3715
	0.021	2972	2922

[a] The ellipticity values are from Figure 3. The CD spectra were recorded with two independent preparations of the polypeptide fragments. Each spectrum is an average of four repeated recordings. The variation between the two independent experiments is within an error of 5%.

DISCUSSION

In the present investigation, we have been mainly concerned with two aspects of mammalian spermatidal protein TP2. First is a comparative study of its ability to bring about condensation of various synthetic polynucleotides to examine whether it shows any sequence preference. Second is the role of zinc in the TP2-mediated condensation process. The circular dichroic spectra obtained with nucleoprotein complexes of TP2 with different nucleic acids have been shown that the synthetic alternating copolymers poly(dG−dC)·poly(dG−dC) and poly(dA−dT)·poly(dA−dT) are condensed much more effectively by TP2 than either of the homoduplexes poly(dG)·poly(dC) or poly(dA)·poly(dT) or even random sequence-containing rat oligonucleosomal DNA. A quantitative analysis of the net condensation observed at an equivalent protein to nucleotide ratio shows that the fold differences for poly(dG−dC)·poly(dG−dC) and poly(dA−dT)·poly(dA−dT) are 11.9 and 3.7, respectively, over rat oligonucleosomal DNA, indicating that TP2 does show sequence preference in bringing about condensation. Among the two alternating copolymers, poly(dG−dC)·poly(dG−dC) is condensed at least 3-fold more effectively than poly(dA−dT)·poly(dA−dT). Another interesting observation is with respect to the condensation of poly(dI−dC)·poly(dI−dC) which is much lower in comparison to that of poly(dG−dC)·poly(dG−dC). In this context, it is worth noting that the shape of the spectrum of poly(dI−dC)·poly(dI−dC) resembles that of left-handed DNA. The original suggestion that it adapts a left-handed helix has been questioned by more recent experiments (Wang & Keiderling, 1993). It is now believed that this nucleic acid does exist in a B or a closely related B form. If one assumes that there is not much deviation of the helical nature of poly(dI−dC)·poly(dI−dC) from that of B-DNA, then our results showing that the condensation of poly(dI−dC)·poly(dI−dC) by TP2 is much less than poly(dG−dC)·poly(dG−dC) suggest that a guanine base is preferred for interaction with TP2 and the ensuing condensation of the polynucleotides. Therefore these results clearly suggest that the condensation brought about by TP2 is not a random process but shows a sequence preference.

Another important observation made in the present study is the effect of EDTA preincubation on the condensation property of TP2. This EDTA pretreatment resulted in a significant decrease (up to 40%) in TP2-mediated condensation of poly(dG−dC)·poly(dG−dC), poly(dA−dT)·poly(dA−dT), and also rat oligonucleosomal DNA (Table 1). In one of our recent studies we have shown that TP2 has predominantly a type I β turn structure contributed by several proline residues in the N-terminal region (Kundu & Rao, 1994). There was a small but significant alteration in the dichroic spectrum of TP2 upon treatment with EDTA. Therefore it is quite likely that the zinc-stabilized secondary structure of TP2 is involved in the sequence preference which we have noted in the condensation process. However, it can be noted that the condensation was not completely abolished upon pretreatment with EDTA. We consider two possibilities for this partial effect of EDTA pretreatment. Firstly, it is possible that zinc might be tightly coordinated which is difficult to be quantitatively removed by EDTA treatment. A second more likely possibiliity is that the condensation observed in the presence of EDTA is due to the basic amino acid stretches present at the C-terminal one-third of the TP2 molecule (see below).

Our recent demonstration that zinc-binding sites are localized to the V8 protease-derived N-terminal polypeptide fragment (Kundu & Rao, 1994) gave us an opportunity to individually study the effects of both the N- and C-terminal polypeptide fragments on DNA condensation. These results have revealed several interesting features. Firstly, it is clear that the N-terminal domain does not condense either poly(dG−dC)·poly(dG−dC) or poly(dA−dT)·poly(dA−dT). On the other hand, the C-terminal fragment condenses both the alternating copolymers. Between the two, copolymer poly(dA−dT)·poly(dA−dT) is condensed more efficiently than poly(dG−dC)·poly(dG−dC). More importantly, the condensation brought about by the C-terminal domain is insensitive to EDTA. These results therefore suggest that the C-terminal fragment is probably the condensing domain of TP2. A careful analysis of the amino acid sequence of TP2 reveals that most of the lysine and arginine residues are localized in this region of the TP2 molecule. It may be worth mentioning here that histone H1 and more specifically its C-terminal domain cause DNA condensation with a preference to oligo(dA−dT) tracts in the DNA duplex (Moran *et al.*, 1985). Therefore, it is very likely that the interaction of the C-terminal domain of TP2 is similar to that of histone H1.

While considering the condensation of DNA brought about by the C-terminal domain of TP2, we also have to compare the relative extent of condensation with that brought about by intact TP2. At an equivalent protein/polypeptide to nucleotide ratio, intact TP2 condenses both poly(dG−dC)·poly(dG−dC) and poly(dA−dT)·poly(dA−dT) much more efficiently than just the C-terminal domain. However, it is clear from our studies that the N-terminal domain per se does not possess a significant DNA-condensing property. Therefore, we would like to interpret these observations as follows. The zinc-induced/stabilized structure in the N-terminal domain of TP2 is probably used as a recognition polypeptide fold for primary interaction with DNA with a preference for GC-rich sequences. Such an interaction might generate subtle alterations in the secondary structure of DNA so as to be condensed more effectively by the C-terminal domain. We are aware of the fact that the CD spectral analysis gives information only about the secondary and

tertiary structures of DNA in the nucleoprotein complexes and does not throw any light on the primary recognition process. However, we would like to add that recently we have carried out a series of experiments on the interaction of TP2 with a synthetic oligonucleotide containing a human CpG island sequence using gel mobility shift assays. These experiments have clearly shown that TP2 forms a specific complex with this oligonucleotide in the presence of poly(dI)·poly(dC) as a competitor. Pretreatment of the protein with 5 mM EDTA decreased the specific complex by more than 90% (manuscript in preparation).

What is the biological significance of such a hypothetical two domain structure of TP2? As mentioned in the introduction, the appearance of TP2 during mammalian spermiogenesis coincides with (a) transformation of nucleosome type of chromatin into a smooth chromatin fiber, (b) initiation of chromatin condensation, and (c) cessation of transcription. Since TP2 preferentially condenses poly(dG−dC)·poly(dG−dC) more efficiently than other types of nucleic acids, we can speculate that chromatin condensation is initiated at GC-rich sequences in the genome. One of the well-defined and well-characterized GC-rich sequences in the eukaryotic genome is CpG islands (Antequera & Brid, 1993; Craig & Bickmore, 1994) which are widely and randomly distributed in the eukaryotic genome and occur at 5′-flanking, intronic, and 3′-flanking regions of several structural genes. Thus, these CpG islands may provide the initiation sites for chromatin condensation by TP2. A subclass of CpG islands is the GC-rich promoter sequences present in most of the mammalian housekeeping genes (Bernardi, 1989). Consequently, recognition of these promoter elements and ensuing condensation of DNA may also lead to cessation of transcription, the other physiological event that is coincidental with the appearance of TP2 during mammalian spermiogenesis.

ACKNOWLEDGMENT

We would like to thank Prof. K. R. K. Easwaran of the Molecular Biophysics Unit for permitting us to use the spectropolarimeter and the skillful technical assistance of Mr. M. Govindaraju in recording the CD spectra.

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BI9424003