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Interaction of Nucleosome Core DNA with Transition Proteins 1 and 3 from Boar Late Spermatid Nuclei

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The DNA binding properties of boar transition proteins 1 and 3 (TP1 and TP3) were studied by means of physicochemical techniques. The ultraviolet difference absorption spectra upon TP1 and TP3 binding to rat liver nucleosome core DNA (double-stranded DNA) showed TP1- and TP3-induced hyperchromicity at 260 nm, which is suggestive of local melting of DNA. CD measurements of TP1-DNA and TP3-DNA complexes indicated that the binding of TP1 and TP3 induced different conformational changes in DNA, probably including local melting of DNA. Thermal melting studies on the binding of TP1 and TP3 to DNA showed that although at 1 mM NaCl TP1 and TP3 caused slight stabilization of the DNA against thermal melting, destabilization of the DNA was observed at 50 mM NaCl. From the results of quenching of the tyrosine fluorescence of TP1 and the tryptophan fluorescence of TP3 upon their binding to double-stranded and single-stranded boar liver nucleosome core DNA at 50 mM NaCl, the apparent association constants for the binding of TP1 to double- and single-stranded DNA were calculated to be 8.0×10^4 and 1.3×10^5 M^{-1}, respectively, and those for the binding of TP3 to double- and single-stranded DNA to be 7.1×10^4 and 1.8×10^5 M^{-1}, respectively. These results suggest that TP1 and TP3, having higher affinity for single-stranded DNA, induce local destabilization of DNA, probably through the stacking of Tyr32 and Trp18 with nucleic acid bases, respectively.

Key words: DNA-protein interaction, late spermatid nuclei, transition protein.

The mechanism of transformation of the nucleosome type of chromatin into nucleoprotamine fibers is rather poorly understood. Direct transition from the nucleosome type of chromatin organization to nucleoprotamine fibers occurs in most species, exceptions being mammals (*1*, *2*). In mammals, spermatidal nuclear transition proteins (TP1-4) appear transiently in elongating spermatids during the process of transformation from the nucleosome type of chromatin to nucleoprotamine fibers (*3-6*). The amino acid sequences of TP1 and TP2, and the nucleotide sequences of their genes are known (*7-11*), and attempts have been made to elucidate how they are transcriptionally and translationally regulated (*12*, *13*). It has been postulated that rat TP1 is a DNA melting protein, its action being mediated through the intercalation of its tyrosine residue between the nucleic acid bases (*14*), and destabilizes compact nucleosome core particles (*15*), and that rat TP2 with two possible zinc fingers is a DNA stabilizing protein (*16*, *17*). Recently, we developed methods for isolating boar TP1, TP3, and TP4 (*18*, *19*), and reported that boar TP3 exhibits 27% sequence similarity to boar TP1 (*20*). The ram and boar TP3s migrate similarly on acid urea- and SDS-PAGE (*5*, *21*). The function of TP3, however, has not been elucidated. Probes like micrococcal nuclease and DNase I have been reported to be inaccessible to the chromatin structure of elongating spermatids (*22*). Hence, we have investigated the nature of the interaction of boar TP1 and TP3 with nucleosome core DNA, *in vitro*, by means of physicochemical techniques, which should yield valuable information from which one can deduce their biological significance. In this paper, we report that boar TP1 and TP3 have higher affinity for single-stranded DNA, and induce local destabilization of DNA, probably through the stacking of Tyr32 and Trp18 with nucleic acid bases, respectively.

MATERIALS AND METHODS

Purification of TP1 and TP3—TP1 and TP3 were isolated and renatured by our methods (*18*): Acid-extracted basic proteins from boar late spermatid nuclei were chromatographed on Fractogel EMD SO_3^- 650 (M) with a linear 0.2-3.0 M NaCl gradient in 0.1 M sodium acetate-HCl buffer, pH 2.0, at 4°C. The TP-containing fractions were subjected to HPLC on Nucleosil 300 7C18 (*21*). The fractions containing TP1 and TP3 were further purified by HPLC on Diol-120 in 0.1% trifluoroacetic acid/10% acetonitrile at room temperature, and on Hitachi #3057 with a linear 0-40% acetonitrile gradient in 0.1% trifluoroacetic acid at 40°C, respectively. The purified TP was dissolved in

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Abbreviations: T_m, melting temperature; TP, transition protein.

32 mM dithiothreitol/8 M urea/10 mM Tris-HCl, pH 8.8, and then incubated for 1 h at 40°C. After the pH of the incubated solution had been adjusted to 2.0 with 10% trifluoroacetic acid, TP was dialyzed against 1 mM NaCl/0.1 mM EDTA/1 mM Tricine-NaOH, pH 7.4.

Preparation of Rat and Boar Liver Nucleosome Core DNA—Rat and boar liver nucleosome core particles were prepared from micrococcal nuclease digests of their liver nuclei by a slight modification of the method of Rill *et al.* (*23*). The nucleosome core particle DNA was obtained from the nucleosome core particles by treatment with pronase E [EC 3.4.24.4], phenol/chloroform extraction, and subsequent ethanol precipitation. This DNA was chromatographed on Sepharose 4B (1.5×75 cm) in 0.15 M NaCl/15 mM sodium citrate buffer, pH 7.0. An aliquot of each eluted fraction was analyzed by 3.5% polyacrylamide-0.5% agarose gel electrophoresis in 2 mM EDTA/20 mM sodium acetate/40 mM Tris-HCl buffer, pH 7.8. The fraction containing about 145 base pair length DNA was dialyzed against 1 mM NaCl/0.1 mM EDTA/1 mM Tricine-NaOH, pH 7.4. The dialyzed DNA was used as the source of double-stranded DNA. Single-stranded DNA was prepared by heating the nucleosome core particle DNA at 90°C for 15 min and then rapid cooling on ice.

Preparation of Complexes of TP with DNA—Rat liver nucleosome core DNA-TP complexes were prepared by direct mixing. To a solution of DNA in 1 mM NaCl/0.1 mM EDTA/1 mM Tricine-NaOH buffer, pH 7.4, the same buffer or 500 mM NaCl/0.1 mM EDTA/1 mM Tricine-NaOH buffer, pH 7.4, and TP in 1 mM NaCl/0.1 mM EDTA/1 mM Tricine-NaOH buffer, pH 7.4, were added with gentle stirring. Each mixture was incubated at 37°C for 12 h. The final concentrations of the nucleic acids added were calculated using the following absorption coefficients/mol phosphate at 260 nm and 25°C: double-stranded DNA, 6.5×10^3; single-stranded DNA, 9.4×10^3. The concentration of DNA was 60 μM with respect to phosphorus. The concentrations of TP1 and TP3 were determined by amino acid analysis. The TP/DNA molar ratios were calculated using 6,219 and 9,134 as the M_r of TP1 and TP3, respectively, and 97,150 as the M_r of the 145 base pair length DNA.

CD Studies—CD spectra for binding of TP1 and TP3 to DNA were measured with a Jasco J-500 spectropolarimeter at 25°C with nitrogen flushing. The results were expressed as the mean residue ellipticity $[\theta]$ in deg·cm²·dmol⁻¹.

Ultraviolet Difference Absorption Spectroscopy—The ultraviolet difference absorption spectra for binding of TP to DNA were recorded with a double beam Hitachi U-2000 spectrophotometer at 25°C.

Thermal Melting of DNA and the DNA-TP Complexes—Thermal denaturation of DNA and its complex with TP at low (1 mM NaCl) and high (50 mM NaCl) ionic strength was recorded with a Beckman DU-8 spectrophotometer. The heating rate was set at 1.5 min for every degree increase in the temperature. Absorbance was recorded at 1°C intervals in the temperature range of 37-90°C. The concentrations of TP1 and TP3 were below the ranges which resulted in the precipitation of nucleoprotein complexes. There was no change in the A_{320}/A_{260} ratio during the course of the experiment. The first derivative values were calculated from the absorbance data by the 3-point average method described by Li (*24*) and Ansevin (*25*).

Fluorescence Measurements—The fluorescence spectra of TP1 and TP3, and their complexes with double-stranded and single-stranded boar liver nucleosome core DNA were recorded with a Hitachi 204 fluorescence spectrophotometer at 25°C. The tyrosine fluorescence spectrum for free TP1 in solution (50 mM NaCl/0.1 mM EDTA/1 mM Tricine-NaOH, pH 7.4) was recorded in a cuvette of 1-cm path length with excitation at 276 nm. The maximum fluorescence emission was 305 nm. The tryptophan fluorescence spectrum for free TP3 in the same solution was recorded in a cuvette of 1-cm path length with excitation at 295 nm. The maximum fluorescence emission was 340 nm. The spectra were recorded for a fixed amount of each TP with increasing concentrations of nucleic acids in 50 mM NaCl/0.1 mM EDTA/1 mM Tricine-NaOH buffer, pH 7.4. The screening effects of DNA on tyrosine and tryptophan fluorescence were corrected for by using *N*-acetyltyrosine ethylester and *N*-acetyltryptophan ethylester at concentrations that had the same relative fluorescence intensity as the TP1 and TP3 solutions, respectively. The final concentrations of the nucleic acids added were expressed as the molar concentrations of bases. In order to calculate the binding constant, K, the fluorescence quenching data were plotted according to the equation described by Kelley *et al.* (*26*):

$$1/\Delta F = 1/(K[N]\Delta F_\infty) + 1/\Delta F_\infty$$

where ΔF = decrease of fluorescence intensity at the emission maximum in the presence of concentration N of DNA, and ΔF_∞ = decrease in fluorescence intensity at infinite ligand concentration. When $1/\Delta F$ is plotted against $1/[N]$, a straight line is obtained for which the slope = $1/K\Delta F_\infty$ and the intercept = $1/\Delta F_\infty$.

RESULTS

To study the binding of TP1 to DNA and TP3 to DNA by ultraviolet difference absorption spectroscopy, CD, and thermal denaturation, we used rat liver nucleosome core DNA as a model DNA, since TP1-boar liver nucleosome core DNA and TP3-DNA complexes were precipitated at a TP/DNA molar ratio of over 0.5.

Ultraviolet Difference Absorption Spectroscopic Studies—In the presence of 1 mM NaCl, TP1-DNA, and TP3-DNA complexes showed positive bands at about 262 and 260 nm, respectively (subsidiary materials Fig. S1). There was a linear increase in absorbance at 260 nm with increasing TP1/DNA molar ratio (Fig. 1). In the presence of 50 mM NaCl, TP1-DNA and TP3-DNA complexes showed positive bands at 270 and 265 nm, respectively (subsidiary materials Fig. S2). There were increases in absorbance at 260 nm of 5 and 12% at TP1/DNA molar ratios of 0.97 and 1.9, respectively, and increases in absorbance at 260 nm of 3 and 10% at TP3/DNA molar ratios of 0.90 and 1.8, respectively. At the same time there was a negligible spectral contribution of TP itself. At higher TP/DNA molar ratios, however, the light scattering contribution was not negligible.

These results suggest that each TP exhibits DNA-melting activity. The different peak positions of the ultraviolet difference absorption spectra for the TP1-DNA and TP3-DNA complexes may reflect changes in the environment of

the bases upon interaction with TP1 and TP3.

Effects of TP1 and TP3 on CD Spectra of DNA—TP binding to double-stranded DNA shifted the CD crossover point to a slightly longer wavelength in the presence of 1 mM NaCl (Figs. 2A and 3A). The CD positive band of the TP1-DNA complex at a TP1/DNA molar ratio of 1.9 increased slightly at wavelengths longer than 272 nm (Fig. 2A, curve 2), and that of the TP3-DNA complex at a TP3/DNA molar ratio of 1.8 increased at wavelengths longer than 258 nm (Fig. 3A, curve 2). In the presence of 50 mM NaCl, the CD positive band of the TP1-DNA complex increased slightly at wavelengths longer than 268 nm at a TP1/DNA molar ratio of 0.97 (Fig. 2B, curve 2). At the ratio of 1.9, the CD positive band increased slightly at wavelengths longer than 284 nm (Fig. 2B, curve 3). On the other hand, the CD positive band of the TP3-DNA complex increased slightly at wavelengths longer than 278 nm at a TP3/DNA molar ratio of 0.9 (Fig. 3B, curve 2). At the ratio of 1.8, the CD positive band increased considerably at wavelengths longer than 274 nm (Fig. 3B, curve 3). The spectral contributions of TP itself were negligible at wavelengths longer than 245 nm at these concentrations (*18*). The CD spectra of the TP1-double-stranded DNA complexes were like those of the TP1-single-stranded DNA complexes in part at wavelengths longer than 272 nm in the presence of 1 mM NaCl, and at wavelengths longer than 284 nm in the presence of 50 mM NaCl, while the CD spectra of the TP3-double-stranded DNA complexes were like those of the TP3-single-stranded DNA complexes in part at wavelengths longer than 267 nm in the presence of 1 mM NaCl, and at wavelengths longer than 280 nm in the presence of 50 mM NaCl (subsidiary materials Fig. S3). These results suggest that the CD changes observed for each TP-double-stranded DNA complex are due to the formation of TP complexes with DNA containing a locally induced single-strand, and that TP3 binding to double-stranded DNA brought about the local formation of single-stranded DNA in a different manner from TP1 binding to double-stranded DNA.

Effect of Binding of TP1 and TP3 on the Thermal Melting of DNA—At low ionic strength, T_m of DNA increased slightly from 59 to 63°C at a TP1/DNA molar ratio of 3.9, with the appearance of another small peak at 81°C (Fig. 4, curve 4). In addition, there was a 4-6% decrease in the overall hyperchromicity of the TP1-DNA

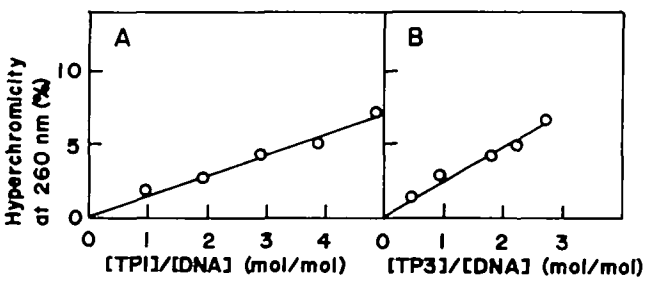

Fig. 1. **Effect of binding of TP1 and TP3 on ultraviolet absorption at 260 nm of double-stranded DNA.** The observed increase in A_{260} for TP binding to double-stranded DNA was plotted as a function of increasing TP/DNA molar ratio. A and B, effects of TP1 and TP3 in 1 mM NaCl/0.1 mM EDTA/1 mM Tricine-NaOH buffer, pH 7.4, respectively.

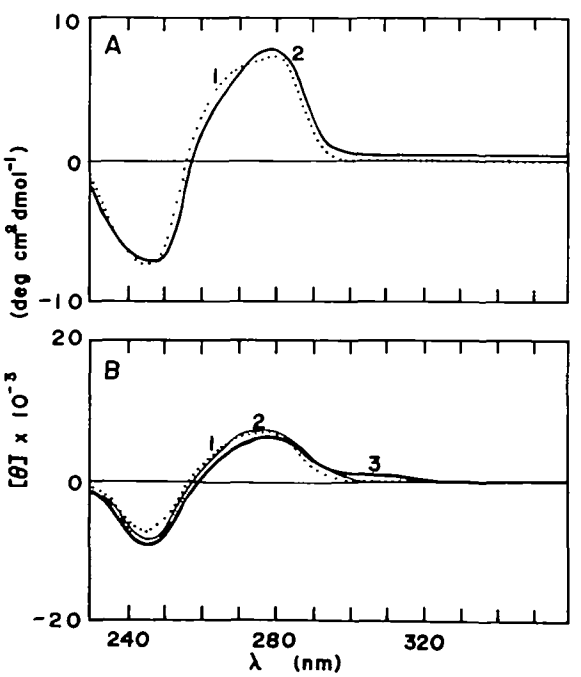

Fig. 2. **Effect of TP1 on the CD spectrum of double-stranded DNA.** A: In 1 mM NaCl/0.1 mM EDTA/1 mM Tricine-NaOH buffer, pH 7.4; 1 (·····), DNA alone; 2 (—), TP1/DNA molar ratio of 1.9. B: In 50 mM NaCl/0.1 mM EDTA/1 mM Tricine-NaOH buffer, pH 7.4; 1 (·····), DNA alone; 2 (—) and 3 (—), TP1/DNA molar ratios of 0.97 and 1.9, respectively.

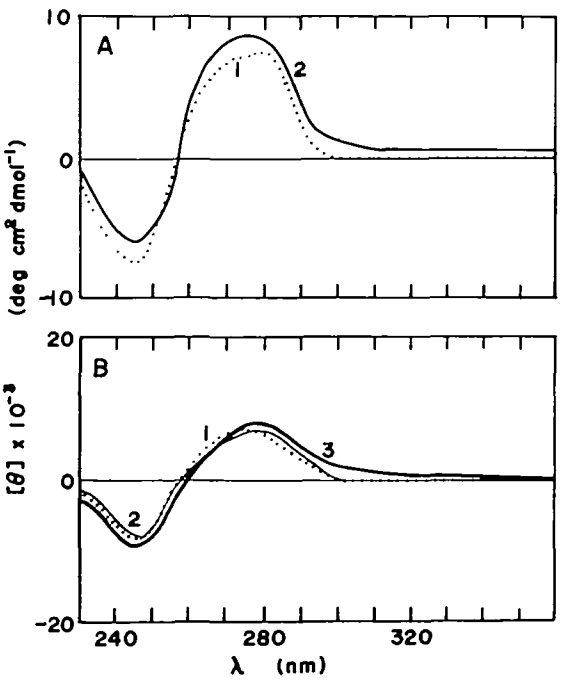

Fig. 3. **Effect of TP3 on the CD spectrum of double-stranded DNA.** A: In 1 mM NaCl/0.1 mM EDTA/1 mM Tricine-NaOH buffer, pH 7.4; 1 (·····), DNA alone; 2 (—), TP3/DNA molar ratio of 1.9. B: In 50 mM NaCl/0.1 mM EDTA/1 mM Tricine-NaOH buffer, pH 7.4; 1 (·····), DNA alone; 2 (—) and 3 (—), TP3/DNA molar ratios of 0.90 and 1.8, respectively.

complex (Fig. 4A, curves 2-4). T_m of DNA increased slightly from 59 to 62°C at a TP3/DNA molar ratio of 2.7 with the appearance of another peak at 74°C (Fig. 5, curve 4), and a 1-3% decrease in the overall hyperchromicity of the TP3-DNA complex (Fig. 5A, curves 2-4). These results suggest that DNA is slightly stabilized against thermal

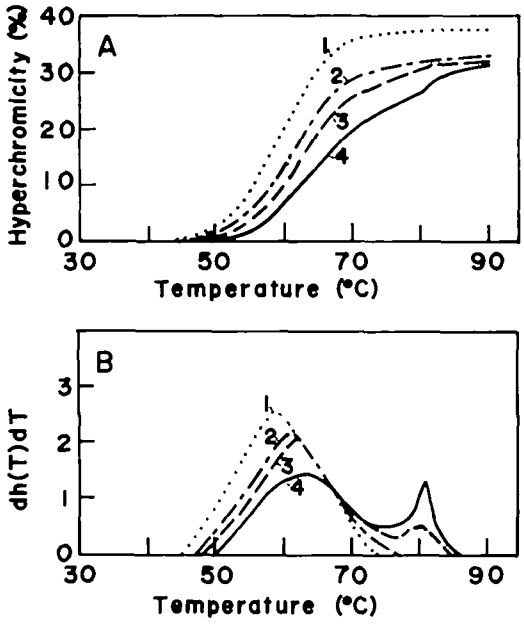

Fig. 4. **Effect of binding of TP1 on the thermal melting of double-stranded DNA under low salt conditions (1 mM NaCl/ 0.1 mM EDTA/1 mM Tricine-NaOH buffer, pH 7.4).** A, hyperchromicity profile; and B, first derivative profile. 1 (·····), DNA alone; 2 (–·–), 3 (- - -), and 4 (——), TP1/DNA molar ratios of 0.97, 1.9, and 3.9, respectively.

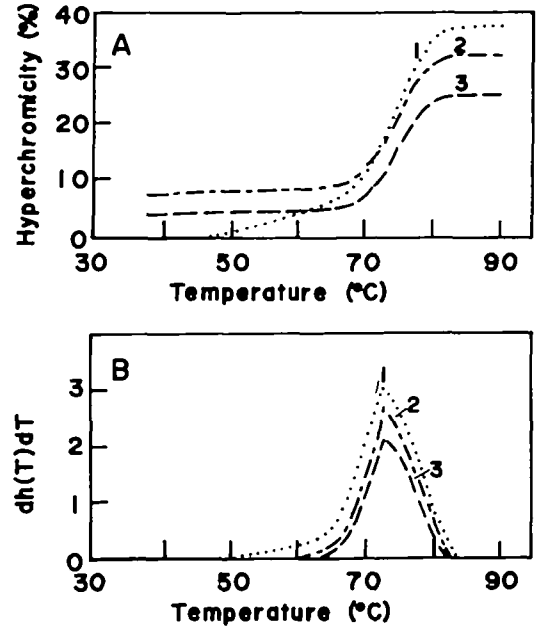

Fig. 6. **Effect of binding of TP1 on the thermal melting of double-stranded DNA under high salt conditions (50 mM NaCl/ 0.1 mM EDTA/1 mM Tricine-NaOH buffer, pH 7.4).** A, hyperchromicity profile; and B, first derivative profile. 1 (·····), DNA alone; 2 (–·–) and 3 (- - -), TP1/DNA molar ratios of 0.97 and 1.9, respectively.

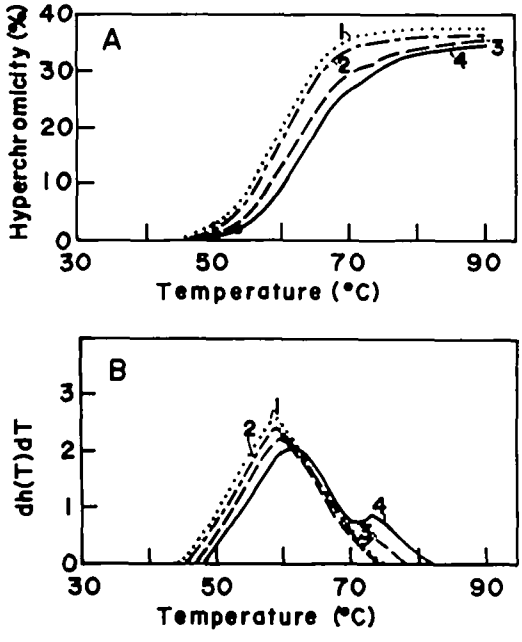

Fig. 5. **Effect of binding of TP3 on the thermal melting of double-stranded DNA under low salt conditions (1 mM NaCl/ 0.1 mM EDTA/1 mM Tricine-NaOH, buffer, pH 7.4).** A, hyperchromicity profile; and B, first derivative profile. 1 (·····), DNA alone; 2 (–·–), 3 (- - -), and 4 (——), TP3/DNA molar ratios of 0.90, 1.8, and 2.7, respectively.

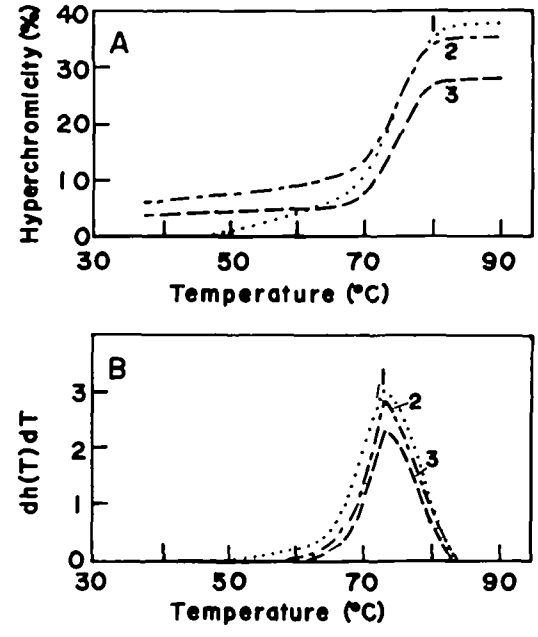

Fig. 7. **Effect of binding of TP3 on the thermal melting of double-stranded DNA under high salt conditions (50 mM NaCl/ 0.1 mM EDTA/1 mM Tricine-NaOH, buffer, pH 7.4).** A, hyperchromicity profile; and B, first derivative profile. 1 (·····), DNA alone; 2 (–·–) and 3 (- - -), TP3/DNA molar ratios of 0.90 and 1.8, respectively.

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                    1              10             20             30 *
TP1  ------------ST----SRKLKSHGMRRGKNRAPHKGVKR---GGSKRKY-RK
                 ::     ::  :    ::: :   : :::     :  ::  :
TP3  AKVTEKSWQPQTTSTKRWKKRKTPSQPRSRGKVRKIYKKVKRPLHVCSRKKYSPK
     1          10        * 20          30          40          50

          40          50
TP1  GSLKSR--KRCDDANRNYRSHL
          ::  ::   ::
TP3  VITTSRRQKRARRAN-KFETIP
        60        70
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Fig. 8. **Comparison of the amino acid sequence of boar TP3 with that of boar TP1.** Dashes represent gaps introduced to optimize the alignment. Identical amino acids are shown by : between corresponding letters. The residue that is probably involved in the interaction of the TP with nucleic acids is indicated by *. These sequences were compared using the GENETYX PROGRAM (*20*).

denaturation upon the binding of each TP at low ionic strength.

On the other hand, in the presence of 50 mM NaCl, the observed thermal melting markedly showed the biphasic character of the thermal melting transition of the DNA-TP1 and DNA-TP3 complexes (Figs. 6 and 7). In addition, there were 5-12 and 3-10% decreases in the overall hyperchromicity of the DNA-TP1 and DNA-TP3 complexes, respectively (Figs. 6A and 7A, curves 2 and 3). The decrease in the overall hyperchromicity of the TP-DNA complex at 50 mM NaCl corresponded to the increase in absorbance at 260 nm in the ultraviolet difference absorption spectra for the TP-DNA complex, as described above. These results indicate that the fraction of DNA saturated with TP (with T_m lower than 37°C, which was the temperature at which the TP-DNA complexes were prepared in the experiment) had melted almost completely, the melting of the remaining DNA being virtually unperturbed (with T_m of 73°C). Jensen *et al.* (*27*) reported that the biphasic thermal melting transitions of the DNA-melting gene 32 protein complexes with polynucleotides are dominated by tight and cooperative binding of the gene 32-protein to the single-stranded polynucleotide lattice, on the basis of the good fit to the data obtained on theoretical analysis of the changes in the shapes of melting transitions in the presence of melting ligands, as binding of the ligands becomes cooperative (*28*). Each of TP1 and TP3, whose complexes with DNA showing biphasic thermal melting transitions, destabilizes DNA, possibly through tight and cooperative protein binding.

Quenching of the Tyrosine Fluorescence of TP1 and of the Tryptophan Fluorescence of TP3 upon Binding to Nucleosome Core DNA—TP1 has two tyrosine residues (Fig. 8). When TP1 was irradiated at 276 nm, it yielded a fluorescence emission spectrum with a maximum wavelength of 305 nm, which corresponded to the emission maximum of free tyrosine. When TP3, having two tyrosine and two tryptophan residues (Fig. 8), was irradiated at 295 nm, under which condition only tryptophan residues are excited, TP3 yielded a fluorescence emission spectrum with a maximum wavelength of 340 nm, while the emission maximum of free tryptophan is 350 nm. Each of the fluorescence intensity at 305 nm of TP1 and that at 340 nm of TP3 was quenched upon binding to double-stranded and single-stranded boar nucleosome core DNA. No peak

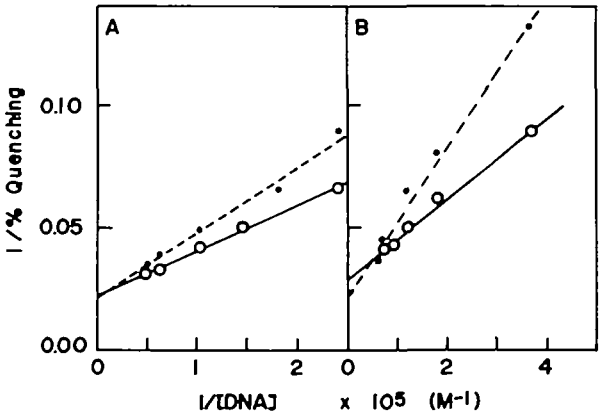

Fig. 9. **Dependence of the fluorescence quenching of TP1 and TP3 on the concentration of nucleic acids.** To calculate the binding constant, 1/% quenching was plotted against the reciprocal molar concentration of nucleic acid bases. A, TP1; B, TP3. The final concentrations of TP1 and TP3 were 5.4 and 2.4 μM, respectively. ●, double-stranded DNA; ○, single-stranded DNA.

shifted when increasing amounts of double-stranded and single-stranded DNA were added. The fluorescence quenching data were plotted according to the method of Kelley *et al.* (*26*) (Fig. 9). The association constants, K, for the binding of TP1 to double-stranded and single-stranded DNA were estimated to be 8.0×10^4 and 1.3×10^5 M^{-1}, respectively, while the K for the binding of TP3 to double-stranded and single-stranded DNA were 7.1×10^4 and 1.8×10^5 M^{-1}, respectively. These results indicate that the affinity of TP1 and TP3 for single-stranded DNA is higher than that for double-stranded DNA. By extrapolating the data obtained from the double-reciprocal plots of the fluorescence quenching of TP1 to infinite nucleic acid concentration, it can be calculated from the y intercept that the quenching is 49% with both double-stranded and single-stranded DNA. Tyr32 of TP1 is flanked by two basic clusters, and the 28-34 sequence of TP1 including Tyr32 is conserved among the TP1 family (*8*), while Tyr50 of boar TP1 is neither flanked by basic clusters nor conserved (*7*, *8*, *29*). Accordingly, Tyr32 of TP1 probably interacts with these nucleic acids. By the same procedure as in the case of TP1, it can be calculated from the y intercept that the fluorescence quenching of TP3 is 49% with double-stranded DNA and 38% with single-stranded DNA. TP3 as well as TP1 had higher affinity for single-stranded DNA, as described above. Trp18 of TP3 is flanked by two basic clusters such as in the TP1 family, and the 16-22 sequence of TP3 including Trp18 exhibits 43% similarity to the conserved 28-34 sequence of TP1, while Trp8 of TP3 is not flanked by basic clusters, nor does the 5-11 sequence of TP3 including Trp8 exhibits significant similarity (*20*) to the 28-34 sequence of TP1. Therefore, Trp18 of TP3 probably interacts with these nucleic acids.

DISCUSSION

Ultraviolet difference absorption and CD spectroscopy suggest that TP1 has DNA-melting activity (Figs. 1 and 2). Thermal denaturation studies substantiated the DNA destabilization by TP1 at 50 mM NaCl (Fig. 6). The quenching of the tyrosine fluorescence of TP1 (Fig. 9A) at

50 mM NaCl indicates that TP1 has higher affinity for single-stranded DNA than double-stranded DNA, and that Tyr32 of TP1 in the conserved 28-34 sequence (*7*, *8*, *29*) probably interacts with nucleic acids. Since boar TP1 exhibits 93% sequence similarity to rat TP1, these results indicate that boar TP1 probably interacts with nucleic acids in a similar manner to rat TP1 (*14*). Boar TP1 brings about local melting of DNA through intercalation of its tyrosine residue (Tyr32) with the DNA bases, while the basic residues, binding electrostatically to DNA phosphates, have a DNA-stabilizing effect. Although TP1 is more basic than histone H1 (*7*, *29*), the increase of 4°C in T_m of DNA upon binding to TP1 at 1 mM NaCl (Fig. 4) is less than that brought about by binding of histone H1 (32°C) (*14*). The net result of the local melting and stabilizing effects of interaction may be reflected in this slight increase in the T_m value upon binding to TP1 at 1 mM NaCl. However, the boar TP1-DNA complex exhibited a rather weak CD signal (Fig. 2), while addition of rat TP1 induced a marginal increase in the positive band at 275 nm and a slight decrease in the negative band at 246 nm at the molar ratios of both 0.5 and 1 (TP1/*Eco*RI linearized pBR322 DNA). The biphasic thermal melting transitions of the TP1-DNA complex at 50 mM NaCl (Fig. 6) indicate that TP1 destabilizes DNA, possibly through tight and cooperative protein binding with a T_m lower than 37°C, as described under "RESULTS," while rat TP1 brings about a decrease in T_m of DNA from 77 to 71°C at 50 mM NaCl, which has been explained as equilibrium destabilization (*14*). These somewhat different binding properties may be due to the difference in the preparation of DNA and TP-DNA complex.

On the other hand, boar TP3 exhibits 27% sequence similarity to boar TP1. TP3 is composed of an N-terminal region (residues 1-19), having Trp 18 flanked by two basic clusters, which is absent in the known TP1 group, and a C-terminal region (residues 20-76), exhibiting a close resemblance to boar TP1 (Fig. 8) (*20*). The quenching of the tryptophan fluorescence of TP3 (Fig. 9B) indicates that Trp18 of TP3 in the 16-22 sequence, exhibiting 43% similarity to the very conserved 28-34 sequence of TP1, probably interacts with nucleic acids. Tyr52 of TP3 in its 48-54 sequence, exhibiting 43% similarity to the conserved 28-34 sequence of TP1, is not flanked by two basic clusters such as in the TP1 family. Whether or not Tyr52 of TP3 is involved in the interaction with nucleic acids remains to be investigated. Thermal denaturation studies substantiated local melting of DNA by TP3 at 50 mM NaCl (Fig. 7). The biphasic thermal melting transitions of the TP3-DNA complex indicate that TP3 as well as TP1 destabilizes DNA, possibly through tight and cooperative protein binding. TP3-DNA complexes showed clear differences from TP1-DNA complexes in the CD spectra (Fig. 3), and ultraviolet difference absorption spectra with peak positions at 265 nm for TP3-DNA complexes and at 270 nm for TP1-DNA complexes. These results indicate that TP1 and TP3 induce different conformational changes in DNA. The mode of binding of TP3 to DNA is probably different from that of TP1.

The affinity of TP3 for single-stranded DNA was about 1.6-fold higher than that of TP1. The size of the indole ring of tryptophan is similar to that of purine bases, so that the stacking of tryptophan with DNA bases possibly involves only one strand of DNA (*30*). TP3 brought about local destabilization of DNA through interaction of its tryptophan residue (Trp18) with the DNA bases, while the basic residues, binding electrostatically to DNA phosphates, have a DNA-stabilizing effect. The net result of the local melting and stabilizing effects of interaction may be reflected in the slight increase of 4°C in the T_m value upon binding to TP3 at 1 mM NaCl (Fig. 5), as well as to TP1.

Baskaran and Rao suggested that TP2, having zinc finger structures, interacts with DNA in a sequence-specific manner, which in turn can influence the other two major events, namely, cessation of the transcription process and initiation of chromatin condensation, and that TP1, having basic amino acid residues evenly dispersed over its molecule, interacts with chromatin randomly, facilitating local destabilization of the nucleosome core particles through intercalation of Tyr32 with the DNA bases (*16*, *17*). Boar TP3, having basic amino acid residues evenly dispersed over the C-terminal two-thirds of its molecule, is present in late spermatids along with TP1 and TP2 (*20*, *21*). The binding of TP3 to DNA induced conformational changes in the DNA molecules, including local destabilization of the DNA, probably through the stacking of Trp18 with the DNA bases, as described above. Accordingly, TP3 is probably involved in the modulation process of the structure of nucleosome core particles to facilitate chromatin transformation into a nucleoprotamine structure in a somewhat related but different manner from TP1.

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Supplemental Materials

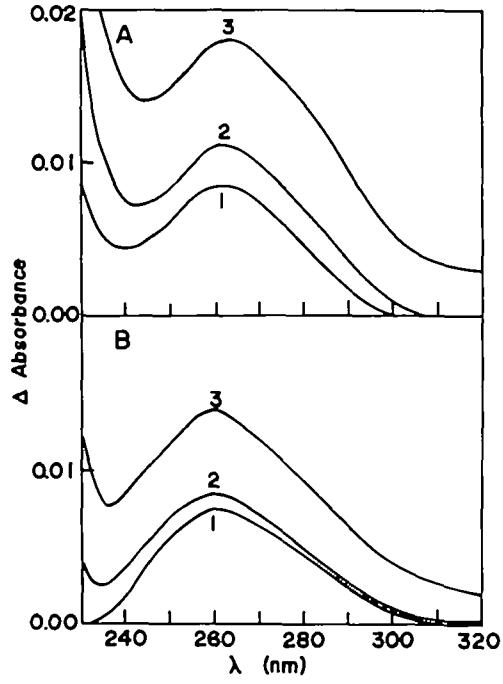

Fig. S1. **Ultraviolet difference absorption spectroscopy of double-stranded DNA upon binding to TP1 and TP3 in 1 mM NaCl/0.1 mM EDTA/1 mM Tricine-NaOH buffer, pH 7.4.** A: Curves 1-3 are the difference spectra at TP1/DNA molar ratios of 0.97, 1.9, and 2.9, respectively. B: Curves 1-3 are the difference spectra at TP3/DNA molar ratios of 0.9, 1.8, and 2.7, respectively.

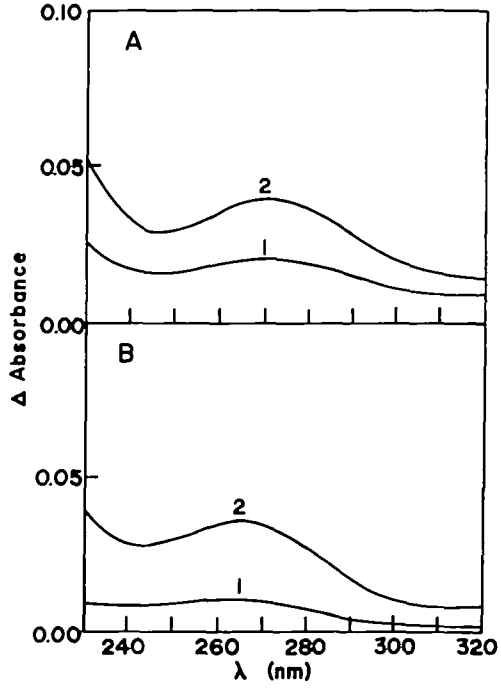

Fig. S2. **Ultraviolet difference absorption spectroscopy of double-stranded DNA upon binding to TP1 and TP3 in 50 mM NaCl/0.1 mM EDTA/1 mM Tricine-NaOH buffer, pH 7.4.** A: Curves 1 and 2 are the difference spectra at TP1/DNA molar ratios of 0.97 and 1.9, respectively. B: Curves 1 and 2 are the difference spectra at TP3/DNA molar ratios of 0.90 and 1.8, respectively.

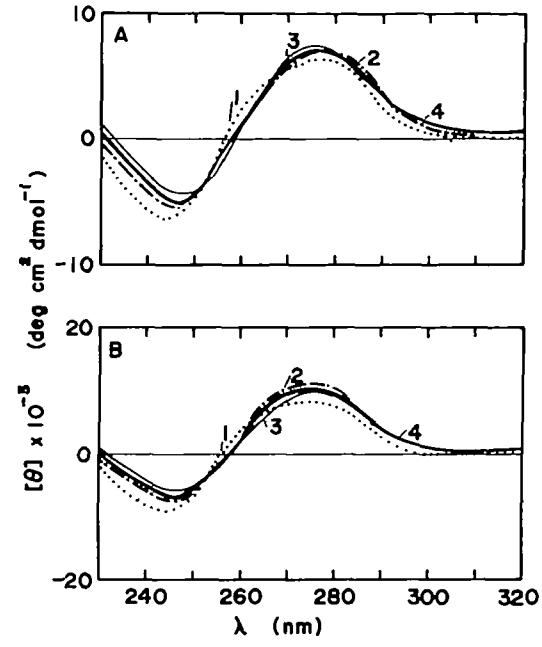

Fig. S3. **Effects of TP1 and TP3 on the CD spectrum of single-stranded DNA.** A: In 1 mM NaCl/0.1 mM EDTA/1 mM Tricine-NaOH buffer, pH 7.4; 1 (····), double-stranded DNA alone; 2 (—·—), single-stranded DNA alone; 3 (——), TP1/single-stranded DNA molar ratio of 1.9; 4 (——), TP3/single-stranded DNA molar ratio of 1.8. B: in 50 mM NaCl/0.1 mM EDTA/1 mM Tricine buffer, pH 7.4; 1 (·····), double-stranded DNA alone; 2 (—·—), single-stranded DNA alone; 3 (——), TP1/single-stranded DNA molar ratio of 1.9; 4 (——), TP3/single-stranded DNA molar ratio of 1.8.