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Interaction of Rat Testis Protein, TP, with Nucleic Acids *in Vitro*

FLUORESCENCE QUENCHING, UV ABSORPTION, AND THERMAL DENATURATION STUDIES*

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The nucleic acid binding properties of the testis protein, TP, were studied with the help of physical techniques, namely, fluorescence quenching, UV difference absorption spectroscopy, and thermal melting. Results of quenching of tyrosine fluorescence of TP upon its binding to double-stranded and denatured rat liver nucleosome core DNA and poly(rA) suggest that the tyrosine residues of TP interact/intercalate with the bases of these nucleic acids. From the fluorescence quenching data, obtained at 50 mM NaCl concentration, the apparent association constants for binding of TP to native and denatured DNA and poly(rA) were calculated to be 4.4×10^3 M^{-1}, 2.86×10^4 M^{-1}, and 8.5×10^4 M^{-1}, respectively. UV difference absorption spectra upon TP binding to poly(rA) and rat liver core DNA showed a TP-induced hyperchromicity at 260 nm which is suggestive of local melting of poly(rA) and DNA. The results from thermal melting studies of binding of TP to calf thymus DNA at 1 mM NaCl as well as 50 mM NaCl showed that although at 1 mM NaCl TP brings about a slight stabilization of the DNA against thermal melting, a destabilization of the DNA was observed at 50 mM NaCl. From these results it is concluded that TP, having a higher affinity for single-stranded nucleic acids, destabilizes double-stranded DNA, thus behaving like a DNA-melting protein.

Mammalian spermatogenesis is characterized by dramatic changes in the chromatin structure. The nucleosome type of chromatin structure is present in the spermatids until the stage of spermiogenesis in the rat, after which time the condensation of chromatin is initiated. The nucleosomes of round spermatids (stages 1–12) contain the somatic histones (H1a, H1bde, H1c, H2A, X2 (H2A variant), H2B, H3, and H4) as well as the testis-specific histones (H1t, TH2A, TH2B, and TH3) (1, 2). These histones are replaced by the testis-specific transition proteins TP and TP2 during stages 13–15 (3). These proteins are in turn finally replaced by protein S1 (equivalent to fish protamine) and TP3 during stages 16–19 (4, 5); protein S1 is the only basic protein present in the mature spermatozoa of the epididymis.

Among the testis-specific transition proteins, TP is a major and a rather interesting protein in that it is a small but highly basic protein made up of 54 amino acids (6). It contains 19% lysine and 21% arginine, thus falling in between the histones on the one hand and protein S1 (60% arginine) on the other. Although this protein, including its amino acid sequence (7), has been known for the last 10 years, nothing is known about the physiological significance of its transitory appearance during spermiogenesis. Since TP does interact with the DNA within the spermatid chromatin, we felt that the physicochemical nature of its interaction with DNA *in vitro* should give valuable information about its biological function. In this context, we have studied, *in vitro*, the interaction of TP with various nucleic acids by employing several physicochemical techniques. The results of investigations presented in this communication clearly show that TP is a DNA-melting protein, probably mediated through the interaction of its tyrosine residues with the nucleic acid bases.

MATERIALS AND METHODS

Bovine serum albumin, poly(rA), *N*-acetyl tyrosinamide, Sephadex G-100, and phenylmethylsulfonyl fluoride were purchased from Sigma.

Purification of TP—TP was purified from rat testes by a simplification of the procedure described by Platz *et al.* (8). About 50 g of testes obtained from male albino rats of I.I.Sc. strain were processed per batch. Initially, the sonication-resistant spermatid nuclei were purified from rat testes as described by Platz *et al.* (8). The sonication-resistant spermatid nuclei were extracted with 50–75 ml of 0.4 N HCl at 0–4 °C for 30 min, and centrifuged at 5,000 rpm for 5 min in a Sorvall RC5B centrifuge. The supernatant was made 5–25% with respect to trichloroacetic acid, and the precipitated proteins were collected by centrifuging at 10,000 rpm for 5 min. The precipitate was washed sequentially with chilled acid-acetone (prepared by adding 200 μl of concentrated HCl to 100 ml of acetone), acetone, and ether and dried under vacuum. The dry powder was dissolved in 1 ml of 7.5 M urea, 2% 2-mercaptoethanol, 0.9 N acetic acid and loaded on a Sephadex G-100 column (bed volume = 400 ml; 105 × 2.2-cm), which was pre-equilibrated with 1 mM HCl, and 4-ml fractions were collected. The absorbance of the eluate was measured at 230 nm. The elution profile showed three peaks, I, II, and III. The proteins in these peaks were identified as TH1, TP2, and TP, respectively, by analyzing them on an acid-urea 15% polyacrylamide gel (9).

Purification of Histone H1 from Rat Liver—Livers were removed after perfusion with normal saline, and the nuclei were purified as described earlier (10, 11). After the final purification step, the nuclei were washed with buffer containing 0.34 M sucrose, 10 mM Tris-HCl, pH 7.5, 25 mM KCl, 5 mM $MgCl_2$, 50 mM sodium bisulfite, 0.1% Triton X-100. The Triton-washed nuclei were first suspended by gentle hand homogenization with a loose fitting pestle in 0.3 M NaCl solution containing 10 mM Tri-HCl, pH 7.4, 50 mM sodium bisulfite at a ratio of 2 volumes per original weight of the liver. After keeping at 4 °C for 30 min, the nuclear suspension was centrifuged at 15,000 rpm for 30 min. The pellet was re-extracted by gentle homogenization in 2 volumes of 0.55 M NaCl, 10 mM Tris-HCl, pH 7.4, 50 mM sodium bisulfite, and after keeping overnight at 4 °C, the suspension was dialyzed overnight against cold water containing 0.2 mM phenylmethylsulfonyl fluoride with two changes after every 6 h. The dialyzed supernatant was lyophilized, and the dry powder was dissolved in 1 ml of 20 mM HCl, 50 mM NaCl, 5 M urea and loaded on a Bio-Gel P-100 column (bed volume = 150 ml; 85 × 0.75-cm) and eluted with 20 mM HCl, 50 mM NaCl. 1-ml fractions were collected, and the absorbance was measured at 230 nm. The fractions under the major peak were pooled, dialyzed as above, and lyophilized. The purity of histone

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H1 was confirmed by acid-urea polyacrylamide gel electrophoresis.

Fluorescence Measurements—The fluorescence measurements for TP and TP·nucleic acid complexes were made on the Hitachi 650-60 fluorescence spectrophotometer. The tyrosine fluorescence spectra for TP solution (in 1 mM sodium cacodylate, pH 7.4, 0.1 mM Na_2EDTA, 1–100 mM NaCl) were recorded in cells of 1-cm path length, with excitation wavelength of 276 nm. The fluorescence emission was found to be 305 nm. The slit widths for excitation and emission beams were 5 nm. The spectra were recorded in the presence of increasing concentrations of nucleic acids (DNA and poly(rA) in 1 mM sodium cacodylate pH 7.4 buffer containing 0.1 mM Na_2EDTA and 50 mM NaCl). The correction for the screening effect of DNA and poly(rA) on fluorescence was applied by using *N*-acetyl tyrosinamide at a concentration that had the same relative fluorescence intensity as the TP solution. The final concentrations of the nucleic acids added were calculated by using the following molar extinction coefficients (per mole of phosphate at 260 nm and 25 °C) and expressed as molar concentrations of bases: double-stranded DNA, 6.5×10^3; denatured DNA, 9.4×10^3; and poly(rA), 9.1×10^3. The concentration of TP was determined by the turbidometric method as described by Platz *et al.* (8). In order to calculate the binding constant, *K*, the fluorescence quenching data were plotted according to the equation described by Kelley *et al.* (12):

$$\frac{1}{\Delta F} = \frac{1}{K[N]\Delta F_\infty} + \frac{1}{\Delta F_\infty}$$

where ΔF = decrease of fluorescence intensity at emission maximum in the presence of concentration N of DNA or poly(rA) 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 = $1/\Delta F_\infty$.

Ultraviolet Difference Absorption Spectroscopy—The ultraviolet difference absorption spectra for binding of TP to DNA and poly(rA) were recorded as follows. Aliquots of stock solutions of poly(rA) and rat liver nucleosome core DNA (11) were diluted in the buffer solution (1 mM sodium cacodylate, pH 7.4, 0.1 mM Na_2EDTA, 1 or 50 mM NaCl) to give the required final concentrations. 1 ml of the solution was taken in the sample cuvette and its UV absorption spectrum was recorded on the double beam Shimadzu UV-190 spectrophotometer. The TP solution of known concentration was added in equal amounts to both the reference and sample cuvettes, and the spectra were recorded after mixing. The difference of the absorption of TP·poly(rA)·DNA complexes and poly(rA)/DNA were calculated at 5-nm intervals, and after taking the dilution factor into account, the difference spectra were drawn.

Thermal Melting Studies—The thermal melting studies on the binding of TP and liver histone H1 to calf thymus DNA at low and high ionic strengths were carried out on the Beckman DU 8 spectrophotometer. The absorbance values were recorded after every 1° interval in the temperature range of 30–90 °C. The DNA concentration used was in the range of 0.8–1.2 A_{260} units/ml. Before use, calf thymus DNA was sonified and subjected to pronase digestion and phenol/chloroform extraction in order to remove any impurities. The first derivative values were calculated from the absorbance data by the three-point average method described by Ansevin (13) and Li (14). The A_{320}/A_{260} ratio remained unchanged during the course of the experiment, indicating the absence of any precipitation of nucleoprotein complexes upon heating the sample.

RESULTS

The polyacrylamide gel electrophoretic pattern of purified TP is shown in Fig. 1*A* along with the pattern obtained for total acid-soluble proteins from sonication-resistant spermatid nuclei. It can be seen that the isolated TP was electrophoretically homogenous. This preparation was used for all the *in vitro* binding studies described below. The gel electrophoretic pattern of purified rat liver histone H1 is shown in Fig. 1*B*.

Quenching of Tyrosine Fluorescence of TP upon Binding to Nucleic Acids—Fig. 2 shows the fluorescence emission spectra of TP at different salt concentrations. Since TP contains only tyrosine residues among the aromatic amino acids (Fig. 3), it is found to yield a fluorescence emission spectra with λ_{max} at 305 nm when excited at 276 nm, which corresponds to the

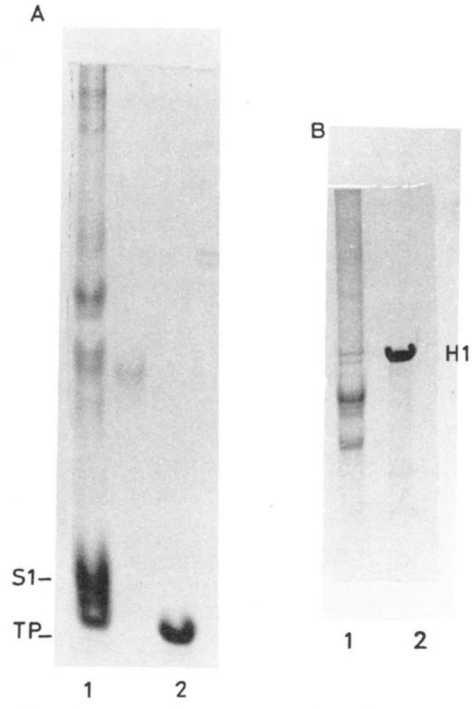

FIG. 1. *A*, polyacrylamide gel electrophoretic pattern of purified TP (*lane 2*) and total acid-soluble proteins from sonication-resistant spermatid nuclei (*lane 1*). *B*, polyacrylamide gel electrophoretic pattern of purified rat liver histone H1 (*lane 2*) and total acid-soluble proteins from rat testes (*lane 1*).

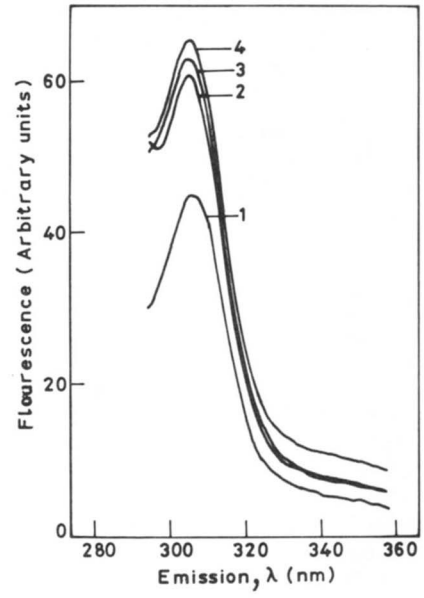

FIG. 2. **Fluorescence emission spectra of TP in a buffer solution (1 mM sodium cacodylate, pH 7.4, 0.1 mM Na_2EDTA) containing 1 mM NaCl (*1*); 10 mM NaCl (*2*); 50 mM NaCl (*3*); 100 mM NaCl (*4*).** Excitation was at 276 nm.

emission maximum of free tyrosine. It can also be seen from Fig. 2 that increasing the NaCl concentration from 1 to 10 mM also increased the fluorescence intensity of TP by nearly 30%. An increase in the NaCl concentration to 50 and 100 mM did not further increase the fluorescence intensity. These results show that a minimum of 10 mM NaCl concentration is necessary for the optimum fluorescence of tyrosine residues in the TP molecule to be observed. Since the addition of NaCl up to a concentration of 100 mM did not affect the fluorescence yield, it can be concluded that the salt does not induce

1 10
Ser—Thr—Ser—Arg—Lys—Leu—Lys—Thr—His—Gly
20
Met—Arg—Arg—Gly—Lys—Asn—Arg—Ala—Pro—His
30
Lys—Gly—Val—Lys—Arg—Gly—Gly—Ser—Lys—Arg
40
Lys—Tyr—Arg—Lys—Ser—Ser—Leu—Lys—Ser—Arg
50
Lys—Arg—Gly—Asp—Ser—Ala—Asp—Arg—Asn—Tyr
Arg—Ser—His—Leu

FIG. 3. **Amino acid sequence of TP as determined by Kistler *et al.* (7).**

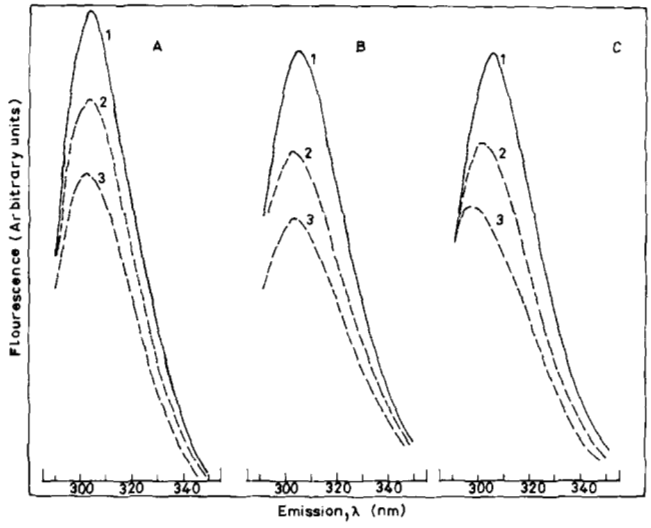

FIG. 4. **Fluorescence quenching of TP by nucleic acids.** The Tyr fluorescence spectra of TP were recorded in the absence (*solid lines*) and in the presence (*dashed lines*) of increasing concentrations of nucleic acids. *A*, double-stranded rat liver core DNA (*1*, TP = 1.25×10^{-5} M; *2*, TP + 1.8×10^{-5} M DNA; *3*, TP + 4.45×10^{-5} M DNA). *B*, denatured core DNA (*1*, TP = 1.25×10^{-5} M; *2*, TP + 1.47×10^{-5} M DNA; *3*, TP + 2.65×10^{-5} M DNA). *C*, poly(rA) (*1*, TP = 1.25×10^{-5} M; *2*, TP + 0.75×10^{-5} M poly(rA); *3*, TP + 2.6×10^{-5} M poly(rA)).

any globular structure in the TP molecule so as to bury the tyrosine residues. In fact, we have observed by circular dichroism studies that the TP molecule exists predominantly as a random coil with very little α-helical structure in it.[1] A concentration of 50 mM NaCl was, therefore, used in all further experiments involving the binding of nucleic acids to TP.

Fig. 4 shows the fluorescence emission spectra for TP in the presence and absence of native nucleosome core DNA (Fig. 4*A*), denatured nucleosome core DNA (Fig. 4*B*), and poly(rA) (Fig. 4*C*). It is evident from the figure that the relative fluorescence intensity at 305 nm of TP was quenched upon binding to all three types of nucleic acids. Fig. 5, *A–C* shows the effect of increasing concentrations of native DNA (Fig. 5*A*), denatured DNA (Fig. 5*B*), and poly(rA) (Fig. 5*C*) on the fluorescence intensity of TP. A saturation in the fluorescence quenching could not be obtained since the addition of higher concentrations of nucleic acids than shown in these figures resulted in the precipitation of nucleoprotein complexes. This is quite expected because TP is a highly basic protein. The fluorescence quenching data were replotted according to Kelley *et al.* (12), and the association constants, *K*, for binding of TP to native DNA (Fig. 5*D*), denatured DNA (Fig. 5*E*), and poly(rA) (Fig. 5*F*) were calculated as 4.40×10^3 M^{-1}, 2.86×10^4 M^{-1}, and 8.50×10^4 M^{-1}, respectively. These binding constants should be taken as only rough estimates because of the insoluble nature of nucleoprotein complexes at high nucleic concentrations. However, these results do indicate that TP has a higher affinity for single-stranded nucleic acids as compared to double-stranded DNA.

Ultraviolet Difference Absorption Spectroscopic Studies—According to the studies of Helene and Lancelot (15), the quenching of tyrosine fluorescence of proteins upon binding to DNA is indicative of an intercalative insertion of tyrosine residues between the adjacent bases of the nucleic acids or a charge transfer complex formation. Since either mode of interaction of tyrosine residues with the nucleic acid bases is expected to bring about changes in the absorption spectra of the nucleic acids on complex formation, the effect of TP binding on the UV absorption spectra of native rat liver nucleosome core DNA and poly(rA) was checked.

The difference absorption spectra for complexes of TP with native DNA in the presence of 2 mM Na^+ concentration is shown in Fig. 6*A*. A peak at 280 nm and a shoulder of 265 nm were observed. It can also be noted that there was an increase in absorbance at 260 nm with increasing concentrations of TP, while at the same time there was no spectral contribution by TP itself. The increase in absorbance at 260 nm was found to be linear when plotted against TP concentration, as shown in Fig. 6*B*, where a maximum of 15% hyperchromicity change at 260 nm was observed before precipitation of the TP·DNA complex occurred.

The difference absorption spectra for binding of TP to poly(rA) in the presence of 2 mM Na^+ concentration, as shown in Fig. 7*A*, indicates a main peak at 272 nm with a small shoulder between 255 and 260 nm. The plot of the hyperchromicity at 260 nm against TP concentration shown in Fig. 7*B* indicates a maximum of 15% hyperchromicity before the complex precipitated. Since poly(rA) is a helically stacked single polynucleotide chain, the induced hyperchromicity upon binding to TP may arise from destacking of the adenine bases, as has been suggested by Helene and Lancelot (15) from their studies on binding of oligopeptides Lys-*X*-Lys (*X* = Tyr, Trp, Phe) with nucleic acids. The observed peak positions of the difference spectra at 270 nm for poly(rA) and 280 nm for double-stranded DNA binding may reflect a change in the environment of the bases upon interacting with tyrosine residues.

The difference spectra were also recorded for the binding of TP to DNA and poly(rA) in the presence of 50 mM NaCl concentration. These results are presented in Fig. 8, *A* and *B*, respectively. It is clear that TP did induce hyperchromicity of both DNA (Fig. 8*A*) and poly(rA) (Fig. 8*B*) at this salt concentration also, and the shapes of the difference spectra were very similar to those observed at 2 mM Na^+ concentration.

Effect of TP Binding on the Thermal Melting of the DNA—The effect of binding of increasing concentrations of TP (below the concentration that brought about precipitation, Figs. 9 and 11) and rat liver histone H1 (Figs. 10 and 12) on the thermal melting of calf thymus DNA were studied at low (2 mM Na^+ concentration, Figs. 9 and 10) as well as high (50 mM Na^+, Figs. 11 and 12) ionic strength conditions. It can be seen that at low ionic strength, the T_m of calf thymus DNA was slightly increased from 51 to 53 °C at a final TP to DNA weight ratio of 1:2, with the appearance of another small peak at 76 °C (Fig. 9). In addition, there was a 5–6% decrease in the overall hyperchromicity in the TP·DNA complexes. In contrast, liver histone H1 was found to stabilize the DNA. As shown in the derivative profile, histone H1 was found to increase the T_m of calf thymus DNA from 50 to 82–87 °C.

On the other hand, at 50 mM Na^+, the T_m value for DNA

[1] J. Singh and M. R. S. Rao, unpublished observations.

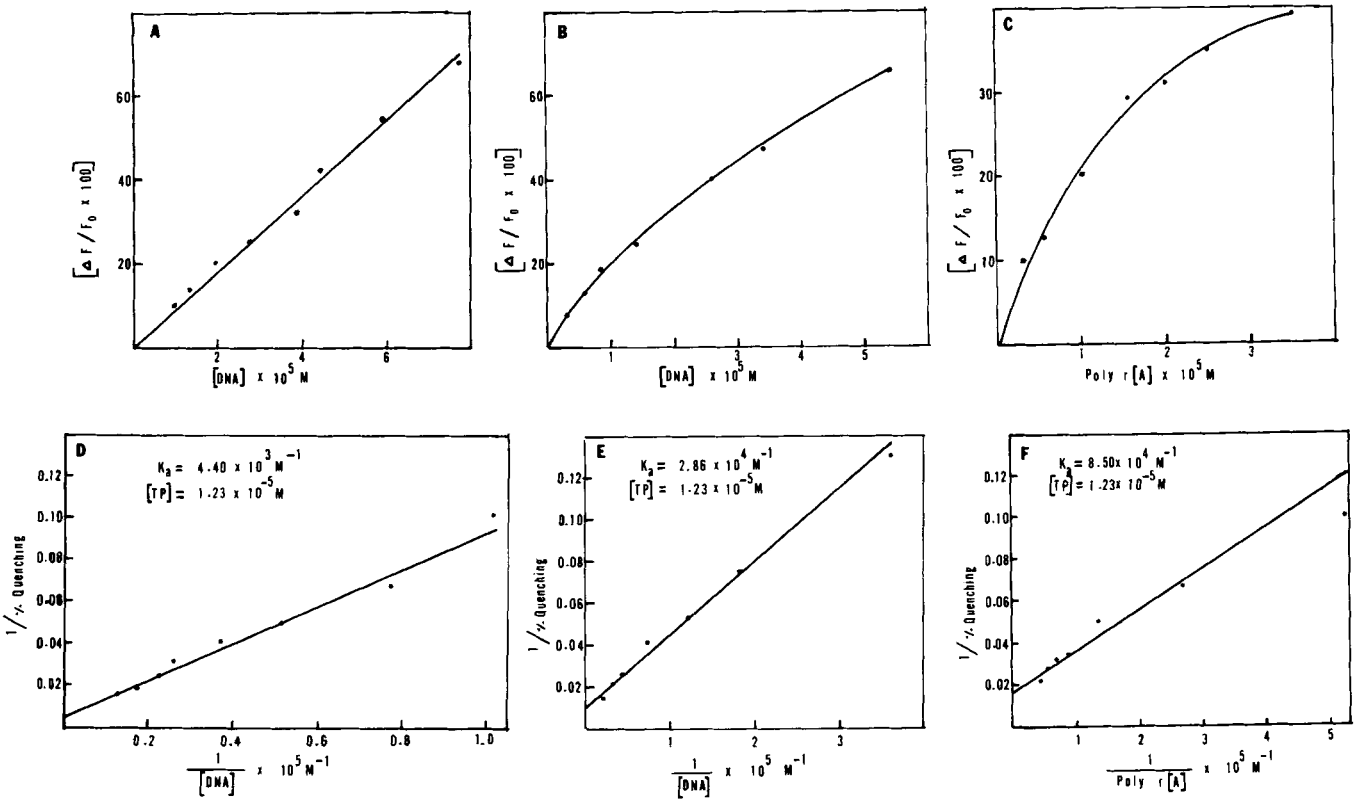

FIG. 5. **Dependence of fluorescence quenching on the concentration of nucleic acids (*A–C*) and determination of binding constants, K_a (*D–F*) of TP for double-stranded DNA (*A, D*), denatured DNA (*B, E*), and poly(rA) (*C, F*).** For calculating the binding constant, 1/% quenching was plotted against the reciprocal nucleic acid concentration.

was decreased by TP binding from 77 to 71 °C, as shown in Fig. 11. This decrease was observed even at low concentrations of TP, whereas at higher concentrations a decrease in the overall hyperchromicity, as well as the first derivative peak area, was also observed. This observation is in striking contrast to the observed effect of histone H1 on the thermal melting of calf thymus DNA at 50 mM NaCl, as shown in Fig. 12. Histone H1 from rat liver was used as a control here since it is known that it binds to DNA primarily through electrostatic interactions, as has been proposed here for TP. Even at 50 mM NaCl, histone H1 was found to stabilize the DNA, as indicated by the biphasic first derivative profile in Fig. 12*B*, showing that the T_m was shifted from 75 to 85–86 °C. There was, however, no decrease in the overall hyperchromicity of the DNA·histone complex formed with increasing concentration of histone H1 with respect to that for DNA alone (Fig. 12*B*), the hyperchromicity curve indicating a relatively unmelted DNA at about 90 °C. The results obtained with thermal denaturation studies are summarized in Table I.

From these results it can be safely inferred that DNA is slightly stabilized against thermal denaturation upon TP binding at low ionic strength. However, at 50 mM salt concentration, TP decreases the T_m of the DNA, indicating a destabilization of the DNA. The decrease in the overall hyperchromicity on the addition of increasing amounts of TP to DNA at high salt strength as shown in Fig. 11 is interesting, especially in view of the fact that the slope of the hyperchromicity curves for DNA and TP·DNA complexes at higher temperatures are similar. According to the results of Jenson *et al.* (16), from their studies on gene 32 protein, such an observation could imply that protein TP induces a pronounced equilibrium destabilization of the DNA.

DISCUSSION

This study of nucleic acid binding properties of TP was undertaken because it would provide valuable information needed to understand its possible function in the process of nucleohistone to nucleoprotamine transition in rat testis. The amino acid sequence of TP, as determined by Kistler *et al.* (7) and shown in Fig. 3, reveals that TP is a highly basic protein with high lysine (19%) and arginine (21%) contents. These lysine and arginine residues are distributed uniformly all along the length of the TP molecule. Therefore, it is likely to interact all along the length with the nucleic acids through electrostatic interactions of its positively charged lysine and arginine residues with the negatively charged phosphate groups of the DNA backbone. In addition, the sequence also indicates the presence of two tyrosine residues at positions 32 and 50, both of which are flanked by the basic amino acid residues. The Tyr-32 residue is located in the highly basic amino acid stretch Lys-Arg-Lys-Tyr-Arg-Lys, and the Tyr-50 residue is present in the stretch Arg-Asn-Tyr-Arg. Therefore, the interaction of TP with the nucleic acids can be expected also to involve the tyrosine residues, possibly as a consequence of the *a priori* binding of the highly basic amino acid stretches with DNA. For this reason, the fluorescence of tyrosine residues of TP was used as a probe to study the possible interaction of tyrosine residues with nucleic acids. This was also facilitated by the fact that TP lacks the other two aromatic amino acid residues, phenylalanine and tryptophan.

The results presented in this paper clearly show that the tyrosine fluorescence of TP is quenched upon binding to native and denatured DNA and poly(rA). A comparison of the association constants, K, for binding of TP to these

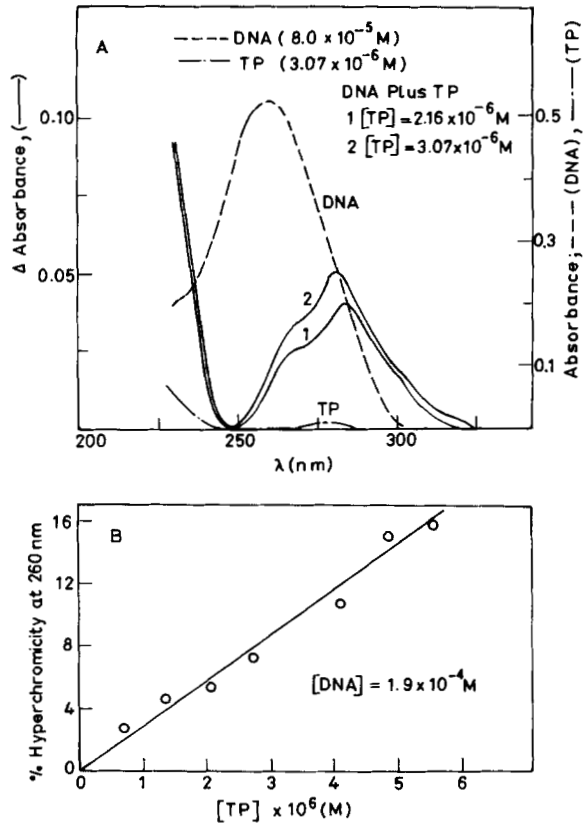

FIG. 6. *A*, ultraviolet difference absorption spectroscopy of double-stranded DNA upon binding to TP. The difference spectra were recorded as described under "Materials and Methods" in a buffer containing 1 mM sodium cacodylate, pH 7.4, 0.1 mM Na_2EDTA, 1 mM NaCl at 25 °C. *B*, the observed increase in A_{260} (ΔA_{260}) for TP binding to double-stranded DNA was plotted as a function of increasing concentration of TP.

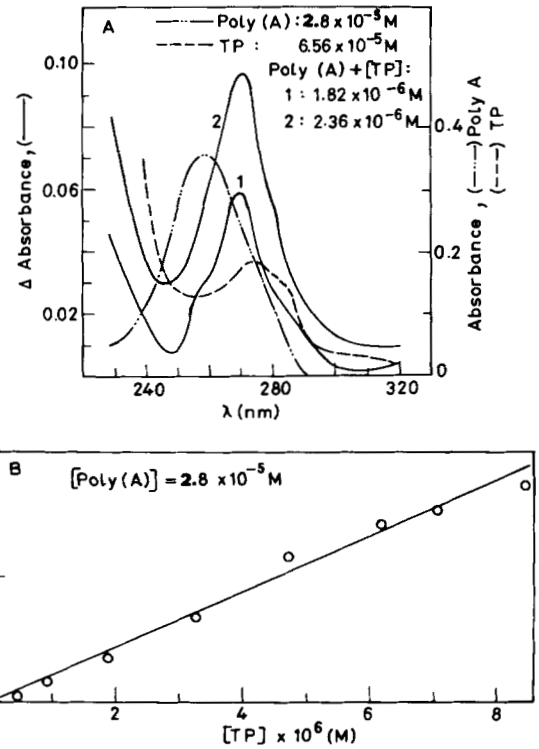

FIG. 7. **Ultraviolet difference absorption spectroscopy of poly(rA) upon binding to TP.** The difference spectra are indicated by *solid lines* (*1* and *2*). The absorbance of poly(rA) (—·—) and TP (- - -) are indicated on the right-hand scale of the *y axis*. The buffer conditions and temperature are the same as described in the legend to Fig. 6. The observed increase in A_{260} (ΔA_{260}) for TP binding to poly(rA) was plotted as function of increasing concentration of TP.

nucleic acids clearly showed that the affinity of TP toward single-stranded nucleic acids is almost 8- to 20-fold higher than for native DNA. By extrapolating the data obtained from the double-reciprocal plots of the fluorescence quenching (Fig. 4) to infinite nucleic acid concentrations, it can be calculated from the *y* intercept that quenching is nearly 100% with both native and denatured DNA. This suggests that probably both of the tyrosine residues of TP were interacting with these nucleic acids. However, with respect to poly(rA), only 55% quenching was observed at infinite nucleic acid concentration, suggesting that probably only one of the two tyrosine residues in TP may interact with poly(rA). It has been shown by Helene and Lancelot (15) that the interaction of the tripeptide Lys-Tyr-Lys with DNA and poly(rA) also resulted in the quenching of the tyrosine fluorescence, which could be ascribed to either partial intercalation of the tyrosine residues with the bases, or hydrogen bonding, or charge transfer complex formation between the bases and the tyrosine residue. They ruled out the possibility of hydrogen bond formation because fluorescence quenching was obtained even upon interaction of an *O*-substituted tyrosine-containing oligopeptide with nucleic acids (15). Furthermore, the NMR results of Dimicoli and Helene (17), using the upfield shift of the tyrosine resonance to monitor the intercalation of tyrosine residue on binding of Lys-Tyr-Lys, indicated that the tyrosine residue got intercalated selectively with the bases of only single-stranded polynucleotides like poly(rA) and not between the bases of double-stranded DNA. On the other hand, the

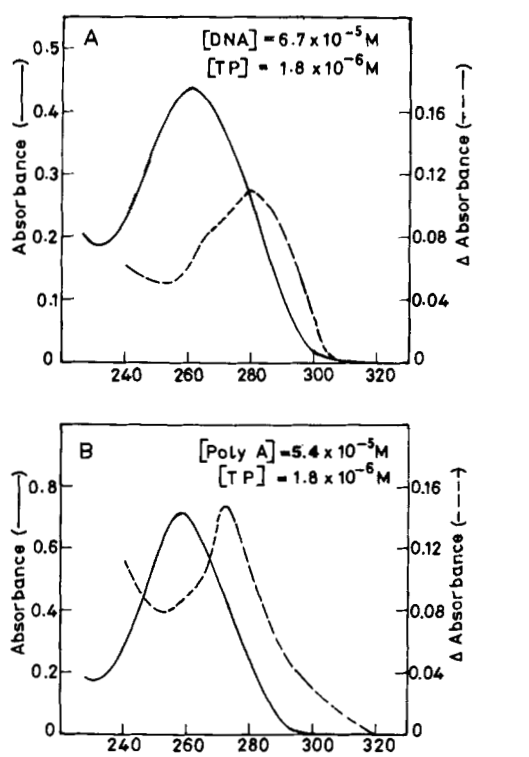

FIG. 8. **Ultraviolet difference absorption spectroscopy of double-stranded DNA (*A*) and poly(rA) (*B*) upon binding to TP in a buffer containing 1 mM sodium cacodylate, pH 7.4, 0.1 mM Na_2EDTA, 50 mM NaCl at 25 °C.**

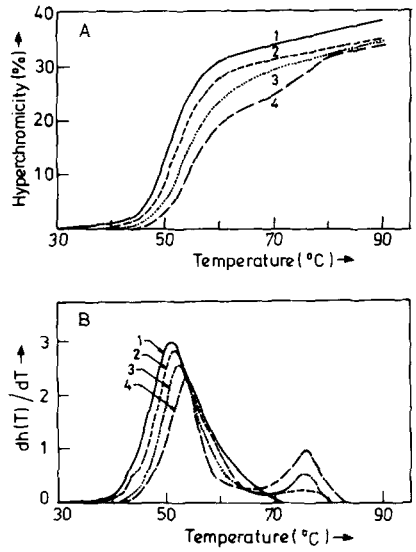

FIG. 9. **Effect of binding of TP on the thermal melting of calf thymus DNA at low salt concentration (1 mM NaCl, 0.1 mM Na_2EDTA, 1 mM sodium cacodylate, pH 7.4).** *A*, hyperchromicity profile and *B*, first derivative profile. The numbers refer to different weight ratios of DNA/TP. *1*, DNA alone; *2*, DNA/TP ratio of 9.6:1; *3*, DNA/TP ratio of 4.3:1; *4*, DNA/TP ratio of 3:1.

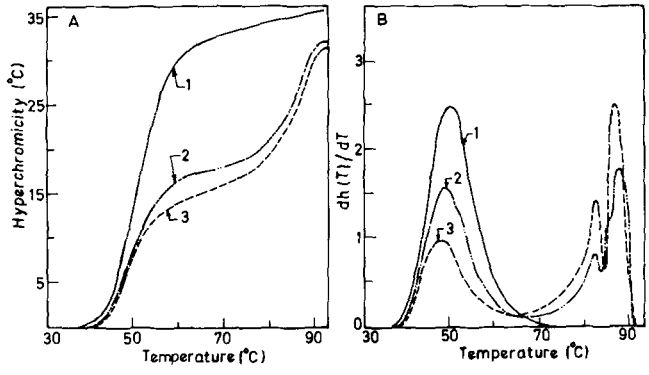

FIG. 10. **Effect of binding of rat liver histone H1 on the thermal melting of calf thymus DNA at low salt concentration.** *A*, hyperchromicity profile and *B*, first derivative profile. The numbers refer to different weight ratios of DNA/H1. *1*, DNA alone; *2*, DNA/H1 ratio of 3:1; *3*, DNA/H1 ratio of 2:1.

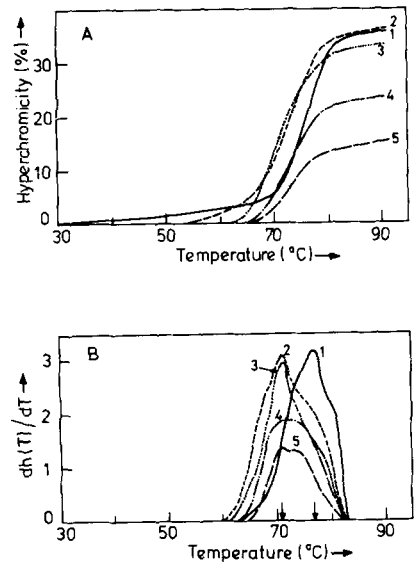

FIG. 11. **Effect of binding of TP on the thermal melting of calf thymus DNA at high salt concentrations (50 mM NaCl, 0.1 mM Na_2EDTA, 1 mM sodium cacodylate, pH 7.4).** *A*, hyperchromicity profile and *B*, first derivative profile. The numbers refer to different weight ratios of DNA/TP. *1*, DNA alone; *2*, DNA/TP ratio of 12:1; *3*, DNA/TP ratio of 6:1; *4*, DNA/TP ratio of 3.5:1; *5*, DNA/TP ratio of 2:1.

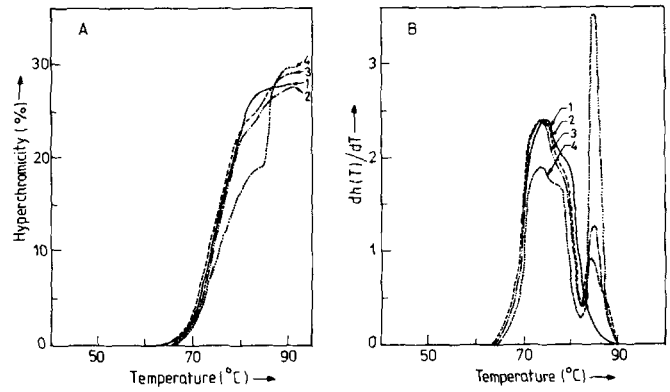

FIG. 12. **Effect of binding of rat liver histone H1 on the thermal melting of calf thymus DNA at high salt concentration.** *A*, hyperchromicity profile and *B*, first derivative profile. The numbers refer to different weight ratios of DNA/H1. *1*, DNA alone; *2*, DNA/H1 ratio of 3.6:1; *3*, DNA/H1 ratio of 2.5:1.

NMR results of Gabbay *et al.* (18) supported the intercalation of the tyrosine residue with the bases in double-stranded DNA. It has also been demonstrated by Sheardy and Gabbay (19) that intercalation occurred only with the basic oligopeptides containing L-Tyr but not D-Tyr. The observations of Tyagalov *et al.* (20) supported the inference of Gabbay and co-workers. They showed that Lys-Tyr-Lys stabilized the DNA against thermal denaturation to a much lesser extent than Lys-Ala-Lys and therefore inferred a local melting of DNA by the tyrosine intercalation between the bases.

According to Helene and Lancelot (15), unequivocal evidence for intercalative mode of interaction of tyrosine with nucleic acid bases is provided by an upfield shift of the tyrosine resonance in the NMR spectra. Such upfield shifts have indeed been reported in the case of interaction of gene 5 protein of bacteriophage fd with single- and double-stranded polynucleotides (21, 22). However, the NMR analysis of binding of TP to DNA or poly(rA) could not be carried out because TP·DNA and TP·poly(rA) complexes were found to precipitate whether prepared by direct mixing or slow dialysis.

A consequence of the intercalative mode of binding of tyrosine has been suggested to be the local melting of nucleic acids. This is evidenced by the thermal melting studies on Lys-Tyr-Lys and DNA-melting protein binding to DNA (20, 23) and observed hyperchromicity effect upon binding of T4-coded gene 32 protein to single- and double-stranded polynucleotides (16, 24). The results obtained in the present investigation using UV difference absorption spectroscopy clearly indicate that TP has DNA-melting property. There was a significant increase in absorbance or hyperchromicity at 260 nm upon binding to double-stranded DNA and poly(rA). These results are similar to those of Mayer *et al.* (25) for binding of Lys-Tyr-Lys to poly(rA), suggesting that the tyrosine residues were intercalated with the bases in poly(rA), resulting in the destacking of adenine bases.

The melting property of TP is further substantiated by the results obtained with thermal denaturation studies. Relatively less stabilization of calf thymus DNA by TP at low ionic strength is surprising in view of the fact that TP is much more basic than histone H1. In view of the observations of

TABLE I

The effect of TP and histone H1 on the thermal melting of calf thymus DNA at low and high salt concentrations

Sample	DNA/protein		T_m values		
	Weight ratio	Molar ratio	1	2	3
		base pairs/ protein		°C	
Low salt (1 mM NaCl)					
Fig. 8					
Curve 1 (DNA)			51.0		
2 (DNA + TP)	9.6:1	92	51.5		
3 (DNA + TP)	4.3:1	40	52.0	76.0	
4 (DNA + TP)	3.0:1	28	54.0	76.0	
Fig. 9					
Curve 1 (DNA)			50.0		
2 (DNA + H1)	3:1	103	49.0	82.0	87.0
3 (DNA + H1)	2.0:1	66	49.0	82.0	86.0
High salt (50 mM NaCl)					
Fig. 10					
Curve 1 (DNA)			77.0	80.0	
2 (DNA + TP)	12.0:1	109	71.0		
3 (DNA + TP)	6.0:1	55	71.0		
4 (DNA + TP)	3.5:1	33	71.0		
5 (DNA + TP)	2.0:1	21	71.0		
Fig. 11					
Curve 1 (DNA)			75.0	79.0	
2 (DNA + H1)	3.6:1	114	75.0	78.0	
3 (DNA + H1)	2.5:1	80	75.0	78.0	

Tyagalov *et al.* (20) indicating relatively less stabilization of DNA by Lys-Tyr-Lys as compared to Lys-Ala-Lys, the present results favor the possibility that TP may indeed bring about a local melting of the DNA through intercalation of its tyrosine residues with the DNA bases, with the basic residues binding electrostatically to the DNA phosphates, which has a DNA-stabilizing effect. The net result of the intercalative and electrostatic modes of interaction may manifest in the slight increase in the T_m value of calf thymus DNA upon binding by TP. DNA destabilization by TP was indeed observed at the higher ionic strength (50 mM NaCl) where it was observed that TP brought about a decrease in the T_m of DNA from 77 to 71 °C (Fig. 11). An additional effect of TP binding was a decrease in the overall hyperchromicity in the final phases of melting, profiles being similar for DNA and DNA·TP complexes. This effect of TP is similar to that reported for binding of gene 32 protein to native DNA, which has been explained as to equilibrium destabilization of DNA.

Thus, the results indicating a DNA-melting property of TP in spite of its highly basic nature lead us to believe that this property may be involved in the mechanism of nucleoprotein transition that occurs during spermiogenesis in the rat. As the process of nucleoprotein transition from nucleohistone to nucleoprotamine in the rat is encompassed by the transient association of spermatid chromatin with TP and TP2, it is likely that the process by which the nucleosomal chromatin is transformed into the fibrous, mid-spermatid chromatin containing TP and TP2 must involve a drastic destabilization of the nucleosome structure. It is worth mentioning here that histone H1 is the last histone to be replaced during spermiogenesis in trout and rat testes, and therefore the histone replacement process is not a simple one involving electrostatic competition (26, 27). Thus, it may be assumed that TP, because of its DNA-melting property, may modulate the structure of nucleosomes in such a way as to facilitate the replacement of histones. Recently, we have observed that addition of TP to nucleosome core particle *in vitro* does destabilize the core particle.[2]

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