A twenty-two-fold increase in the relative affinity of estrogen receptor to poly (dA-dC)·poly (dG-dT) in the presence of polyamines

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ABSTRACT

We studied the relative efficacy of polyamines to facilitate the binding of estrogen receptor to poly(dA-dC).poly(dG-dT). In the absence of polyamines, 1,400 µg/ml of this polynucleotide eluted 50% of bound estrogen receptor from DNA-cellulose. In contrast, 50% estrogen receptor was eluted by 65 µg/ml of poly(dA-dC).poly(dG-dT) complexed with 150 µM spermidine. Putrescine and spermine also enhanced the ability of poly(dA-dC).poly(dG-dT) to elute estrogen receptor, but the magnitude of the effect was not as high as that of spermidine. Control experiments with calf thymus DNA and poly(dA-dT).poly(dA-dT) showed 6- and 3-fold increase, respectively in their affinity for estrogen receptor in the presence of spermidine. The dramatic increase in the affinity of poly(dA-dC).poly(dG-dT) for estrogen receptor in the presence of polyamines might be a result of the conversion of the polynucleotide to the left-handed Z-DNA form. These results show that polyamines are capable of participating in estrogenic regulation of gene expression by altering the affinity of the receptor for specific DNA sequences.

INTRODUCTION

Estrogen receptor is a gene regulatory protein present in the female sex-accessory tissues of vertebrates (1). The receptor protein is dormant in the cell until it is transformed to a functional state after its interaction with estrogen (2-4). Early biochemical studies indicated the presence of the receptor in the cytoplasmic compartment of the cell (5, 6). It was thought that the estrogen-receptor complex translocated to the nucleus and exerted its effects by binding to specific DNA sequences. Recent immunological studies, however, suggest that the majority of the receptor is localized in the nucleus (7, 8). The mechanism by which estrogen receptor triggers gene expression is poorly understood at present. Several endogenous and exogenous factors are known to modulate the transformation and DNA binding of estrogen and other steroid receptors (9-13).

We recently showed (14) that naturally occurring polyamines, putrescine ($\text{H}_2\text{N}(\text{CH}_2)_4\text{NH}_2$), spermidine ($\text{H}_2\text{N}(\text{CH}_2)_3\text{NH}(\text{CH}_2)_4\text{NH}_2$), and spermine ($\text{H}_2\text{N}(\text{CH}_2)_3\text{NH}(\text{CH}_2)_4\text{NH}(\text{CH}_2)_3\text{NH}_2$) are capable of stabilizing rabbit uterine estrogen-receptor complex. These polyamines are present in all living cells and their levels are highly regulated by cell growth cycles and differentiation processes (15-18). In general, intracellular polyamine levels are in the millimolar range. Spermidine levels are reported to be 0.5 mM in uterus, 1.5 mM in growing mammary tumors, and 1 mM in regressing mammary tumors (16). The spermine levels of these tissues are about 0.3, 0.4, and 0.5 mM, respectively. Under physiological ionic conditions, polyamines are positively charged. Cellular macromolecules, DNA, RNA, and negatively charged proteins are therefore targets for the interaction of polyamines by electrostatic and hydrophobic forces. Recent studies show that the interaction of polyamines with certain polynucleotides provokes a conformational transition in the polynucleotides from their usual right-handed B-DNA form to a left-handed Z-DNA conformation (19-21).

The Z-DNA conformation is induced in alternating purine-pyrimidine sequences by molar concentrations of NaCl (19), micromolar concentrations of polyamines (20, 21), or by negative supercoiling (23, 24). Z-DNA-forming sequences, especially tracts of up to 50 base pairs of $(\text{dA-dC})_n \cdot (\text{dG-dT})_n$ sequences have been detected in the regulatory regions of human cardiac muscle actin gene (25), human fetal globin gene (26) and in the human (27) and rat somatostatin gene (28). Nordheim and Rich (29) showed that potential Z-DNA-forming sequences are present in the transcriptional enhancer elements of several viral DNAs. Presence of these sequences in the critical control regions of the genes and their ability to assume Z-DNA conformation under physiologically relevant ionic conditions (22-24) suggest a possible involvement of Z-DNA in gene regulation. In order to gain further insight into the mechanism of estrogenic regulation of gene expression, we studied the binding of estrogen receptor to $\text{poly}(\text{dA-dC}) \cdot \text{poly}(\text{dG-dT})$ in the presence and absence of polyamines. $\text{Poly}(\text{dA-dT}) \cdot \text{poly}(\text{dA-dT})$, a polynucleotide that does not undergo the B to Z transition in the presence of polyamines, was used as a control. Our results show that polyamines cause 3- and 22-fold increase in the binding of estrogen receptor to $\text{poly}(\text{dA-dT}) \cdot \text{poly}(\text{dA-dT})$ and $\text{poly}(\text{dA-dC}) \cdot \text{poly}(\text{dG-dT})$, respectively.

MATERIALS AND METHODS

Chemicals.

17 β -[2,4,6,7-³H]-Estradiol ([³H]-E₂) (95 Ci/mmol), and ¹⁴C-labelled bovine serum albumin (BSA) and human γ -globulin were purchased from New England Nuclear (Boston, MA). Putrescine.2HCl, spermidine.3HCl, spermine.4HCl, and diethylstilbestrol were obtained from Sigma Chemical Co. (St. Louis, MO). Bovine pancreas RNase A was obtained from Worthington Biochemicals (Freehold, NJ). The enzymatic activity of RNase was 5,400 units per mg protein.

Poly(dA-dC).poly(dG-dT) (S_{20,w} = 10.9; M_w = 1.5 x 10⁶) and poly(dA-dT).poly(dA-dT) (S_{20,w} = 8.07; M_w = 5.4 x 10⁵) were purchased from Pharmacia, Inc. (Piscataway, N.J.). The polymers were dissolved in a buffer containing 10 mM Tris.HCl (pH 7.4), 150 mM NaCl, and 1 mM EDTA to form a stock solution containing 1-2 mg/ml polynucleotide. For most of the experiments, this solution was diluted to 300 μ g/ml in 10 mM Tris.HCl (pH 7.4), 1 mM NaCl and 0.1 mM EDTA (Tris buffer) and dialysed extensively from the same buffer using Spectrapor tubing (Spectrum Medical Industries, Inc., Los Angeles, CA) with a molecular weight cut off of 6,000-8,000.

Calf thymus DNA (M_w ~ 19 x 10⁶) was obtained from Sigma Chemical Co. It was dissolved in a buffer containing 10 mM Tris.HCl (pH 7.4), 150 mM NaCl, and 1 mM EDTA, and purified by phenol extraction and dialysis from the same buffer. A stock DNA solution was kept at a concentration of 1 mg/ml at 4°C. For the receptor binding assays, this solution was diluted and dialysed as described for poly(dA-dC).poly(dG-dT).

Preparation of Cytosolic Estrogen Receptor.

Immature rabbits (5-6 lbs, New Zealand White) were purchased from Birchwood Farms, Hudson, WI. Rabbits were sacrificed by an injection of Nembutal (Abbot Laboratories, Chicago, IL). The uteri were removed and dissected free from surrounding fat (30). The tissue was minced and homogenized at 4°C in 10 volumes of a buffer containing 10 mM Tris.HCl, pH 7.4, 1 mM EDTA, and 1 mM dithiothreitol (TED buffer) using a Polytron PT-10 homogenizer (Brinkmann Instruments, Westbury, NY). The tissue homogenate was centrifuged at 105,000 x g for 45 min to obtain cytosol. The cytosol was incubated with 2 nM [³H]-E₂ for 2 h at 4°C and purified by sucrose gradient centrifugation. The receptor content of the cytosol was determined by dextran-coated charcoal (DCC) assay. For this assay, aliquots of cytosol were incubated with 2 nM [³H]-E₂ for 2 h at 4°C in the presence or absence of 400 nM diethylstilbestrol to determine nonspecific binding and total

binding, respectively. The cytosol containing the hormones was then treated with an equal volume of DCC suspension (final concentration: 0.025% dextran and 0.25% charcoal) for 10 min at 4°C. The reaction mixture was centrifuged at 5,000 x g and the radioactivity of the supernatant was determined using Aquasol-2 scintillation fluid in a Beckman LS2800 scintillation counter. Specific binding of [³H]-E₂ to the receptor was calculated by subtracting nonspecific binding from total binding. Protein concentration of the cytosol was 3.7 ± 0.5 mg/ml as determined by the Lowry assay (31).

Partial Purification of Estrogen Receptor.

The cytosolic estrogen receptors were partially purified by preparative sucrose gradient centrifugation. To prepare the 4S receptor, cytosol was equilibrated with 2 nM [³H]-E₂ and incubated with RNase A at 100 µg/ml for 1 hr at 4°C (9). RNase treatment was used to transform estrogen receptors to the DNA binding 4S form (9). After the RNase treatment, cytosol was added to a pellet of DCC (0.025% dextran and 0.25% charcoal) and incubated for 10 min at 4°C. The mixture was then centrifuged at 5,000 x g and the supernatant was collected. The DCC-treated cytosol (5 ml) was layered on top of 10-30% linear sucrose gradients in 40 ml polyallomer tubes (14). The gradients were centrifuged at 135,000 x g in a SW 27 rotor for 24 h at 4°C. Subsequently, 0.5 ml fractions were collected from the bottom of the gradient by gravity flow, and the radioactivity of 50 µl aliquots of each fraction was determined to locate the receptor peak. The peak fractions were pooled and incubated with 2 nM [³H]-E₂ for 2 h at 4°C to compensate for the loss of the ligand during ultracentrifugation. The receptor content was determined by DCC assay. In a typical purification, the specific binding of estradiol in the cytosol increased from 370 to 1,150 fmoles/mg protein. The relabelled receptor solution was stored as 5 ml aliquots at -20°C. Sedimentation constants, dissociation rates and DNA binding of the receptor were not affected during storage for 6 weeks.

Analytical Sucrose Gradient Centrifugation.

Partially purified receptor preparation was treated with a pellet of DCC to remove free estradiol. This receptor solution was layered on top of linear sucrose gradients (10-30%) containing TED buffer in 3.8 ml polyallomer tubes. The gradients were centrifuged at 256,000 x g for 16 h. Five-drop fractions were collected and their radioactivity was determined by scintillation counting. Sedimentation constants were determined by adding ¹⁴C-labelled BSA and γ-globulin as internal markers.

NaCl Gradient Elution of Estrogen Receptors Bound to DNA-cellulose.

Calf thymus DNA coupled to cellulose was obtained from Sigma Chemical Company (St. Louis, MO). The amount of DNA in cellulose was 6 mg/gm as determined by diphenylamine assay (32). The resin (0.25 g) was soaked overnight in TED buffer and the supernatant was decanted. The resin was suspended in 2 ml TED buffer, centrifuged at 5,000 x g for 10 min and the supernatant was removed. Partially purified receptor preparation (1 ml) was added to the DNA-cellulose and incubated for 30 min at 22°C with frequent mixing (33, 34). The receptor preparation consisted of [³H]-E₂ in equilibrium with [³H]-E₂R complexes. The amount of [³H]-E₂R complexes in this mixture was determined by DCC assay. The DNA-cellulose slurry was packed in a 3 ml minicolumn (Isolab Inc., Akron, OH). The column was washed 3 times with 1 ml of TED buffer to remove unbound receptor and free [³H]-E₂. The column was then connected to a Buchler automatic gradient former. An identical control column was prepared with cellulose instead of DNA-cellulose. Both columns were simultaneously eluted with 1 mM to 1 M NaCl gradient in TED buffer. The flow rate was 0.5 ml/min. We collected 0.5 ml fractions and the radioactivity of each fraction was determined by scintillation counting. The percentage of receptors bound to the DNA was calculated from the amount of eluted receptor and the amount of total receptor incubated with the DNA-cellulose.

DNA-cellulose Competitive Elution Assay.

One hundred milligrams of DNA-cellulose was soaked overnight in TED buffer and the supernatant was decanted from the resin. The resin was resuspended in 10 ml TED buffer. The DNA-cellulose suspension was aliquoted into a series of tubes (200 µl/tube). Each tube contained 12 µg DNA. The tubes containing the DNA-cellulose suspension was centrifuged at 5,000 x g for 10 min and the supernatant was removed. Aliquots (200 µl) of partially purified estrogen receptor solution was added to DNA-cellulose pellets in the tubes. The receptor-DNA-cellulose mixture was incubated at 22°C for 60 min with frequent mixing. The reaction mixture was centrifuged at 5,000 x g for 10 min and the supernatant was removed. The DNA-cellulose pellet containing bound receptor was washed twice with 1 ml TED buffer to remove unbound receptor and free estradiol.

We prepared calf thymus DNA and polynucleotide solutions in separate tubes for eluting estrogen receptor. Different concentrations of polyamines were added to 300 µl DNA/polynucleotide solutions and incubated for 30 min

Figure 1: Sucrose density gradient profile of partially purified estrogen receptors. Cytosolic estrogen receptor was partially purified by preparative sucrose gradient centrifugation. RNase-treated (○) and -untreated (●) receptors were then examined by analytical sucrose gradient centrifugation. Non-specific binding (▲) was determined by adding 400 nM diethylstilbestrol to the partially purified receptor and incubating at 4°C for 24 h. Internal markers were BSA (4.5S) and γ -globulin (7S).

at 22°C. DNA dilution by the polyamine solution was minimized by using a 100-fold concentrated stock solution. The DNA/polynucleotide solutions complexed with the polyamines were added to triplicate receptor-DNA-cellulose pellets and incubated for 60 min at 22°C with frequent mixing. A set of tubes containing receptor-DNA-cellulose pellets was eluted with 0.5 M NaCl to estimate the total amount of receptor bound to the DNA-cellulose pellet. Another set of controls included elution with appropriate concentrations of polyamines, without DNA/polynucleotides. The reaction mixture was then centrifuged and the radioactivity of the supernatant was determined by scintillation counting.

RESULTS

Characterization of Estrogen Receptor.

Estrogen receptor was extracted from rabbit uteri in hypotonic buffer (10 mM Tris.HCl (pH 7.4), 1 mM EDTA, 1 mM dithiothreitol) at 4°C. Figure 1 shows the sedimentation pattern of native and RNase-treated receptors after their partial purification. The untreated and RNase-treated receptors sedimented at 7S and 4S regions of sucrose gradient, respectively. Measurements of DNA binding and ligand dissociation rates showed that the 4S

Figure 2: NaCl gradient elution profile of estrogen receptor from a DNA-cellulose minicolumn. Partially purified, [³H]-E₂-labelled estrogen receptor was incubated with DNA-cellulose (●) or cellulose alone (○) for 30 min at 22°C, and packed into separate minicolumns. The receptors were eluted by a gradient of 1 mM to 1 M NaCl in TED buffer.

receptor was in the transformed state (9, 14). Hence, we used the RNase-induced 4S form of estrogen receptor to study the effect of polyamines on the DNA binding of estrogen receptor.

Figure 2 shows the elution profile of estrogen receptor from DNA-cellulose column using 1 mM to 1 M NaCl in TED buffer. The receptor bound to the column eluted at 0.4 M NaCl concentration. This result shows the presence of only one class of high affinity DNA binding form of estrogen receptor in our preparations. The amount of estrogen receptor bound to DNA-cellulose was 49% in this experiment. The unbound receptors (~ 50%) were detected in flow through and wash fractions collected before the elution. The DNA binding capacity of four different receptor preparations was $42 \pm 7\%$. In control experiments, estrogen receptor showed no significant binding to cellulose that was not coupled to DNA.

Effects of Polyamines on Competitive Elution of Estrogen Receptor With Polynucleotide/DNA.

In order to study the effect of polyamines on the binding of estrogen receptor to poly(dA-dC).poly(dG-dT), we used a competitive elution assay (33-35). In this assay, we incubated the receptor with DNA-cellulose to form a complex of DNA-cellulose with estrogen receptor. After removing unbound receptor by centrifugation, the complex was treated with

Figure 3: Effect of polyamines on the ability of poly(dA-dC).poly(dG-dT) to elute estrogen receptor from the DNA-cellulose-receptor pellet. Poly(dA-dC).poly(dG-dT) (200 μ g/ml) was complexed with putrescine (Δ), spermidine ($\circ$), and spermine ($\bullet$) and used to elute the estrogen receptor from the pellet. Error bars indicate standard deviation in three separate experiments.

poly(dA-dC).poly(dG-dT) and polyamines. The polyamines were added to poly(dA-dC).poly(dG-dT) and incubated for 30 min at 22°C before adding to the DNA-cellulose-receptor pellet. Figure 3 shows the effect of putrescine, spermidine, and spermine on the ability of poly(dA-dC).poly(dG-dT) to elute estrogen receptor from DNA-cellulose pellet. The percentage elution in these experiments was normalized with respect to the elution of estrogen receptor from DNA-cellulose using TED buffer containing 0.5 M NaCl. The 100% value eluted by 0.5 M NaCl was about 8,000 cpm per tube. About 10% of the radioactivity was eluted in Tris buffer or Tris buffer containing polyamines. This background was subtracted before calculating percentage of receptors eluted by the polynucleotides. For the experiment shown in Figure 3, we used 200 μ g/ml poly(dA-dC).poly(dG-dT). The concentration of polyamines varied from 100 to 800 μ M putrescine, 25 to 300 μ M spermidine, and 25 to 125 μ M spermine. Control tubes contained the same concentration of polyamines in Tris buffer. In the absence of polyamines, poly(dA-dC).poly(dG-dT) was capable of eluting 16% estrogen receptor from DNA-cellulose after equilibrating the solutions for 60 min at 22°C. In the

Figure 4: Effect of polyamines on the ability of calf thymus DNA to elute estrogen receptor from the DNA-cellulose-receptor pellet. Calf thymus DNA (200 $\mu\text{g/ml}$) was complexed with putrescine (Δ), spermidine ($\circ$), and spermine ($\bullet$) and used to elute estrogen receptor from the pellet. Standard deviation in three experiments is shown by error bars.

presence of 150–300 μM spermidine, however, 100% of the receptor was eluted by the same concentration of polynucleotide.

With 125 μM spermine, a maximum of 80% estrogen receptor could be eluted with 200 $\mu\text{g/ml}$ poly(dA-dC).poly(dG-dT). The 50% elution of the receptor occurred at 75 μM spermine. Precipitation of the polynucleotide occurred above 125 μM of spermine. Putrescine was least effective and eluted only 70% of the receptor at an optimum concentration of 800 μM . In control experiments in the absence of poly(dA-dC).poly(dG-dT), none of the polyamines could elute a significant amount of estrogen receptor from DNA-cellulose. These results show that polyamines exerted their effect on the binding of estrogen receptors only after their complexation with poly(dA-dC).poly(dG-dT). Time-course experiments on the preincubation of polyamines with polynucleotide indicated that poly(dA-dC).poly(dG-dT) acquired 80% of its ability to elute estrogen receptor in 5 min of incubation with 150 μM spermidine at 22°C.

Figure 4 shows the effect of polyamines on the elution of estrogen receptor from DNA-cellulose with soluble calf thymus DNA. In these experiments, we mixed 200 $\mu\text{g/ml}$ of calf thymus DNA with 0.1 to 1 mM putrescine, 25 to 300 μM spermidine, and 25 to 100 μM spermine and incubated the solutions for 30 min at 22°C. We then added these solutions to

Figure 5: Concentration dependence of poly(dA-dC).poly(dG-dT) to elute estrogen receptor from DNA-cellulose-receptor pellet. Poly(dA-dC).poly(dG-dT) was used in the presence (●) or absence (○) of 150 μ M spermidine to elute estrogen receptor from the pellet.

DNA-cellulose-receptor complex prepared in different tubes and incubated for 60 min at 22°C. In the absence of polyamines, soluble calf thymus DNA eluted 16% of estrogen receptor from DNA-cellulose. There was an increase in the percentage of receptor eluted by calf thymus DNA after its incubation with spermidine or putrescine. In contrast to the efficacy of poly(dA-dC).poly(dG-dT) to elute 100% of estrogen receptor complex in the presence of 150 μ M spermidine, only 55% of the receptor was eluted by calf thymus DNA in the presence of 200 μ M and 1 mM spermidine and putrescine, respectively. Spermine was ineffective in enhancing the elution of estrogen receptor up to 100 μ M concentration. At higher concentrations, spermine caused precipitation of the soluble calf thymus DNA and hence it could not be used for elution. Thus, spermidine and putrescine have a stimulatory effect on the binding of estrogen receptor to calf thymus DNA.

Relative Binding Affinity of Estrogen Receptors to DNA in the Presence and Absence of Polyamines.

In order to further define the effect of polyamines on the binding of estrogen receptor to poly(dA-dC).poly(dG-dT), we determined the amount of polynucleotide required to elute 50% of the receptor in the presence and absence of 150 μ M spermidine. Figure 5 shows the concentration dependence

Figure 6: Concentration dependence of poly(dA-dT).poly(dA-dT) to elute estrogen receptor from DNA-cellulose-receptor pellet. Poly(dA-dT).poly(dA-dT) was used in the presence (●) or absence (○) of 150 μ M spermidine to elute the receptor from the pellet.

of poly(dA-dC).poly(dG-dT) on the elution of estrogen receptor from DNA-cellulose. The elution of 50% estrogen receptor from DNA-cellulose was achieved at $1,400 \pm 100$ μ g/ml ($n = 3$) of poly(dA-dC).poly(dG-dT) in the absence of polyamines. In contrast, the same amount of estrogen receptor was eluted by 65 ± 5 μ g/ml of poly(dA-dC).poly(dG-dT) in the presence of 150 μ M spermidine. This result shows a dramatic increase in the affinity of estrogen receptor for poly(dA-dC).poly(dG-dT) in the presence of spermidine.

Poly(dA-dC).poly(dG-dT) has been shown to undergo the B to Z transition in the presence of polyamines (22). Therefore, a polyamine-induced conversion of the polynucleotide to the Z-DNA conformation might be a possible explanation for the increased affinity of estrogen receptor to poly(dA-dC).poly(dG-dT). In order to examine this possibility, we used poly(dA-dT).poly(dA-dT) as a control. This polynucleotide contains an alternating purine-pyrimidine sequence, but it does not form Z-DNA conformation in the presence of 150 μ M spermidine (36). Figure 6 shows the concentration dependent elution of estrogen receptor from DNA-cellulose by this polynucleotide in the presence and absence of 150 μ M spermidine. In the absence of spermidine, 400 ± 25 μ g/ml poly(dA-dT).poly(dA-dT) was necessary to elute 50% of the receptor. In the presence of spermidine, only 120 ± 10 μ g/ml was required for 50% elution of the receptor. This result shows that spermidine is capable of facilitating the binding of estrogen

Figure 7: Concentration dependence of calf thymus DNA to elute estrogen receptor from receptor-DNA-cellulose pellet. Calf thymus DNA was used in the presence (●) and absence (○) of 150 μ M spermidine to elute estrogen receptor from the pellet.

receptor to poly(dA-dT).poly(dA-dT); but the magnitude of this increase is much lower than that of poly(dA-dC).poly(dG-dT).

To examine the effect of polyamines on the binding of a native DNA to estrogen receptors, we determined the concentration-dependent elution of estrogen receptor with calf thymus DNA in the presence and absence of 150 μ M spermidine (Figure 7). The elution of 50% receptor was achieved at 135 ± 10 μ g/ml ($n = 4$) calf thymus DNA in the presence of 150 μ M spermidine. In contrast, 750 ± 50 μ g/ml of the same DNA was required to elute 50% of the receptor in the absence of spermidine.

DISCUSSION

This study demonstrates that natural polyamines are capable of inducing a remarkable increase in the binding affinity of estrogen receptor to native calf thymus DNA, poly(dA-dT).poly(dA-dT) and poly(dA-dC).poly(dG-dT). In the presence of 150 μ M spermidine, we observed a 22-fold increase in the relative affinity of estrogen receptor for poly(dA-dC).poly(dG-dT) compared to its binding affinity in the absence of spermidine. The increase in the binding affinity of estrogen receptor to poly(dA-dT).poly(dA-dT) and calf thymus DNA were, however, much lower (3- and 6-fold, respectively). These

results show that polyamines have a differential effect on the recognition of estrogen receptor to DNA, depending on the base sequence distribution.

A possible mechanism for the increased binding of estrogen receptor to synthetic polynucleotides and calf thymus DNA in the presence of polyamines might involve structural alterations of the receptor, DNA, or both. We showed earlier that polyamines stabilize estrogen receptor complex from ligand dissociation (14). The lower dissociation rate of the transformed receptor is usually correlated with an increased binding of the receptor to DNA (37). A three-fold increase in the half-life of dissociation ($t_{1/2}$) was observed in the presence of 1 mM spermidine. Thus, one of the contributing factors in the increased binding of the receptor to calf thymus DNA and polynucleotides seems to be the stabilization of estrogen receptor by polyamines.

The differential effects of polyamines on the binding of estrogen receptor to polynucleotides and calf thymus DNA might be the result of a conformational transition in the polynucleotide. Thomas and Messner (22) showed that the circular dichroism spectrum of poly(dA-dC).poly(dG-dT) underwent inversion in the presence of spermidine and spermine. This is a characteristic change associated with the transition of polynucleotides from their usual right-handed B-DNA form to the left-handed Z-DNA form. This result suggests that the increase in the affinity of estrogen receptors to poly(dA-dC).poly(dG-dT) is a result of the induction of its Z-DNA form in the presence of polyamines.

We used poly(dA-dT).poly(dA-dT) as a control since this polynucleotide does not undergo the B to Z transition in the presence of spermidine (36). In the absence of polyamines, poly(dA-dT).poly(dA-dT) was more effective than calf thymus DNA in eluting the receptor from DNA-cellulose. This result is in agreement with the report of Kallos and Hollander (33) who compared the affinity of different DNA's and polynucleotides for estrogen receptor. In the presence of 150 μ M spermidine, poly(dA-dT).poly(dA-dT) had a 3-fold increase in its binding affinity for estrogen receptor. This increase represents the contribution from the general stabilization effect of polyamines on receptor-DNA interaction. Consequently, the 6-fold increase in the binding affinity of calf thymus DNA could partly be attributed to the formation Z-DNA conformation in tracts of alternating purine-pyrimidine sequences.

Among the potential Z-DNA-forming sequences, (dA-dC)_n.(dG-dT)_n are particularly important because these sequences are widely dispersed in

native DNAs. Several stretches of $(dA-dC)_n$ sequences have been discovered in the regulatory regions of human fetal globin (26), rat somatostatin (28), and prolactin gene (38). In the prolactin gene, this sequence is 1,000 bp upstream from the coding region. In a survey of the 5' flanking sequences of eukaryotic genes, it was found that potential Z-DNA-forming alternating purine-pyrimidine sequences were present in the enhancer elements of several genes (29). Enhancers are DNA sequences within or near various genes transcribed by RNA polymerase II and are required for full activity of their promoters (39). It has been suggested that the receptor protein binds to the enhancer element and facilitate gene transcription (3). Recent studies (40, 41) show that hormone regulatory elements, which are specific DNA sequences that recognize steroid receptors occur at varying distances (as far as 2,700 base pairs) upstream from the gene. Although many of the hormone regulatory elements characterised so far do not contain perfect blocks of alternating purine-pyrimidine sequences, it may be noted that left handed helices have also been detected in non-alternating base sequences (42). In this context, the high affinity binding of estrogen receptor to poly(dA-dC).poly(dG-dT) in the Z-form suggests the involvement of DNA conformational polymorphism in estrogenic regulation of gene expression.

The specificity of estrogen receptor interaction with Z-DNA conformation compared to other proteins is also of interest. Thomas and Messner (43) have shown that the binding affinity anti-DNA autoantibodies present in the sera of systemic lupus erythematosus patients increased when the polynucleotides were presented in the Z-DNA conformation in a solid phase enzyme immunoassay. On the other hand, when nucleosomes reconstituted on the B-form of poly(dG-m⁵dC).poly(dG-m⁵dC) were converted to the Z-conformation, free polynucleotides were formed suggesting an instability of histone-DNA interactions (44). Thus, positive and negative control mechanisms involving Z-DNA are possible.

Intracellular polyamine levels are delicately controlled by ornithine decarboxylase, spermidine and spermine synthetases and acetylases. The fluctuations in the concentration of polyamine levels might affect the binding of regulatory proteins with DNA and consequently gene expression. We (45) and others (46, 47) have shown that polyamines are required for estrogenic regulation of cell growth in breast cancer cells. The present results suggest a mechanism for the action of polyamines involving modulation of the binding of estrogen receptor to specific DNA sequences.

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