Polyamine induced aggregation of DNA

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ABSTRACT

Polyamine induced aggregation of various DNAs has been studied under conditions usually employed in many enzymatic assays where DNA is one of the substrates. Spermine was by far the most efficient polyamine in causing aggregation followed by spermidine and cadaverine. All double-stranded and naturally occurring single-stranded DNAs were found to aggregate. No aggregation of single-stranded homodeoxypoly- mers could be detected under the same conditions. The concentration of polyamine at which the aggregation commenced was found to be a linear function of the DNA concentration. The slope of the curves depended on the nature of the poly- amine, DNA the concentration of Mg** and the ionic strength.

INTRODUCTION

Polyamines are known to play an important role in a number of intracellular processes. For recent reviews on polyamines see references 1 and 2. The interaction between nucleic acids and polyamines has been studied in a number of laboratories. Polyamines bind to nucleic acids and may cause precipitation of these at least in vitro when both are present in high concentrations 3-5. Most of this work, however, has been carried out using rather unphysiological conditions such as absence of Mg** and salts.

A number of enzymes in the nucleic acid metabolism such as various RNA and DNA polymerases have been shown to be affected by polyamines 6,7. We have previously described the effect of polyamines on the enzymes T₄ polymucleotide ligase and kinase 8,9. Recently we have also shown that polyamines have a dramatic effect on the activity of DNA polymerase I from E.coli 10. In order to be able to dis- guish between polyamine induced effects on kinetic para- meters, effects on the enzyme structure and effects on or
aggregation of the DNA substrates we have carried out an extensive study on polyamine induced aggregation of various DNAs. The results obtained in this study should be applicable to a number of different enzyme systems.

MATERIALS

DNAs. [³H] T₇ DNA was a gift from K. Solberg. It was isolated as described in reference 11 with a specific activity of 650 cpm/nmoles. [¹⁴C] d(A-T)ₙ was made essentially according to the procedure of Burd and Wells 12, the specific activity being 960 cpm/nmoles. [¹⁴C] d(A-T)ₙ of different molecular weights were made by treating the DNA by pancreatic DNase in 50 mM Tris buffer, 5 mM MgCl₂ pH 7.5, at 37°C for various lengths of time. The pancreatic DNase was inactivated by heating the samples at 80°C for 10 minutes. Unlabelled d(A-T)ₙ was a product of Sigma Chemical Company, the average sedimentation coefficient being s₂₀,ₘ = 5.6 S. (dA)ₙ, s₂₀,ₘ = 6.5 S, (dT)ₙ, s₂₀,ₘ = 5.9 S and d(T)₁₀ were purchased from P.L. Biochemicals. [³H] labelled φX174 and φX174 RF I form were gifts from Dr. I.F. Nes.

Polyamines. All polyamines were purchased from Sigma Chemical Company, except spermine which was from Koch Light. The purity of the polyamines was examined by paper chromatography as previously described 1.

Enzymes. Pancreatic DNase was obtained from Sigma Chemical Company. Bacterial phosphatase was a product of Worthington Biochemical Corporation. T₄ polynucleotide kinase was a gift from Dr. J.R. Lillehaug.

METHODS

Test for aggregation. The primary objective of this work was to study polyamine induced aggregation of various DNAs using conditions normally employed in enzymatic assays where DNA is one of the substrates. The concentration of MgCl₂ in such assays is usually between 5-10 mM whereas the concentration of DNA may vary considerably. Thus in the case of the standard assay of DNA polymerase I from E.coli 13 and T₄ polynucleotide ligase 8 the standard DNA concentration used are 30 and 1.4 µM DNA, respectively. With that in mind the concentration of MgCl₂ used in the standard
assay in the present work was taken to be 7 mM whereas the concentration of DNA varied from 5 to 30 μM. The standard assay contained 70 mM Hepes/KOH pH 7.0 and 7 mM MgCl₂; the total ionic strength was estimated to be 0.080. The concentrations of DNA are given in the legend to each figure. The solutions were mixed at 0°C in Eppendorf 1.5 ml polyethylene reaction vials, the total volume being 150 μl or 300 μl depending on whether radioactivity or absorbance of the supernatant was to be measured. The reaction mixture was incubated at 37°C for 10 minutes. At the end of this period it was subjected to centrifugation for 2 minutes in a Wifug microcentrifuge at 9000 rpm (3500 x g) and room temperature. The amount of DNA left in the supernatant was then determined either from radioactivity or absorbance measurements. In the case of the former 100 μl of the supernatant was pipetted onto 3 MM Whatman filter paper discs. These were then subsequently dried and counted in a liquid scintillation counter. The absorbance measurement of DNA was carried out using 0.3 ml cuvettes having a light path of 1 cm.

The time of sedimentation of 2 minutes used in the standard assay was chosen so as to be able to distinguish between the size of the aggregates at various DNA concentrations. In most experiments 20 % or less of the DNA remained in the supernatant after a centrifugation period of 2 minutes.

Determination of molecular weight and sedimentation coefficients. The average molecular weight of \([^{14}C] d(A-T)_n\) was determined using T₄ polynucleotide kinase essentially as described in reference 14.

The sedimentation coefficients were determined from data obtained using Spinco Model E Analytical centrifuge or MSE Centriscan ultracentrifuge.

RESULTS

Effect of different polyamines. Aggregation experiments with T₇ DNA were performed in the presence of different polyamines using standard conditions described in Methods, and the results are shown in Figure 1. Spermine was found to be the most efficient polyamine with regard to aggregation of DNA followed by spermidine and cadaverine. Putrescine,
Figure 1. Influence of different polyamines on aggregation of [\( ^{3}H \)] T7 DNA. The standard assay system as described in Methods was employed. The concentration of T7 DNA was 15 \( \mu \)M. Polyamine concentration varied as shown above.

However, had little effect. The concentrations of the different polyamines where aggregation commenced, \([\text{polyamine}]_{agg}\) (see Figure 1), which is taken to be the half-point concentration between zero aggregation and the plateau value obtained, was 0.08 mM for spermine, 1.5 mM for spermidine and 15 mM for cadaverine. It should be pointed out that no precipitate or flocculation could be detected after the amines had been mixed with DNA. It appears that the sedimentation coefficient for the aggregates formed in the presence of spermine, spermidine or cadaverine must be approximately the same.

Effect of DNA Structure and molecular weight. The \([\text{polyamine}]_{agg}\) values in the case of spermine and spermidine were determined for various DNAs using standard conditions and 15 \( \mu \)M DNA and the results are shown in Table I. The supercoiled DNA, \( \phi X 174 \) RF I, gave the lowest \([\text{polyamine}]_{agg}\) both with spermine and spermidine. The highest \([\text{polyamine}]_{agg}\) values were obtained with single-stranded DNAs. The \([\text{polyamine}]_{agg}\) values of spermidine for the double-stranded DNAs
T₇ and d(A-T)ₙ were found to lie between the values for the supercoiled and single-stranded φX174 DNAs. With spermine, on the other hand, φX174 aggregated easier than T₇ and d(A-T)ₙ.

Aggregation experiments were also carried out with the synthetic polymers (dA)ₙ and (dT)ₙ. When present as single-stranded DNA no aggregation was detected as shown in Table II using spermidine. Similar results were also observed when these DNAs were subjected to centrifugation in the analytical centrifuge. Aggregation was, however, detected when (dA)ₙ and (dT)ₙ were mixed in equal molar ratio thus forming the double-stranded DNA (dA)ₙ • (dT)ₙ. When (dT)₁₀ was added to (dA)ₙ little aggregation was detected when the ratio between (dA)ₙ and (dT)₁₀ was high. Aggregation commenced as this ratio decreased and at a ratio of 1:1 only 33% of the DNA remained in the supernatant.

Table I

[Polyamine]ₐₚₚ values for various nucleic acids.

<table>
<thead>
<tr>
<th>DNA</th>
<th>[Polyamine]ₐₚₚ (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spermine</td>
</tr>
<tr>
<td>T₇</td>
<td>0.079</td>
</tr>
<tr>
<td>d(A-T)ₙ, 5.6 S</td>
<td>0.057</td>
</tr>
<tr>
<td>φX174 RF I</td>
<td>0.010</td>
</tr>
<tr>
<td>φX174</td>
<td>0.015</td>
</tr>
<tr>
<td>T₇, heat-denatured</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Standard conditions were employed and the concentration of DNA was in all cases 15 μM (phosphate).

Table II

Aggregation of different synthetic DNAs in the presence of 3.3 mM spermidine.

<table>
<thead>
<tr>
<th>DNA</th>
<th>Absorbance 260nm</th>
<th>Absorbance 260 nm % DNA in super-</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>before centri-</td>
<td>after centrifugation</td>
</tr>
<tr>
<td>(dA)ₙ</td>
<td>0.21</td>
<td>0.20</td>
</tr>
<tr>
<td>(dA)ₙ</td>
<td>0.54</td>
<td>0.52</td>
</tr>
<tr>
<td>(dT)ₙ</td>
<td>0.25</td>
<td>0.24</td>
</tr>
<tr>
<td>(dA)ₙ • (dT)ₙ (1:1)</td>
<td>0.30</td>
<td>0.03</td>
</tr>
<tr>
<td>(dA)ₙ • (dT)ₙ (20:1)</td>
<td>0.19</td>
<td>0.18</td>
</tr>
<tr>
<td>(dA)ₙ • (dT)ₙ (1:1)</td>
<td>0.36</td>
<td>0.12</td>
</tr>
<tr>
<td>d(A-T)ₙ</td>
<td>0.18</td>
<td>0.05</td>
</tr>
</tbody>
</table>

The DNAs were allowed to anneal prior to addition of spermidine. All experiments were carried out at 25 °C.
The influence of molecular weight in the [polyamine]_{agg} was investigated using d(A-T)_{n} samples of varying molecular weight. The latter DNAs were prepared as described in Methods. The results of these experiments are shown in Table III. In the molecular weight range studied the [polyamine]_{agg} increased slightly with decreasing molecular weight. It is interesting to note that a DNA of average molecular weight as low as 9000 will aggregate under the conditions employed.

Effect of pH. At pH 7 spermine has close to 4 and spermidine close to 3 positive charges. An increase in pH brings about a concomitant decrease in the number of charged amino groups of these molecules, and thus at pH 9 spermine has only 2-3 positive charges. It should be pointed out, therefore, that higher concentrations of polyamines are needed for aggregation to occur at higher pH values. The aggregation of d(A-T)_{n} by spermine at three different pH values, pH 7.0, pH 8.0 and pH 8.8 was investigated in detail using the standard assay and 10 μM of the polymer. The [spermine]_{agg} at the respective pH values were 0.057 mM, 0.25 mM and 1.15 mM.

Influence of MgCl_{2} and salt. Aggregation experiments were carried out using different concentrations of MgCl_{2} and as expected the concentration of MgCl_{2} had a profound influence on the aggregation of d(A-T)_{n} as shown in Figure 2. The aggregating concentration of spermine increased almost linearly with increasing concentrations of MgCl_{2} and at 20 mM MgCl_{2} it was approximately 20 times higher than that obtained in the absence of MgCl_{2}. Somewhat similar results were obtained with spermidine and also in the presence of increasing concentration of KCl. Thus when 0.05 and 0.2 M KCl were added

<table>
<thead>
<tr>
<th>DNA</th>
<th>Average mol. weight</th>
<th>[Spermine]_{agg} (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>d(A-T)_{n} I</td>
<td>4 x 10^{5}</td>
<td>0.050</td>
</tr>
<tr>
<td>d(A-T)_{n} II</td>
<td>9 x 10^{4}</td>
<td>0.095</td>
</tr>
<tr>
<td>d(A-T)_{n} III</td>
<td>9 x 10^{3}</td>
<td>0.110</td>
</tr>
</tbody>
</table>

Standard assay conditions were employed, the concentration in all cases being 10 μM (phosphate).
Figure 2. Influence of MgCl₂ concentration on spermine induced formation of [¹⁴C] d(A-T)ₙ aggregates. Standard assay conditions were employed except that the concentration of MgCl₂ varied as shown above. The concentration of d(A-T)ₙ was 10 μM (phosphate).

to the reaction mixtures the [spermine]ₐᵍᵍ increased from 0.057 to 0.6 and 2.0 mM, respectively.

Aggregation at different DNA concentrations. As expected the [polyamine]ₐᵍᵍ values were also found to depend very much on the DNA concentrations employed in the different experiments. Figure 3 shows the influence of DNA concentration on the [spermidine]ₐᵍᵍ for two different DNAs, namely φX174 and φX174 RF I. For both DNAs the experiments were carried out in the absence and presence of 0.1 M KCl. In all cases a linear relationship was obtained between the [spermidine]ₐᵍᵍ and the DNA concentration. This relationship can be expressed by the following equation:

\[
[spermidine]_{agg} = \alpha [DNA] + \beta
\]

α and β are constants and the DNA concentration is expressed in mM. These constants were determined for some DNAs as shown in Table IV. For a given ionic strength α was found to be 5-6 fold larger for φX174 than for φX174 RF I, whereas β differed only maximally 1 fold. Addition of 0.1 M KCl gave an increase in α of approximately 6 fold for both DNAs and in the case of β this increased only for φX174 RF I. When
Figure 3. Effect of DNA concentration on [spermidine]. Experiments with $\phi X174$ and $\phi X174$ RF I were carried out in the absence and presence of 0.1 M KCl. Otherwise standard conditions were used.

Table IV

Values of $\alpha$ and $\beta$ constants for different nucleic acids with spermidine.

<table>
<thead>
<tr>
<th>DNA</th>
<th>$\alpha$</th>
<th>$\beta$ (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\phi X174$</td>
<td>110</td>
<td>0.4</td>
</tr>
<tr>
<td>$\phi X174 + 0.1$ M KCl</td>
<td>600</td>
<td>0.4</td>
</tr>
<tr>
<td>$\phi X174$ RF I</td>
<td>20</td>
<td>0.2</td>
</tr>
<tr>
<td>$\phi X174$ RF I + 0.1 M KCl</td>
<td>110</td>
<td>0.6</td>
</tr>
<tr>
<td>$\phi X174$ RF I + 15 mM MgCl$_2$</td>
<td>50</td>
<td>0.2</td>
</tr>
</tbody>
</table>

15 mM MgCl$_2$ was used instead of the customary 7 mM $\alpha$ increased 2.5 fold whereas $\beta$ was unchanged.

Similar results to those observed with spermidine was also obtained with spermine. Using standard conditions the values calculated for $T_\gamma$ were $\alpha = 1.1$ and $\beta = 0.064$ mM.
DISCUSSION

The present work deals with polyamine induced aggregation of various DNAs using conditions normally employed in many enzymatic reactions. The aggregation was found to be dependent on the ionic strength, pH and the concentration of Mg\(^{++}\) and DNA. An empirical relationship was established between the \([\text{polyamine}]_{\text{agg}}\) and the concentration:

\[
[\text{polyamine}]_{\text{agg}} = \alpha[\text{DNA}] + \beta
\]

Under defined reaction conditions \(\alpha\) and \(\beta\) are constants which are characteristic of a certain DNA. The \(\alpha\) constant reflects the ease of aggregation of a given DNA whereas \(\beta\) gives the \([\text{polyamine}]_{\text{agg}}\) at infinite DNA dilution. The latter constant does not appear to vary much for the different DNAs tested. If \(\alpha\) and \(\beta\) are known for a given DNA under certain experimental conditions then it follows that if

\[
\frac{\alpha[\text{DNA}] + \beta}{[\text{polyamine}]} < 1
\]

taggregation will take place. If, on the other hand, this expression is larger than 1 aggregation will not occur.

Polyamines bind to negatively charged phosphate groups in the DNA molecules thereby neutralizing the charges on these. \(^{16}\) Liquori et al.\(^{17}\) have presented evidence suggesting that in the case of double-stranded DNAs polyamines such as spermine and spermidine bind preferentially across the narrow groove in the molecule thereby creating a tighter interaction between the strands. Aggregation of DNA molecules are thought to occur as a result of the charge neutralization. It might also be enhanced when polyamines bind between strands of different molecules. Such intermolecular cross-linking would be easier to visualize with rod-like double-stranded DNAs than with the random-coil single-stranded DNAs. The marked difference in aggregation behaviour between double- and single-stranded DNAs might be explained by such an hypothesis.

The influence of polyamines on the activities of various enzymes involved in DNA and RNA metabolism such as DNA and RNA polymerases has been studied in many laboratories.
many cases an activation is observed in the presence of low concentration of polyamines followed by a pronounced inhibition at higher concentrations\textsuperscript{6,7,18}. The inhibition noted is probably the result of aggregation of the DNA substrates rather than an effect on the kinetic parameters. It is important to realize that under the conditions used in many of these experiments aggregation of double-stranded DNAs will take place without any visible precipitate being formed in the reaction vessels.

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REFERENCES

