nitrogen deficiences may prevail and where adequate nitrogen supplies might limit future crop production. The maintenance of high protein seed for a given genotype may be imperative for the progressive development of agronomic crops.

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Actinomycin Binding to DNA: Inability of a DNA Containing Guanine To Bind Actinomycin D

Abstract. *Polydeoxy (adenylyl-thymidylyl-cytidylyl) · polydeoxy(guanylyl-adenylyl-thymidylyl), a double-stranded DNA polmer of high molecular weight containing 33 percent guanine plus cytosine, binds little or no actinomycin D, as measured by five different techniques. In contrast, the sequence isomer of this DNA, polydeoxy(thymidylyl-adenylyl-cytidylyl) · polydeoxy(guanylyl-thymidylyl-adenylyl), does bind the antibiotic. Thus, the presence of guanine in a DNA is not a sufficient requisite for the binding of actinomycin D.*

Poly d(A-T-C) · poly d(G-A-T) (*1*) was prepared by a combination of chemical and enzymatic techniques (*2*); it is a DNA polymer of high molecular weight (approximately 0.3 million daltons) containing a strictly repeating deoxyadenylyldeoxythymidylyldeoxycytidylyl sequence in one strand and a strictly repeating deoxyguanylyldeoxyadenylyldeoxythmidylyl sequence in the complementary strand. The DNA is double-stranded (*3*) and contains equal quantities of the two strands (*2*). The ability of this DNA, which contains 33 percent G+C, to bind actinomycin D was judged by spectroscopy, equilibrium dialysis, buoyant density in the analytical ultracentrifuge, absorbance-temperature transitions, and the inhibition in vitro of DNA-dependent synthesis of RNA. Little or no antibiotic is bound to this DNA under the conditions studied. This is the first example of a DNA containing guanine which does not bind actinomycin D. Thus, contrary to predictions (*4*), the presence of guanine in a DNA is not a sufficient requisite for binding.

The metabolic basis for the bacteriostatic and antitumor activities of actinomycin has been widely studied (*4*). The antibiotic binds to DNA and preferentially inhibits the DNA-dependent synthesis of RNA. The binding of actinomycin is specific for double-stranded helical DNA which contains guanine (*4–7*); single-stranded or heat-denatured DNA binds the drug only poorly, and a variety of RNA's will not bind actinomycin. In addition, a hybrid molecule consisting of the single-stranded DNA of bacteriophage alpha and its complementary RNA shows no detectable binding. Hence, it has been concluded (*4, 6*) that actinomycin binding is specific for polynucleotides containing guanine which exist in a DNA B configuration.

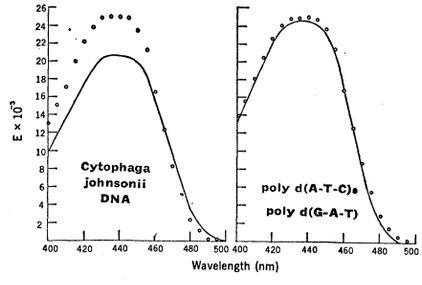

Fig. 1. Spectra of actinomycin D before and after mixing with DNA from *Cytophaga johnsonii* and poly d(A-T-C) · poly d(G-A-T). Spectra were obtained with a split compartment mixing cell (Pyrocell Manufacturing Co.) in a Cary 15 spectrophotometer. A composite spectrum was first determined with DNA (16 to 20 μM) in one compartment and actinomycin D (22 μM) in the other compartment (open circles); the cell was then inverted and mixed, and the perturbation spectra (solid lines) were determined after 10 minutes. All substances were dissolved in 0.01*M* sodium chloride and 0.001*M* sodium phosphate (*p*H 7.4).

Figure 1 shows the capacity of two DNA's, poly d(A-T-C) • poly d(G-A-T) and naturally occurring DNA from *Cytophaga johnsonii* (both containing 33 to 34 percent G+C), to perturb the visible spectrum of actinomycin D. The DNA from *C. johnsonii* induces a slight hyperchromic shift at 480 nm and a stronger hypochromic shift at 440 nm, both shifts resembling those caused by other DNA's containing guanine (*4*). Conversely, poly d(A-T-C) • poly d(G-A-T) causes no spectral perturbation outside of experimental error.

The most sensitive and thermodynamically sound technique used for detecting the binding of actinomycin to DNA is equilibrium dialysis with tritiated actinomycin. Twenty equilibrium dialysis experiments were performed on two different preparations of poly d(A-T-C) • poly d(G-A-T); the experiments were performed at 23°C in cells designed to contain as little as 0.1 ml of solution on each side of the membrane (*8*). The DNA concentration was held at $8 \times 10^{-6} M$ throughout, and the concentration of tritiated actinomycin D (Schwarz BioResearch; specific activity, 3.4 c/mmole) was varied from 0.05 to $5.0 \times 10^{-6} M$; experiments were performed in a buffer consisting of 0.01*M* sodium chloride and 0.001*M* sodium phosphate (*p*H 7.4). Equilibrium was achieved in 120 hours in the dark, with gentle shaking. The experiment was designed so that it was possible to easily detect as little as one actinomycin D molecule bound per 100 DNA nucleotides. At an actinomycin concentration ($3.2 \times 10^{-6} M$) that is saturating for a variety of DNA polymers as well as for two naturally occurring DNA's, poly d(T-A-C) • poly d(G-T-A) (*1, 2*) bound approximately 0.07 mole of actinomycin per mole of DNA phosphorus (that is, over 60,000 count/min). Under identical conditions, poly d(A-T-C) • poly d(G-A-T) showed no detectable binding of the antibiotic (less than 500 count/min). Binding isotherms for this DNA were indistinguishable from those obtained (*8*) under identical conditions for poly d(A-T) • poly d(A-T) and

poly d(I-C) • poly d(I-C) (*9*), two DNA's which do not bind the antibiotic (*4, 8*).

In analytical ultracentrifuge experiments, the shift in buoyant density of a DNA induced by the presence of some antibiotics serves as another tool for detecting binding (*4, 10*). In the presence of actinomycin, the buoyant density of a DNA that binds the antibiotic in a cesium sulfate gradient is markedly depressed. Figure 2 shows the results of experiments where poly d(A-T-C) • poly d(G-A-T) and its sequence isomer poly d(T-A-C) • poly d(G-T-A) (*1, 2*) are titrated with actinomycin D. Experiments were performed as described (*11*) except that a given concentration of actinomycin D was added and poly d(A-T) • poly d(A-T) was the marker DNA. The buoyant density of poly d(T-A-C) • poly d(G-T-A) is reduced by as much as 30 mg/cm^3 in the presence of the antibiotic, whereas the buoyant density of poly d(A-T-C) • poly d(G-A-T) is changed only slightly. This slight density decrease observed for poly d(A-T-C) • poly d(G-A-T) is easily detectable and is reproducible for different preparations of this DNA. Hence under these conditions, some small amount of possibly nonspecific binding was detected. Two major differences between these experiments and the equilibrium dialysis experiments should be pointed out: (i) by necessity the centrifugation experiments were performed in very concentrated salt solution, and (ii) actinomycin D concentrations of up to 8 moles per mole of DNA deoxyguanylic acid were used. Thus this observed binding may be related to the weak binding of actinomycin to DNA (*5*) and not to the strong binding believed to cause inhibition of DNA transcription.

The binding of actinomycin to DNA causes an elevation in the temperature necessary to convert the helix to a random coil (*4, 6, 10*). The absorbance-temperature transition for poly d(A-T-C) • poly d(G-A-T) in the presence of 23 μM actinomycin D was indistinguishable (1°C increase outside of experimental error) from that observed in the absence of the antibiotic at 0.01M sodium ion concentration. Under similar conditions, the melting temperature (T_M) of pneumococcal DNA (containing 39 percent G+C) was raised 16°C (*4*).

The mode of action of actinomycin as an inhibitor of RNA synthesis in intact cells has been elucidated as an interference with the ability of DNA to act as a template for the DNA-dependent RNA polymerase in vitro (*4*). There is evidence that the antibiotic inhibits primarily the RNA chain elongation process and, to a lesser extent, the initiation of new RNA chains (*12*). Since poly d(A-T-C) • poly d(G-A-T) is the first DNA found that contains guanine but does not bind actinomycin D (at least at the low concentrations normally studied), the effect of the antibiotic on the transcription in vitro of this DNA by the *Escherichia coli* RNA polymerase was studied. The conditions used for these studies were similar to those described (*11*), except that poly d(A-T-C) • poly d(G-A-T) was the DNA used and actinomycin was added at various concentrations; all four ribotriphosphates were supplied, with riboadenosine triphosphate (rATP) containing a radioactive isotope, in order to permit the transcription of both DNA strands. In a 30-minute reaction period, the incorporation of labeled substrate into acid-insoluble RNA in the presence of 0.45 and 4.5 μM actinomycin D was 119 and 85 percent, respectively, of the incorporation into the control. Incorporation into the control indicated approximately twofold transcription. Under identical conditions, but with salmon sperm DNA as the template, the incorporation of substrate was 47 and 7 percent, respectively, of that of the control.

The reason for the inability of poly d(A-T-C) • poly d(G-A-T) to bind the drug is unknown at this time. Physical and enzymatic studies (*2, 3, 8*), show that this DNA has slightly different characteristics from its sequence isomer, poly d(T-A-C) • poly d(G-T-A), which does bind the antibiotic (Fig. 2). Thus the two DNA's may have sufficiently different configurations, dictated by their base sequences, to determine this difference in their behavior. That poly d(A-T-C) • poly d(G-A-T) may not possess a DNA B configuration is apparently unimportant as regards actinomycin D binding, since other DNA's, which have been shown to contain configurations other than the B type, will bind (*8*). Conversely, perhaps the binding of actinomycin D requires a specific type of base sequence which is not present in poly d(A-T-C) • poly d(G-A-T). It is impossible at this time to choose between the alternative explanations. The reason for this behavior may have to await a complete structural elucidation.

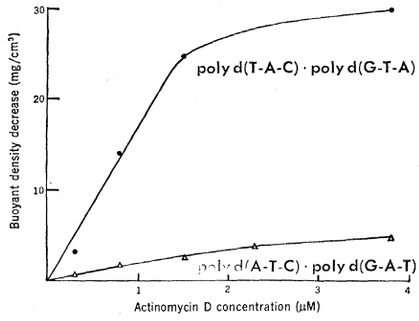

Fig. 2. Decrease in buoyant density of poly d(T-A-C) • poly d(G-T-A) and poly d(A-T-C) • poly d(G-A-T) in the presence of actinomycin D. Cesium sulfate density gradients (*p*H 7.4) were established in a model E analytical ultracentrifuge. The DNA densities in the absence of actinomycin are 1.422 and 1.418 g/cm^3, respectively.

It is concluded that the presence of guanine moieties in a DNA is not, in itself, a sufficient requisite to cause the binding of actinomycin D. The DNA structural considerations may be of paramount importance. Indeed in most cases, the presence of guanine in a DNA may induce a suitable configuration to permit the binding of actinomycin, hence explaining the observed (*4*) necessity for guanine.

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