THE BINDING OF ACTINOMYCIN TO CRAB dAT; THE NATURE OF THE DNA BINDING SITE

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The present communication deals with the nature of the binding site on DNA for actinomycin D (AMD). We find that with the component of Cancer antenarrius crab DNA known as crab dAT, for which the ratio of GC to AT base pairs is 1:28, the strong binding saturates at one actinomycin per 56 base pairs. This result most probably excludes the hypothesis that a GpG sequence on one strand is required for strong binding. Several alternate hypotheses, which are consistent with the results, are examined.

The strong binding sites for AMD appear to occur only in native two-stranded DNA (Goldberg, Rabinowitz, and Reich, 1962; Cavalieri and Nemchin, 1964; Gellert, Smith, Neville, and Felsenfeld, 1965). Since synthetic dAT does not bind at all (Goldberg, et al., 1962; Gellert, et al., 1965; Kahan, Kahan, and Hurwitz, 1963) a GC base pair must be present in these sites. Studies with nucleosides suggest that G rather than C is the critical residue for binding (Kersten, 1961). However, in a number of DNA's with a GC content varying from 75 to 25%, the number of AMD binding sites is fairly constant at about 1 per 7 base pairs (Gellert, et al., 1965). Thus, either there is some further requirement for the sequence around a G in order for binding to occur, or repulsive

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interactions (either directly steric or indirectly by local distortion of the DNA structure) between neighboring actinomycins limit the amount of binding. Hamilton, Fuller, and Reich (1963) estimate that the direct steric effect of the cyclic peptide chains of actinomycin would extend for about three base pairs.

Crab dAT is mainly an alternating AT copolymer, with about 3% of GC base pairs. A method for the preparation of this material in its fully native form, without disruption of the GC base pairs, has been developed in this Laboratory (Davidson, Widholm, Nandi, Jensen, Olivera, and Wang, 1965). In this DNA, GC base pairs are sufficiently rare so that steric interactions are less likely to be important; furthermore, the sequence GpG occurs in a very low frequency. It therefore seemed of interest to investigate the binding of actinomycin to native crab dAT.

Experimental

Cancer antenarrius DNA was prepared essentially by the method of Smith (1963) except that all steps were carried out in the cold. The component called crab dAT was separated by the mercury-cesium sulfate buoyant density method of Davidson, et al. (1965). This DNA is an alternating copolymer with 3.5% GC base pairs (Widholm, 1965); its properties are generally very similar to those of the better known Cancer borealis dAT (2.7% GC). Actinomycin D was a gift from Merck, Sharp, and Dohme.

Equilibrium binding studies were made by sedimenting the DNA-AMD complex and measuring the concentration of free AMD remaining in the supernatant solution. Three ml of solution containing known amounts (spectrally determined) of DNA and AMD were placed in a carefully washed SW39 polyallomer tube and covered with two ml of mineral oil. The bottom ml also contained 20% sucrose. Salt concentrations were 10^{-3} M phosphate, 10^{-2} M NaCl, pH 7. The rotor was spun at 42,000 rpm for six hours at 20°C thereby pelleting the AMD-DNA complex. The

polyallomer tubes were dripped from the side, one ml from the bottom, and two ml of supernatant solution collected. The free AMD concentration was then measured by spectrophotometry, using 1 and 5 cm path lengths. Actinomycin readily adsorbs to glass surfaces and was handled with polypropylene tubes and pipets.

Results and Discussion

Plots of the binding of actinomycin to native calf thymus DNA and crab dAT are shown in Fig. 1. The vertical coordinate, r, is the ratio of

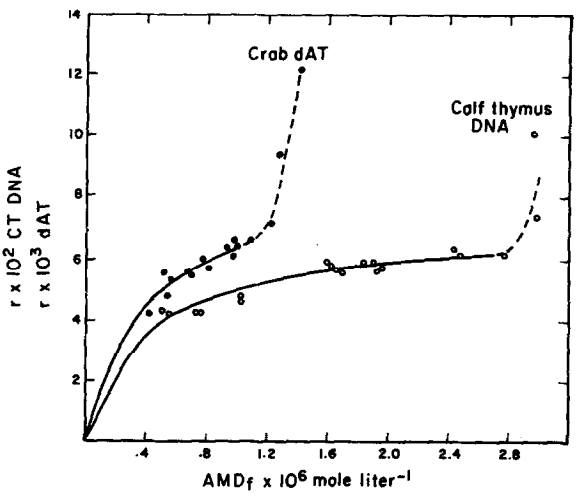

Fig. 1

bound AMD to total nucleotides; the horizontal coordinate is the concentration of free AMD. The curves indicate a strong binding reaction at low free actinomycin concentration followed by further uptake of AMD at a higher free AMD concentration. A least-squares analysis of a Scatchard plot of these data in the strong binding region gives $K = 2.3 (\pm .5) \times 10^6$ $mole^{-1}$ liter, and one site per 14 (±1) nucleotides, for calf thymus DNA. These values agree well with those reported by Gellert, et al. (1965).

For crab dAT, the intrinsic binding constant is $2.5 (\pm .5) \times 10^6$

mole^{-1} liter, and there is one site per 112 (±4) nucleotides, or one per 56 base pairs. Since the binding constant is of the same order of magnitude as for calf thymus and other typical DNA's, the binding site on crab dAT appears to be a typical strong binding site. There is approximately one such binding site for every two GC base pairs in crab dAT.

The nearest neighbor frequencies for Cancer borealis dAT (2.7% GC) have been determined by Schwartz, Trautner, and Kornberg (1962). These data are: ApA = 0.0127, TpT = 0.0126; CpA = 0.0100, TpG = 0.0089; GpA = 0.0042, TpC = 0.0015; CpT = 0.0004, ApG = 0.0018; GpT = 0.0081; ApC = 0.0069; GpG = 0.0009, CpC = 0.0009; TpA = 0.504; ApT = 0.429; CpG = 0.0007; GpC = 0.0015. From these data, 0.939 of the G residues are followed by non-G; 0.927 of the G residues are preceded by a non-G residue. If there is no correlation between the probability of a base preceding a G and the probability of a base following a G, 0.87 of the G residues do not have a G residue on either side as a nearest neighbor. If these data for Cancer borealis dAT apply to Cancer antennarius dAT, and if all the binding sites involve a G residue, the data exclude the possibility that adjacent G's on the same strand are needed to define a strong binding site.

Professor Don Crothers has pointed out the following argument to us. The nearest neighbor frequencies for Cancer borealis show that 0.64 of the G's are followed by a pyrimidine and 0.74 of the G's are preceded by a pyrimidine. Thus, assuming no correlation between the nucleotides preceding and following the G, $1-(0.64 \cdot 0.74) = 0.52$ of the G's have a purine as a nearest neighbor. Thus, the assumption that a binding site consists of a G plus an adjacent purine is consistent with the observation of one site per two G's.

Apart from the nearest neighbor frequencies, nothing is known about the distribution of GC base pairs along the chain. If the distribution is random, and if p = frequency of occurrence of an AT base pair, the

probability that a sequence following a given GC pair consists of (n - 1)AT pairs followed by a GC pair is $p^{n-1}(1 - p)$. The average length of such a sequence is $\langle n \rangle = 1/(1 - p)$. The value of n such that half the sequences are equal to or shorter than n is $\ln 2/(1 - p)$. Thus, if $\langle n \rangle = 28$, half the sequences are longer than 19 base pairs. It seems unlikely that a steric interaction between actinomycins would extend this large distance.

Therefore, if every GC base pair is intrinsically a binding site, the observation that only 1/2 of them are available for strong binding implies that the GC base pairs are not distributed at random along the crab dAT chain but there is sufficient bunching to decrease the fractional number of sites to 1/2. Alternatively, there is an additional sequence requirement around a G; for example, the one suggested by Crothers that the G must have a neighboring purine.

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