THE REQUIREMENT OF INTACT DOUBLE HELICAL STRUCTURE FOR PRIMARY BINDING IN QUINACRINE-NUCLEIC ACID COMPLEXES

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SUMMARY: Exciton-like splitting is observed in the ultraviolet difference absorption spectra of complexes of quinacrine and polynucleotides with intact double helical structure but not in denatured DNA. These results indicate that an intact double helical structure of the polynucleotide is a prerequisite for exciton-like splitting and primary binding in quinacrine-nucleic acid complexes.

Introduction:

In general, two types of complexes formed between aminoacridine dyes and nucleic acids can be distinguished; a strong 'primary' binding which occurs at high nucleotide phosphate-dye ratios (P/D), and a weaker 'secondary' binding which obtains at low P/D ratios (1,2). Lerman (3,4), proposed a model for intercalation in which the cationic acridine dye was enclosed between adjacent base pair layers in the nucleic acid double helix. However, similar binding constants and visible absorption and fluorescence spectral properties were observed for the strong 'primary' binding of acridine dyes to both native and denatured DNA (2,5,6,7,8). Blake and Peacocke (9) presented a modification of the Lerman model to account for these similarities. They proposed that the positive charge of the dye remained in the neighbourhood of the negatively charged phosphate backbone of the DNA and the dye inserted between adjacent nucleic acid bases on the same strand. The important distinction between these two models is that the former requires an intact double stranded helical structure whereas the latter only requires base stacking within the same strand of the polynucleotide.

Recently we reported the observation of exciton-like interaction in

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the ultraviolet difference absorption spectra (250-300 nm) of quinacrine (QAC)[3]-DNA and QAC-poly d(AT)·poly d(AT) complexes (10). We have shown that such an interaction, which results in a clearly defined splitting of the absorption band, occurs only if there is a close approach between the polynucleotide bases and QAC. The lack of distinct splitting in the spectra of QAC-poly d(G)·poly d(C) complexes was consistent with this interpretation.

In this report we have studied the ultraviolet difference absorption spectra of QAC complexes of native and denatured DNA and demonstrate that the observation of exciton splitting requires intact double stranded polynucleotides thus supporting Lerman's original intercalation model for QAC binding to nucleic acids.

Materials and Methods:

Calf Thymus DNA was purchased from Worthington Biochemicals. Solutions of DNA were prepared in 0.05M cacodylate buffer (pH 7) and dialyzed for 24 hours prior to use. The DNA was denatured by heating the solution to 100°C for 5 minutes and then rapidly cooling in ice. The hyperchromic effect of the native DNA, measured at 260 nm was 42%. Thermal stability measurements were performed using a modified Gilford 240 spectrophotometer equipped with a Model 2427 thermo-programmer. Denatured, sheared DNA was fractionated on pre-equilibrated hydroxyapatite columns (60°C, 0.15M phosphate buffer pH 7) according to the method described by Bernardi (11). The slow reassociating fraction recovered from the eluant was rechromatographed in the same manner to ensure proper separation of the DNA components. Rapid reassociating or double stranded DNA was recovered from the columns after three washes with 0.15M phosphate buffer followed by 0.5M phosphate. It had a C_0t value of 1.3 s.M.1^{-1}. These fractionated DNAs were dialyzed against 0.05M cacodylate buffer prior to use and the dialyzed single stranded DNA (slow reassociating fraction) was again heat denatured before use.

Quinacrine dihydrochloride was purchased from Sigma Chemicals and recrystallized in the anhydrous form from methanol/2-butanol (1/1). Fresh stock solutions of QAC were prepared each day and solutions of QAC and DNA were used within 1 hour of preparation.

The difference absorption spectra were run as previously described (10) on a Cary 15 spectrophotometer.

Results and Discussion:

In order to ensure that the difference absorption spectra of the QAC-native DNA and QAC-denatured DNA complexes were measured at P/D ratios where strong 'primary' binding was dominant, spectrophotometric titrations were performed keeping the QAC concentration constant and varying the DNA

[3]Abbreviation: QAC, quinacrine.

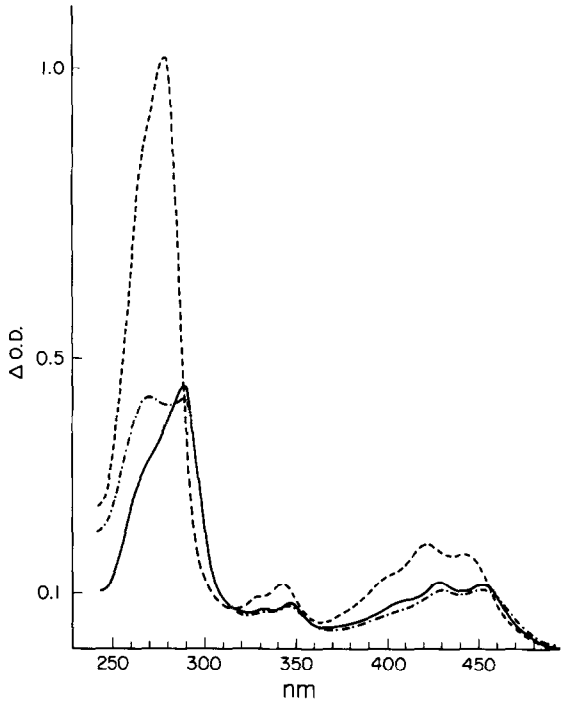

Figure 1: Absorption spectrum of QAC ---; difference absorption spectrum of QAC and native DNA -·-·-·; and denatured DNA ——; against the respective polynucleotide solutions. [QAC] = 1.76×10^{-5}M; [native or denatured DNA] = 3×10^{-4}M; cacodylate buffer, 0.05M, pH 7. Spectra were run on a Cary 15 spectrophotometer (dispersion 17 A°/mm) using 1 cm cells at 23°C. The reference cell contained a concentration of native or denatured DNA equal to that in the sample cell which contained the QAC-DNA solution. Identical maxima were observed for reference O.D.'s of 0.6 (slits, 1 mm) and 1.85 (slits, 2.5 mm).

concentration. The limiting absorption spectrum for QAC-native DNA was reached at ~ P/D = 7 whereas the limiting spectrum for QAC-denatured DNA was reached at ~ P/D = 15. These spectra (Fig. 1) were superimposable in the 400-500 nm region and additionally a distinct isosbestic point at 455 nm was observed in the titrations for both systems. Increasing the P/D ratios beyond these limiting values did not alter the visible absorption band. Scatchard representations of these results (Fig. 2) indicated that the apparent number of strong binding sites (β_{app}) for QAC increased substantially in denatured DNA compared to native DNA. This result agrees with that reported by Drummond et al (2) who found that β_{app} increased for other aminoacridines in denatured DNA. The similarity of the initial steep slopes of the Scatchard plots, which are indicative of a strong binding process, and the visible absorp-

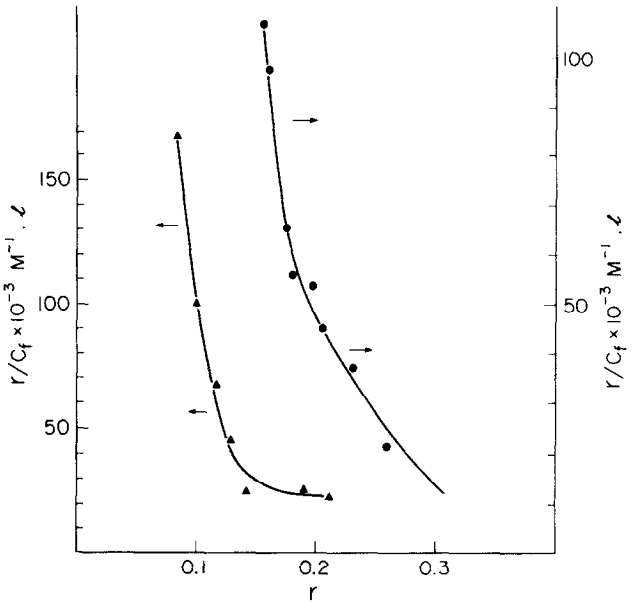

Figure 2: Scatchard plots for QAC binding determined from absorption measurements at 420 nm. C_f is the molar concentration of free QAC and r is the molar concentration of bound QAC per nucleotide phosphate; left ordinate, native DNA ▲; [QAC] = 1.03×10^{-5}M: right ordinate, denatured DNA ●; (42% hyperchromicity) [QAC] = 9.74×10^{-6}M: cacodylate buffer, pH 7, 0.05M.

tion bands for both native and denatured complexes with QAC do not require that the nature of the strong binding complexes should be the same in both cases. However, because of these similarities, particularly that of the binding parameters, Pritchard, Blake and Peacocke (9) proposed a modification of Lerman's original intercalation model that would not require an intact double helical structure of the DNA for strong dye binding.

Examination of the difference absorption spectra in the 250-300 nm region (Fig. 1) reveals important differences between the spectra of QAC-native DNA and QAC-denatured DNA complexes. These difference spectra were of samples having a high P/D ratio (P/D = 15) where according to the Scatchard plot the residual amount of free QAC was negligible. Additionally, the concentration of QAC was low enough and the P/D high enough that dye-dye interactions were also negligible. The previously reported exciton-like splitting which is observed in the difference spectra of QAC-native DNA is lacking in the difference spectra of QAC-denatured DNA. As discussed above the visible absorption bands were superimposable for the two complexes.

The distinct splitting in the ultraviolet difference spectrum of the QAC-native DNA complex has been assigned (10) as being due to a dipole-dipole interaction between the S_3 (280 nm) state of QAC and the S_1 (260 nm) state of DNA. The difference spectrum of the QAC-denatured DNA complex in the same region exhibits a broadened absorption band at 290 nm, whose intensity is reduced compared to that of free QAC, with a weak shoulder at 270 nm. The lack of splitting in the spectrum of QAC-denatured DNA clearly indicates that the structure of the complexes of QAC with native DNA and denatured DNA are significantly different. This difference is not apparent when one compares the visible absorption bands only.

These structural differences are obviously determined by the degree of helicity of the DNA samples. Thermal stability measurements indicate that the residual degree of helicity of the denatured DNA was only 16% that of the native double stranded DNA. These results indicate that exciton splitting in the spectra of QAC-DNA complexes requires an intact double stranded helical structure of the polynucleotide. Moreover, the additional requirement that there be a close approach between the transition dipoles for exciton interaction confirms that QAC intercalates between adjacent base pair layers in complexes with DNA, as Lerman originally proposed.

The increase in the β_{app} in the Scatchard plot for the QAC-denatured DNA complexes and the similarity in the visible absorption spectra for the QAC-DNA complexes is consistent with a model in which the electrostatic interaction between the anionic phosphate backbone of the DNA and the cationic side chain of QAC is an important feature. Besides this electrostatic interaction in the complexes of QAC and double stranded helical polynucleotides, QAC intercalates between adjacent base pair layers. Undoubtedly the electrostatic binding of the side chain of QAC to native DNA limits the accessibility of the phosphate groups to additional dye molecules. Thus on denaturation the availability of the phosphate groups to the dye is increased and this is reflected in the increased β_{app} obtained from the Scatchard plot.

The shoulder at 270 nm and the significant hypochromicity in the ultraviolet difference absorption spectrum of QAC-denatured DNA complexes could be due to QAC complexes with residual double helical regions of the denatured DNA. This possibility was ruled out by fractionating the denatured DNA, under controlled conditions, into two parts, double stranded ($C_o t$ 1.3

s.M.l^{-1}) and single stranded DNA. The latter fraction binds poorly to hydroxyapatite (0.15M phosphate) and lacks complete co-operativity in melting, particularly after it was heat denatured again in low ionic strength buffer prior to use. The difference absorption spectrum of the complex of QAC and this single stranded DNA was superimposible on that of QAC-unfractionated denatured DNA. Therefore the ultraviolet difference absorption spectrum in this case indicates that there must be an interaction between the transition dipoles of the nucleic acid bases and of QAC in these complexes but of a lesser magnitude than that between QAC and the nucleic acid bases in double stranded helical DNA. Singlet energy transfer experiments (12) are in agreement with this observation.

We have shown that exciton-like splitting is only observed in the ultraviolet difference absorption of QAC-double stranded helical polynucleotide complexes and that this observation supports Lerman's original model for intercalation in these polynucleotides. Comparison with the results obtained with denatured DNA indicate that the observation of exciton-like splitting in the ultraviolet spectral region can be an important diagnostic for helix-coil transitions. The visible absorption spectra do not allow this distinction.

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