Absence of Hyperchromic Effect in DNA Heated Above Tm., in the Presence of Tryptophan

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A strong interaction between DNA and Tryptophan in water solutions is described. This interaction determines the absence of hyperchromic effect in heated DNA above its melting point, when Tryptophan is present (base mol/tryptophan mol). At lower concentrations of Tryptophan only a shift of the Tm. of the DNA-Tryptophan solutions and a change in the shape of the thermal denaturation curves occurs. These effects are neither due to the ionic strength of the Tryptophan, nor to pH variations. P.M.R. spectroscopic studies confirm the interaction of the uracil ring of Uridine and the indol ring of Tryptophan.

There are several evidence of interactions between proteins and nucleic acids 1-12. This paper describes the absence of hyperchromic effect when an aqueous solution of DNA is heated above its melting point, in the presence of Tryptophan (base mol/tryptophan mol.) We have also found, that at lower concentrations of Tryptophan a shift in the Tm. of the DNA-Tryptophan mixtures occurs. Finally a molecular interaction between Uridine and Tryptophan at room temperature is reported.

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Material and Methods

Salmon sperm DNA and Uridine were obtained from Calbiochem, and l-Tryptophan from Schwarz Bioresearch Inc.

All spectrophotometric measurements were done in a Beckmann DU Spectrophotometer with temperature regulator.

Proton Magnetic Resonance data were obtained using a 60 Mc/s Hitachi-Perkin Elmer instrument at 30 °C. All spectra were run using Deuterium (99.7 per cent isotope excess) (Calbiochem) as solvent. Proton chemical shifts are expressed in ppm from Benzene as external standard. The pH (pD) of solutions in D$_2$O were adjusted with HCl to a value of 2.

Results

Fig. 1 shows the absence of an hyperchromic effect, when DNA-Tryptophan solutions are heated above the melting point of the control DNA, which in turns shows a typical pattern of thermal denaturation. No changes of absorbancy are observed in the Tryptophan control solutions when heated.

At a ratio of (DNA base:tryptophan) 4 : 1 (see Fig. 2) the melting point of the mixture increases by about 20 °C, its denaturation curves being of a shorter width (1/3 of the width of the control curve).

This interaction is not due to the effect of the ionic strength of Tryptophan itself, as can be seen in Fig. 3, which shows a comparison between the variations in the melting point of DNA and of DNA plus Tryptophan solutions, at different molarities of sodium chloride. Only small changes in the Tm. of DNA solutions occurs when the aminoacid is present, whereas the variation in the control DNA is proportional to the logarithm of the ion strength. The pH of all solutions was 6.
ed with Proton Magnetic Resonance Spectroscopy and the most relevant data are summarized in Fig. 4. In this figure we observe that the signals of protons corresponding to C₅ and C₆ of the base, are shifted to higher fields when the concentration of Tryptophan increases. These shifts are of the order of 0.22 ppm. when measured between infinite dilution to 0.4 M concentration of added Tryptophan. On the other hand the signals corresponding to ribose protons (C₅') of Uridine are not shifted in the presence of Tryptophan.

![Chemical shift of Uridine protons](image)

**Fig. 4.** Proton chemical shift of Uridine in the presence of different concentration of Tryptophan. ○ Protons of C₆ of Uridine base; ● Protons of C₅ of Uridine base; + Protons of C₅' of Uridine ribose.

**Discussion**

The absence of hyperchromic effect in DNA-Tryptophan mixtures, when heated above its melting point, can be interpreted as due to the formation of a strong complex between DNA and Tryptophan, which could hinder the thermal denaturation of DNA. At lower concentration of Tryptophan this interaction should be partial, manifesting itself only through a shift of the Tm. of the mixtures to higher values and a change in the shape of the absorbancy curve.

The effects of this interaction can not be understood as a consequence of the ionic strength of Tryptophan itself, an account of the magnitude of these effects, see Fig. 3.

The P.M.R. data mentioned above, can be interpreted in terms of the existence of an interaction between the uracil ring of Uridine and the indol ring of Tryptophan. This interaction suggested by the fact that the protons of C₅ and C₆ of the Uridine base are shifted to higher fields, but not so the C₅' protons of the ribose. Furthermore the alanine chain of Tryptophan apparently does not interact with Uridine. This follows from the fact that its protons are not shielded in the presence of Uridine, when the concentration of Tryptophan increases from 0.1 to 0.4 M.

The Spectrophotometric analysis, at room temperature, of the absorbancy of Uridine-Tryptophan solutions, in the range of 200 nm to 700 nm, shows a hypochromism of about 10% in the zone of 200 to 230 nm, for which we do not have an interpretation as yet. The Tryptophan-DNA solutions do not show appreciable changes in a Spectrophotometric analysis between 200 and 700 nm, at room temperature.

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