pH INDUCED CHANGES IN THE UV-ABSORPTION BAND OF DEOXYRIBONUCLEIC ACID

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The 260 nm-absorption band of deoxyribonucleic acid (DNA) shows no sharp fine structure at room temperature and pH 7. Nevertheless, weak shoulders at different points of the UV-spectrum appear to be real and they have been described as n $-\pi^*$ transitions (Fresco, 1961; Falk, 1964), similar to those seen in several polynucleotides (Rich and Kasha, 1960, Gellert, 1961), The thermal denaturation of the DNA is in general accompanied by the well known increase in the UV-absorption band at 260 nm, but the shape and spectral position of this band remains practically unchanged during the melting procedure.

This paper reports the effect of variation of pH upon the absorption band at 260 nm of two DNA's with extremely different GC-contents. For this purpose DNA's from Sarcina maxima (29 mole % GC) and Streptomyces chrysomallus (72 mole % GC) were employed.

It follows from the measurements described below that the pH induced spectral changes of the UV-absorption are mainly caused by protonation of the cytosine residues.

Materials and methods

The samples used were preparations described elsewhere (Sarfert and Venner, 1965 and 1966). The DNA was dissolved up to concentrations of 5.10^{-5} - 1.10^{-4}M DNA phosphate in deionized water, containing 0,02 M KCl. DNA solutions were dialysed at 1 °C against acidic KCl solutions. No UV absorption could be measured outside the dialysis bag, which indicates that the depurination found at higher temperatures did not occur (Venner, unpublished). To diminish the influence of secondary structure resulting from partial denaturation with decreasing pH, the samples were generally heated 10 min in a boiling water bath and cooled down by quenching in ice-water mixture. In some cases DNA was heated 10 min in a sealed tube at 130 °C and cooled down quickly. This DNA material is denoted as "strongly heat denatured". The spectra were recorded on a Unicam SP 700 spectrophotometer and in some cases with an Ultrascan (Hilger and Watts Ltd., London) using silica cells with 1 cm light path.

Results and discussion

Fig. 1 demonstrates that the spectral shapes of the native and denatured state of the DNA's from both sources are very similar at neutral pH, except for the hyperchromic effect. The maximum is located at 257 nm for *Str. chrysomallus* DNA and at 260 nm for *S. maxima* DNA; the minima were found to be at 230 and 233 nm respectively. In the acidic pH range, below pH 3, the GC-rich DNA shows in the normal form, as well as in the "heat denatured" form, drastic changes in the UV-absorption band with a broadening

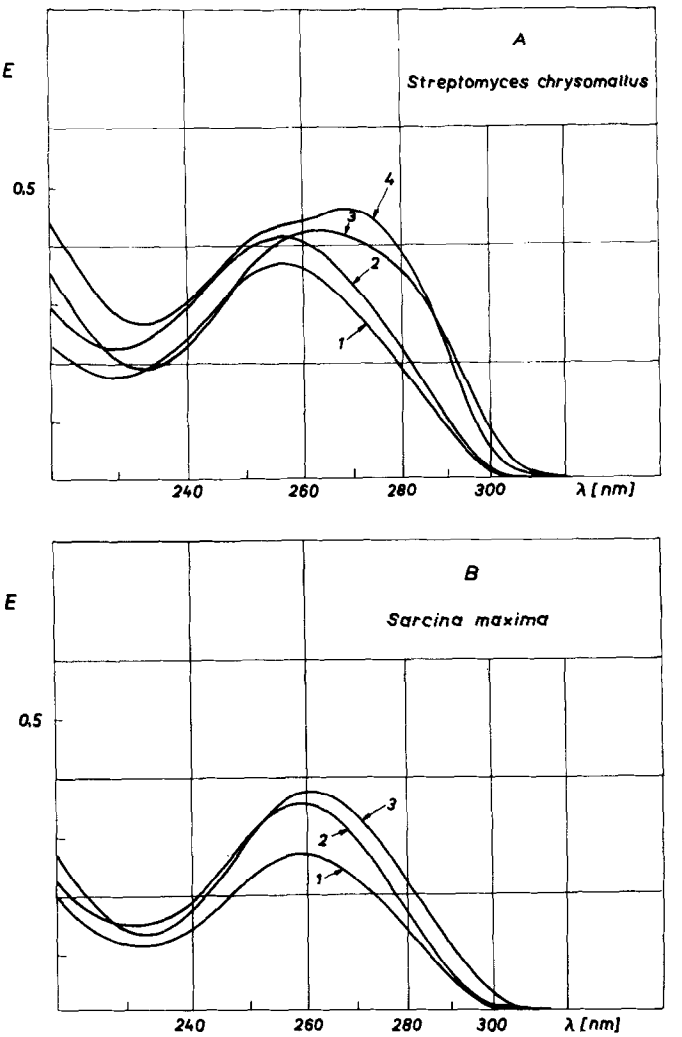

Fig. 1: UV absorption spectra of DNA's from two sources in 0.02 M KCl at different pH and room temperature. A, DNA from Streptomyces chrysomallus; B, DNA from Sarcina maxima; curve 1, native DNA, pH 7; curve 2 "heat denatured" DNA, pH 7; curve 3, "heat denatured DNA", pH 3; curve 4, acid form of "strongly heat denatured DNA", pH 3.

towards the longer wavelength side. When using "strongly heat denatured" DNA a material of reduced molecular weight with some loss of purines, which is to be expected from the de-

purination studies of Greer and Zamenhof (1962), a new peak appeared at 270 nm which results primarily from protonation of the bases but also to a lesser extent from secondary structural changes (curve 4) (Zimmer et al. in press). The absence of this pronounced displacement in the case of AT-rich DNA indicates clearly that the GC content of the DNA is responsible for its occurrence. To obtain more information on this observation the UV-absorption spectra were measured down to pH 2.4 (not shown in Fig. 1). The changes in the spectra are very pronounced in the band height at 270 nm and in the half wavelength, $\lambda_{1/2}$, at the longer wavelength side. If the absorbance and $\lambda_{1/2}$ are plotted versus pH, drastic changes are observed between pH 5.5 and 2.5 as shown in Fig. 2. It is known that the spectra of deoxycytidylic acid and deoxycytidine change predominately between pH 7 and 2.4, whereas

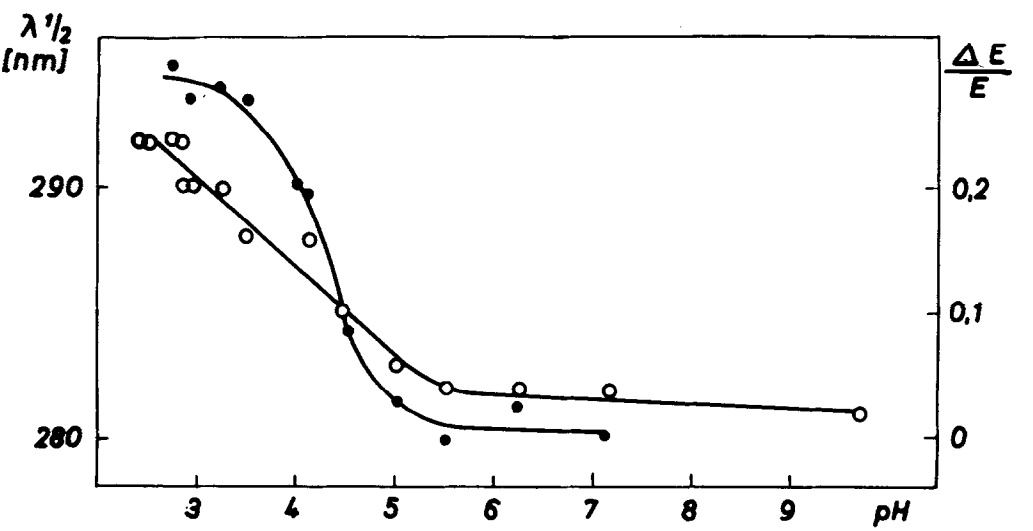

Fig. 2: pH dependent changes of the UV-absorption spectra of DNA from Streptomyces chrysomallus in 0.02 KCl at room temperature; ••• ; changes in optical density at 270 nm; ooo ; shift of $\lambda_{1/2}$.

that of deoxyadenosine is little changed and that of thymidine practically unaffected by lowering the pH (Voet et al., 1963). A slight change in the deoxyguanylic acid spectrum and in the titration curves at 290 nm appears below pH 3.5 (Zimmer and Venner, unpublished). However, this effect is meaningless since the pK is approximately 2.7 for deoxyguanylic acid and changes at 280 nm are zero. In accordance with earlier conclusions (Zimmer and Venner, 1966) on cytosine protonation in DNA the origin of the observed spectral changes (Fig. 1 and 2) and the band near 270 nm result from protonation of cytosine residues. From the results in Fig. 2 the apparent pK for cytosine dissociation in the denatured form of DNA was determined to about 4.3, which is comparable with the value of 4.65 resulting from titration studies at 280 nm.

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References

Falk, M. J. Amer. Chem. Soc. 86, 1226 (1964)
Fresco, J. R. Tetrahedron, 13, 185 (1961)
Gellert, M. J. Amer. Chem. Soc. 83, 4664 (1961)
Greer, S. and S. Zamenhof, J. Mol. Biol. 4, 123 (1962)
Rich, A. and M. Kasha, J. Amer. Chem. Soc. 82, 6197 (1960)
Sarfert, E. and H. Venner, Z. Physiol. Chem. 340, 157 (1965)
Sarfert, E. and H. Venner, report at the symposium "Isolation and structure of DNA from Microorganisms", Jena (1966)
Voet, D., W. D. Gratzer, R. A. Cox and P. Doty, Biopolymers, 1, 193 (1963)
Zimmer, Ch., G. Luck, H. Venner and J. Frič, Biopolymers, in press
Zimmer, Ch. and H. Venner, Biopolymers 4, 1073 (1966)