THE BINDING OF COPPER(II) IONS TO DNA

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Eichhorn (1962), Eichhorn and Clark (1965) and Hiai (1965) have shown that Cu(II) ions have a specific effect in decreasing the stability of the DNA helix to thermal denaturation. The decrease of T_m with increase of Cu^{++} concentration at constant ionic strength is markedly different from the effect observed with other ions and the denaturation at relatively low temperatures induced by the presence of Cu(II) ions has been shown by Eichhorn and Clark (1965) and by Hiai (1965) to be reversible, addition of electrolyte (KCl, KNO_3) to produce a high ionic strength renaturing the denatured DNA. The assumption that the loss of hypochromicity at relatively low temperatures in the presence of Cu(II) ions is consequential on a denaturation process is supported by the evidence [Hiai (1965)] that under the same conditions there is observed a marked decrease in viscosity, a loss of biological activity and a decrease in molecular asymmetry.

The mechanism whereby Cu(II) ions specifically decrease the thermal stability of the DNA helix is obscure. Eichhorn and Clark (1965) interpret their data by assuming that the Cu(II) ions are interposed between the complementary strands of DNA by co-ordination to the bases in such a manner that the hydrogen bonds are broken and the secondary structure of each strand destroyed. However, in view of the ready renaturation

of the denatured DNA on increasing the concentration of simple electrolyte, it is assumed by Eichhorn and Clark that the Cu(II) ions must somehow keep the bases in register even in the denatured state. This view is also held by Hiai (1965) who further considers that the bases in the DNA helix only become available for interaction with Cu(II) ions at elevated temperatures although the interaction can readily take place at lower temperatures after the helix has been disrupted. No direct evidence for the interaction of Cu(II) ions and the base-pairs of DNA was given by either of these groups of workers.

Our experiments on the thermal stability of DNA in the presence of Cu(II) ions in general confirm the experimental results of Eichhorn and Clark (1965) and of Hiai (1965). However, we wish to present direct evidence that there is interaction between Cu(II) ions and nitrogen atoms of the DNA bases after denaturation in the presence of Cu(II) ions at elevated temperatures, but not at room temperature. Cu(II) ions in the presence of non-complexing anions (e.g. NO_3^-) exhibit an absorption spectrum in the region 600 to 900 mμ with a

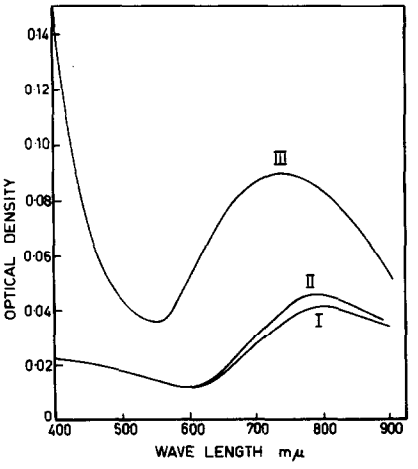

Fig. 1. Absorption spectra of Cu(II) ions in the presence of DNA. Concentrations: Cu^{++} (as nitrate), 4×10^{-4} M; DNA, 7.2×10^{-4} M; KNO_3, 2×10^{-3} M. I, Cu^{++} at 25°C; II, Cu^{++} and DNA at 25°C; III, Cu^{++} and DNA after heating at 55°C, measured at 25°C.

maximum at 800 mμ (curve I, Fig. 1) which is characteristic of the $[Cu(H_2O)_6]^{++}$ ion. This spectrum is little changed on the addition of DNA at 25°C (curve II, Fig. 1). After heating DNA at 55°C in the presence of Cu(II) ions for as little as one minute, a procedure sufficient to produce denaturation, there is a marked increase in absorption and a shift of the maximum to 740 mμ (curve III, Fig. 1). This increased absorption and shift of the maximum to shorter wavelengths is similar to the effect observed on co-ordination of Cu(II) with ammonia and ethylene diamine, the stronger ligand field due to the nitrogen derivative causing the shift and the increase in intensity of the absorption band. The maximum for the ion $[Cu(NH_3)(H_2O)_5]^{++}$ occurs at 740 mμ and for $[Cu(NH_3)_2(H_2O)_4]^{++}$ at 680 mμ. These spectral data thus afford direct evidence for the bonding of the Cu(II) ions to the nitrogen atoms in the heterocyclic bases after denaturation.

The U.V. spectrum of DNA, as is well known, shows a maximum at 258 mμ. The position of this maximum remains unchanged by the addition of Cu(II) ions at 25°C but on increasing the temperature there is a small shift in the maximum towards longer wavelengths and an increase in absorption due to the decrease in hypochromicity as shown in Table 1. Accompanying the shift in the position of the absorption

Table 1. Shift of U.V. absorption maximum of calf thymus DNA in presence of Cu(II) ions after heating at various temperatures.
(Concentrations: DNA, 2.99×10^{-4} M; Cu^{++}, 0.95×10^{-4} M)

Temperature °C	Position of maximum absorbance mμ	$O.D._{max.}\ T/O.D._{max.\ 25}$
25	258	1.00
35	259	1.04
45	260	1.45
55	261	1.55

maximum is a change in the absorption in the region 260 to 520 mμ towards longer wavelengths (this may be seen in Fig. 1 in the region 400 to 520 mμ) and which may best be described as the formation of a shoulder in the absorption curve in the region 265 to 280 mμ. The appearance of this shoulder may be attributed to the perturbation of the allowed energy levels of the bases by the co-ordinating Cu(II) ions. This behaviour is similar to that observed by Yamane and Davidson (1961) for the spectral shift associated with the interaction of DNA with mercury(II) ions, which was also interpreted as an interaction between the Hg^{++} ions and the ring nitrogen atoms of the heterocyclic bases.

It is noteworthy that no bonding of Cu(II) ions to heterocyclic nitrogen atoms occurs until the thermal denaturation process occurs. At 25°C, determination of the extent of binding of Cu(II) ions to DNA shows that binding increases with concentration and that there is only one type of binding site, the same is true for DNA denatured in the absence of Cu(II) ions and subsequently placed in an environment containing these ions; however, at 55°C there is a marked increase in the absorption compared with the values at 25°C and the number of binding sites is greater (Coates, Jordan and Srivastava, to be published). It is thus concluded that the Cu(II) ions at room temperature bind to the phosphate sites only, but at higher temperatures when some relative motion of the two strands in the DNA helix is possible, such as occurs at the "annealing temperature" [Marmur, Rownd and Schildkraut (1963)], penetration of the helix by the Cu(II) ions can occur which results in binding of the Cu(II) ions to nitrogen atoms of the bases such as to bring about marked distortion or partial disruption of the helix to produce denaturation.

EXPERIMENTAL

Calf thymus DNA was prepared by the method of Kay, Simmons and

Dounce (1952) and E. coli DNA by the method described by Marmur (1961). U.V. spectra were obtained using a Gilford model 2000 absorbance recorder used in association with a Beckman DU monochromator and visible spectra were determined with a Shimadzu spectrophotometer model QR.50 using 10 cm. cells.

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