Charge-Transfer Interactions between Nucleobases and Metal Ions: ESR and Optical Absorption Studies

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The charge-transfer interaction of different nucleobases with Cu^{2+} has been investigated by means of ESR and optical absorption studies. It could be shown that cytosine forms the strongest complex. The greatest reduction of Cu^{2+} (that is, spin concentration) was obtained, however, with adenine. Based on these results the following order for the electron donor strength of the nucleobases has been obtained: Ade>Cyt>Thy>Ura.

Introduction

Recent investigations have shown that metal ions have profound effects on the structure and function of nucleic acids [1]. For a better unterstanding of such an influence, it is necessary to obtain some more detailed information about the type and loci of the metal ion-nucleic acid interaction. In this regard, the metal ion-nucleobase association is of special interest. Since the affinity to metal ions seems to correlate with the theoretical electron donor properties of the nucleobases [2, 3], it may be concluded that the metal ion-nucleobase interaction is due, at least partly, to a charge-transfer interaction.

In an interesting investigation, Machmer and Duchesne [4] have determined the rel. electron donor strength of nucleobases using chloranil as an acceptor and dimethylsulfoxide (DMSO) as a solvent. The wavelengths of the optical absorption bands obtained were the larger the smaller the π-ionization potentials of the bases revealing, in this way, the good electron donor property of the purines.

Since metal ions and water are biologically more relevant than chloranil and DMSO the electron donor properties of the nucleobases have been investigated in an aqueous system. Cu^{2+} was used as an electron acceptor since most of the investigations concerning metal ion-nucleic acid interactions have

been done with this metal ion. Its charge-transfer interaction with nucleobases and the relative strength of such an association have been determined spectrophotometrically and by means of electron spin resonance technique.

Materials and Methods

The nucleobases adenine, cytosine, thymine, and uracil as well as $Cu(NO_3)_2$ were purchased from Merck AG, Darmstadt. Bi-distilled water was used as a solvent. Guanine couldn't be investigated because of its very low solubility in water. All substances were of reagent-grade quality and were used without further purification. The test solutions were always prepared shortly before the spectra were recorded.

The electron spin resonance (ESR) spectra were determined with a Varian E9 100-kc ESR spectrometer using a liquid sample accessory. All spectra presented were obtained at identical experimental conditions. A Wang 600 computer was used for determining the relative spin concentration. The S.D. of the results obtained in this way is about $\pm 1.5\%$.

The optical absorption studies were carried out with a Zeiss DMR 21 spectrometer. Difference spectra (nucleobase and Cu^{2+} vs. nucleobase) were recorded in the wavelength region between 250 and 800 nm.

Results and Discussion

In Figs 1 a and 1 b the effect of increasing concentrations of adenine and cytosine, resp., on an

5.0 mM Cu^{2+} in H$_2$O

H ⟶

50 G

Control

Ade

3.0 mM

5.0 mM

7.7 mM

20 mM

30 mM

Fig. 1 a

5.0 mM Cu^{2+} in H$_2$O

H ⟶

50 G

Control

Cyt

2.0 mM

5.0 mM

10 mM

20 mM

30 mM

Fig. 1 b
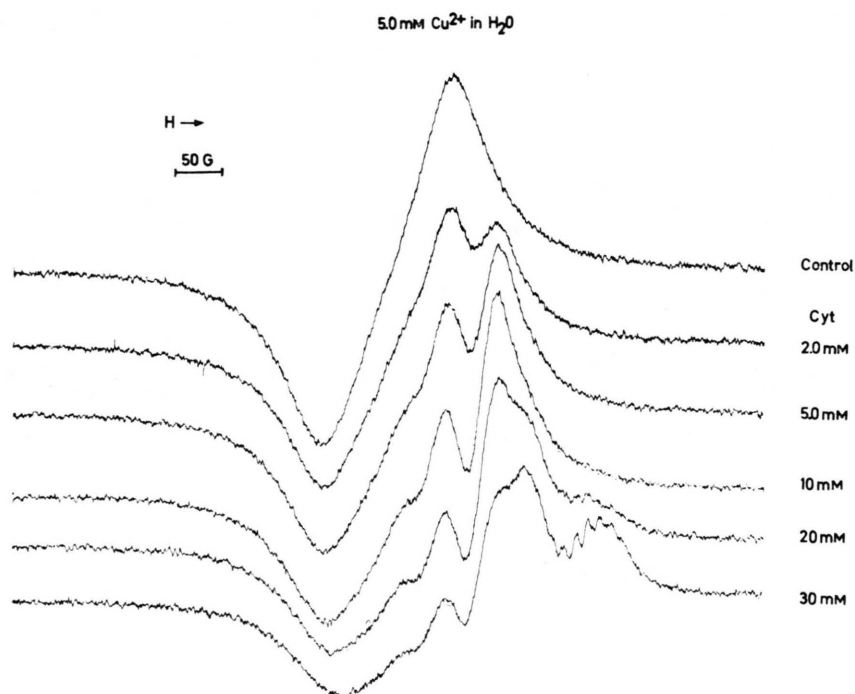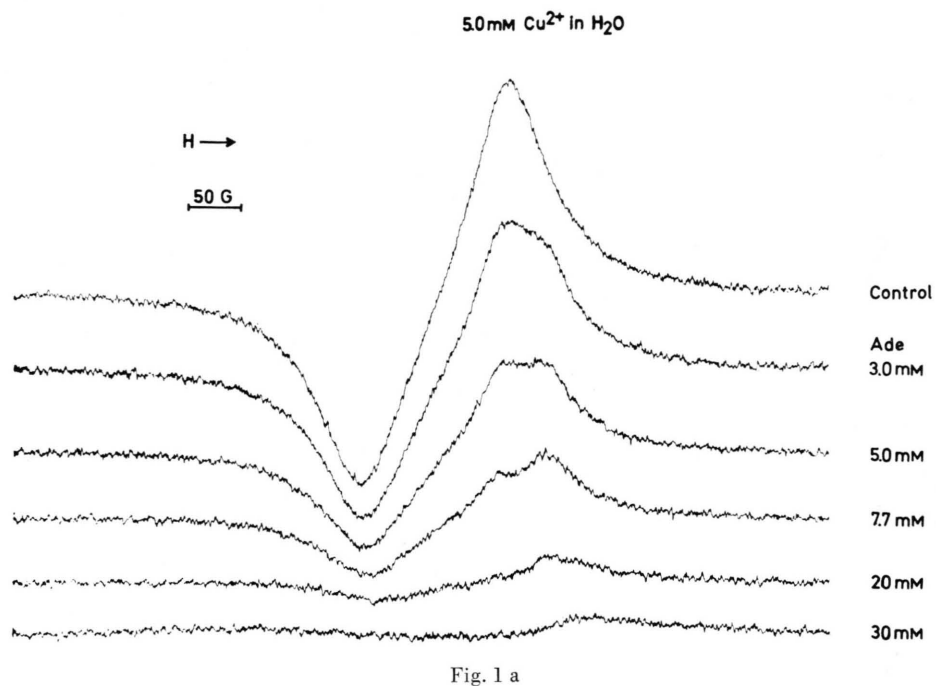
Fig. 1. The effect of varying concentrations of nucleobases on an aqueous 5.0 mM Cu^{2+} ESR spectrum. a. Adenine; b. cytosine.

aqueous Cu^{2+} ESR spectrum is shown. Two interesting facts should be noted: The reduction in spin concentration and the appearance of a hyperfine structure. As can be seen, the hf structure is much more pronounced in the case of cytosine. Such a hyperfine splitting was originally observed by McGarvey[5] when he determined the ESR spectra of copper salts in different solvents. It could be shown[5, 6] that anisotropic nuclear hyperfine interactions can contribute to paramagnetic line broadening in solution and that this line broadening can arise from both spin-lattice relaxation and transverse relaxation effects. Moreover, the difference in line widths in a hyperfine multiplet is caused by the dependence of these two relaxation times on the nuclear orientation.

It is interesting to note that in the present case an hf splitting is obtained by adding a nucleobase, *e. g.*, to an aqueous copper solution. From this, one might conclude that the two nucleobases form stronger bonds with copper than the water molecules with the result of higher oscillational frequencies giving a smaller Fourier component in the region of the resonance frequency. Thus, nucleobase addition to an aqueous copper solution results in an increase in relaxation times. The stronger interaction of cytosine with copper over that of adenine is indicated by the greater changes in the resonance pattern.

The partly resolved signal observed in the case of cytosine at higher field cannot be explained as yet.
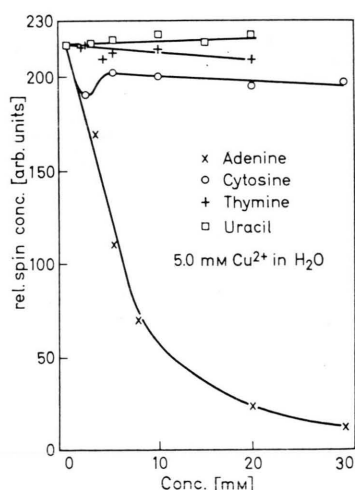

Fig. 2. The relative spin concentration of different Cu^{2+}-nucleobase complexes *vs* the nucleobase concentration used.

In regard to the reduction of the Cu^{2+} spin concentration, adenine is, however, much more effective than cytosine (s. Fig. 2). At small concentrations, cytosine behaves like adenine. At higher cytosine concentrations, however, the spin concentration increases again followed by a gradual decrease. The shape of this curve or, with other words, the smaller effectiveness of cytosine in reducing Cu^{2+} to cuprous ions might be explained as follows: At small concentrations, the strength of the complex formed between copper and cytosine or adenine is about the same since only a small portion of the ligand places in a square planar or tetrahedral structure is occupied. At higher concentrations, that is, if all ligand places are occupied, cytosine forms a stronger complex with copper than adenine. In the case of adenine, this complex is so weak that the electron having been transferred from the nucleobase to the metal ion cannot interact with the nucleobase entity any more. The two signals at higher field, not belonging to the four line hf structure of copper, exhibit some type of additional interaction in the case of cytosine.

No hyperfine splitting of the Cu^{2+} ESR signal was observed when either thymine or uracil were added to the aqueous copper solution. The addition of thymine resulted in a small reduction in spin concentration. In the case of uracil, a very minute increase could be observed, if at all.

Since only adenine and cytosine exhibit a strong interaction with Cu^{2+}, it might be assumed that the nitrogen of the amino group is the loci of interaction in these complexes. An attachment of oxygen to the cupric ion can be excluded since neither uracil nor thymine modify the Cu^{2+} ESR spectrum.

Further confirmation of this interpretation was obtained by optical absorption studies. The absorption spectra (s. Fig. 3 for adenine; in the case of cytosine similar bands were observed at slightly higher wavelengths) consist of two bands one of which is located in the near UV region and the other one in the visible region. The charge-transfer bands were obtained only for copper-adenine or cytosine complexes; no CT-bands were observed in mixtures of copper-thymine or uracil in the wavelength region investigated.

The change in wavelength as well as in intensity of the CT-band at about 280 nm with increasing concentration of adenine or cytosine is shown in Fig. 4. As can be seen, the red shift with increasing
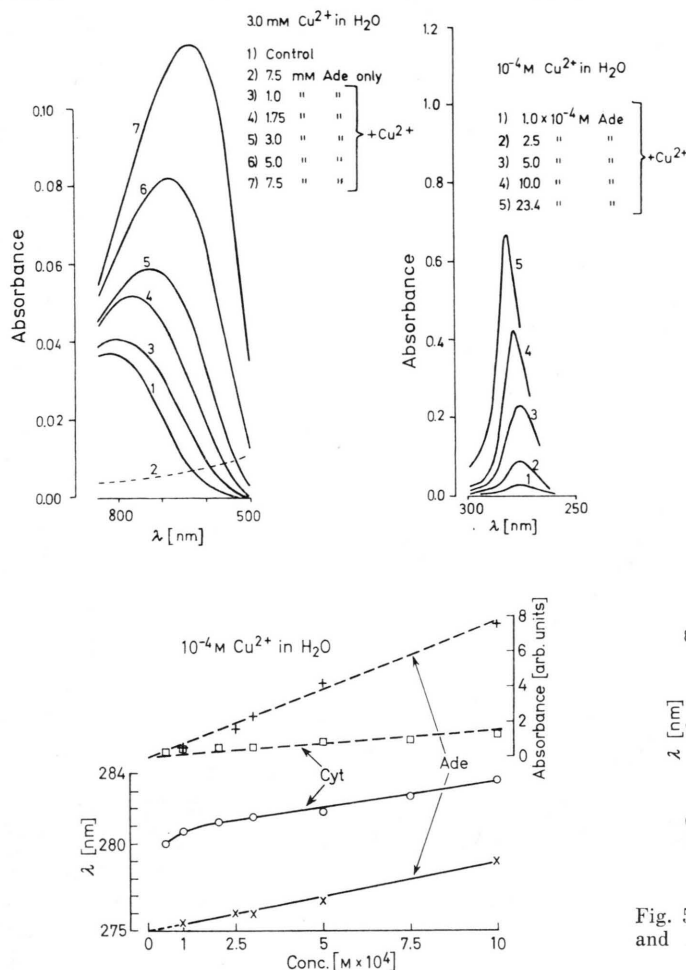
Fig. 3. Difference spectra of adenine and mixture of Cu²⁺ and adenine in an aqueous solution.

3.0 mM Cu²⁺ in H₂O

1) Control
2) 7.5 mM Ade only
3) 1.0 " "
4) 1.75 " "
5) 3.0 " " } +Cu²⁺
6) 5.0 " "
7) 7.5 " "

10⁻⁴ M Cu²⁺ in H₂O

1) 1.0 × 10⁻⁴ M Ade
2) 2.5 " "
3) 5.0 " " } +Cu²⁺
4) 10.0 " "
5) 23.4 " "

Fig. 4. Plot of the UV CT-absorption band (wavelength and intensity) *vs* the concentration of adenine (λ: ×, OD: +) and cytosine (λ: ○, OD: □).
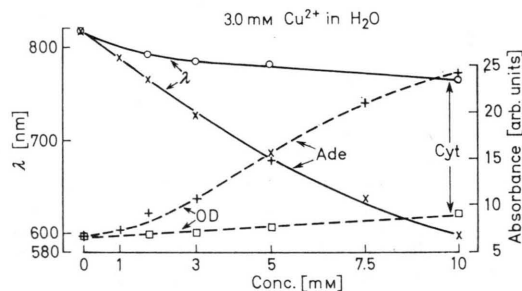
Fig. 5. Plot of the visible CT-absorption band (wavelength and intensity) *vs* the concentration of adenine (λ: ×, OD: +) and cytosine (λ: ○, OD: □).

concentration is about the same for both of the nucleobases investigated. The increase in intensity is, however, more pronounced in the case of adenine.

Similar results were obtained for the visible region (s. Fig. 5). The wavelength shift was, however, more pronounced in the case of adenine and occurred towards the shorter wavelength.

Two important facts should be pointed out: The CT-bands of cytosine are located at longer wavelengths if compared with the same concentration of adenine. The change in intensity of these CT-bands with increasing concentration is, however, much more pronounced for adenine if compared with cytosine.

The results obtained are in very good agreement with the CT-theory: The larger the wavelength of the CT-band, the smaller the dissociation constant of the complex. Thus, the optical absorption studies confirm the ESR results according to which cytosine forms a stronger complex with copper than adenine.

Since no CT-band could be observed when thymine or uracil had been added to the Cu²⁺ solution, one might conclude that the NH₂ group of the nucleobases adenine and cytosine is an important factor for forming the CT-complexes with metal ions. This has already been discussed in the chapter dealing with the ESR results. From the strength of the copper-cytosine complex it can be concluded, that the pk-value of the NH₂ group of cytosine must be larger than the one of adenine. In this case, the intensity of the CT-band of adenine has to be greater, an effect obtained recently when the CT-

interactions of aliphatic amino acids with Cu^{2+} have been determined [7]. According to these findings, a low pk-value of the NH_2 group seems to correspond to a larger change in intensity of the CT-band and to a large reduction in spin concentration, that is, to a large electron donor strength. In general, the optical results obtained support the assumption that the amino group participates in the interaction.

Finally, the sequence adenine > thymine > uracil, obtained experimentally, agrees with the theoretical electron donor strengths of the nucleobases [3]. According to the calculations, the effectiveness of cytosine should be comparable with the one of uracil. The experimental results obtained show,

however, that, in regard to complex formation, cytosine exerts the strongest effect while, in regard to reduction of Cu^{2+}, it is placed between adenine and thymine. The complex formation ability of cytosine can be explained by provoking the presence, at least partly, of its tautomeric form, the k-value of which indicates a stronger electron donor strength than the one of adenine. A possible explanation for the sequence Ade > Cyt > Thy > Ura, obtained for Cu^{2+} reduction, is the presence of a mixture of the two tautomeric forms of cytosine.

[1] N. A. Berger and G. L. Eichhorn, Biochemistry **10**, 1857 [1971].

[2] R. M. Izatt, J. J. Christensen, and J. H. Rytting, Chemical Reviews **71**, 439 [1971].

[3] A. and B. Pullman, Quantum Theory of Atoms, Molecules, and the Solid State, p. 353, Academic Press, New York 1966.

[4] P. Machmer and J. Duchesne, Nature **206**, 618 [1965].

[5] B. R. McGarvey, J. Phys. Chem. **60**, 71 [1956].

[6] H. M. McConnell, J. Chem. Phys. **25**, 709 [1956].

[7] W. Lohmann, Z. Naturforsch. **26 b**, 1098 [1971].