Charge-Transfer Interactions of Aliphatic Amino Acids with Metal Ions

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The effect of various non-SH-containing amino acids on an electron spin resonance spectrum as well as on an optical absorption spectrum of an aqueous copper(II) solution was investigated. The data obtained indicate that a charge-transfer complex is formed with amino acids acting as electron donors. A plot of the wavelength of the CT-band vs. the pK values of the carboxyl group exhibits a straight line with the larger pK values appearing at shorter wavelengths. Similar results were obtained when the intensity was plotted vs. the pK values of the amino group; again, higher pK values correspond to lower intensities. Thus, the method allows the determination of the pK values of prosthetic groups of amino acids. The data obtained are in good agreement with the CT-theory.

For several years there have been speculations about the possible role of charge-transfer (CT) complexes in different biological systems. Most of the investigations dealt with $\pi-\pi$ CT-complexes; however, complexes of the $n-\pi$ type are also possible since many biomolecules contain suitable lone-pair electrons. Considerable progress was achieved in the case of amino acids and proteins; their electron donor properties seem to be well established. Studies on nucleic acids and their components (bases, nucleosides, nucleotides) showed purine derivatives to be electron donors, too. Moreover, stability constants of the complexes obtained indicated that the pyrimidine derivatives are weak donors only. In the case of uracil and its halogenated derivatives acceptor properties cannot be excluded. This would support the theoretical predictions by Brillouin who assumed that the arginine-thymine and guanine-cytosine interactions are possibly donor-acceptor interactions.

Most of the investigations have been done with chloranil (tetrachloroquinone), iodine, oxygen or flavins acting as acceptors. Little attention has been given to the involvement of metal ions, although many biomolecules are known to contain them. Their structural incorporation as well as their functions are still unsolved in many instances.

The observations that some metal ions are present in deoxyribonucleic acid (DNA) and that the addition of copper(II) ions reversibly denatures DNA stimulated the interest in the interactions between the bases and metal ions. These interactions might have a marked effect on the key hydrogen bonds in biological systems. In addition, the importance of metal ions for some enzyme reactions and for electron transfer processes, such as in photosynthetic units and in mitochondria, stimulated our interest to investigate the interactions between aliphatic amino acids and copper(II) ions in regard to charge-transfer complexing.

Recently we have shown that SH-containing substances, such as cysteine and red glutathione, form CT-complexes with copper(II) ions. In the present work we have extended these investigations to non-SH-containing amino acids.

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Materials and Methods

The amino acids used for this investigation were obtained from Mann Research Laboratories, Inc., New York, New York, and were of reagent-grade quality. They were used without further purification. Different concentrations of the amino acids were prepared by dissolving them in aqueous solutions of 0.15 mM CuCl$_2$ (optical absorption measurements) and 150 mM CuCl$_2$ [electron spin resonance (ESR) studies]. Doubly distilled water was used as the solvent throughout the experiments.

The optical absorption spectra were determined with a Cary 15 spectrophotometer. In each case the difference spectra (mixture of amino acid and copper(II) ions vs. amino acid) were recorded. The ESR spectra were obtained with a Varian V 4502 100-kc ESR spectrometer using a liquid sample accessory. A DPPH (diphenylpiorylhydrazil) standard ($g = 2.0036$) was used as a reference for marking resonance positions. The first derivative of the absorption curve was recorded.

Results and Discussion

Fig. 1 shows the effect of different amino acids on the copper(II) ESR signal used as a control. The spectra obtained were identical for all non-SH-containing aliphatic amino acids. The hf splitting occurring with increasing concentrations of amino acids strongly suggests an interaction between the two constituents.

The formation of such a charge-transfer complex is also confirmed by optical absorption studies (see Fig. 2). As can be seen in these difference spectra, a new broad band appears with increasing concentration of glycine; the copper(II) ion concentration was kept constant: 0.15 mM. An increase in intensity and a red displacement occur with increasing glycine concentration. It should be pointed out that these complexes are rather stable since there was no change with time in the spectra.

In general, the same results were obtained with other aliphatic amino acids. However, a comparison of the CT-bands of different amino acids of the same concentration (5.0 mM) showed that the bands were located at different wavelengths and exhibited different intensities depending on the amino acid used.

A decrease in maximum absorption frequency is, according to Mulliken's CT-theory, equivalent to a decrease in the dissociation constant of the complex. Since the functional groups of the amino acids are involved in the formation of complexes with metal ions it should be of interest to find out the existence of a possible correlation between the observed optical data and the pK values of amino acids.

In Fig. 3, the pK values of the carboxyl group are plotted vs. the wavelength of the CT-bands using a constant concentration of the various amino acids (5.0 mM). The values of glycine and serine were used for calibrating the curve. Whenever the values agreed with those ones, which were also obtained by other methods, they were marked with a dot (•).
Fig. 3. Plot of the CT-absorption band (wavelength) vs. the pK values of the carboxyl group (explanation see text). $\Delta$ characterizes the values which differ from the ones previously reported. Finally, open circles (O) represent values previously unknown.

As can be seen, most of the values are located on a straight line which can be expressed as $\lambda_{CT\text{-band}} = -0.7536 \, \text{pK} + b$ with 2 nm $\pm$ 0.05 pK units. The exceptions are the diamino, monocarboxylic acids (ornithine, lysine) and the monoamino, dicarboxylic acids (aspartic acid, glutamic acid). The deviations obtained with ornithine and lysine are probably caused by the presence of HCl. It is interesting to note that the other diamino, monocarboxylic acids, arginine and citrulline, act like monoamino, monocarboxylic acids indicating that the amino group in $\delta$ position does not participate, to any larger extent, in the formation of the complex.

The results obtained are in very good agreement with the CT-theory: an increase in the wavelength of the CT-band is equivalent to a decrease in the dissociation constant. Moreover, methylation causes a decrease in the ionization potential resulting in an increase in the wavelength of the CT-band and, thus, in a decrease in the pK values. The data obtained confirm this prediction: the CT-band of sarcosine, a methylglycine, is located at a considerably higher wavelength than the one for glycine.

A plot of the intensity of the CT-band vs. the pK values of the amino group, using alanine and serine for calibration, exhibits again a straight line for most of the amino acids tested (see Fig. 4). Again, deviations were obtained with the same amino acids mentioned in Fig. 3. The intersection of the measured intensity of ornithine and lysine with the lower dashed line and the solid line, resp., results in the pK values of the two amino groups of those two amino acids. The same applies for asparagine and glutamine using the solid line and the upper dashed line.

The values obtained by this method agree with the ones obtained by other methods with the exception of leucine, isoleucine, and threonine (see Figs. 3 and 4). Based on our results, the following pK values were obtained (the average values obtained by other methods are given in parenthesis):

- $\text{pK}_1$: Thr 2.16 (2.088), Leu 2.195 (2.344), Ileu 2.17 (2.339).

An explanation cannot be offered for this deviation as yet. In a few cases, it has been possible to determine the pK values for the first time.

An interesting result is obtained when the copper(II) ion concentration, at constant amino acid concentration, is varied. In this case (see Fig. 5), the wavelength of the CT-band remained unchanged (at 211 nm); only the intensity increased proportionally to the copper concentration.
From the results obtained it might be concluded that the amino acids investigated form a charge-transfer complex with copper(II) ions. These complexes are rather weak as indicated by the ESR spectra. This is in agreement with Pearson's classification according to which the two prosthetic groups (NH$_2$, COO$^-$), available for complex formation, are hard ligands. With metal ions, they undergo electrostatic or $\delta$ covalent interactions. This interaction is the stronger the smaller the ionization potential of the donor (amino acid). Thus, the most stable complexes have the largest absorption wavelength. As demonstrated above, this method is useful for determining pK values of amino acids. The values obtained are very accurate; the standard deviation is less than 2 percent.

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Das Schwingungsspektrum der Trichlorcyanursäure, (CINCO)$_3$

The Vibrational Spectrum of Trichloroisocyanuric Acid, (CINCO)$_3$

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The vibrational spectrum (IR and Raman) of trichloroisocyanuric acid, (CINCO)$_3$ is recorded and assigned on the basis of a planar molecule with symmetry D$_{3h}$.

Die Ausdehnung unserer Untersuchungen über Substitutionsreaktionen mit Verbindungen, die elektronegatives Halogen enthalten, auf CINCO und (CINCO)$_3$ ließen es wünschenswert erscheinen, Informationen über die Strukturen dieser Verbindungen zu erhalten. Während über CINCO und (CINCO)$_2$ bereits vollständige Schwingungsspektren vorliegen, ist für die trimere Form dieses Systems bislang nur das IR-Spektrum im Bereich von 4000 — 650 cm$^{-1}$ bekannt. In der vorliegenden Arbeit berichten wir über die Ausdehnung der Messung des IR-Spektrums bis 200 cm$^{-1}$ sowie über das bisher unbekannte Raman-Spektrum des (CINCO)$_3$, weil nur aus der Kenntnis des gesamten Schwingungsspektrums eine sichere Entscheidung über das Vorliegen eines planaren Moleküls (D$_{3h}$) oder eines nichtebenen Moleküls (C$_{3v}$, C$_s$) getroffen werden kann.

Tab. 1 unterrichtet über Anzahl, Verteilung und Auswahlregeln der Schwingungen auf die einzelnen Klassen für die beiden in Betracht kommenden Modelle.

Man entnimmt Tab. 1, daß sich die Erwartungsspektren in der Zahl der IR-aktiven Schwingungen unterscheiden (10 bzw. 14). Dieses Kriterium ist jedoch von nur geringer Beweiskraft, da gelegentlich mit zufälligen Entartungen zu rechnen ist, die eine höhere Symmetrie vortäuschen können. Hin-