Charge Transfer Interactions between Isonicotinic Acid Hydrazide and Cupric Ions

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The interaction of Cu²⁺ with isonicotinic acid hydrazide (INH) and some of its derivatives has been investigated by means of electron spin resonance, nuclear magnetic resonance, and optical absorption studies. It could be shown that an interaction exists between the metal ions and the hydrazine side chain in addition to the very weak interaction with the ring nitrogen. The results obtained indicate the formation of a charge transfer complex.

Introduction

During the last few years isonicotinic acid hydrazide (INH) (or also known as isoniazide) has been used with great success and almost exclusively in the treatment of tuberculosis. In animal experiments, however, there were some indications of a possible carcinogenic effectiveness of this drug. Until now, in human beings treated with INH such an effect could not be observed thus far. Moreover, INH is mutagenic in the host-mediated assay, but not in the dominant lethal test of mice.

Several hypothesis have been proposed in order to explain the mechanism of action of INH. None of them seems to describe adequately the results obtained. There are, however, some indications that INH forms a complex with copper ions resulting in a decrease of the copper level in blood and liver as well.

Because of the importance of INH for the treatment of tuberculosis and in order to eliminate possible side effects it is necessary to elucidate its mechanism of action. For this reason, the interaction of INH and some of its derivatives with cupric ions has been investigated by means of electron spin resonance, nuclear magnetic resonance, and spectrophotometric techniques.

Material and Methods

Isonicotinic acid hydrazide (INH) was kindly supplied by Dr. Adler. The derivatives nicotinic acid amide (NAA) and isonicotinic acid (INA) as well as copper nitrate were purchased from Merck Co., Darmstadt. Isonicotinic acid amide (IAA) and nicotinic acid diethyl amide (NDA) were obtained from EGA-Chemie, Steinheim/Albuch. All substances used were of reagent-grade quality and were used without further purification. Different concentrations of these substances were prepared by dissolving them in 5.0 mM Cu(NO₃)₂ (for ESR experiments; 2.5 mM for optical studies) solution using bidestilled water as a solvent. For the NMR experiments, deuterated dimethylsulfoxide (d₆-DMSO, 99.5%) was used. In the latter case, varying concentrations of Cu(NO₃)₂ (up to 6 × 10⁻⁵ M) were added to a constant concentration of INH or its derivatives (0.2 M) just prior to the measurements. The spectra were recorded at room temperature and didn’t change with time.

The electron spin resonance (ESR) spectra were determined with a Varian E9 100 kc ESR spectrometer using a liquid sample accessory cell.

The optical absorption studies were carried out with the Zeiss DMR 21 spectrophotometer using 1.0 cm cells. Immediately after preparing the samples difference spectra were recorded in the wavelength region between 500 and 900 nm.

Nuclear magnetic resonance (NMR) spectra were recorded on an HA 100 Varian spectrometer. Chemical shifts were measured with a frequency counter relative to an internal (CH₃)₄Si standard (TMS) and are given in parts per million (ppm).

Results and Discussion

The influence of different concentrations of INH on the Cu²⁺ ESR spectrum in aqueous solution is shown in Fig. 1. As can be seen, a hyperfine structure appears with increasing concentrations of
INH. Such an hf splitting was originally observed by McGarvey $^9$ when ESR spectra of copper salts were determined in different solvents. It could be shown $^9, ^{10}$ 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 differences in line widths in a hyperfine multiplet are caused by the dependence of these two relaxation times on the nuclear orientation.

It is interesting to note that in the present case the hf splitting is obtained by adding INH to an aqueous copper solution, that is using the same solvent. From this, one might conclude that INH forms stronger bonds with the cupric ions than with the water molecules resulting in an increase in relaxation times.

In order to determine the loci of interaction in these Cu$^{2+}$-INH complexes, several INH derivatives have been investigated (s. Fig. 2). At comparable concentrations (100 mM), the changes obtained
with IAA, NAA, and NDA are about the same; however, they differ considerably from the one obtained with INH. The hf splitting well pronounced in the case of INH is indicated only weakly in the case of the other substances used. Moreover, the similarity of the spectra obtained with IAA and NAA suggests that the position of the substitution of the side chain (e.g., position 3 or 4 of the pyridine ring) is without any influence. Furthermore, the stronger complex formation with INH as compared to the derivatives, especially IAA, is due to the NH group. The very weak complex formation between Cu2+ and the other compounds investigated supposedly occurs at the ring nitrogen as based on the experimental results. It should be pointed out that the spin concentration remains about unchanged with increasing concentration of the substances. 

Further confirmation of this interpretation was obtained by optical absorption studies. Difference spectra of 2.5 mM Cu2+ and increasing INH concentrations in an aqueous solution are shown in Fig. 3. As can be seen, a new band appears at about 700 nm, the extinction of which increases with increasing INH concentration. At an about 10-fold concentration of INH, the intensity remains unchanged at a wavelength of about 660 nm. In the case of the other derivatives investigated only minute changes occurred, an effect which can be neglected and is obviously caused by the very weak interaction between Cu2+ and the ring nitrogen. Thus, the CT band observed with the Cu2+-INH system is due to the hydrazine side chain and emphasizes again the importance of the NH entity.

The spectrophotometrical values confirm the ESR results according to which only INH forms a CT complex with Cu2+. In order to obtain some more information about the loci of interaction NMR investigations have been conducted.

The influence of increasing concentrations of Cu2+ on the NMR spectrum of INH is shown in

**Fig. 3.** Difference spectra of 2.5 mM Cu2+ and increasing INH concentrations using H2O as a solvent.

![Difference spectra of 2.5 mM Cu2+ and increasing INH concentrations using H2O as a solvent.](image)

**Fig. 4.** Influence of different Cu2+ concentrations on the NMR spectrum of 0.2 M INH using DMSO as a solvent.

![Influence of different Cu2+ concentrations on the NMR spectrum of 0.2 M INH using DMSO as a solvent.](image)
Fig. 4 using DMSO as a solvent. Already very small amounts of Cu\(^{2+}\) affect the NH- as well as the NH\(_2\)-protons. At larger concentrations, there is an influence on the peaks of the H\(_a\)-protons, while the H\(_b\)-protons are the most insensitive ones. Again, these results imply that the NH – NH\(_2\) side chain is the most important loci of interaction between Cu\(^{2+}\) and INH. From the line broadening of the H\(_a\)-peaks it can be concluded that the probability for Cu\(^{2+}\) of being close to the pyridine nitrogen must be relatively high. The life time of such a Cu\(^{2+}\)-pyridine complex can be estimated to be of the order of 10\(^{-4}\) sec. From this and the small chemical shift obtained after Cu\(^{2+}\) addition it can be concluded that a so-called collision complex is formed.

The spectra obtained with some other INH derivatives, e.g. IAA, NAA, and INA exhibit a line broadening of the H\(_a\)-protons only. These findings indicate a weak interaction between Cu\(^{2+}\) and the pyridine nitrogen. Obviously, there is no interaction between the metal ions and the NH\(_2\) entity of the compounds investigated unless there is another NH group adjacent to the NH\(_2\) group, e.g. in the case of INH. Unfortunately, an interaction between Cu\(^{2+}\) and the COOH group could not be observed since the acid proton could not be detected.

From the experimental data obtained it can be concluded that in addition to the very weak interaction of Cu\(^{2+}\) with the ring nitrogen (of all substances), a decisive and important interaction of the metal ions occurs via the hydrazine side chain (of INH). Such an interaction is presumably a charge-transfer interaction, an assumption which is supported by the fact that only in the case of INH a new CT absorption band is formed. Furthermore, ESR investigations have shown that a rather considerable hf splitting is obtained with INH only. The mechanism provoked for the biological action of INH in the treatment of tuberculosis is probably occurring via complexing cupric ions.

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