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Structural and Antimicrobial Studies of Coordination Compounds of Phenylalanine and Glycine

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Abstract
Coordination compounds of glycine and phenylalanine were synthesized, characterized using electronic and infrared spectroscopy, magnetic susceptibility measurement and mass spectrometry. The ligands coordinated to the metal in a bidentate fashion using N and O donor atoms. Square planar geometry is proposed for the Mn(II), Co(II), Ni(II) and Cu(II) complexes based on the results obtained from their characterization. Tetrahedral geometry is however proposed the Cd(II) complexes. The *in-vitro* biological effect of the synthesized compounds was investigated. The compounds were tested against three gram-negative bacteria, *E.coli*, *P. aeruginosa*, *P. vulgaris*, three gram positive bactria, *S. aureus, B. subtilis,* MRSA and a fungus *C. Albicans*. A comparative study of the zones of inhibition observed for the ligand and coordinated synthesized compounds indicated that the synthesized compounds, in some cases, showed higher zones of inhibition than that of the free ligands. It is therefore suggested that increasing the number of chelate rings may increase the lipophilicity of the coordinated complex and therefore its antimicrobial activity.

Introduction

The study of coordination compounds has received much attention in recent years. This interest was generated by the discovery of the anti-bacterial, -fungal and -cancer activities of several coordination compounds. As a result, studies have been carried out on the structure and chemical behavior of several metal complexes (Chohan et al., 2006). Various *in-vivo* studies have shown that biologically active compounds become more bacteriostatic and carcinostatic upon chelation (Chohan et al., 2006; Husseiny et al., 2008). Amino acids, which are also components of proteins, offer excellent ligands for binding to metal ions (Zhang & Lippard, 2003; Kostova, 2006). The properties of coordination compounds are influenced to a considerable extent by the nature and the oxidation state of the central metal atom. A method of studying this influence is by comparing the compounds formed by a series of metal atoms in a given oxidation state with a particular ligand (Komiyama et al., 2008).

Although coordination compounds of amino acids, such as histidine (Nomiya et al., 2000), arginine, glutamic acid (Legler et al., 2001) have been synthesized and their antimicrobial properties studied, little attention has been focused on hydrophobic amino acids, such as phenylalanine. Chelation of bulky ligands to metal cations reduces the polarity of the ion. Due to the glycolipophilic nature of the cell wall, an increase in the lipophilicity of a coordination compound enhances its ability to penetrate bacterial cell membrane. This concept has been

applied to the molecular design of coordination complexes of phenylalanine **(1)**. Phenylalanine is a hydrophobic amino acid with a bulky benzyl R side chain. The results obtained are then compared with that of similar coordination complexes of glycine **(2)**, (the simplest amino acid).

This paper reports the synthesis and characterization of coordination compounds of manganese (Mn), cobalt (Co), copper (Cu), cadmium (Cd) and nickel (Ni) with phenylalanine **(1)** and glycine **(2)**, metal to ligand ratio 1:2 and the evaluation of their antimicrobial activities.

Materials and Methods

All reagents and solvents used were of analytical grade. The complexes were prepared according to a modification of literature procedure (Nomiya & Yokoyama, 2002). The infrared spectra were recorded on a Genesis II FTIR spectrophotometer in the range 450-4200 cm-1 (KBr discs). The solid reflectance electronic absorption spectra of the complexes in the range 200-1000 nm were obtained with a Genesys UV-Vis spectrophotometer. Melting points or decomposition temperatures (M.P/D.T.) was measured using open capillary tubes on a Gallenkamp (variable heater) melting point apparatus. The magnetic susceptibility for some of the complexes was measured at room temperature using a MSB-AUTO (Sherwood scientific) Gouy balance. Mass spectra were obtained with a GCT premier mass spectrometer, by direct insertion electron impact ionization with a time of flight (TOF) analyzer. The general equations for the reactions are as follows:

Preparation of Coordination Complexes

The coordination compounds were prepared by the addition of 0.01 M (2.46, 2.61, 2.38, 1.72 and 2.11 g) of the appropriate metal salt (manganese, cobalt, nickel, copper, and cadmium respectively) to a solution of the ligand (**1**) 0.02 M, 3.37 g and (**2**) 0.02 M, 1.52 g. The mixture was then heated to reflux for 1 h, using a water bath. Precipitates were formed within the refluxing time for majority of the complexes, while some required concentration on a water bath and cooling before solid products were obtained. The products obtained were filtered and washed with methanol. Products were then dried *in vacuo* at 60 °C.

Antimicrobial Activity using Disc Diffusion Assay

The *in vitro* antimicrobial properties of the complexes were performed at the Pharmaceutics laboratory at the department of Pharmaceutics, Faculty of Pharmacy Obafemi Awolowo Univerfsity, using a modification of literature procedure (Murray et al., 1995). The strains used were *Escherichia coli* NCTC 8196, *Pseudomonas aeruginosa* ATCC 19429, *Staphylococcus aureus* NCTC 6571, *Proteus vulgaris* NCIB, *Bacillus subtilis* NCIB 3610 and Methicillin resistant *S. aureus* clinical isolate for bacteria and *C. albicans* NCYC 6 for fungi. The standard strains were from stocks of culture collections maintained in the laboratory. Bacteria were maintained on nutrient agar slants and fungi on Sabouraud Dextrose Agar slants at 4 °C and subcultured monthly. Each test agent (20 mg) was dissolved in 1 ml sterile distilled water boiled gently in a Bunsen flame. Discs of Whatman No 1 filter paper (φ 6 mm) were soaked with 2 drops of the test agent using a sterile Pasteur pipette and allowed to dry at room temperature.

Two colonies of a 24-hour plate culture of each organism were transferred aseptically into 10 ml sterile distilled water in a test tube and mixed thoroughly, using an electric shaker, for uniform distribution. A sterile cotton swab was then used to spread the resulting suspension uniformly on the surface of oven-dried Mueller Hinton Agar (Oxoid) and Sabouraud Dextrose Agar plates (Sterillin) for bacteria and fungi, respectively. These were incubated for an hour at 37 and 25 °C for bacteria and fungi, respectively. Sterile forceps were used to aseptically place each of the discs on the agar plates and the plates were then refrigerated for 30 min at 4 °C following which, the inoculated plates were incubated at 37 °C for 24 hours for bacteria strains and at 25 °C for 72 hours for the fungal strain. Antimicrobial activity was evaluated by noting the zone of inhibition against the test organisms (Murray et al., 1995).

Conclusion

The coordination complexes of Mn(II), Co(II), Ni(II), Cu(II), Cd(II) with two amino acids glycine and phenylalanine were synthesized and characterized. The ligands coordinated the metal ions through N and O donor atoms. A square planar geometry was proposed based on the result of the various analyses. A comparative study of the zones of inhibition indicated that chelation may increase the antimicrobial activity of biological ligands such as amino acids. Also the antimicrobial activity of the coordination compounds is also a function of the metal.

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