CHAPTER I

INTRODUCTION

1.1 General Introduction

It has been known before 1950 that naturally occurring substances namely pterins, riboflavins and various purins form stable complexes with the ions of heavy metals. Although the metal ions in these complexes no longer react as simple inorganic ions, the complexes are in equilibrium with a (usually exceedingly minute) proportion of inorganic ions from which they are formed (1). Around 1950, it was found that other biochemically important substances have a similar avidity for heavy metal ions, namely the porphyrins, proteins and amino acids (1).

Accordingly, it has been desirable to know quantitatively, (i) the avidity of each biochemically important complex-forming agent for the ions of various heavy metals, and (ii) the avidity of each biologically significant heavy metal for various complex-forming agents (including foreign toxic substances).

Considering amino acids in particular, their interactions with heavy metal ions were found to affect their activities and properties. Similarly, the metal-binding powers of the amino acids are of a relative magnitude sufficient to play an important part in the metabolism of trace metal ions (2). The connection among metal

ions, ligands and cancer was recently underlined by Williams (3) with particular emphasis on complexes of amino acids and metal ions (4).

Because of their biological importance, complex stability constants for many metal ions with amino acids have been repeatedly published and redetermined with the variation of methods , the variation of temperatures and the variation of ionic strengths.

1.2 The Previous Work

The avidities of the α - aminoacids for the heavy metal ions by pH-metric titration with glass electrode at 20°C were studied by Albert (1,2) but, the ionic strength of the solutions was uncontrolled. The overall stability constants (log β_2) of the complexes of Mn (II) ion with DL-tryptophan and L-lysine $2HCl.H_2O$ reported were ca.5 and 2.0, respectively, and Cd (II) ion with DL-tryptophan was 8.1(1). Mn (II) ion complexes with L-leucine was investigated by Kroll (5), pH-metric titrations were carried out in the solutions of an ionic strength of 0.1 M KCl and a temperature of 25°C with glass electrode. The 1:1 Mn (II) ion to L-leucine complexes appeared to be the predominant form with the stability constant (log K_1) of 2.15 (5). In order to examine the maximum degree of formation or maximum average ligand number ($\bar{n}_{max}$) for the complexes of Mn (II) ion with L-valine, Childs and Perrin (6) have studied pH-metrically with glass electrode for the solutions of an ionic strength of 0.15 M KNO_3 and a temperature of 37.00 $\pm$ 0.05°C in a pH range of 7.1-9.3. Reported $\bar{n}_{max}$ was 0.9 and stability constant (log K_1) was 2.337 $\pm$ 0.085 (6).

Metal chelates of some sulfer-containing amino acids were studied by Lenz and Martell (7). They introduced the new computerized method to calculate the potentiometric data obtained from the solutions of an ionic strength of 0.10 M KNO_3 and a temperature of 25.05 $\pm$ 0.05°C. The reported stability constants, log K_1 and log K_2 respectively, were 2.77 and 1.80 for Mn (II)-methionine system, 4.38 and 4.24 for Pb (II)-methionine system (7).

The polarography of glycine-metal complexes and the determination of stepwise formation constants were studied by McKenzie and Mellor (8). The reported values of stepwise formation constants, log K_1 and log K_2 respectively, for the solutions of an ionic strength of 0.10 M KNO_3 and a temperature of 25°C were 8.0 and 7.3 for Cu (II) -glycine complexes. The stepwise stability constants were not determined for Zn (II) -glycine complexes and the precipitate occurred in Pb(II) -glycine solutions (8). Rao and Subrahmanya (9) studied only the polarographic behavior of Zn (II) -amino acid complexes. Effect of pH and concentration of amino acid anion on polarographic half-wave potentials were studied for Zn (II) -glycine complexes, Zn (II) -alanine complexes and Zn (II) -valine complexes (9). The same effect was also found for Cd (II) -glycine complexes, Cd (II) -alanine complexes and Cd (II) -valine complexes (10). The influence of pH on half-wave potentials and diffusion currents were established for Pt (II) -amino acid complexes of glycine and valine (11). Polarographic study of L-tryptophan complexes with Zn (II), Ni (II) and Co (II) was performed and the overall stability constant were found to be log β_2 = 14.70 for Zn(II) -L-tryptophan system, $\beta_3 = 6.8 \times 10^9$ for Ni (II)-L-tryptophan system and log β_1 = 4.72 for

Co (II) -L-tryptophan system (12). The coordination number for polarographic study of glycine complexes with antimony (III) was found to be one, thus the formation of 1:1 metal-ligand chelate was indicated and the value of the stability constant was $\beta_1 = 3.98 \times 10^{10}$ for Sb (II) -glycine system (13).

1.3 The Present Work

There have been a few instances on the literature survey for the complex formation of the essential amino acids with the heavy metal ions, especially Cd (II) ion, Pb (II) ion and Mn (II) ion. Essential amino acids are required by the body for the production of protein but, differing from the others, there are no biochemically pathways available for their syntheses, so they are supplied from the diet only. Mn (II), Cd (II) and Pb (II) ions are the heavy metal ions which are believed to be toxic and these metal ions were representatively sampled from the first, second and third rows of the transition elements, respectively. It should be very interesting to study about their complex formations.

In the present study, essential amino acids for human viz. L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-threonine, L-tryptophan and L-valine were chosen for complex formation study with Mn (II), Cd (II) and Pb (II) ions. The conditions used were a constant temperature of $37.0 \pm 0.5^{\circ}C$ and a constant ionic strength (μ) of 0.10 M KCl. The acid-dissociation constants (K_a) of the essential amino acids were firstly determined and their complex formations were secondly studied by pH-metric

titration technique. The complex formations were also studied polarographically by varying pH of the solutions and varying the ligand concentrations. The polarographic behaviors, compositions and stability constants of the complexes were finally reported.

1.4 Introduction to Amino Acids

Proteins are essential components of all living cells. During hydrolysis, proteins break down to amino acids. The significance of amino acids is no limited to their being components of proteins but, in metabolism, they undergo many other reactions and supply precusor for other endogeneous substances (e.g. hemoglobin of blood) (14).

Amino acids are the substances that possess two characteristic functional groups : the amino group ($-NH_2$) and the carboxyl group (-COOH). All amino acids that occur as components of proteins have their amino group in α-position to the carboxyl group, namely α-amino acids. The α-amino acids are the most significant in the biological world, for they are the fundamental units from which proteins are constructed. The general formula of an α-amino acid is shown in Figure 1.1 (a).

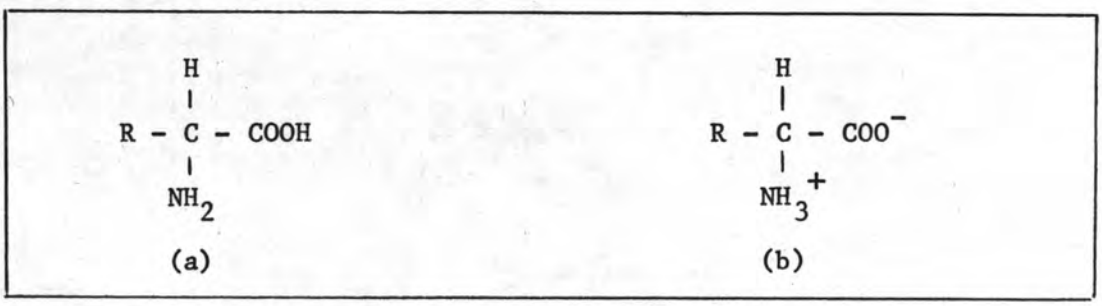
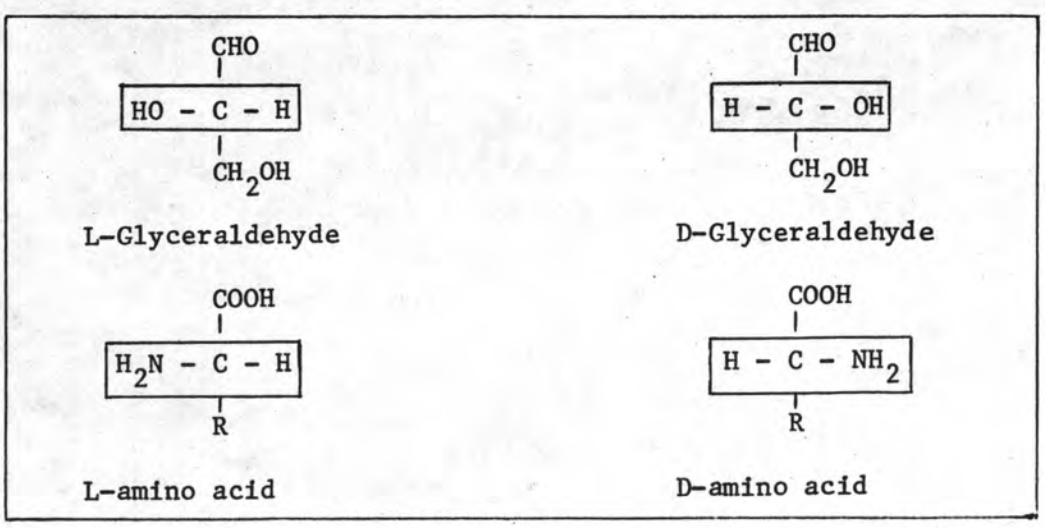


Figure 1.1 General formula for α-amino acids.
(a) un-ionized form ; (b) zwitter ion form

The acidic carboxyl group of an amino acid can lose a proton by dissociation and the basic amino group can pick up a proton. If both groups are ionized, the result is the "dipolar ion" or "zwitter ion", meaning hybird ion as shown in Figure 1.1(b). Now much evidence supports that zwitter ions are predominant in solution. Among the facts of evidence are high dipole moments and high solubility in polar solvents. At the pH of cellular fluids and blood plasma or the physiological pH (pH 7.4), the predominant form of amino acids are also zwitter ion form (15).

If the R-group of an α-amino acid is something other than hydrogen, the amino acid contains a chiral carbon adjacent to the carboxylic acid, they may exist in two forms, one the mirror image of the other. With the exception of glycine (the R-group is hydrogen), the amino acids are optically active compounds, and those found in naturally occuring proteins are all L-amino acids. The absolute configurations of the amino acids are related back to the configuration of glyceraldehyde. L-amino acids are those written so that the amino group is to the left when the carboxyl group is written up as shown below.



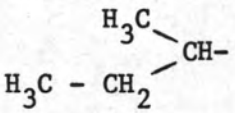
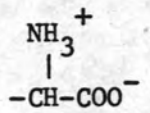
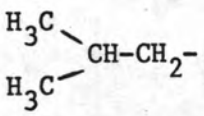
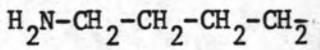
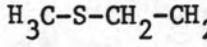
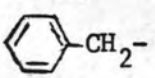
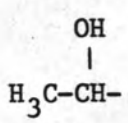
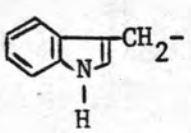
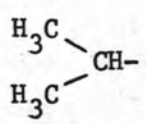
D-amino acids are not found in proteins and are not part of the metabolism of higher organisms. However, several D-amino acids are important in the structure and metabolism of lower forms of life (15).

1.5 Essential Amino Acids (16,17)

Of the 20 amino acids required by the body for the production of proteins, adequate amounts of about 10 can be synthesized by enzyme-catalyzed reactions starting from carbohydrate of lipid fragments and a source of nitrogen. The remaining amino acids, are either no biochemical pathways available for their synthesis, or the available pathways do not provide adequate amounts for proper nutrition. Accordingly, these amino acids must be incorporated into the body in the food we eat, and are therefore called essential amino acids. In reality, all amino acids are essential for normal tissue growth and debelopment. However, the term "essential" is reserved for those that must be supplied in the diet.

For fully grown young men, eight amino acids are essential. The names, standard three-letter abbreviations and structural formulas for each of the 8 essential amino acids are shown in Table 1.1.

Table 1.1 The 8 essential amino acids for humans (15)

name	abbreviation	structural formula	
		R-group	functional group
L-Isoleucine	L-Ile		
L-Leucine	L-Leu		
L-Lysine	L-Lys		
L-Methionine	L-Met		
L-Phenylalanine	L-Phe		
L-Threonine	L-Thr		
L-Tryptophan	L-Trp		
L-Valine	L-Val		

In recent study, histidine was proved to be essential for growth in infants, there is some evidence that it may be needed by adults as well. Arginine can be synthesized by adults, but apparently the rate of internal synthesis is not fast enough to meet the needs of the body during periods of rapid growth and protein synthesis. Therefore,

depending on the age and state of health, either eight, nine or ten amino acids may be essential for humans. Note that since phenylalanine is the precusor of tyrosine, the figures given for phenylalanine assume the presence of adequate tyrosine. Similarly, the figures for methionine assume the presence of adequate cysteine. Anyone who consumes about 40-55 grams of protein daily in the form of meat, fish, cheese or eggs certainly satisfies his need for these essential amino acids. The estimated minimum daily requirements of these essential amino acids are given in Table 1.2.

Table 1.2 Estimated minimum daily requirements of the essential amino acids for fully grown, healthy young men and women (15).

Essential amino acid	Minimum daily requirement (grams)	
	women	men
L-Isoleucine	0.45	0.70
L-Leucine	0.62	1.10
L-Lysine	0.50	0.80
L-Methionine	0.55	1.01
L-Phenylalanine	1.12	1.40
L-Threonine	0.30	0.50
L-Tryptophan	0.16	0.25
L-Valine	0.65	0.80

The relative usefulness of a dietary protein depends on how well its amino acid pattern matches that required for the formation of tissue protein in humans. For proper tissue maintenance and growth, all amino acids, both essential and non-essential, must be

present at the same time. In this sense, tissue growth is an all-or-nothing process; if even one amino acid is missing, no protein at all is made.