Co-ordination of Trace Metal Ions with Glycine Amnio Acid a Neuroreceptor in Biological System

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Summary: Glycine is a simplest non essential amino acid. It is present in high concentration in central nervous system (CNS), involved in neural excitability. Inhibitory neurotransmissions in the mammalian CNS is mainly mediated by glycine. In the present investigation studied the complexing properties of glycine molecule with trace metal ions such as Fe^{+3}, Al^{+3}, Cr^{+3}, Mg^{+2}, Zn^{+2}, Ca^{+2}, Cu^{+2}, Ni^{+2} and Co^{+2}. Potentiometrically and spectrophotometrically the mole ratio method were applied for the calculation of mole ratio between metal ions and ligand molecules.

Introduction

Glycine is the simplest amino acid. It is non essential synthesized by human body from carbon dioxide and ammonia the amino nitrogen of glycine can easily be exchangeable for this reason it is used to synthesize other biomolecules such as Serine threonine Heme, Purine ring and Glutathione. It is directly or indirectly involved in one carbon metabolism. Glycine is present in high concentration in central nervous system and it is involved in neural excitability it's concentration is high in the grey matter of the spinal cord and destruction of the neuron of this region result fall in the concentration [1]. Inhibitory neurotransmissions in the mammalian CNS is mainly mediated by some amino acids. These multiple functions of Glycine transmission correlate with the localization of different types of Glycine receptors in the respective brain regions [2] Compound that selectively potentiate Glycine responses are therefore potential therapeutics for muscle relaxation and pain relief. Glycine causes the opening of the anion selective channel of the receptor thereby allowing chloride ions into the cytoplasm the resulting hyperpolarizing of the membrane stabilizes the resulting potential of the cell. [3] Non-Ketonic hyperglycemia disorder occur in neonates due to deficiency of glycine cleavage results severe neurological disturbances often life threating, glycine accumulates in the body fluid [4]. Increased urinary excretion of the glycine because defect in transport inherited as an autosomal recessive trait. In vitamin B6 deficiency excess of glycine when feded result the oxalates excreted in the urine [5]. Collagen major component of connective tissue contain high components of glycine. In the view of importance of glycine [6]. The health of human beings depends largely on a normal supply of essential trace elements and changes in trace element concentration in body tissues may lead to sever reactions. Thus it is obvious that lack of essential elements as cofactors of enzymes may have serious consequences of orderly chain of enzyme reaction in metabolism and may lead to physiological disorders. Deficiency of essential trace elements produce multiple and diverse clinical symptoms. These may arise from inadequate dietary intake decreased bio-availability, in which decreased absorption, excessive excretion utilization occurs [7]. Calcium is biologically an important alkaline earth metal. It is present in the body mainly in the form of bones and teeth. Ionized calcium is involved in blood coagulation, function of heart, muscles, nerves and release of neurotransmitter. Calcium metabolism influences the parathyroid and permeability of a membrane. Its deficiency causes rickets [8]. Magnesium is involved in multiple of biochemical function mainly related to enzyme systems. It play role in metabolism of nuclear protein, DNA and uric acid synthesis. Its deficiency causes cardio vascular diseases. Iron is metabolically active, in human, plants and microbes. It plays a vital role in oxygen transport and erythrhropoiessis. It is a major component of hemoglobin and myoglobin and transferring oxygen. Its deficiency causes anemia and myocardial infraction and excess amount causes Iron overload [9]. Manganese is essential for glucose and fat metabolism; its deficiency leads to growth retardation, disorder of reproduction and nervous system. The toxic effect leads to carcinogeneses. Chromium exists in two-oxidation state trvalant and hexavalan, the lower oxidation state is stable and non-toxic while hexavalent chromium is reported to

be toxic to human body on lungs, liver and kidney. It is important for the maintenance of glucose, lipid and protein metabolism, deficiency results elevation of glucose and cholesterol level [10]. Cobalt is the component of Vitamin B_{12} It is essential for the production of red blood cells, deficiency causes hematological disorder, toxic effects lead to polycythemia [11]. Nickel is essential for the synthesis of insulin and activates some other enzymes; it has some role in RNA. Deficiency leads to dermetitus and deformilities of bones [12]. Copper is important part of plasma protein cereluplasmin and many other metaloprotein such as cytochrome oxidases, it is responsible for the metabolism of tyrosine and production of skin pigments. Zinc is one of the most abundant trace element about eighty enzymes in human being are zinc containing which are involved in animal, plants and bacterial growth. Zinc deficiency may cause growth retardation infertaility delay wound healing, hair growth night blindness [13]. The aim of our investigation was to study the complexing properties of glycine with trace metal ions such as Fe^{+3}, Al^{+3}, Cr^{+3}, Mn^{+2}, Zn^{+2}, Ca^{+2}, Cu^{+2}, Ni^{+2} and Co^{+2}which are present in the human body. For this purpose we studied the complexes of glycine potentiometrically and spectrophotometrically the mole ratio method were applied for the calculation of mole ratio between metal ions and ligand molecules.

Result and Discussion

The potentiometric titration curves (pH Vs Vol. of NaOH added) for glycine and its metal complexes i.e. Fe^{+3},Cr^{+3}, Al^{+3}, Cu^{+2}, Co^{+2}, Ni^{+2}, Zn^{+2}, Ca^{+2} and Mg^{+2} are shown in fig.1. The pH values of metal ions complexes and their absorbance are shown in Table 1. These data explain that tripositive ions forms most stable complexes at acidic pH, Fe^{-2} at pH 4.5, Al^{+3} at pH 5.5 and Cr^{+3} at pH 5.0. Ca^{+2} and Mg^{+2} ions do not form complexes with glycine because they have identical potentiometric curve as the glycine has, among dipoistve ions Cu^{+2} forms most stable complexes at pH 6.5. The complex formation of Ni^{+2} takes place at pH 9.0. In case of Co^{+2} the complex formation starts nearly at neutral pH that is 7.5. Published and original data on the stability of complexes formed by bivalent ions of first transition series have been collected. The order of stability is Mn< Co< Ni< Cu> Zn of nearly all types of complexes irrespective of nature of the coordinated ligand or of the number of the ligand molecules involved. A theoretical justification for the stability

Table-1:.Absorbance of Glycine Complexes at the pH of Complex Formation

Metal Ions	pH	M : L	λ_{max}	Absorbance
Al^{+3}	5.50	------	------	------
Fe^{+3}	4.50	1:1	500	0.214
Cr^{+3}	7.50	1:1	520	0.152
Cu^{+2}	6.50	1:1	480	0.170
Ni^{+2}	9.00	1:1	400	0.140
Co^{+2}	7.50	1:1	500	0.132
Ca^{+2}	----	---	---	------
Mg^{+2}	---	---	---	------
Zn^{+2}	8.2	1:1	---	-----

order follows from consideration of the reciprocal for ionic radii and the second ionization potentials of the metal concerned [8]. Our results follow the reported order of stability. Table 2 shows the first stability constant log β_1 and second log β_2 of Glycine metal complexes calculated by computer program and comparision with some othe ligands. Important result observed when we compared the potentiometric curve of Al^{+3} and Fe^{+3} ions. The Al^{+3} ions show the competition with Fe^{+3} ions at the binding site. As the reported data showed that Al^{+3} blocks binding site of Fe^{+3} in biological systems [2]. Our research show that Al^{+3} form very stable complexes with glycine, as we know that glycine is very important amino acid of neurotransmitter so if we use aluminium utensils for household purposes most of the glycine of food will destroy. The presence of Al^{+3} in biological system disturb the nervous system this is the reported cause of Alzheimer disease [10]. The other importance of study of metal ions complexation is that it is reported, Zn^{+2} ions effect the glycine receptor activity even at low concentration of Zn^{+2}10 micro mole and enhance glycine mediated currents at higher concentrations that is of Zn^{+2} 1 milli mole, have an opposite inhibitory effect [11]. It means that the excesses of Zn^{+2} ions in the biological system has inhibitory effect and deficiency of Zn^{+2} ions have positive effects on the nervous system. Table-3 shows the data of mole ratio method, observed that in equimolar concentration of glycine and metal ion 1: 1 complex formed but at higher concentration of glycine 1: 2 complex are formed, 1: 1complex is more stable than 1: 2 complex.

Amino acids and proteins are used as a carrier or as a binding sites for different drugs. This type of chelating studies of amino acids are very important in drug metabolism. If changing the concentration of metal ions, abolishes activity of drug against the microorganisms. When amino acids are used in metal free system they are also not absorbed and drug

Table-2: Some Reported β values with other ligands

	2,4 dihydroxy-benzoic acid			3,4 dihyroxy-benzoic acid			8-hydroquinolin 5-sulphonic acid		
	β_1	β_2	β_3	β_1	β_2	β_3	β_1	β_2	β_4
Cd (II)	--	--	--	6.0	12	---	---	---	---
Co (II)	--	--	--	7.0	12	15	--	--	--
Cr (III)	--	---	--	--	--	--	11	22	--
Cu (II)	--	18	--	--	--	--	--	20	--
Fe (III)	18	--	--	14	27	37	--	23	--
Mg (II)	--	--	--	6.3	--	--	--	--	--
Mn (II)	--	--	--	--	--	--	7	13	-
Zn (II)	--	--	--	6	17	--	8	10	--

Metals ions	Glycine		Analine		Benzoyltolyl-hydroxylamine	
	β_1	β_2	β_1	β_2	β_1	β_2
Mn (II)	3.44	6.63	3.04	6.02	5.51	10.25
Co (II)	5.06	8.94	4.82	8.48	6.61	12.25
Ni (II)	6.14	11.15	5.96	10.66	7.22	12.93
Cu (II)	8.47	15.38	8.51	15.37	9.35	17.04
Zn (II)	5.33	9.72	5.21	9.54	6.74	12.33

Table-3: Jobs plot of iron Glycine Complexes

M: L	Absorbance λ_{max} 500
1: 1	0.214
1: 2	0.15
1: 3	0.12
1: 4	0.10
1: 5	0.90
1: 6	0.80

became unaffected. Immune system are also protein which work against antigens, this system is effective when only traces of metal ions are present [14]. Among People of Pakistan self-medication is common. They use to take protein extracts for common diseases. The disadvantage of this is that people used them without precautions and limits, the excessive intake may cause abnormalities in the body. There are many different types of complexation of amino acids with bio available trace metal or micronutrients occurs in living system. Copper, Iron, Cobalt, Zinc, Nickel, Chromium, Calcium and Magnesium are micro nutrients present in living system. These metal ions are involved in different metabolic path ways of biological processes, they also act as inhibitors for different enzymes therefore all the vital role of the body may be disturbed by deficiency of metal ions, such as electrolytic disturbance, alteration in membrane permeability [15, 16]. Taking certain precautionary measures can reduce these interactions. Our studies showed that glycine has positive interaction with Iron and Copper. At stomach pH, which is very low near about two at this pH complexation do not occur with trace metals. The intestinal pH is alkaline most of the interactions occur at intestinal pH.

Determination of Log β Values and Thermodynamic Values Through Potentiometric Method by Computer Program Best

The data obtained from pH titrations will be utilized for the calculations of log β values. For this purpose computer program BEST will be used. Data files FOR004.DAT will be prepared for each titration. Calculated β values will be refined several times, till the sigmafit values reduced upto 0.04. The date file of this program will be required the following information:-

1. Total volume of the solution.

2. Molarity of the base used for pH titration.

3. Change in pH after each step.

4. No of millimole of metal ions present in the solution

5. No. of millimole of ligand present in the solution.

The whole calculations in this program will be based upon the expected β values for each species present in the solution by refining these values to get sigmafit values, the goodness of sigmafit will be reflected on accuracy of K values. The K values of the complexes at different temperature will be used to calculate the thermodynamics values of complexes.

$$\Delta G = -Rt \ln \beta$$

$$\Delta G = \Delta H - T\ \Delta S$$

$$\ln B = \Delta H/T + \Delta S/R$$

A plot in β vs 1/ T, slope = $-\Delta H/ R$, intercept = $\Delta S/ R$ [6].

Experimental

All reagents were of AR grade. Solutions were made in deionized water freed from CO_2. For all pH measurement Orion pH meter model SA 720 was used. For pH Calibrations 0.05 M solution of potassium hydrogen pthalate pH value 4.01, for pH 7.00 (Sodium Hydrogen Phosphate Buffer) and for pH 10.00 ($NaHCO_3$ and Na_2CO_3 Buffer) at 25 °C were used, made from BDH standard chemicals [7]. For potentiometric titrations a double walled glass cell was used. The temperature of the cell was kept constant throughout the experiments by circulating

water. All the titrations were done at temperatures i.e. 30 °C, 20 ml of 0.01 M metal ion solution mixed with 20 ml of 0.01 M glycine solution and titrated with 0.1 M NaOH solution. The change in pH was noted with the small increment (0.05 ml) of base. The solution was stirred with magnetic stirrer constantly. For each metal Glycine solution, these titrations were performed twice to minimize the probable error [8]. For spectrophotometric measurements, spectra were recorded on Shimadzu UV 160 a Spectrophotometer. The absorbance peeks of the complex at different pH were scanned [9].

References

1. M. N. Hughes, and R. K. Poole, "Metals and Micro Organism" First Ed. Published by Chapman and Hall, 167 (1989)
2. A. K. Dass, Medicinal Aspects of Bio-Inorganic Chemistry. First Ed. CBS Publisher, Delhi, India, 7 (1990)
3. T. Hussain and I. Naved, Clinical Pharmacology, 4th Ed. Carvan Book Center, 321 (1993).
4. J. Kuhse, *Curr. Opin. Neurobiol.*, **5**, 318 (1995).
5. L. Bodo and G. Maksay, *Trends in Pharma Sci.*, **23** (11), 220 (2002).
6. P. Legendre, *Cell. Mol. Life Sci.*, **58** (2001).
7. A. I. Vogel, Quantitative Chemical Analysis, 2nd Ed. W.H. Freeman and Company, NewYork, 53 (1990).
8. A. E. Martell and A. J. Motekaitis, The Determination and Use of Stability Constants, 1st Ed. Publisher V.C.H (1988).
9. J. Bassette, Vogel's Text Book of Quantitatie Inorganic Analysis, ELBS and Longman, U.K., 1st Ed. 86 (1979).
10. D. A. Phipps, Metal and Metaboloism. 2nd Ed. Oxford University, 63 (1978).
11. S. Fucile, *Neuron*, **28**, 571 (2000).
12. P. J. Collipp, *Ann. Nutr.*, **50** (3), 607 (1990)
13. M. C. Linder and M. Hazegh-Azam, *Amer., J. Clinic., Nut,* **63**, 797 (1996) *Acta Psychair. Scand. Suppl.*, **303,** 49 (1983).
14. M. C. Linder, and M. Hazegh-Azam, *American Journal Clinical Nutrition.*, **63**, 797 (1996).
15. F. Zhou, T. Kunihiku, *J. Mol. Pharmacol.* **65**, (5), 1141 (2004).
16. Y. Z. Hamada, C. Barndon, and D. Joseph, *J. Synth., Inorga nic, Nano-Metal Chemistry.* **35**, (7), 515 (2005).