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Synthesis of Metal Complexes with Amino Acids for Animal Nutrition

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Abstract: Metal complexes of amino acids are important in the biological system, for both nutritive and catalytic chemical reactions. A series of metal (II) complexes have been synthesized by the reaction of chloride salts of magnesium, calcium, iron, cobalt, copper and zinc with amino acids DL-alanine, L-glutamic acid and leucine. Complex formation occurred at the proximal sites of the carboxyl moiety and the alpha amino nitrogen by 1:2 stoichiometric reactions which were also confirmed by elemental analysis and FTIR data. The metal complexes of DL-alanine and L-glutamic acid are water soluble while metal-leucine complex has solubility in DMSO. The purposed structures of complexes were confirmed by ^{1}HNMR studies.

Key words: Amino Acids · ^{1}HNMR · FTIR · Metals

INTRODUCTION

Metals and amino acids exhibit various function in biological systems. Tissue fluids, living cells, skeleton and teeth are comprised of calcium as essential constituent as well has a prime importance for enzymatic system to transmit the nerve impulses and muscles contractions. Magnesium is enzyme activator closely associated with calcium as cofactor with thiamin pyrophosphate and magnesium deficiency reduces the oxidative phosphorylation. Magnesium receptors on bone and kidney cell responsible for calcium metabolism by the interaction of parathyroid hormone. Calcium and magnesium bis-glycinate are being used nutritionally functional chelates. Haemoglobin is most important oxygen-transport metalloproteinase which contain most of iron in body. Transferrin and ferritin proteins also contain iron which transports it from one part of the body to another and provided storage respectively. The diets containing iron from amino acids complexes have better metabolism, absorption as well placental and mammary iron transfer than any other form. Ferrous bis-glycinate complex nutritionally meets all the requirements [1-4]. Antioxidant and enzymatic activator zinc is intracellular trace mineral found abundantly in animals. Sexual organs activity both in male and females, maintaining protein metabolism and vision, synthesis of nucleic acids, immune activation and process of repairing is controlled by virtues of it. The body contains many enzymes and proteins which are driven by copper like cuproenzymes and chaperone proteins also have neuronal and hepatic functions. Zinc methionine in combination with copper lysine has been reported for increased retention of fetal membranes in lactating cows as compared to others without these treatments [5-8]. The synthesis of vitamin B_{12} is done by the cobalt physiologically, the amino acid complex with cobalt are not usually used for nutritional purpose rather cobalt complex with arginine, histidine, lysine and serine have viral inhibitory effects [9-11]. The present work emphasis the synthesis of [Magnesium, Mg(II)], [Calcium, Ca(II)], [Iron, Fe(II)], [Cobalt, Co(II)], [Copper, Cu(II)] and [Zinc, Zn(II)] complexes with amino acids DL-Alanine, L-Glutamic acid and Leucine. The obtained complexes were characterized by their elemental analysis, Fourier transform infra-red (FTIR) and proton nuclear magnetic resonance (^{1}HNMR) studies.

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MATERIALS AND METHODS

Chemicals used in present work, were of analytical grade obtained from E-Merck and BDH and chloride salts of metals were used. 100 mL of equimolar quantities of each metal salt and amino acid (0.1M) were mixed in a flask fitted with a water condenser and a magnetic stirrer and a hot plate. The pH of the content was maintained at 4-6 for Fe, Cu, Zn and Co, while for Ca and Mg the pH was 8-10. The contents of the flask were refluxed for 1-4 hrs and the change in color was noted. In order to isolate the complex, the contents of the flask were evaporated in a china dish to 15-25mL and cooled in a refrigerator for crystallization. Lumps of crystals were observed in different shapes and were washed with small amount of water and acetone mixture (1:4). The crystals were dried on filter paper and stored in sample bottles. Alpha IR spectrometer, Bruker and NMR spectrometer, Bruker were used to record the IR and NMR spectra respectively. PG-T80$^+$ UV-Vis spectrophotometer, PG-990 atomic absorption spectrometer (AAS), Flash HT Plus elemental analyzer, Thermo were used for λ_{max}, metal contents and concentration of carbon (C), hydrogen (H) and nitrogen (N) of respective synthesized complexes respectively while the melting point was measured by Gallenkamp apparatus.

RESULTS AND DISCUSSION

Physiochemical Properties: The magnesium, calcium, iron, cobalt, copper and zinc complexes with DL-Alanine, Leucine and L-Glutamic acid using equimolar concentration were synthesized. All the complexes (1-18) Metal-Alanine, Metal-Glutamic acid complexes are water soluble while Metal-Leucine has dimethylsulfoxide (DMSO) solubility, each metal complex has a definite crystal shapes. The metal to amino acid ratio was 1:2 confirmed by AAS. Most of the amino acid complexes were formed at a pH of 4-6, however calcium and magnesium complexes were formed at a pH of 8-10. The propped structures of metal complexes with alanine (a), leucine (b) and glutamic acid (c) are presented in Figure 1 and elemental data is well agreed with proposed formula. The analytical and physical data is summarized in Table 1.

UV-Vis and IR Spectra: All the complexes (1-18) showed the prominent colors which absorb in visible region of spectrum, the absorption maximum (λ_{max}), well resolved bands in both UV and visible region and molar extinction coefficient (ε_{max}) using concentration of 8x10^{-3} M by using the cell with path length of 1cm are presented in Table 2. The formation of metal alanine complex is evidenced by the formation of five member lactones type structure for these complexes as shown in Figure 1. The FTIR Spectra of these compounds can thus be correlated to five member lactones. So the complex showing minimum transmittance in the range 1000-1300 cm^{-1} [C-O stretching (asymmetric & symmetric)] can be considered to have maximum chances of metal to alanine bonding. Fe in this regards show minimum transmittance of all metal alanine complexes in this region i.e. 1100 cm^{-1}. From this it can be concluded that Alanine has the maximum tendency for bonding with Fe and 79.7 % yield of Fe-Alanine complex prove our observation, on the other hand the maximum transmittance of Cu-Alanine complex at 1151 cm^{-1} exhibit the minimum tendency of alanine to bind with Cu and it is also evident from its yield of only 15.4%. In other metal-alanine complexes the situation is in-between these two extremes as it is evidence by their % transmittances in the range of C-O stretching for lactones. The order of strength of metal-alanine bond for different metals is as follows: Fe>Co>Ca>Zn>Mg>Cu. The complex of metals with glutamic acid showing minimum transmittance in the range of 1000-1300 cm^{-1} [C-O stretching (asymmetric & symmetric)] can be considered to have maximum chances of metal to L-glutamic acid bonding. 18.2% transmission by Ca-glutamic acid at 1130 cm^{-1} showed the minimum transmittance among all metal-glutamic acid complexes. So glutamic acid have maximum tendency to bind with Ca and it is also evident from its 46.17% yield. On the other hand maximum transmittance is shown by Fe-glutamic acid complex which was 78.5% at 1205 cm^{-1}, thus exhibiting minimum tendency of glutamic acid to bind with Fe and it is evident from its yield of 2.96% while the transmittance exhibit by other metal-glutamic acid complexes in-between these two extremes. The bond of different metals with leucine follow the order as Ca>Co>Cu>Zn>Mg>Fe. 1000-1300 cm^{-1} [C-O stretching (asymmetric & symmetric)] can be considered to have maximum chances of metal to leucine bonding because of showing minimum transmittance. In case of leucine, Zn showed the minimum transmittance of 1102 cm^{-1} with 23.01% transmittance among all other metal-leucine complexes in this region. So leucine have maximum tendency to bind with Zn and it is also evident from its 26.77% yield. On the other hand maximum transmittance is shown by Mg-leucine complex which was 64.8% at

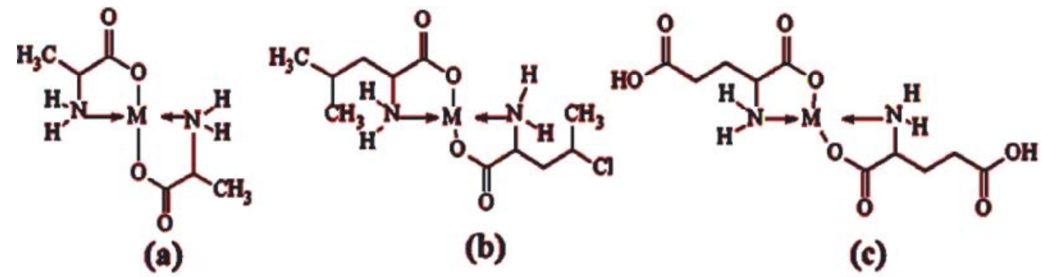

Fig. 1: Proposed structures of metal (II) complexes

Table 1: Analytical and physical data of metal complexes (1-18)

Complex	Calc. (Found)% H	N	C	M	Yield (%)	MP(°C)
Metal-Alanine						
$C_6H_{12}MgN_2O_4$ [200.48]	5.98(5.62)	13.97(13.88)	35.91(35.87)	12.12(12.32)	23.9	90
$C_6H_{12}CaN_2O_4$ [216.26]	5.50(5.32)	12.95(12.98)	33.29(33.56)	18.53(18.32)	30.1	103
$C_6H_{12}FeN_2O_4$ [232.03]	5.17(5.34)	12.07(12.21)	31.03(31.45)	24.07(24.23)	79.7	78
$C_6H_{12}CoN_2O_4$ [235.11]	5.10(4.99)	11.90(11.65)	30.62(30.12)	25.07(25.11)	32.5	112
$C_6H_{12}CuN_2O_4$ [239.73]	5.01(4.76)	11.68(11.43)	30.03(30.34)	26.51(26.45)	15.4	120
$C_6H_{12}ZnN_2O_4$ [241.56]	4.97(4.87)	11.59(11.76)	29.81(29.28)	27.07(27.11)	28.8	80
Metal-Glutamic acid						
$C_{10}H_{16}MgN_2O_8$ [316.56]	5.05(5.23)	8.84(8.78)	37.91(37.88)	7.68(7.66)	9.65	235
$C_{10}H_{16}CaN_2O_8$ [332.34]	4.81(4.77)	8.43(8.35)	36.11(36.22)	12.06(12.23)	46.17	230
$C_{10}H_{16}FeN_2O_8$ [348.11]	4.60(4.82)	8.04(8.11)	34.47(34.32)	16.04(16.11)	2.96	240
$C_{10}H_{16}CoN_2O_8$ [351.19]	4.56(4.89)	7.97(7.88)	34.17(34.11)	16.78(16.49)	23.81	235
$C_{10}H_{16}CuN_2O_8$ [355.81]	4.50(4.34)	7.87(7.76)	33.73(33.66)	17.86(17.98)	22.46	233
$C_{10}H_{16}ZnN_2O_8$ [357.64]	4.47(4.67)	7.83(7.91)	33.55(3365)	18.28(18.21)	11.33	232
Metal-Leucine						
$C_{12}H_{24}MgN_2O_4$ [284.65]	8.43(8.33)	9.84(9.78)	50.59(50.78)	8.54(8.59)	8.27	308
$C_{12}H_{24}CaN_2O_4$ [300.42]	7.99(7.88)	9.32(9.44)	47.93(47.66)	13.34(13.44)	16.65	220
$C_{12}H_{24}FeN_2O_4$ [316.18]	7.59(7.43)	8.86(8.54)	45.54(45.45)	17.66(17.34)	8.91	210
$C_{12}H_{24}CoN_2O_4$ [319.27]	7.52(7.34)	8.77(8.45)	45.10(45.22)	18.46(18.33)	12.12	260
$C_{12}H_{24}CuN_2O_4$ [323.87]	7.41(7.34)	8.65(8.55)	44.46(44.43)	19.62(19.34)	10.43	240
$C_{12}H_{24}ZnN_2O_4$ [325.72]	7.37(7.56)	8.60(8.45)	44.21(44.23)	20.07(20.19)	26.77	303

Table 2: Physical and spectral data of metal complexes (1-18)

Complex	Color	IR (υ cm^{-1})	λ_{max} (nm)	ε_{max} (L moles cm^{-1})
Metal-Alanine				
$C_6H_{12}MgN_2O_4$ [200.48]	Light brown	3150.5-3665.8 (N-H), 1587.1-1650 (C-O),1138.5-1351.1 (C-O), 794.0 (M-O), 655.5 (M-N)	239, 349	24.6
$C_6H_{12}CaN_2O_4$ [216.26]	Off white	3154.8-3655.8 (N-H), 1582.1-1656 (C-O),1119.5-1353.1 (C-O), 791.0 (M-O), 651.5 (M-N)	238, 352	19.6
$C_6H_{12}FeN_2O_4$ [232.03]	Brick red	3151.5-3657.5 (N-H), 1577.1-1648 (C-O),1100.0-1353.1 (C-O), 790.0 (M-O), 651.5 (M-N)	233, 500	46.2
$C_6H_{12}CoN_2O_4$ [235.11]	Tea pink	3152.7-3662.5 (N-H), 1585.1-1646 (C-O),1109.5-1354.1 (C-O), 792.0 (M-O), 651.5 (M-N)	227, 560	22.4
$C_6H_{12}CuN_2O_4$ [239.73]	Blue	3153.5-3661.8 (N-H), 1582.1-1653 (C-O),1151.0-1356.1 (C-O), 787.0 (M-O), 658.0 (M-N)	241, 630	30.6
$C_6H_{12}ZnN_2O_4$ [241.56]	White	3152.5-3661.8 (N-H), 1581.1-1654 (C-O),1123.5-1358.1 (C-O), 791.0 (M-O), 650.5 (M-N)	198, 329	4.6
Metal-Glutamic acid				
$C_{10}H_{16}MgN_2O_8$ [316.56]	Light brown	3101-3494 (N-H), 1601-1663 (C-O),1391-1361 (C-O), 841.0 (M-O), 560.2 (M-N)	212, 349	24.3
$C_{10}H_{16}CaN_2O_8$ [332.34]	Off white	3102-3480 (N-H), 1609-1658 (C-O),1130-1351 (C-O), 842.0 (M-O), 561.2 (M-N)	223, 352	19.1
$C_{10}H_{16}FeN_2O_8$ [348.11]	Brick red	3109-3494 (N-H), 1601-1662 (C-O),1205-1361 (C-O), 841.0 (M-O), 558.2 (M-N)	202, 500	46.09
$C_{10}H_{16}CoN_2O_8$ [351.19]	Tea pink	3101-3497 (N-H), 1612-1658 (C-O),1141-1355 (C-O), 840.5 (M-O), 561.5 (M-N)	219, 560	22.1
$C_{10}H_{16}CuN_2O_8$ [355.81]	Blue	3115-3492 (N-H), 1616-1661 (C-O),1152-1351 (C-O), 841.0 (M-O), 560.1 (M-N)	238, 630	30.8
$C_{10}H_{16}ZnN_2O_8$ [357.64]	White	3101-3492 (N-H), 1601-1663 (C-O),1172-1352 (C-O), 846.0 (M-O), 556.2 (M-N)	218, 329	4.2
Metal-Leucine				
$C_{12}H_{24}MgN_2O_4$ [284.65]	Light brown	3150.5-3665.8 (N-H), 1587.1-1650 (C-O),1195-1351.1 (C-O), 794.0 (M-O), 655.5 (M-N)	228, 352	24.2
$C_{12}H_{24}CaN_2O_4$ [300.42]	Off white	3140.3-3645.2 (N-H), 1593.1-1635 (C-O),1127.5-1372.1 (C-O), 782.0 (M-O), 635.21 (M-N)	210, 500	19.7
$C_{12}H_{24}FeN_2O_4$ [316.18]	Brick red	3165.5-3635.3 (N-H), 1584.1-1630 (C-O),1159.5-1341.9 (C-O), 789.0 (M-O), 652.5 (M-N)	226, 560	46.1
$C_{12}H_{24}CoN_2O_4$ [319.27]	Tea pink	3157.5-3642.8 (N-H), 1567.1-1670 (C-O),1137.5-1361.5 (C-O), 798.0 (M-O), 658.5 (M-N)	239, 630	22.2
$C_{12}H_{24}CuN_2O_4$ [323.87]	Blue	3158.5-3675.8 (N-H), 1567.1-1670 (C-O),1147.5-1373.1 (C-O), 797.0 (M-O), 665.0 (M-N)	243, 329	30.1
$C_{12}H_{24}ZnN_2O_4$ [325.72]	White	3160.5-3655.8 (N-H), 1583.1-1657 (C-O),1102-1359.1 (C-O), 788.0 (M-O), 649.5 (M-N)	224, 349	4.1

Table 3: [1]HNMR data of amino acid and metal complexes

Compound	^{1}HNMR (δ, ppm)
DL-alanine	1.71 (d, 3H, CH_3), 5.11 (q, 1H, CH), 6.96 (s, 1H, OH), 6.13 (s, 2H, NH_2)
[M(Ala)$_2$]	1.21 (d, 3H, CH_3), 3.03 (q, 1H, CH), 1.89 (s, 2H, NH_2)
L-glutamic acid	3.78 (t, 1H, CH), 1.99 (t, 2H, CH_2), 2.43 (t, 2H, CH_2), 9.21 (s, 2H, 2xOH), 9.13 (s, 2H, NH_2)
[M(Glu)$_2$]	3.72 (t, 1H, CH), 1.69 (t, 2H, CH_2), 2.13 (t, 2H, CH_2), 5.21 (s, 1H, OH), 5.13 (s, 2H, NH_2)
Leucine	0.98 (d, 6H, 2xCH_3), 1.49 (d, 2H, CH_2), 4.43 (t, 1H, CH), 2.19 (q, 1H, CH), 8.21 (s, 1H, OH), 8.03 (s, 2H, NH_2)
[M(Leu)$_2$]	0.78 (d, 6H, 2xCH_3), 1.62 (d, 2H, CH_2), 2.83 (t, 1H, CH), 2.31 (q, 1H, CH), 2.09 (s, 2H, NH_2)

1195 cm^{-1}, thus exhibiting minimum tendency of leucine to bind with Mg and it is evident from its 8.27% yield as well. In other metal-leucine complexes the transmission is in-between these two extremes following the binding order of Zn>Ca>Co> Cu>Fe>Mg. Some important IR bands of metal complexes are presented in Table 2.

^{1}H NMR Spectra: ^{1}HNMR assignments of deuterated solution of amino acids and their metal complexes (DL-alanine and L-glutamic acid in D_2O while leucine in DMSO, d_6) are summarized in Table 3. The recorded spectra (^{1}H NMR, 400 MHz) for all the free ligands and their metal complexes (1-18) were identified with help of literature cited, were found in their expected regions [12]. The chemical shift value of amino group and hydroxyl value was shifted to higher field region because of coordination bonding and deprotonation respectively which confirm the involvement of both groups in bonding. The groups which are away from metal in complex showed a little bit difference in their chemical shifts.

CONCLUSIONS

The metal amino acid complexes are of extreme importance from nutritive point of view for both animals and plants because of their easy absorption due to their smaller size. Their bonding strengths are strong enough for the molecules to remain intact through application and absorption, but not as strong as to resist breakdown for metabolic usage to the metal atoms because they are not synthetic or foreign to living system.

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