



International Journal of Pharmaceutical Research and Development (IJPRD)

Platform for Pharmaceutical Researches & Ideas

www.ijprd.com

STUDY OF PREFERENTIAL BINDING OF LEAD CATION IN LEAD -PROTEIN COMPLEXES

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ABSTRACT

An analysis of the geometry of interaction of the toxic metal Pb(II) with proteins reveals preferential ligands that are functional group of side chain of proteins (Viz. carboxylate, imidazole group). It is also observed that the water molecules play a decisive role in the interaction geometry. In the study we have carried out, it has been observed that the commonest interactions of lead cation are with glutamate and with carboxylate group in general. The next commonest interactions are with water molecules. The mean distances of metal ligand interaction have been summarized.

Key words: *Interaction geometry, carboxylic ligand, ligand water, toxic metal, coordination number.*

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INTRODUCTION

Metal atoms or ions occur widely with proteins and have variety of functions. They can be a part of active sites in catalytic process in biological systems; in other cases they play an important role of maintaining structure. In literature, it is found that lot of effort has been put by research scholars to study and investigate the role of metal ions in biological system.

The preeminence of interaction of toxic metal (Co, Hg, Pb, Cd, As etc.,) and non toxic metal (Na, Ca, Fe, Zn etc.,) ions with amino acid/protein is well documented in literature (A. Sigel & H. Sigel, 2006). Metals at higher than allowed concentrations, generally produce toxicity

by forming complexes with organic complexes (called ligands). The modified molecules lose their ability to function properly which leads to the malfunction or death of affected cells. (Ballwin Dr. Marshall W.J, 1999). In the present study one of toxic metal lead (II) and its complexes is considered. The divalent lead could either be hard or soft acid depending upon pH of the biological medium. Lead resembles the divalent sub group IIA metals in chemical behaviors more than its own sub group IVA metals. Pb(II) with its electronic configuration (Xe) $4f^{14}5d^{10}6s^2$ is one of the post transition elements that exhibit inert-pair. The relatively inert outer lone pair electrons affect the stereo-chemistry with the preferred ligands (Liat

shimoni et al., 1998). It appears that Pb(II) preferentially binds to thiol, carboxylate groups in proteins, which can inactivate certain enzyme system or affect proteins structure. For detailed understanding of these effects, it is necessary to know the nature and environment of the protein binding sites with the metal. The Pb (II) complexes of protein are comparatively few and usually difficult to study due to their extreme insolubility.

This environment pollutant lead which is widely used in paint industry and storage battery industry shows its toxic effect through inhibition of hemoglobin – causing anemia, affects nervous system, especially in the case of children. To counteract the effect of lead poisoning more effectively, drugs may be designed for which it is necessary to establish the preferred ligands of lead and their interaction geometry.

Materials and Methods

In this study three-dimensional data on crystal structures of small molecules with carbon atom from CSD (Cambridge structural database) and information on biological macromolecules contained in PDB had been used, the list of molecules is given.

M.M Harding has given the metal ligand target distances & angles at metal atom, focusing distortion of the coordination geometry from regular polyhedral geometry (M.M.Harding 2000, 2001).J.P.Glusker(1999) has given detail analysis of coordination number, percentage of preferential interaction of ligands,energy aspects of coordination sphere of Mg^{2+} , Ca^{2+} , Mn^{2+} and Pb^{2+} Cations. Based on this reference, Pb (II) – Ligand environment is further analyzed here with more number of structures, through distance and angle determination. The aim of this study is to look for the most preferential ligand/s of the side chain of proteins and their interactional mode with toxic metal lead and to see if there emerges any particular rule. To establish the geometry, Metal to donor atomic distances and angles at the metal atom are measured (M.M Harding).

The PDB is accessed by way of the World Wide Web and useful samples (enzyme or protein molecules with Pb^{2+}) of 3-D crystal structures are Available online on www.ijprd.com

retrieved on Java platform. Pb (II) Co-ordination geometry is analyzed only for structures with resolution between 1.2 – 3.0Å. Metal-Ligand distances (M-L) and the inter bond (L1-M-L2) angles are measured using the software Rasmol v. 2.6. The definition of a coordination sphere used here requires the donor atom to be within specified target distances of the metal atom + tolerance (M.M.Harding, 2001). The proposed target distance is found to vary for cysteine lead (CSB) and trimethyl lead (PBM) complexes (Table - 1a).

Result and Discussion

metal- carboxylate interaction

A carboxylate group may coordinate to metal through one oxygen atom (unidentate) or through both oxygen atoms (bidentate) and the two oxygen atoms may be coordinated to two different metal cations (bridging). Metal ion in unidentate as well as bidentate conformation prefers syn direction for coordination. It is found that the M-O distance to a bidentate coordination is longer than that of a unidentate coordination for specific lead ion in the selected sample (more than one lead cation is found in a single lead protein structure) (M.MHarding, 2001). And in all there are 45 cases of bidentate interaction within the first coordination sphere and only 26 cases of unidentate mode of interaction have been noticed.(M-O) distances for both the modes are summarized(Table 2a & 2b).Bridging coordination is observed in few case such as in structures with PDB ID 1e9n,2vo1 and1n0y.

Carboxylates in active sites of enzymes generally take up the more basic syn lone pair's direction for metal chelating rather than the less basic anti lone pairs as noted by Gandour (1981). The relative position of the metal ion is expressed in terms of torsion angle θ (O2- CD- O1- M for glu, O2-CG – O1- M for Asp). The metal ion is broadly clustered in three groups around the syn lone pair direction. For bidentate mode -sp, +sp & +sc (-30° to 0° , 0° to $+30^\circ$ & $+30^\circ$ to $+90^\circ$) directional approach is seen for lead cation, where as for unidentate mode of interaction, the relative position of the cation is along +sc , -sc, -ac locations

(+30° to + 90°, -30° to -90° & -90° to -150°). The scatter plot (Fig .1) shows the preference of syn direction over anti direction. The exceptional case –ac is noticed in lead cation coordination (Table-2 a & 2b).

The arrangement of metal ion in syn direction is more common than that of anti. In all 78 % of interaction happens to be along syn position of lone pair oxygen of the side chain carboxylate ligand. The case of 'direct bonding', where the metal lies equidistant from the two oxygen of carboxylate group (C.J Carrel et al, 1988) could not be found in this analysis. But it can be stated that 'near direct bonding' is observed to be prevalent, wherein M-O1, M-O2 distances are not exactly equal but differ slightly (Table- 2 b).

The metal-oxygen distance and hence the preference is known to depend on the coordination number of the cation (Chakraborti et al 1981, Einspahr & Bugg 1984). The histogram (Fig. 2,) shows the distributions of Pb ion preference for ligand Oxygen in various protein structures considered, with different coordination numbers.

Role of water molecules

Water makes up a major part of typical protein and nucleic acid crystal structures. The metal ions in biological systems bind at the sites of high hydrophobic contrast (Mason.M.Yamashita et al., 1990). This means that there is strong hydrophilic environment just around the metal cation which includes the internal water in proteins crystals. Thus water is an important ligand to the lead cation. Oxygen of water [O(w)] plays a decisive role in shaping of the polyhedron geometry of interaction of lead. There are only five cases of the considered sample structures (PDB Id – 1qnv, 1ps1, 2oq1, 2qd5, 2qkl) that does not show any Pb-O(w) interaction, as shown in the figure 4, 5 , 6 and 7.

Water seems to be an important ligand of the coordination sphere of metal ion lead with a bound Glu residue .In many cases the oxygen of water is approximately perpendicular to the plane formed by metal ion lead with carboxylate group. Presence of water gives an energetically favorable mode of interaction (Charkabarti 1990). One or more than one oxygen of water molecule can serve as ligand

to Pb (II). For example, in 1hqj (2006) Pb (II) cation ligands to three O (w) & one O of Glu to have tetrahedral coordination. In 1hqj (2001) three O (w) & one N of Arg ligates to the cation to form tetrahedral coordination. In 1ocx (1185B) five O (W), one OD, one NE coordinates to form polyhedron. Unusually longer than allowed distances (>3.5Å) observed in this study shows the presence of weak interaction within the inner sphere coordination. Cyclic ring formation is observed with one oxygen of water and two Oxygens of carboxylate group and the lead cation. This kind of chelating is observed in almost all anti and near syn mode of liganding, where water is available within the first coordinating sphere.

Coordination number & ligand preferences

Because of the inert-pair effect of divalent lead 6s electrons, their participation in bond formation requires more energy. The 6s orbital contracts, lone pair electrons present causes non-spherical distribution of charges. Higher CN (coordination number) shows holodirection arrangement of ligands which are influenced by ligand over crowding, lower CN is observed where ligands are hemi directed. This is true in case of small crystal structures (J.P.Gluskar, 1999), it also holds well in the present study. For CN =4, ten structures were found to coordinate nitrogen from protein side chain, five of them coordinate with sulphur of protein side chain. Preferences for all CN of these two ligands are shown in Fig.3. The tetrahedral geometry shows distortion from ideal geometry. (Zabrodsky et al.1993). Similar distortions were observed for CN =5, 6, 7 (M.M Harding, 2000). According to CN, hemi directional arrangement of ligands is more preferred in the case of Pb(II) since CN=4 occur 38 times out of a total of 73 lead ion environment analyzed. Rare case of CN=2 that is observed can be attributed to uncertainties in the refinement of crystal structure, could have missed some ligands. Table 3, shows that tetrahedral coordination is preferred. Rare case of linear (bent) co-ordination is seen. CN=7 is observed in case of 1qr7 where Pb (II) interacts with oxygen of an inorganic molecule (PEP) and in 1ocx (Trimethyl lead) the interaction is with 6 oxygen ligands and

one nitrogen of Asn. CN=9, CN=10 is observed in 1n0y (508), 1n0y (503) respectively.

Table-1a: Metal- ligand distance of oxygen, nitrogen, sulphur of side-chain and oxygen of water is given below: (mean distance in Å)

Pdb ID	R(Å)	M-O(Asp/glu)	M-O(w)	M-N	M-S
1b6g(CSB)	1.15	-	4.002	-	3.671
1 hqj	1.2	2.595	2.123	2.508	-
2fj9	1.6	2.525	2.864	-	-
1ssy	1.7	2.441	4.267	4.076	-
1zhw	1.7	2.801	5.485	-	-
2oq1	1.9	-	-	-	2.621
2vo1	1.95	2.603	2.282	-	-
1sn8	2	2.23	2.924	-	-
2ani	2	2.607	1.597	-	-
1ocx(PBM)	2.15	2.78	3.344	2.332	5.283
1e9n	2.2	3.395	3.146	3.055	-
1 n0y	2.2	2.609	2.783	-	-
1 fjr	2.3	2.708	3.048	4.976	-
2 fp1	2.3	2.689	2.948	2.465	-
2 qd5	2.3	-	-	2.635	-
2 qkl	2.33	5.356	-	-	-
1qnv	2.5	-	-	-	2.798
2 ch7	2.5	2.901	3.433	-	-
1 qr7	2.6	3.016	3.531	2.722	3.254
1ps1(PBM)	2.6	3.162	-	-	-
1ka4	3.0	3.655	2.216	2.281	-
2 goa	-	2.867	2.887	-	-
1nao	-	2.839	3.305	3.036	-

Table 1b: Mean (M-O) distances for other ligands in Å (CAC=cacodylatelon, PEP=poly enol pyruvate, SIN=succinic acid)

LIGAND	PDB ID	(Pb-O)
(O)		
Carbonyl	1b6g	3.625
	2oq1	5.212
	1xxa	2.443 (4)
Serine	1b6g	3.797
Threonine	1ocx	5.336
	1n0y	3.486
CAC	1n0y	2.654
PEP	1qr7	3.736
SIN	1hqj	2.930 (6)

Table 2a: METAL- CARBOXYLATE INTERACTION. (DISTANCES IN Å) C in torsion angle is CD for Glu & CG for Asp. M-O1 is the nearest distance of metal cation to carboxylate oxygen

Unidentate Mode			
			Torsion Angle(θ)
PDB ID	Residue	M-O ₁	O ₂ -C-O ₁ -M
1xxa	Asp 88F	3.028	24.3
1qr7	Asp 326A	2.921	106.9
	Asp 326 B	2.965	106.3
1ka4	Glu 270	4.185	31.2
2 vo1	Asp 131 A	2.002	-19.8
	Asp 133 A	2.002	22.0
	Asp 129 A	1.997	-98.5
	Asp 95 A	2.693	39.5
	Asp 95 A	1.998	-64.0
	Asp 24 A	2.608	77.3
	Asp 20 A	2.063	-85.1
2 vo1	Asp 56 A	2.023	-96.3
	Asp 58 A	3.002	47.8
2o3C	Asp 63 A	2.718	-112.7
	Asp 63 B	2.604	-92.3
	Asp 63 C	2.412	-104.6
2ch 7	Glu 506	3.031	-170.3
2 goa	Asp 258 A	2.145	-43.2
1 nao	Glu 98A	3.450	69.3
	Glu 64	2.218	35.7
1n0y	Asp 20A	2.297	-126.5
	Asp 56A	2.245	-83.9
	Asp 58 A	2.412	-118.2
	Glu 67 A	3.281	-117.5
	Asp 129 B	2.370	-127.5
	Asp 80B	2.754	93.8

TABLE 2b: METAL-CARBOXYLATE INTERACTION. (DISTANCES IN Å) C in torsion angle is CD for Glu & CG for Asp. M-O1 is the nearest Distance of metal cation to carboxylate oxygen

Bidentate Mode				
PDB ID	Residue	M-O1	M-O2	Torsion angle O1-C-O2-M
1 ssy	Glu 270A	2.441	2.534	1.6
1 zhw	Asp 364	2.801	2.827	-2.0
1sn8	Glu 72A	2.23	2.859	-1.0
1ocx	Glu 6A	2.842	2.979	-13.2
	Glu 6B	2.718	2.791	-16.5
1 xxa	Glu 150 C	3.153	4.443	-77.7

1 efn	Asp 108 B	4.736	5.460	84.2
1 qr7	Glu 302 A	2.975	3.447	-13.4
	Glu 302 B	2.944	3.394	-15.7
1 ps1	Glu 89A	3.169	3.881	-23.9
2 vo1	Glu 140A	2.944	2.985	-2.6
	Asp 122A	2.005	2.709	0.2
	Asp 118 A	2.488	2.685	-4.0
	Glu 104 A	2.549	2.816	15.4
	Glu 14A	2.69	3.661	1.6
	Glu 31A	1.994	2.737	-1.4
	Asp 22A	2.033	3.1	24.3
2vo1	Asp 58A	2.572	2.987	-128.5
	Glu 97 A	2.670	2.708	-91.5
	Asp 64A	2.021	3.271	-37.9
2fj 9	Asp 69A	2.230	2.821	-2.0
2 ani	Glu 270A	2.559	2.655	-7.3
2qkl	Glu 32B	5.131	5.581	74.5
2o3 c	Glu 89 C	2.285	3.531	19.7
2 fp1	Glu 51 B	2.748	2.776	-3.0
	Glu 48 B	2.534	2.579	-4.6
	Glu 41 B	2.514	2.657	0.2
2ch7	Glu 282	2.812	2.968	-27.5
	Glu 471	2.582	2.727	-166.0
	Glu 247	2.827	3.365	28.6
2goa	Asp 242 A	2.804	3.306	26.6
	Asp 242 B	2.706	3.374	23.5
Inao	Glu 30 A	2.181	3.459	38.8
	Asp 58 B	2.137	3.419	-17.0
	Glu 95B	2.394	3.19	40.7
	Glu 61 A	2.376	3.572	35.2
In0y	Glu 31 A	2.537	2.577	20.3
	Asp 22A	2.515	3.663	16.5
	Asp 24 A	2.621	3.775	63.7
	Glu 11A	2.473	2.655	4.7
	Glu 14A	2.314	3.033	13.1
In0y	Asp 58 A	2.451	3.126	29.8
	Glu 67 A	2.754	2.821	26.0
	Glu 114 B	2.498	2.505	6.2
	Asp 64 A	2.571	3.157	17.2

Table 3: Preferred co-ordination geometry (co-ordination number=CN) of lead as found in all the selected samples (PDB)

Pdb Id	Polyhedron	No. Of Occurrences
1ps1,1zhw,1efn ,2vo1(1152),1n0y(52)	Linear(CN=2)	5
2oq1,2qkl,2ani,1nao(274,276)	Trigonal(CN=3)	5
1hqj(2001-2009), 1fjr601,1fjr604,1xxa418,1xxa417, 1xxa416,1sn8,1qnv,1e9n(1319,1320),1syy, 1ocx1184 (A, B) 1185(PBM), 1b6g233, 150. 2fj9,2vo1(1153),2o3c(402A,403B), 1n0y(275,277,278) 2fp1,2ch7(1530,1531)1n0y(504,507,509)	Tetrahedral (CN=4)	38
1xxa(419)	SquarePlanar (CN=4)	01
1ka4,2vo1(1146),1n0y(505,506)	Trigonal bipyramidal(CN=5)	04
1fjr(602,603) ,2qd5(1101)	Squarepyramidal (CN=5)	03
1ocx1184c,1qr7 (351b),2vo1(1150),2qd5(1102), 2o3c(401c),2fp1(501),2goa(903A,904B), 1n0y(500,501)	Octahedral(CN=6)	11
1ocx(1185b),1qr7 351a,2vo1(1147,1148.1149)	Pentagonal bipyramidal(CN=7)	05

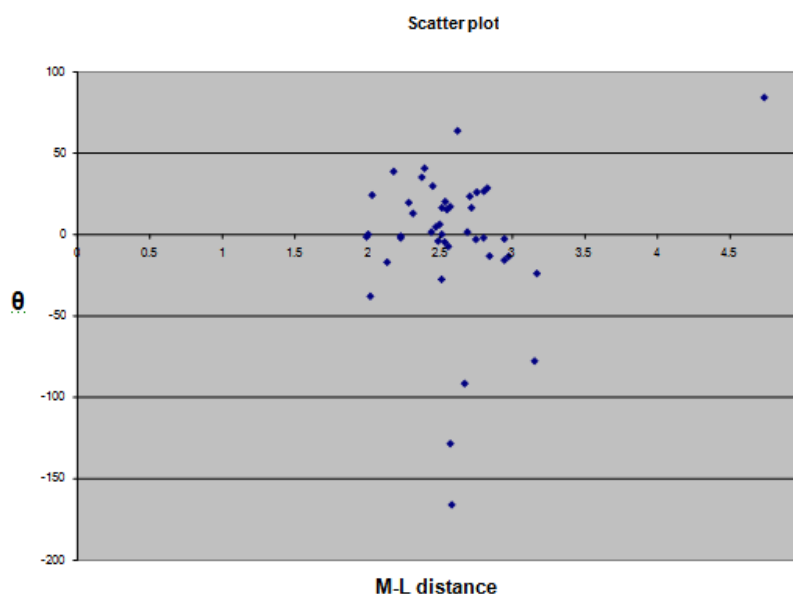
Figure 1. Gives the distribution of torsion angle θ in monodentate and bidentate mode of coordination

Figure2. Shows the preferential binding of lead in different coordination atmosphere. Darker bars shows side chain oxygen preference percentage while less darker bars is that of oxygen of water, as found in the samples analyzed.

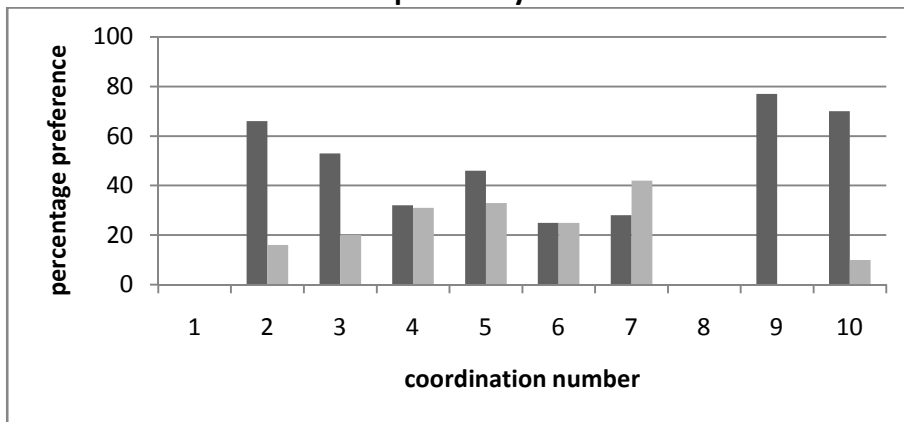


Figure 3. Preferential binding lead with nitrogen of side chain (darker bars) and with sulphur of side chain (less darker bars), as found in the analyzed samples

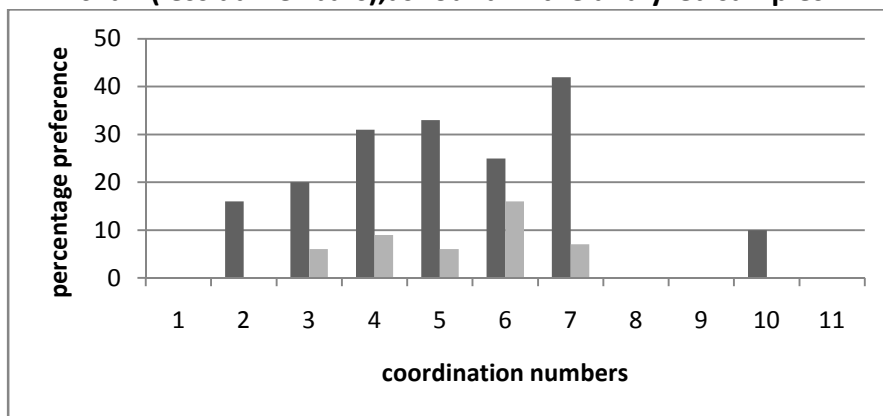


Figure 4. Shows 2qd5; CN=5, SQUARE PYRAMIDAL COORDINATION

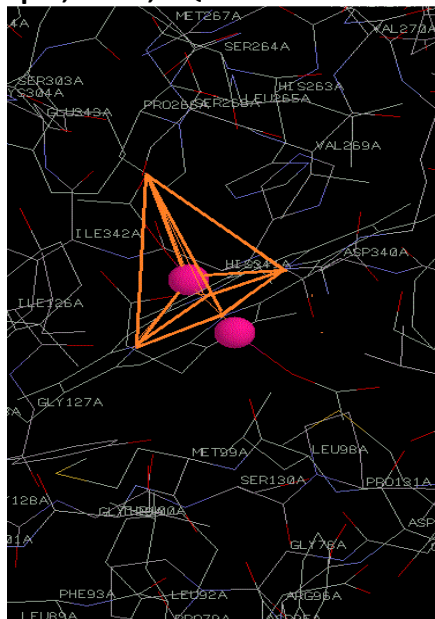


Figure 5. shows 1ps1; CN =2, LINEAR BENT COORDINATION

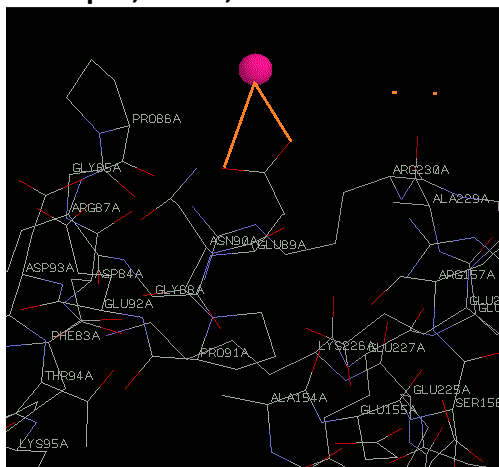


Figure6. shows 2oql; CN=3, TRIGONAL COORDINATION

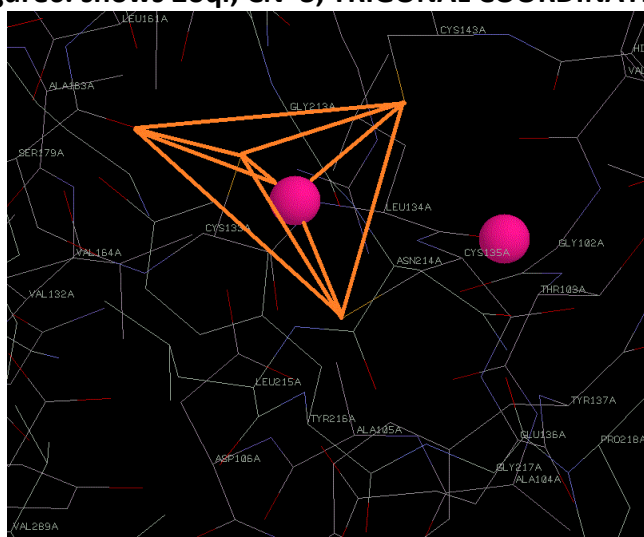
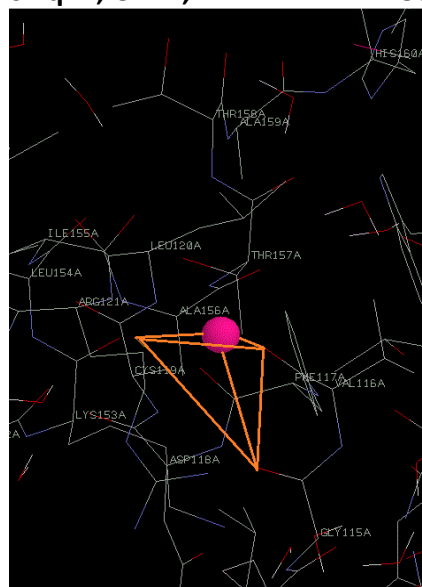


Figure 7. Shows 1qnv; CN=4, TETRAHEDRAL COORDINATION



CONCLUSION

From the analysis it is observed that Pb (II) appears to prefer mostly oxygen atoms of carboxylate group of side chain of proteins especially of Glu along syn direction oxygen lone pair in bidentate mode of interaction. Lead ion prefers equally both syn and anti position of oxygen lone pair, where it exhibits unidentate mode of interaction. Chelating type of coordination of the cation is generally noticed in anti conformation. The next preferable ligand happens to be oxygen of water. Carbonyl oxygen of main chain, nitrogen of imidazole and sulphur of cysteine are least favored, though sulphur is the preferential ligand in glutathione binding of toxins. However more number of structure analyses would be required to draw more authentic conclusions.

This knowledge of ligand preferences and their coordinate geometry are helpful in the design of drugs to counteract the effect of lead poisoning without depleting the essential metals such as Zinc & Calcium. (Kosnett.MJ), (Dart.R.C)

ACKNOWLEDGEMENT

I am grateful to Dr.Rathankar and Dr.Arvidan for useful discussion and for the encouragement.

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