

## Structural Aspects of Metal Ion-Carboxylate Interactions

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**Abstract:** The problem of determining metal cation binding selectivity for the syn and anti lone pair electrons of the oxygen atoms in a carboxylate group has been addressed by an analysis of metal carboxylate structures found in crystal structure determinations listed in the Cambridge Structural Database. Only carboxylate groups that act as isolated ligands were considered; those with neighboring additional metal-binding groups were eliminated. In general it is found that the cation lies in the plane of the carboxyl group which can bind from one to four metal ions. The exceptions to this finding are the larger of the alkali metal and alkaline earth cations which may bind well out of the plane of the carboxyl group; in these cases up to six metal ions may be coordinated to the carboxylate group. The syn lone pair on a carboxylate oxygen atom, the more basic lone pair (the one used in carboxyl dimer formation), is preferred for cation binding, although the very small and very large cations show a higher proportion of anti lone pair binding. Some cations will share the two carboxylate oxygen atoms equally, a situation referred to as "direct binding". This type of binding occurs mostly, although not exclusively, when the metal...oxygen distance is in the range 2.3-2.6 Å.

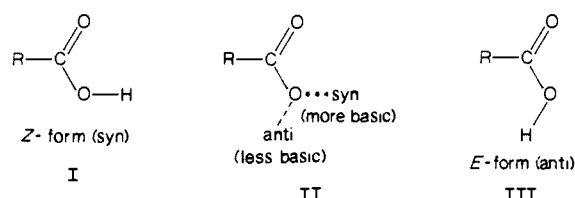
The carboxylate groups of aspartate and glutamate side chains in proteins are a common site for the binding of a metal cation. The relative three-dimensional locations of such metal cations with respect to the carboxylate groups in metal-containing proteins are of interest because this information is needed in the studies of certain enzymic mechanisms and in attempts to stabilize proteins. For example, presently we are investigating the protein electron density map of the enzyme D-xylose isomerase (glucose isomerase)<sup>1</sup> which contains the metal ions cobalt(II), magnesium(II), and manganese(II). There have been several studies of metal ion-carboxyl group participation in catalysis,<sup>2-4</sup> but experimental evidence on the stereochemistry of the interaction is still required. In this article we have considered the problem of finding, for a wide variety of metal cations, the most likely locations of the metal ions relative to carboxylate groups.

The carboxylate ion has one negative charge delocalized within it, and each oxygen atom has two lone pairs disposed at 120° to the C-O bond and in the plane of the carboxyl group. However, it is evident from a variety of crystal structures that the carboxylate group may bind up to four metal cations or that it may, in certain circumstances, share the metal cation between both oxygen atoms, as has been found for calcium carboxylates.<sup>5</sup> The problem may then be restated by the question of the importance of the directionalities of the oxygen lone pairs and also the question of which lone pair of each oxygen atom of a carboxylate group is preferred for metal ion binding.

The geometry of the position of the hydrogen atom in a carboxyl group has been described by Rebek and co-workers. They have designed, made, and studied some compounds in which the carboxylic acid groups, from steric effects caused by the rest of the molecule, are obliged to approach each other in a controlled way.<sup>6-9</sup> Two locations for a proton to lie, when attached to a carboxylate group in the directions of lone pair electrons, are designated syn and anti; they are illustrated in Scheme I. In the syn conformation (Z-form) the proton is on the same side of the C-O bond as the C=O bond while in the anti conformation (E-form) this is not the case. The syn conformation is that which is found when carboxyl groups dimerize by forming two hydrogen bonds. Ab initio SCF studies of formic acid<sup>7</sup> indicate that the syn (Z) conformation is more stable than the anti (E) conformation by 4.5 kcal/mol. This result might be considered to imply that the syn lone pairs are more basic than the anti lone pairs.

Gandour<sup>9</sup> has noted that the carboxylates in active sites of enzymes generally employ the more basic syn lone pairs for metal chelation rather than the less basic anti lone pairs; he estimates that syn protonation is 10<sup>4</sup>-fold more favorable than anti protonation. This means<sup>9</sup> that the carboxylate is a weaker base when constrained to accept a proton in the anti (E) direction.

Scheme I



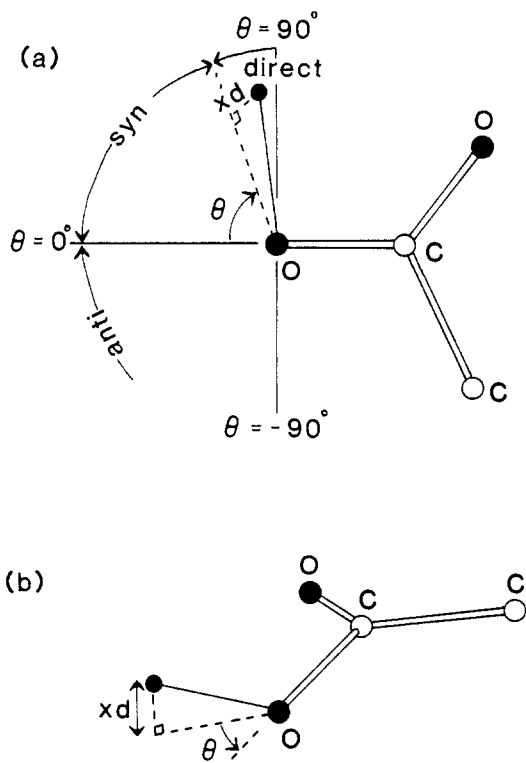
In view of Gandour's results one might presume that metal ions would always prefer the syn conformation for binding. We attempted to determine to what extent this is true by analyzing the metal cation surroundings of carboxylate groups in crystal structures. The positions of metal binding were determined for each appropriate structure by use of the Cambridge Structural Database (CSD),<sup>10</sup> which contains atomic positions of all small organic structures for which X-ray crystallographic analyses have been reported in the literature. The files in this database are in a form that is very convenient for finding those structures that contain the geometrical characteristics required in this study. We extracted data on all carboxylate structures, divided these data into groups depending on the type of metal cation in them, and then eliminated those in which there was a second intramolecular contact in the ligand that interacted with the metal ion. The overall aim was to find the syn/anti ratio for various metal cations.

## Experimental Section

The geometry of metal ion interactions with a carboxyl group that we have used in this study<sup>11</sup> is illustrated in Figure 1. The in-plane angle of 0° lies along the C-O direction and separates syn(positive angles) from anti(negative angles). The location of a hydrogen atom in carboxyl group

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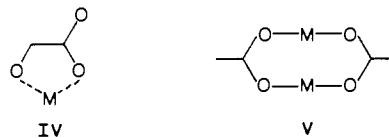
**Figure 1.** Two views of a representation of the C-O...M<sup>n+</sup> plane angle  $\theta$  and the deviation,  $x_d$  in Å, of the metal ion from this plane. Note that syn binding lies in the range  $\theta = 0^\circ$  to about  $80^\circ$ , anti bonding lies at negative values of  $\theta$ , and direct bonding lies in the area  $80$ – $90^\circ$ .

dimerization would be at angles near  $60^\circ$  (syn) while "direct bonding" in which the metal ion is equally shared by both oxygen atoms of the carboxyl group lies at angles near  $90^\circ$ .

The Cambridge Structural Database (CSD) was searched<sup>10,12,13</sup> for compounds containing carboxylate groups by means of the CSD program CONNSER (chemical connectivity search) by use of a request file, CARBOX.CNQ (general carboxylate search, code name CARBOX). This search generated a file CARBOX.COD (six-letter code names for carboxylates) which, for convenience, was divided into smaller code files by an editing procedure. The connectivity of atoms was determined by running the CSD program RETC (retrieve connectivities) on these smaller files and generating a file CARBOX.CON. CONNSER was again run in order to separate all of the compounds containing metals from those that did not; the input (requested by the program) was now CARBOX.CON rather than the entire data file. By variation of the \*.CNQ file, code lists for each metal or group of atoms could be made as required. This gave a series of smaller code files for specific metals or specific groups of metals. Those files with transition metals were further divided in each valence state using the CSD program BIBSER (bibliographic search). The code files from CONNSER were input into the CSD program RETB (retrieval of bibliographic information) to make a file \*.BIB, and then BIBSER was run to separate each valence state. Thus, for each multivalent metal, the carboxylate data were divided into different files. The CSD program RETD (retrieval of data) was then run excluding all compounds without coordinates, or with  $R$  factors above 0.075. The output was a file CARBOX.DAT containing all coordinates and other crystallographic data for use in the CSD program GEOM (molecular geometry), which was then run. Details of input files are given in deposited Table A.

Some fragments had conformations where the metal was bonded to a third oxygen atom or to a nitrogen atom in the same anion containing the carboxylate group. It was felt that this additional chelation would bias the directionality of metal binding by forcing it into anti position IV. In order to exclude these situations, the program GEOM was run twice. It was first run using \*.GEO as input in order to obtain all of the carboxylate fragments. It was run a second time using \*.GEE, as input, in order to obtain fragments with the bias, e.g., carboxylates with another oxygen atom in the same molecule coordinated to the same metal. The program (which we named COMP.DEL) was written to check for dupli-

cation and to delete entries common to both \*.GEO and \*.GEE, that is, it made a coordinate file containing only those coordinate sets without the bias shown in IV. Another factor that would bias the results was that carboxylates that formed dimers with two metal ions forced the metal into syn position V. This second bias, like the first, was eliminated by preparing an appropriate command file and then editing those instances where these dimers (V) were formed out of the first coordinate file (\*.GEO). Codes finally used are listed in deposited Table B.



Once the above routines were used, two further programs were used. A program (which we named FLIP) was written to ensure that all of the metal atoms were on the same side of the line along the C-C bond. A program (which we named ALIGN) was written to reject all coordinate sets in which the carboxyl group deviated by more than  $5^\circ$  from planarity; it also used a Student's  $T$  test to reject those sets with  $T > 3$ . These programs gave a desired average carboxyl structure, the positions of all of the metal atoms with respect to this average structure, distances between the metal (M<sup>n+</sup>) and the oxygen atoms, the C-O distance, the C-O-M<sup>n+</sup> angle, the C-C-O and O-C-O angles, and frequency diagrams of the O-metal distance and the C-O-M<sup>n+</sup> angle.

The metal atom positions were used as input for the ICR program MOLTRS, which will output, with any desired symmetry operation, those coordinates that are given as data input.<sup>14</sup> This program was used to put atoms into four symmetrical sections because of the symmetry of the carboxyl group. The average carboxyl group and metal atom positions were visually examined with the program VIEW, which plots atomic and scatterplot positions so that they can be viewed on a screen or printed on hard copy.<sup>15</sup> Two scatterplots of metal position with respect to a standard carboxylate group position were obtained with this program for each metal; one view was on to the plane of the carboxyl group and another view was along that plane. The scatterplots of metal positions were then smeared by putting a Gaussian function at each metal position and contouring the result.<sup>16</sup> This involved use of the ICR programs (named CELLIO, GENAT, GENMAP, and FIXMAP). Contour maps showing where the metal atoms tend to cluster were obtained and were illustrated by the ICR program DOCK.<sup>17</sup> The results for groups of elements are shown in Figure 2. Individual plots for atoms with varying valence states are illustrated in deposited Figure A. The major deviations from planarity are in group IA elements (alkali metals).

To illustrate the directionality of the C-C-O...M<sup>n+</sup> interactions, histograms were drawn with a program that we wrote (named TORSION2). The representation of directionality of metal binding, shown in Figure 1a, is on to the plane of the carboxyl group with the C-O group (coordinated to M<sup>n+</sup>) as the pivot point. This plot does not contain information on whether the metal ion is in the plane of the carboxylate group. The torsion angle, C-C-O...M<sup>n+</sup>, drawn schematically in Figure 1b, represents the deviation of the metal cation from the plane of the carboxyl group. Scatterplots were made of the bond lengths (O-M<sup>n+</sup>) versus the bond angles (C-O...M<sup>n+</sup>) in the plane of the carboxyl group for each valence state. These results are illustrated in Figure 2. The scatter is random for group IA but, generally for other groups, most scatterpoints are at  $-60^\circ$  (anti),  $60^\circ$  (syn), and, in some cases,  $90^\circ$  (direct).

## Results and Discussion

It is clear from the contoured scatterplots presented in Figures 2 (scatterplots and graphs for groups in the Periodic Table) and 3 (scatterplots for some common elements) that the most likely arrangements of metal cations are those designated syn, anti, and direct. The metal cations generally lie in the plane of the carboxyl group unless constrained out of it by another intramolecular interaction, a situation excluded in this study. A given metal cation may, under the criteria for selection for this study, bind to more than one carboxylate provided it is not in the same liganding molecule. A listing of percentages of syn, anti, and direct locations

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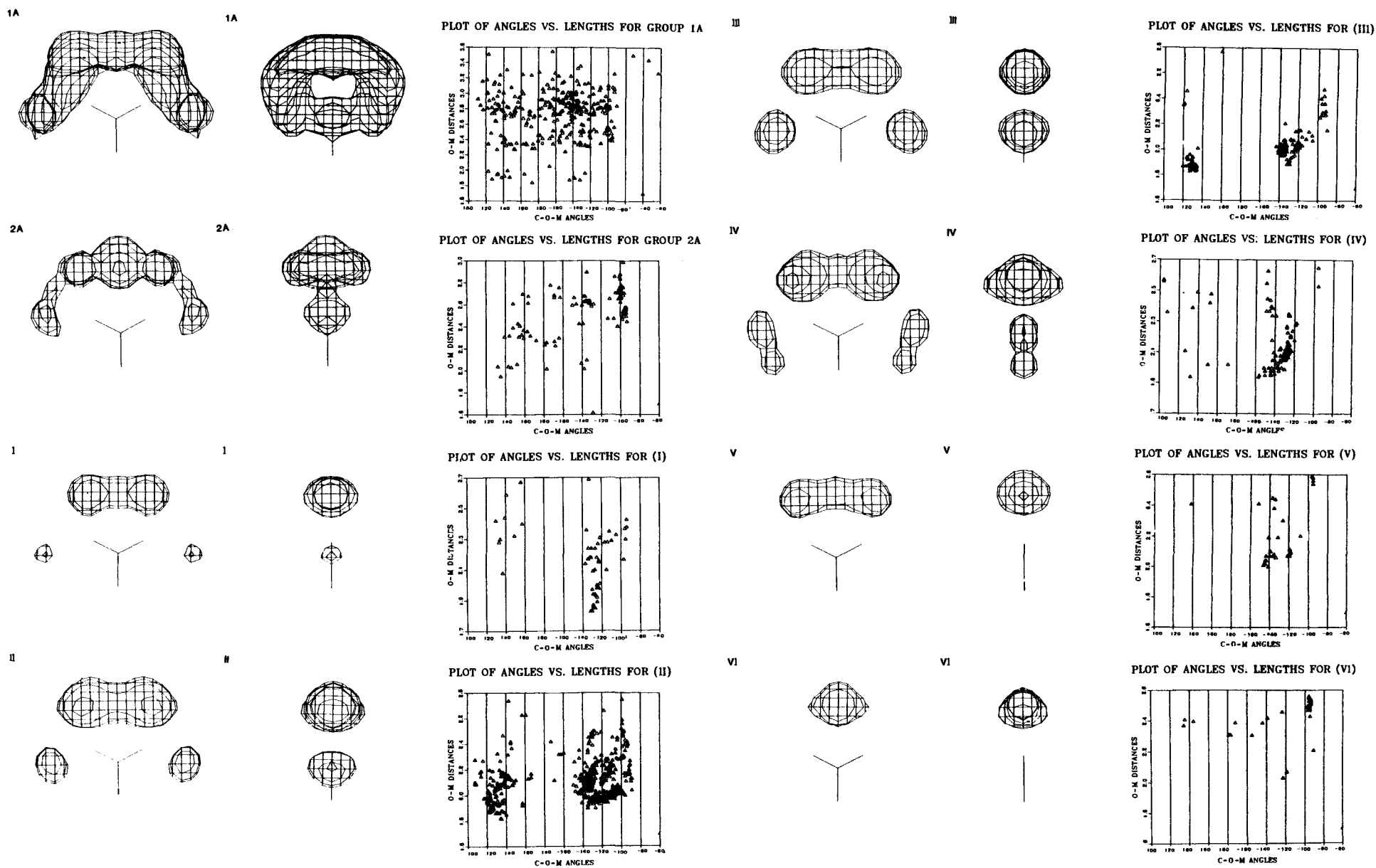
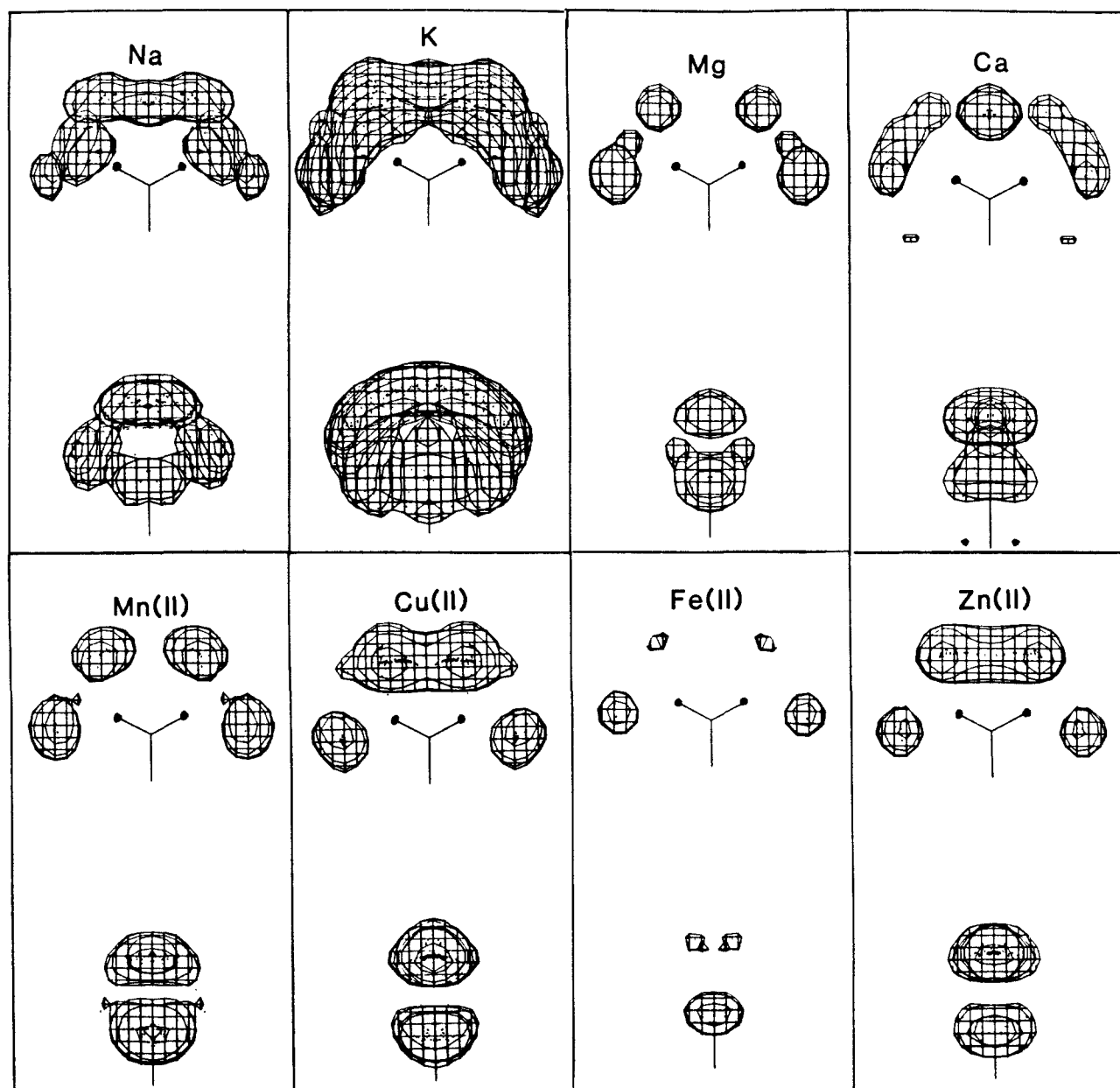
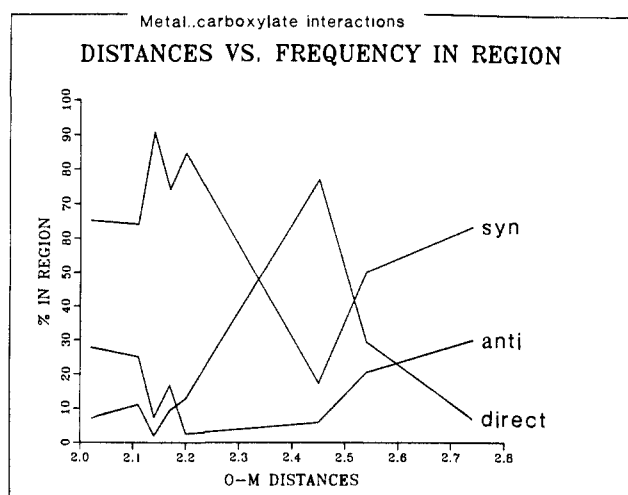


Figure 2. Scatterplots for groups of atoms. Views are contours of locations of metal cations viewed onto the plane of the carboxyl group and along this plane. Also shown is a plot of angle ( $\theta$ ) versus O...M<sup>n+</sup> distance for each group.



**Figure 3.** Individual scatterplots for  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Fe}^{2+}$ , and  $\text{Zn}^{2+}$ . For each ion the upper view is onto the plane of the carboxyl group and the lower view is along this plane with carboxylate oxygen atoms eclipsed.



**Figure 4.** Percentages of the three types of binding, syn, anti, and direct, as a function of  $\text{O}\cdots\text{M}^{n+}$  distance. Note that the peak of direct bonding lies between 2.2 and 2.6 Å for  $\text{O}\cdots\text{M}^{n+}$ .

for metal cations is given in Table I together with  $\text{O}-\text{M}^{n+}$  bond distances (average and minimum). The overall values for a total of 1558 metal-carboxylate interactions are 62.9% syn, 22.7% anti, and 14.4% direct. The elements potassium, sodium, and copper(II) have a high number of entries in this table, and of these, sodium and potassium ions show extensive out-of-plane interactions.

In Figure 4 an approximate analysis of the type of bonding (syn, anti, or direct) as a function of  $\text{M}^{n+}\cdots\text{O}$  distance is given. It is seen that the amount of direct bonding predominates in the narrow  $\text{M}^{n+}\cdots\text{O}$  range (2.3–2.6 Å); otherwise syn is generally preferred to anti in the ratio of 2–3:1, except when the  $\text{M}^{n+}\cdots\text{O}$  distances are short (<1.95 Å) when the ratio drops to less than 1. Elements with a reasonably high percentage of "direct" bonding are mercury(II), cadmium(II), calcium(II),<sup>5</sup> samarium(III), thallium(III), cerium(IV), and uranium(VI). Some selected data on direct bonding to illustrate the  $\theta$  values and the similarities in  $\text{M}^{n+}\cdots\text{O}$  distances are given in deposited Table C. In most cases the  $\text{M}^{n+}\cdots\text{O}$  distances and the  $\theta$  angles are equivalent for both oxygen atoms.

The overall characteristics of metal-carboxylate interactions are diagrammed in Figure 5 and Table II. These show, with

Table I. Binding for Individual Cation Types When 9 or More Entries Were Found

element	% syn	% anti	% direct	(M...O) av	(M...O) min	no. of entries if $\geq 9$	no of entries			predominant finding
							syn	anti	direct	
Mn(III)	0.0	100.0	0.0	1.88	1.83	11	0	11	0	anti
Co(III)	27.0	73.0	0.0	1.89	1.83	37	10	27	0	anti
Li(I)	41.2	58.8	0.0	1.94	1.85	17	7	10	0	anti
Cr(III)	50.0	50.0	0.0	1.95	1.92	10	5	5	0	syn/anti
Cu(I)	81.8	13.6	4.5	1.98	1.83	22	18	3	1	syn
Pt(II)	10.0	90.0	0.0	1.98	1.93	10	1	9	0	anti
V(IV)	94.4	5.6	0.0	1.99	1.94	19	18	1	0	syn
V(III)	100.0	0.0	0.0	2.00	1.97	11	11	0	0	syn
Fe(III)	100.0	0.0	0.0	2.01	1.96	27	27	0	0	syn
Rh(III)	100.0	0.0	0.0	2.02	1.98	18	18	0	0	syn
Cu(II)	73.6	22.7	3.7	2.03	1.82	163	120	37	6	syn
Mg(II)	44.4	55.6	0.0	2.04	1.95	9	4	5	0	anti
Re(III)	100.0	0.0	0.0	2.04	2.02	10	10	0	0	syn
Ru(III)	90.9	0.0	9.1	2.04	1.95	11	10	0	1	syn
Zn(II)	70.1	22.9	7.5	2.05	1.88	67	47	15	5	syn
Fe(II)	33.3	66.7	0.0	2.06	1.87	9	3	6	0	anti
Co(II)	73.0	13.5	13.5	2.06	1.96	37	27	5	5	syn
Ni(II)	63.3	23.7	13.2	2.07	1.93	38	24	9	5	syn
Tc(V)	100.0	0.0	0.0	2.08	2.07	10	10	0	0	syn
Mo(IV)	100.0	0.0	0.0	2.09	2.06	16	16	0	0	syn
W(IV)	100.0	0.0	0.0	2.10	2.05	61	61	0	0	syn
W(II)	100.0	0.0	0.0	2.10	2.08	12	12	0	0	syn
Mo(V)	100.0	0.0	0.0	2.11	2.01	12	12	0	0	syn
Mn(II)	50.0	50.0	0.0	2.16	2.08	50	25	25	0	syn/anti
Nb(V)	84.6	0.0	15.4	2.18	2.13	13	11	0	2	syn
Sn(II)	100.0	0.0	0.0	2.19	2.12	17	17	0	0	syn
Sn(IV)	60.0	40.0	0.0	2.30	2.11	40	24	16	0	syn
Dy(III)	55.6	11.1	33.3	2.32	2.21	9	5	1	3	syn
Ag(I)	73.9	26.1	0.0	2.33	2.14	23	17	6	0	syn
Cd(II)	37.8	5.4	56.8	2.34	2.23	37	14	2	21	direct
Hg(II)	65.0	5.0	30.0	2.36	2.11	20	13	1	6	syn
Tl(III)	0.0	18.2	81.8	2.39	2.26	11	0	2	9	direct
Ca(II)	31.6	34.2	34.2	2.42	2.23	38	12	13	13	anti/direct
Sm(III)	36.7	23.3	40.0	2.44	2.26	30	11	7	12	direct
Na(I)	59.8	28.0	12.2	2.45	2.25	82	49	23	10	syn
Nd(III)	51.2	26.8	22.0	2.46	2.34	41	21	11	9	syn
U(VI)	15.6	6.7	77.8	2.48	2.31	45	7	3	35	direct
Ce(IV)	40.0	20.0	40.0	2.52	2.37	10	4	2	4	syn/direct
La(III)	57.1	14.3	28.6	2.52	2.41	21	12	3	6	syn
Sr(II)	60.3	3.4	36.2	2.69	2.43	58	35	2	21	syn
Ba(II)	64.3	21.4	14.3	2.75	2.61	14	9	3	2	syn
K(I)	68.8	26.9	4.3	2.86	2.46	208	143	56	9	syn
Rb(I)	55.6	33.3	11.1	2.98	2.82	9	5	3	1	syn
Cs(I)	38.9	44.4	16.7	3.24	2.77	18	7	8	3	anti

Table II. Maximum Observed Deviation of the Metal Cation from the Carboxylate Ion Plane

	general	transition metals	lanthanides and actinides
(a) in plane (approximately)	Li; Be; Mg; In(II,III); Sn(II); Sb(III,V); Tl(I,III); Pb(IV)	Sc; Ti(IV); V(II,III,IV); Cr(II,III); Mn(II,III); Fe(II,III); Co(II,III); Ni(II); Cu(I,II); Zn(II); Mo(II,III,IV); Tc(V); Ru(I,II,III); Rh(III); Pd(II); W(II,IV); Re(III); Os(III,IV,VI); Ir(III); Pt(II); Au(II,III)	Pr; Gd; Er; Yb(II); Np(VI)
(b) slightly out-of-plane up to $\sim 0.5$ Å		Nb; Mo(V)	La; Nd; Sm; Dy; Tm; Np(V)
(c) more out-of-plane up to $\sim 1.5$ Å	Ca; Sn(IV)	Cd(II)	Ce; U(VI); Am
(d) greatly out-of-plane over 1.5 Å	Na; K; Rb; Sr; Cs; Ba; Pb(II)	Ag(I); Hg(II)	

identities of the metal cations, their maximum deviations from the carboxylate plane and the pattern of metal ions around the carboxylate group, divided, for convenience, into 16 categories. Since samplings are small in many cases (see Table I), these lists and diagrams are used only to indicate a trend. Those metals with directed atomic orbitals have a greater tendency to lie in the plane of the carboxylate ion. The highly ionic metals are not so constrained to lie in this plane. This may possibly explain why alkali

metal citrates, for example, are easier to crystallize than higher valence metal citrates; the metal is more accommodating to the arrangement of citrate ions. The information presented in this article may provide an additional aid to those already in use<sup>18</sup> for

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## Metal ... carboxylate interactions

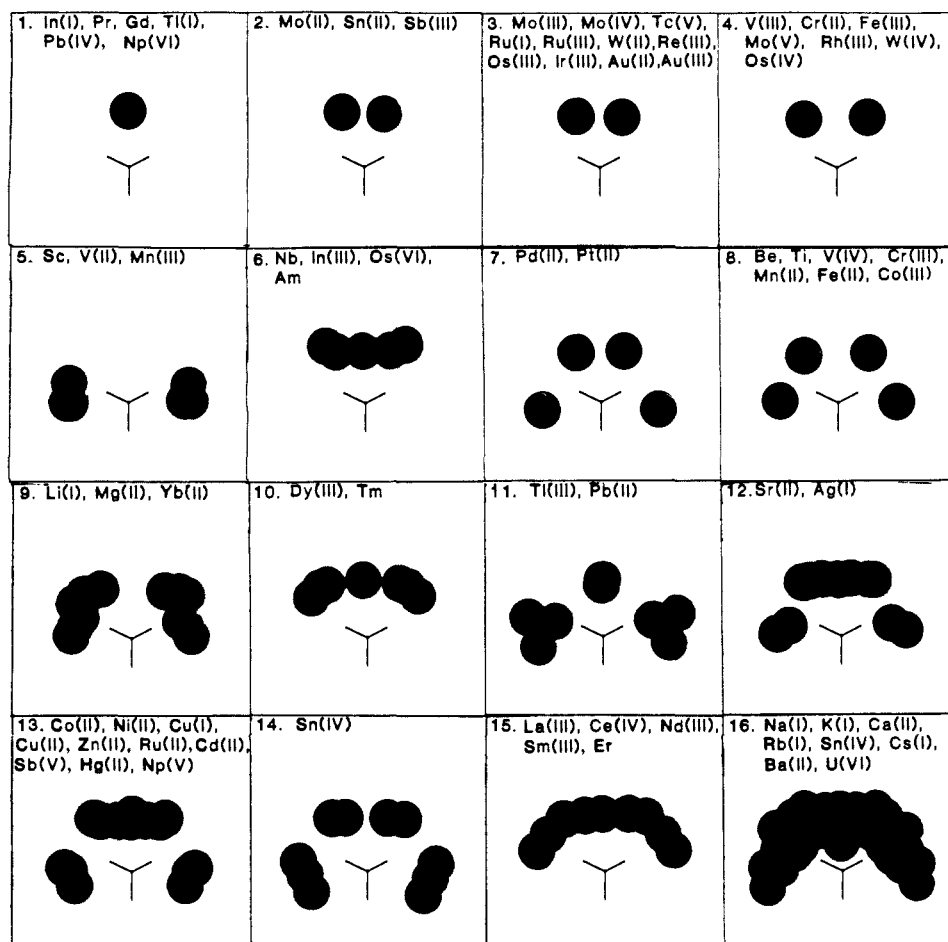


Figure 5. Chemical identities of metals interacting in one of 16 ways with a carboxylate group. View onto the plane of the carboxylate group.

locating metal ions in protein electron density maps.

Three main conclusions may be drawn from this study:

1. The metal cation generally lies in the plane of the carboxyl group. There are some notable exceptions. Alkali metals and some alkaline earth metals, which ionize readily and form strong bases, appear to have less specific locations of binding.

2. In an  $M^{n+}\cdots O$  range of 2.3 to 2.6 Å, it is common to have "direct bonding", such that the metal lies equidistant from the two oxygen atoms of the carboxylate group. At other  $M\cdots O$  distances this "direct bonding" is rare; it is a function of the carboxylate "bite" size (2.2 Å) and of a need to keep  $O\cdots M^{n+}\cdots O$  angles reasonably large (larger than approximately 60°). A listing of distances and angles for the examples of "direct bonding" found are given in deposited Table C.

3. The arrangement with the metal in the syn position is much more common than that for which it is anti. Exceptions are found at very short and very long  $M^{n+}\cdots O$  distances, that is, for very small and very large cations. The overall values are 62.9% syn, 22.7% anti, and 14.4% direct.

The information that there is a high probability that the metal lies in the carboxyl plane, but with possible deviations for specific metals as listed in Table II, provides a mechanism for searching for metal-binding positions in proteins that is complementary to

that suggested by Eisenberg.<sup>18</sup> We have used this in our study of the 1.9 Å resolution map of xylose isomerase from *Streptomyces rubiginosus*<sup>1,19</sup> to locate two metal sites. One metal binding site involves three carboxylates (aspartate and glutamate), histidine and water and the other involves four carboxylate groups and water.

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**Supplementary Material Available:** Table A, input to the computational search; Table B, listing of CSD entries (codes and their journal references) used; Table C, some data on direct bonding; Figure A, contoured scatterplots of metal ion locations viewed both on the plane of the carboxyl group and along this plane (125 pages). Ordering information is given on any current masthead page.

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