Structural Studies of Transition Metal Complexes of Triionized and Tetraionized Citrate. Models for the Coordination of the Citrate Ion to Transition Metal Ions in Solution and at the Active Site of Aconitase[1]

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Abstract: Single crystal x-ray structural characterizations of $[Fe(II)(H_2O)_6][Fe(II)C_6H_5O_7(H_2O)]_2{\cdot}2H_2O$ at room temperature and $\{[N(CH_3)_4]_5[Ni(II)_4(C_6H_4O_7)_3(OH)(H_2O)]{\cdot}18H_2O\}_2$ at −156 °C have been carried out to determine the effect of the ionization state of the citrate ion on its mode of coordination to transition metal ions. Triionized citrate forms a tridentate chelate with Fe(II) in which the protonated hydroxyl group, the central carboxyl group, and one terminal carboxyl group are coordinated to a single Fe(II) ion. Both oxygen atoms of the other terminal carboxyl group are coordinated to two other symmetry-related Fe(II) ions. Hexaquoiron(II) is the counterion. The structure is isomorphous with those determined previously for triionized citrate complexes of magnesium (C. K. Johnson) and manganese(II) (H. L. Carrell and J. P. Glusker). Crystal data: space group $P2_1/n$; $a = 20.400$ (3), $b = 6.710$ (1), $c = 9.173$ (2) Å; $\beta = 96.99$ (1)°; $Z = 2$; $R = 0.027$, $R_w = 0.037$. Tetraionized citrate forms a large anionic complex with Ni(II) ions, in which two nonbonding tetrahedra of Ni(II) ions are joined together through a center of symmetry by bridging citrate carboxyl groups. Two of the faces of the tetrahedra are capped by triply-bridging ionized citrate hydroxyl oxygen atoms and one by a triply-bridging hydroxide ion. The ionized hydroxyl oxygen atom of the third crystallographically independent citrate ion bridges two of the Ni(II) ions of the fourth face and is hydrogen bonded to a water molecule that is coordinated to the other Ni(II) ion of that face. The discrete complex is surrounded by tetramethylammonium ions and hydrogen bonded water molecules. Crystal data: space group $P\bar{1}$; $a = 11.836$ (7), $b = 14.292$ (6), $c = 20.925$ (16) Å; $\alpha = 96.16$ (5), $\beta = 106.36$ (5), $\gamma = 94.89$ (4)°; $Z = 1$; $R = 0.072$, $R_w = 0.078$. Since a significant fraction of the unit cell is occupied by water, the structure of the Ni(II)-tetraionized citrate complex serves as an excellent model for the structure of transition metal citrates in alkaline solution.

Aconitase [citrate (isocitrate) hydrolyase, EC 4.2.1.3] is the Krebs cycle enzyme which catalyzes the stereospecific interconversions of citrate, *cis*-aconitate, and 2*R*,3*S*-(+)-isocitrate. Numerous investigations have demonstrated that aconitase requires ferrous iron for activation of its catalytic activity. In addition, the presence of cysteine, or any of several organic reducing agents which have been tested, greatly increases the activation effect of the ferrous ions, whereas cysteine itself provides no activation in the absence of ferrous ions.[2-5] Activation of aconitase by many other metals has been attempted with no success.[2,4]

Villafranca and Mildvan[4,6] have detected the presence of a substrate-Fe(II)-enzyme ternary complex at the active site of aconitase by ^1H nuclear magnetic resonance studies of Fe(II)-activated aconitase in the presence of citrate in D_2O at pD 7.5. In addition, they have shown that Mn(II) serves as a competitive inhibitor of aconitase by the formation of a substrate-Mn(II)-enzyme ternary complex at the active site.

Although detailed crystallographic investigations have been carried out by Glusker and co-workers[7] on citric acid and three different ionization states of citrate with the utilization of various cations, the only transition metal complex of citrate that was investigated was a manganous complex of triionized citrate, $[Mn(II)(H_2O)_6][Mn(II)C_6H_5O_7(H_2O)]_2{\cdot}2H_2O$,[8] which was shown to be isomorphous with the magnesium salt of triionized citrate.[9] During the course of experiments in this laboratory with various ferrous citrate complexes, good crystalline samples of the complex of iron(II) with triionized citrate were obtained, and the determination of the structure was undertaken for comparison with the previously reported structures of triionized citrate complexes of divalent metal ions.

Titrations of equimolar solutions of transition metal ions and citric acid are characterized by the ionization of 4 equiv of acid,[10-14] which suggests that the coordination of the citrate ion to the transition metal substantially lowers the p*K* of the citrate hydroxyl group. The ionization of this group could significantly alter the metal-ligand interaction of the entire complex. In the case of the 1:1 ferrous and manganous citrate complexes, the tetraionized form of citrate predominates in neutral or alkaline solution.[13,14] Since the pH optimum for enzymatic activity of aconitase from various sources in a variety of buffers falls in the range of 7.2–8.6,[2] structural studies of transition metal citrates from alkaline solution should afford more information in terms of the mechanism of enzyme action. While Villafranca and Mildvan[6] have compared manganese-proton distances in the crystal structure of the manganous triionized citrate complex with corresponding distances that they obtained by paramagnetic ^1H NMR T_1 measurements of ternary aconitase-Mn(II)-citrate and aconitase-Fe(II)-citrate complexes in D_2O, the pD of the solutions used in the NMR study was 7.5.

The difficulties associated with the preparation of easily oxidized Fe(II) complexes are alleviated by the development of techniques with air stable analogues. While efforts to crystallize an iron(II) complex of tetraionized citrate have thus far been unsuccessful, a Ni(II) citrate complex has been crystallized from alkaline solution and its structure has been determined by x-ray crystallographic techniques.

Experimental Section

$[Fe(II)(H_2O)_6][Fe(II)C_6H_5O_7(H_2O)]_2{\cdot}2H_2O$. Preparation. An aqueous solution of 1 M ferrous citrate and 1 M trisodium citrate was continuously purged with nitrogen gas while it was electrolyzed to reduce trace quantities of Fe(III) to Fe(II). A voluminous white precipitate was obtained which was subsequently filtered, washed with water, and dried with acetone. A portion of the precipitate was dissolved in a minimum amount of dilute hydrochloric acid solution. Dilute sodium hydroxide solution was then added very slowly while nitrogen gas was passed over the top of the solution. Crystals formed after approximately 2 weeks.

X-Ray Data Collection. The crystals were found to be isomorphous with magnesium citrate decahydrate and manganous citrate decahydrate (monoclinic space group $P2_1/n$). The needle-like crystal,

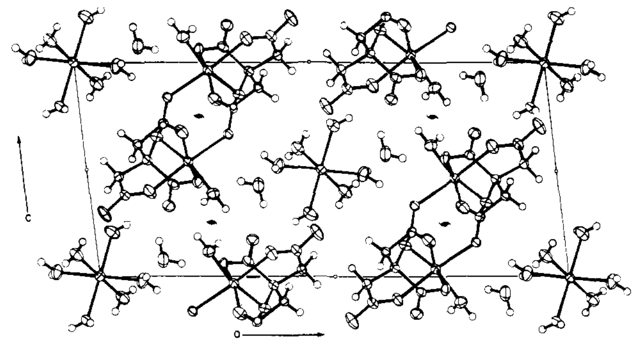


Figure 1. Packing diagram of the unit cell of $[Fe(II)(H_2O)_6][Fe(II)C_6H_5O_7(H_2O)]_2\cdot 2H_2O$ viewed down the *b* axis.

Table I. Final Positional Parameters for All Atoms of $[Fe(II)(H_2O)_6][Fe(II)C_6H_5O_7(H_2O)]_2\cdot 2H_2O$[a,b]

Atom	*x*	*y*	*z*
Fe(1)	0.000 00	0.000 00	0.000 00
Fe(2)	0.217 88 (2)	0.507 64 (6)	0.537 10 (4)
O(1)	0.188 92 (10)	−0.021 40 (29)	0.835 44 (21)
O(2)	0.210 77 (11)	−0.246 85 (34)	0.673 63 (23)
O(3)	0.026 10 (11)	0.494 70 (30)	0.292 83 (25)
O(4)	0.129 17 (10)	0.529 96 (32)	0.392 34 (24)
O(5)	0.233 38 (9)	0.222 67 (32)	0.444 93 (23)
O(6)	0.165 39 (11)	0.000 20 (30)	0.329 23 (23)
O(7)	0.157 99 (10)	0.303 35 (31)	0.649 55 (22)
O(8)	0.261 56 (14)	0.677 13 (41)	0.383 84 (26)
O(9)	0.038 66 (13)	−0.116 55 (41)	0.207 83 (25)
O(10)	0.089 09 (12)	0.158 87 (44)	−0.018 59 (29)
O(11)	−0.043 27 (14)	0.242 18 (37)	0.100 86 (30)
O(12)	0.139 29 (13)	0.552 35 (42)	0.075 26 (34)
C(1)	0.175 16 (13)	−0.113 20 (41)	0.714 32 (30)
C(2)	0.115 49 (15)	−0.039 14 (46)	0.616 50 (34)
C(3)	0.130 96 (13)	0.159 17 (41)	0.542 16 (29)
C(4)	0.066 86 (14)	0.250 90 (45)	0.466 49 (33)
C(5)	0.074 87 (14)	0.436 83 (43)	0.378 26 (30)
C(6)	0.180 26 (13)	0.123 12 (42)	0.429 91 (29)
H(1)	0.083 (2)	−0.015 (5)	0.670 (4)
H(2)	0.100 (2)	−0.125 (6)	0.550 (4)
H(3)	0.039 (1)	0.285 (5)	0.541 (3)
H(4)	0.040 (1)	0.147 (5)	0.404 (3)
H(5)	0.177 (2)	0.246 (6)	0.708 (4)
H(6)	0.283 (2)	0.630 (5)	0.316 (4)
H(7)	0.240 (2)	0.740 (6)	0.346 (4)
H(8)	0.030 (2)	−0.217 (7)	0.219 (4)
H(9)	0.071 (2)	−0.085 (7)	0.242 (5)
H(10)	0.119 (2)	0.099 (6)	−0.066 (4)
H(11)	0.099 (2)	0.259 (7)	−0.006 (5)
H(12)	−0.068 (2)	0.292 (5)	0.063 (4)
H(13)	−0.026 (2)	0.308 (6)	0.163 (4)
H(14)	0.135 (2)	0.566 (8)	0.163 (6)
H(15)	0.1.71 (2)	0.582 (7)	0.060 (5)

[a] In this and all subsequent tables, the estimated standard deviations of the last significant figures are given in parentheses. [b] Thermal parameters are available.[17]

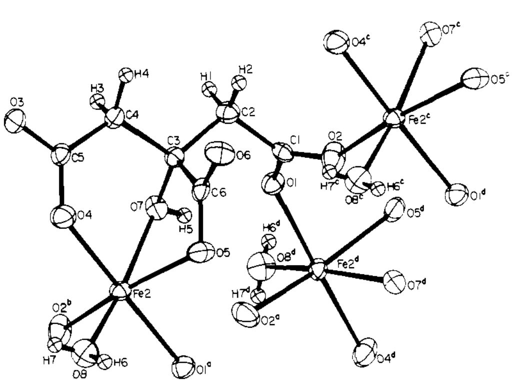


Figure 2. Coordination of triionized citrate to Fe(II) ions with the numbering scheme employed in the discussion of the structure. The superscripts refer to the following symmetry operations: a: $1/2 - x, 1/2 + y, 3/2 - z$; b: $x, 1 + y, z$; c: $x, y - 1, z$; d: $1/2 - x, y - 1/2, 3/2 - z$.

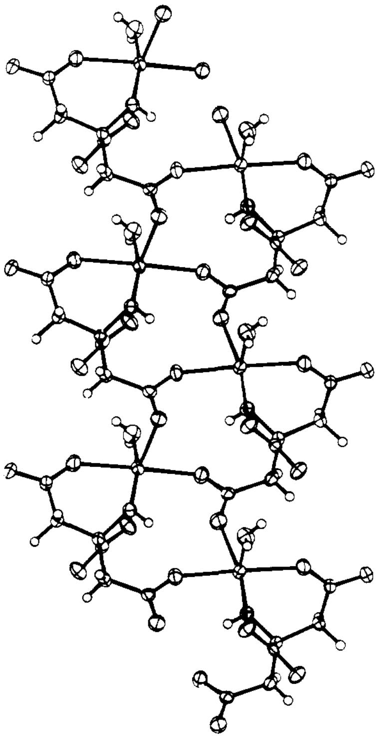


Figure 3. Infinite chain of $Fe(II)C_6H_5O_7(H_2O)$ along a 2_1 axis of the unit cell.

0.18 × 0.08 × 0.08 mm, was placed inside a capillary and mounted on a Syntex $P\bar{1}$ automatic diffractometer with monochromatized Mo Kα radiation (λ 0.710 69 Å). Automatic centering of 15 strong reflections yielded lattice parameters, $a = 20.400$ (3), $b = 6.710$ (1), $c = 9.173$ (2) Å, and $\beta = 96.99$ (1)°. The volume of the unit cell was 1246.4 (4) Å^3. The experimental density of 1.91 g/cm^3 measured by the flotation method is in good agreement with the calculated density of 1.908 g/cm^3 based on two formula units of $[Fe(II)(H_2O)_6][Fe(II)C_6H_5O_7(H_2O)]_2\cdot 2H_2O$ per unit cell.

Intensity data were collected with a θ–2θ scan technique from 0.8° below the Kα_1 peak to 0.8° above the Kα_2 peak. All independent reflections for which $2\theta \leq 50°$ were scanned at a rate of 2.0°/min. The ratio of the total background time to total peak scan time was 0.8.

The calculated transmission coefficients (based on a linear absorption coefficient of 18.7 cm^{-1} for Mo Kα radiation) fell within the range of 0.74 to 0.77 and were applied to the data during the course of the refinement. A total of 1710 reflections for which $I \geq 3\sigma(I)$ were used in the solution and refinement of the structure.[15]

Solution and Refinement of the Structure. The positions of the two crystallographically independent iron(II) ions were obtained from a three-dimensional Patterson map. Fourier syntheses in conjunction with least-squares refinements yielded the positions of all atoms of the unit cell. Absorption corrections were applied to the individual structure factors. A series of least-squares refinements of positional parameters of all atoms, anisotropic thermal parameters of nonhydrogen atoms, and isotropic thermal parameters of hydrogen atoms converged to $R = 0.027$ and $R_w = 0.037$.[15] The final cycles of this series included real and imaginary corrections to the scattering factors of iron due to anomalous dispersion.[16] A final difference Fourier map revealed no peaks larger than 0.3 e/Å^3. The final standard deviation of an observation of unit weight was 1.41.

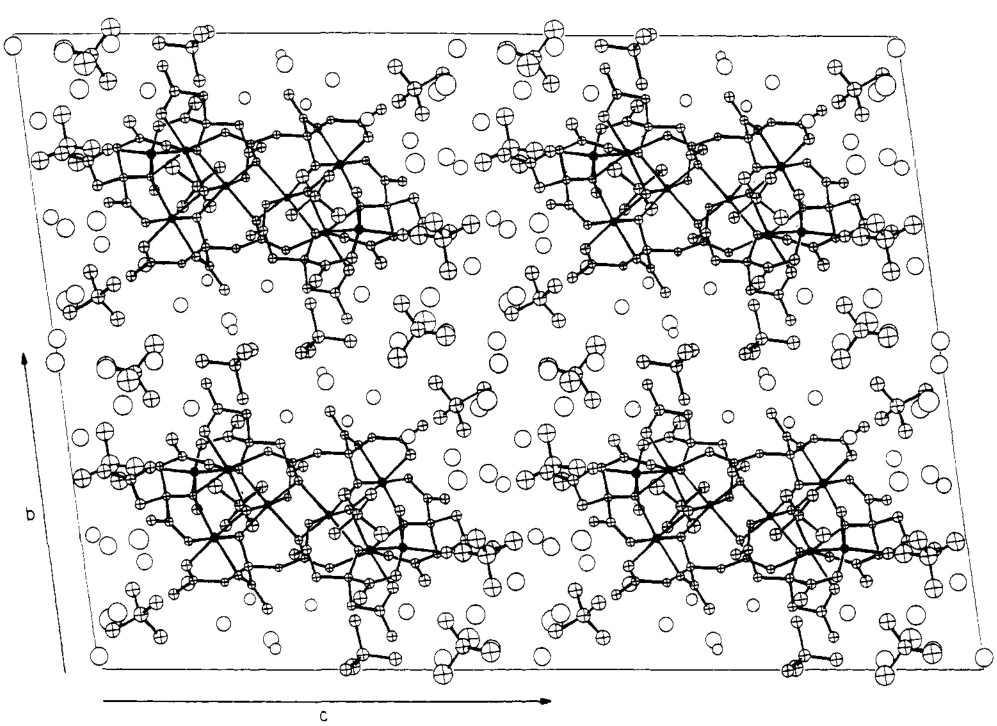


Figure 4. Packing diagram of $\{[N(CH_3)_4]_5[Ni(II)_4(C_6H_4O_7)_3(OH)(H_2O)]\cdot 18H_2O\}_2$ viewed down the *a* axis. The open circles represent the oxygen atoms of the noncoordinated water molecules.

The positional parameters, as determined in the final least-squares cycle, are given in Table I. Bond distances and bond angles with estimated standard deviations calculated from the full inverse matrix are presented in Table II. A listing of observed structure factor amplitudes from the data obtained and calculated structure factor amplitudes based on the parameters of the final least-squares refinement and a table of thermal parameters are available.[17]

$\{[N(CH_3)_4]_5[Ni(II)_4(C_6H_4O_7)_3(OH)(H_2O)]\cdot 18H_2O\}_2$. Preparation. Single crystals of this nickelous citrate complex were obtained from a highly concentrated aqueous solution made by the following pro-

Table II. Interatomic Distances (Å) and Angles (deg) for $[Fe(II)(H_2O)_6][Fe(II)C_6H_5O_7(H_2O)]_2\cdot 2H_2O$[a]

Distances					
C(1)-C(2)	1.506 (4)	C(5)-O(3)	1.251 (3)	Fe(1)-O(11)	2.115 (3)
C(4)-C(5)	1.507 (4)	C(5)-O(4)	1.265 (4)	Fe(2)-O(1)^{a}	2.116 (2)
C(3)-C(6)	1.544 (4)	C(6)-O(5)	1.266 (3)	Fe(2)-O(2)^{b}	2.085 (2)
C(2)-C(3)	1.545 (4)	C(6)-O(6)	1.248 (3)	Fe(2)-O(4)	2.116 (2)
C(3)-C(4)	1.533 (4)	C(3)-O(7)	1.442 (3)	Fe(2)-O(5)	2.130 (2)
C(1)-O(1)	1.272 (3)	Fe(1)-O(9)	2.122 (2)	Fe(2)-O(7)	2.178 (2)
C(1)-O(2)	1.240 (3)	Fe(1)-O(10)	2.132 (3)	Fe(2)-O(8)	2.090 (2)
Angles					
O(1)-C(1)-O(2)	122.6 (3)	C(1)-C(2)-C(3)	110.7 (2)	O(1)^{a}-Fe(2)-O(7)	101.4 (1)
O(3)-C(5)-O(4)	122.0 (3)	C(3)-C(4)-C(5)	115.7 (2)	O(1)^{a}-Fe(2)-O(8)	89.6 (1)
O(5)-C(6)-O(6)	123.9 (3)	C(2)-C(3)-C(6)	110.1 (2)	O(1)^{a}-Fe(2)-O(5)	88.3 (1)
O(1)-C(1)-C(2)	115.9 (3)	C(4)-C(3)-C(6)	110.5 (2)	O(2)^{b}-Fe(2)-O(4)	101.8 (1)
O(2)-C(1)-C(2)	121.3 (3)	C(2)-C(3)-C(4)	109.7 (2)	O(2)^{b}-Fe(2)-O(8)	92.3 (1)
O(3)-C(5)-C(4)	117.6 (3)	O(9)-Fe(1)-O(10)	91.7 (1)	O(2)^{b}-Fe(2)-O(7)	97.4 (1)
O(4)-C(5)-C(4)	120.3 (2)	O(9)-Fe(1)-O(11)	91.1 (1)	O(4)-Fe(2)-O(5)	88.4 (1)
O(5)-C(6)-C(3)	117.6 (2)	O(10)-Fe(1)-O(11)	93.2 (1)	O(4)-Fe(2)-O(7)	81.7 (1)
O(6)-C(6)-C(3)	118.5 (2)	O(1)^{a}-Fe(2)-O(4)	174.6 (1)	O(4)-Fe(2)-O(8)	86.6 (1)
O(7)-C(3)-C(2)	111.0 (2)	O(2)^{b}-Fe(2)-O(5)	166.5 (1)	O(5)-Fe(2)-O(7)	75.0 (1)
O(7)-C(3)-C(4)	105.9 (2)	O(7)-Fe(2)-O(8)	166.1 (1)	O(5)-Fe(2)-O(8)	97.2 (1)
O(7)-C(3)-C(6)	109.6 (2)	O(1)^{a}-Fe(2)-O(2)^{b}	82.2 (1)		
Hydrogen Bond Oxygen-Oxygen Distances					
O(7)-O(1)	2.791 (3)	O(9)-O(3)^{d}	2.743 (3)	O(11)-O(12)^{f}	2.753 (4)
O(7)-O(2)^{a}	2.976 (3)	O(9)-O(6)	2.799 (4)	O(11)-O(3)	2.714 (4)
O(8)-O(6)^{c}	2.856 (4)	O(10)-O(1)^{e}	2.840 (3)	O(12)-O(4)	2.945 (4)
O(8)-O(6)^{b}	2.926 (4)	O(10)-O(12)	2.923 (4)	O(12)-O(5)^{c}	2.865 (4)

[a] The superscripts refer to the following symmetry operations: a: $1/2 - x, 1/2 + y, 3/2 - z$; b: $x, y + 1, z$; c: $1/2 - x, 1/2 + y, 1/2 - z$; d: $x, y - 1, z$; e: $x, y, z - 1$; f: $-x, 1 - y, -z$.

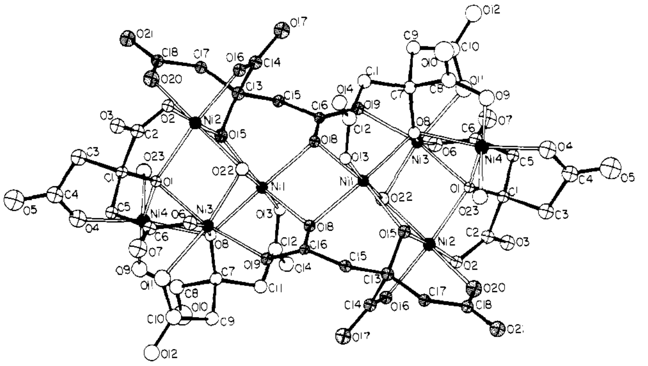


Figure 5. Discrete anionic complex of Ni(II) and citrate(−4) ions with the numbering scheme employed in the discussion of the structure. A center of symmetry is located in the center of the complex.

cedure. Nickel(II) hydroxide was prepared by the addition of excess sodium hydroxide to a solution of 0.005 mol of $NiSO_4$ in water. The precipitate was filtered and washed three times and then dissolved in a solution that contained 0.005 mol of citric acid. Two equivalents (0.010 mol) of tetramethylammonium hydroxide were titrated into the solution. At a concentration of approximately 0.5 M, the pH of the resulting solution was 9.20. The solution was concentrated slowly by evaporation for several days. The crystals that formed were very soft plates with reflective surfaces. However, removal of a crystal from the mother liquor caused it to shatter almost immediately. A dried crystal of this kind resulted in a highly mosaic diffraction pattern. Consequently, a bright green crystal 0.15 × 0.13 × 0.05 mm was drawn into a glass capillary. A small amount of mother liquor was retained in the capillary in close proximity to the crystal.

X-Ray Data Collection. The crystal was mounted on a Syntex $P\bar{1}$ automatic diffractometer and cooled to −156 °C with a locally constructed low-temperature device.[18] Automatic centering of 15 strong reflections with monochromatized Mo $K\alpha$ radiation (λ 0.710 69 Å) yielded lattice parameters for the triclinic crystal of: a = 11.836 (7), b = 14.292 (6), c = 20.925 (16) Å, α = 96.16 (5), β = 106.36 (5), and γ = 94.89 (4)°. The volume of the unit cell was 3352 (4) $Å^3$.

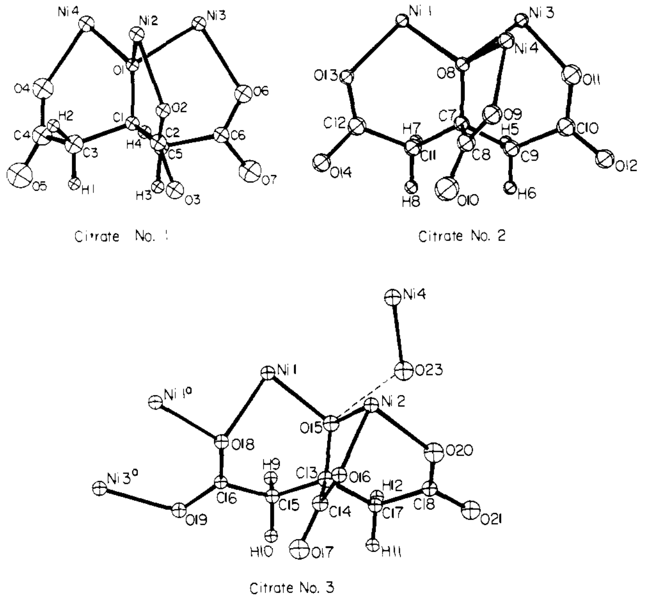
Intensity data were collected with the employment of a θ–2θ scan technique from 0.9° below the $K\alpha_1$ peak to 0.9° above the $K\alpha_2$ peak at a scan rate of 1.85°/min. The total background count time was equal to the total peak scan time. A first set of data was collected in which all independent reflections with $2\theta \leq 40°$ were measured. Four observed reflections were inadvertently omitted from this set during data processing. A second shell of data was then collected for which $40° < 2\theta \leq 45°$. All reflections with $h \leq 7$ within this shell were measured. During the course of measurement of reflections within this set for which $h = 8$, the crystal became detached from the capillary and data collection was terminated. Three intense reflections were monitored after every 97 measured reflections throughout the data collection. No significant fluctuations were observed. The combined data sets yielded 3733 observed reflections for which $I \geq 3\sigma(I)$.

Solution and Refinement of the Structure. A three-dimensional Patterson map was fitted by a model of two tetrahedra of Ni(II) ions related by a center of symmetry, consistent with assignment of $P\bar{1}$ as the space group. A series of Fourier syntheses, phased initially on these heavy atoms, then on more atoms as they were found, yielded the positions of the nonhydrogen atoms of three citrate ions, a water molecule within the coordination sphere of one of the Ni(II) ions, a triply bridging hydroxide ion, and five tetramethylammonium ions within the asymmetric unit.

Disorder among a significant portion of the water molecules and two of the five tetramethylammonium ions was evidenced by large isotropic temperature factors obtained in the least-squares refinements and by several very small interatomic distances which indicated mutually exclusive sets of atoms. Subsequently, idealized coordinates were calculated for the tetrahedral tetramethylammonium ions in which a nitrogen–carbon bond distance of 1.50 Å was utilized. Two independent one-half weighted rigid bodies were substituted for each of the two disordered tetramethylammonium groups. Refinement of the multipliers of the disordered water molecules yielded a complex model for the disorder which involved one-third, one-half, and two-thirds occupancies of several water positions. As a check of this complex model, a set of three least-squares refinements were performed in which the multipliers and/or isotropic temperature factors of the disordered water molecules were varied. A summary of the relevant parameters for these three cycles is available.[17]

The final cycles of the refinement included real and imaginary corrections to the scattering factors of the nickel ions due to anomalous dispersion.[19] Additionally, calculated positions for the methylene protons of the three citrate ions were included but were not varied. The final least-squares cycle converged to R = 0.072 and R_w = 0.078. The standard deviation of an observation of unit weight for this cycle was 1.83.

A final difference Fourier map revealed no peaks larger than 0.7 e/$Å^3$. Of those peaks which were present, it was possible to locate all but one of the methyl protons of the three tetramethylammonium groups which did not exhibit disorder. In addition, the hydrogens of the triply-bridging hydroxide ion and of the coordinated water molecule were located in this map. Because of the extensive disorder of the water molecules, no attempt was made to locate the noncoordinated water hydrogen atoms in this map.



Citrate No. 1

Citrate No. 2

Citrate No. 3

Figure 7. Configurations and conformations of the crystallographically independent citrate ions of the Ni(II)-citrate(−4) complex.

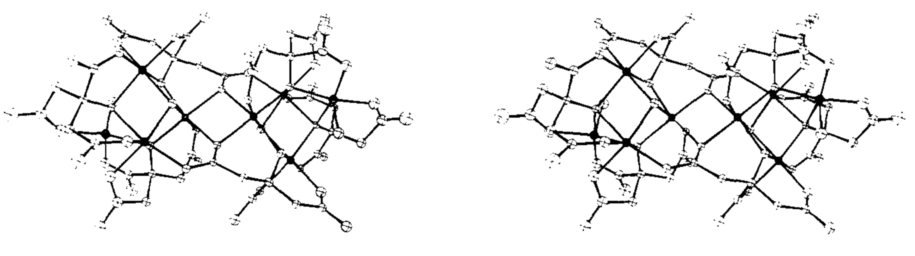


Figure 6. Three-dimensional view of the discrete anionic complex of Ni(II) and citrate(−4) ions.

Table III. Final Positional and Isotropic Thermal Parameters of $\{[N(CH_3)_4]_5[Ni(II)_4(C_6H_4O_7)_3(OH)(H_2O)]\cdot 18H_2O\}_2$

Atom	x	y	z	B
Ni(1)	0.4733 (2)	0.4831 (1)	0.0677 (1)	0.93 (5)
Ni(2)	0.6415 (2)	0.5873 (1)	0.1988 (1)	1.08 (5)
Ni(3)	0.6454 (2)	0.3714 (1)	0.1468 (1)	0.98 (5)
Ni(4)	0.4850 (2)	0.3813 (1)	0.2240 (1)	1.30 (5)
O(1)	0.6421 (8)	0.4547 (6)	0.2339 (5)	0.85 (21)
O(2)	0.8091 (8)	0.5999 (7)	0.2586 (5)	1.05 (21)
O(3)	0.9372 (9)	0.5348 (7)	0.3351 (5)	1.55 (22)
O(4)	0.5221 (10)	0.3661 (7)	0.3207 (6)	2.21 (25)
O(5)	0.6354 (10)	0.3795 (8)	0.4264 (6)	3.26 (28)
O(6)	0.8184 (9)	0.3548 (7)	0.1885 (5)	1.55 (23)
O(7)	0.9318 (10)	0.2778 (8)	0.2638 (5)	2.42 (25)
O(8)	0.4586 (8)	0.3709 (7)	0.1221 (5)	1.01 (21)
O(9)	0.3399 (9)	0.2927 (7)	0.1994 (5)	1.81 (23)
O(10)	0.1954 (10)	0.2203 (8)	0.1107 (6)	2.94 (27)
O(11)	0.6021 (9)	0.2530 (7)	0.1889 (5)	1.90 (24)
O(12)	0.5345 (9)	0.1000 (7)	0.1715 (5)	1.78 (23)
O(13)	0.2975 (8)	0.4572 (7)	0.0198 (5)	1.00 (21)
O(14)	0.1288 (9)	0.3595 (7)	−0.0252 (5)	1.58 (22)
O(15)	0.4680 (9)	0.5831 (7)	0.1424 (5)	1.24 (22)
O(16)	0.6686 (9)	0.7051 (7)	0.1577 (5)	1.15 (21)
O(17)	0.5631 (9)	0.8094 (7)	0.1025 (5)	1.83 (23)
O(18)	0.4775 (8)	0.5932 (7)	0.0091 (5)	0.97 (21)
O(19)	0.3538 (8)	0.6844 (7)	−0.0510 (5)	1.05 (21)
O(20)	0.5858 (9)	0.6746 (7)	0.2707 (5)	1.88 (24)
O(21)	0.4687 (9)	0.7650 (7)	0.3080 (5)	1.72 (23)
O(22)	0.6527 (9)	0.5002 (7)	0.1179 (5)	1.32 (22)
O(23)	0.3932 (9)	0.4967 (7)	0.2297 (5)	1.77 (23)
C(1)	0.7388 (13)	0.4571 (10)	0.2943 (7)	1.00 (32)
C(2)	0.8342 (14)	0.5372 (11)	0.2956 (8)	1.30 (33)
C(3)	0.6965 (14)	0.4820 (11)	0.3558 (8)	1.75 (36)
C(4)	0.6100 (15)	0.4036 (12)	0.3689 (9)	1.95 (37)
C(5)	0.7899 (13)	0.3622 (10)	0.2996 (7)	1.13 (33)
C(6)	0.8481 (14)	0.3319 (11)	0.2464 (8)	1.23 (33)
C(7)	0.3781 (14)	0.2859 (10)	0.0917 (8)	1.14 (32)
C(8)	0.2991 (15)	0.2676 (11)	0.1374 (8)	1.58 (34)
C(9)	0.4422 (14)	0.1963 (11)	0.0887 (8)	1.32 (33)
C(10)	0.5316 (14)	0.1810 (12)	0.1527 (8)	1.69 (35)
C(11)	0.3062 (14)	0.2895 (11)	0.0194 (8)	1.34 (34)
C(12)	0.2414 (15)	0.3762 (11)	0.0053 (8)	1.61 (35)
C(13)	0.4530 (13)	0.6768 (11)	0.1323 (8)	1.19 (33)
C(14)	0.5710 (14)	0.7349 (11)	0.1294 (8)	1.18 (33)
C(15)	0.3600 (13)	0.6806 (10)	0.0631 (7)	0.97 (31)
C(16)	0.3971 (13)	0.6512 (10)	0.0026 (7)	0.82 (31)
C(17)	0.4191 (13)	0.7277 (10)	0.1901 (7)	0.99 (32)
C(18)	0.4972 (14)	0.7223 (10)	0.2601 (8)	1.13 (33)
N(1)	0.0289 (12)	0.4853 (9)	0.1325 (7)	1.93 (29)
Me(11)	0.1173 (16)	0.5480 (12)	0.1100 (9)	2.85 (41)
Me(12)	0.0975 (17)	0.4240 (13)	0.1817 (10)	3.73 (46)
Me(13)	−0.0608 (15)	0.4249 (11)	0.0745 (8)	2.13 (37)
Me(14)	−0.0339 (15)	0.5496 (12)	0.1701 (8)	2.12 (38)
N(2)	0.7977 (12)	0.0434 (10)	0.1004 (7)	2.45 (31)
Me(21)	0.6767 (15)	−0.0052 (12)	0.0582 (9)	2.46 (39)
Me(22)	0.8019 (15)	0.1487 (12)	0.0931 (8)	2.24 (38)
Me(23)	0.8132 (16)	0.0298 (12)	0.1732 (9)	2.73 (40)
Me(24)	0.8945 (17)	0.0022 (14)	0.0751 (10)	3.85 (47)
N(3)	0.4714 (14)	0.0685 (11)	0.3293 (8)	3.79 (37)
Me(31)	0.4347 (21)	0.0495 (17)	0.3895 (12)	6.34 (63)
Me(32)	0.4170 (19)	0.1502 (15)	0.2991 (10)	4.51 (50)
Me(33)	0.4320 (21)	−0.0184 (17)	0.2759 (12)	6.33 (63)
Me(34)	0.6051 (25)	0.0925 (20)	0.3447 (14)	8.48 (78)
W(1)	0.4007 (9)	0.9363 (7)	0.0967 (5)	1.75 (23)
W(2)	0.8740 (9)	0.7804 (7)	0.1388 (5)	2.05 (24)
W(3)	0.0028 (10)	0.2026 (8)	−0.0011 (5)	2.45 (25)
W(4)	0.1697 (11)	0.9072 (9)	0.1024 (6)	4.16 (31)
W(5)	0.0869 (11)	0.1791 (9)	0.2115 (6)	3.92 (30)
W(6)	−0.0095 (11)	0.5818 (8)	0.4706 (6)	3.54 (29)
W(7)	0.2715 (12)	0.0372 (10)	0.5042 (7)	5.17 (35)
W(8)	0.1007 (10)	0.8572 (8)	0.2058 (6)	3.37 (28)
W(9)	0.8286 (12)	0.2742 (9)	0.4693 (7)	4.84 (34)
W(10)	0.1271 (12)	0.0306 (10)	0.2807 (7)	5.25 (35)
W(11)	0.9061 (13)	0.1715 (10)	0.3653 (7)	6.07 (38)

Table III (*Continued*)

Atom	*x*	*y*	*z*	*B*
W(12)	0.2798 (14)	0.8475 (11)	0.4602 (8)	7.37 (44)
W(13A)	0.1257 (34)	0.6657 (28)	0.3646 (19)	4.02 (91)
W(13B)	0.1644 (17)	0.6064 (14)	0.3340 (10)	4.22 (47)
W(14A)	0.4756 (33)	0.5943 (23)	0.3692 (17)	6.37 (89)
W(14B)	0.4027 (32)	0.5859 (23)	0.3520 (17)	6.53 (89)
W(15A)	0.2167 (32)	0.7326 (26)	0.2732 (19)	3.36 (85)
W(15B)	0.2480 (15)	0.7925 (12)	0.3182 (9)	2.46 (38)
W(16A)	0.3244 (36)	0.6159 (29)	0.4415 (21)	5.10 (104)
W(16B)	0.5421 (22)	0.2511 (17)	0.4822 (13)	3.92 (60)
W(17A)	0.5160 (30)	0.8250 (22)	0.4410 (16)	5.97 (81)
W(17B)	0.5936 (26)	0.8138 (19)	0.4394 (14)	4.30 (67)
W(18A)	0.0717 (27)	0.0579 (22)	0.3910 (15)	5.88 (76)
W(18B)	0.1436 (24)	0.1212 (20)	0.4058 (13)	4.45 (65)
H(1)	0.7659	0.4954	0.3969	0.80
H(2)	0.6533	0.5393	0.3502	0.80
H(3)	0.8518	0.3659	0.3440	0.80
H(4)	0.7250	0.3106	0.2959	0.80
H(5)	0.4824	0.2017	0.0529	0.80
H(6)	0.3782	0.1414	0.0739	0.80
H(7)	0.3594	0.2877	−0.0102	0.80
H(8)	0.2431	0.2340	0.0037	0.80
H(9)	0.2864	0.6379	0.0590	0.80
H(10)	0.3407	0.7471	0.0602	0.80
H(11)	0.4204	0.7969	0.1861	0.80
H(12)	0.3377	0.7011	0.1892	0.80
		Disordered Tetramethylammonium Groups[a]		
Group 1A				3.4
N	0.8928	0.8317	0.3896	
Me(1)	0.9702	0.7921	0.3503	
Me(2)	0.9687	0.8806	0.4570	
Me(3)	0.8223	0.9015	0.3521	
Me(4)	0.8098	0.7528	0.3992	
Group 1B				5.9
N	0.8849	0.8439	0.3595	
Me(1)	0.9511	0.8057	0.3129	
Me(2)	0.9629	0.9224	0.4101	
Me(3)	0.7759	0.8814	0.3198	
Me(4)	0.8494	0.7662	0.3953	
Group 2A				5.4
N	0.2404	0.3583	0.4107	
Me(1)	0.3236	0.4032	0.4773	
Me(2)	0.2058	0.2558	0.4137	
Me(3)	0.1315	0.4083	0.3947	
Me(4)	0.3006	0.3659	0.3569	
Group 2B				6.1
N	0.2828	0.3904	0.4169	
Me(1)	0.3502	0.3166	0.4512	
Me(2)	0.3617	0.4828	0.4322	
Me(3)	0.2422	0.3597	0.3424	
Me(4)	0.1771	0.4026	0.4416	
		Hydroxide and Coordinated Water Hydrogen Atoms[b]		
OH	0.6936	0.5035	0.0822	
HOH(1)	0.4306	0.5496	0.1973	
HOH(2)	0.4496	0.5311	0.2862	

Multipliers of Disordered Water Oxygen Atoms

Atom	Multiplier	Atom	Multiplier	Atom	Multiplier	Atom	Multiplier
W(13A)	0.33	W(14B)	0.50	W(16A)	0.33	W(17B)	0.50
W(13B)	0.67	W(15A)	0.50	W(16B)	0.50	W(18A)	0.50
W(14A)	0.50	W(15B)	0.67	W(17A)	0.50	W(18B)	0.50

[a] Each of the two tetramethylammonium groups that exhibited disorder was refined as two independent rigid bodies with multipliers of one-half. [b] The positions of these atoms were obtained from the final difference map and were not used for the structure factor calculation.

Table IV. Interatomic Distances (Å) and Angles (deg) for $\{[N(CH_3)_4]_5[Ni(II)_4(C_6H_4O_7)_3(OH)(H_2O)]\cdot 18H_2O\}_2$

Distances

Citrate No. 1		Citrate No. 2		Citrate No. 3	
C(1)-O(1)	1.44 (2)	C(7)-O(8)	1.44 (2)	C(13)-O(15)	1.40 (2)
C(2)-O(2)	1.25 (2)	C(8)-O(9)	1.25 (2)	C(14)-O(16)	1.27 (2)
C(2)-O(3)	1.27 (2)	C(8)-O(10)	1.29 (2)	C(14)-O(17)	1.26 (2)
C(4)-O(4)	1.26 (2)	C(10)-O(11)	1.29 (2)	C(16)-O(18)	1.30 (2)
C(4)-O(5)	1.25 (2)	C(10)-O(12)	1.26 (2)	C(16)-O(19)	1.26 (2)
C(6)-O(6)	1.25 (2)	C(12)-O(13)	1.25 (2)	C(18)-O(20)	1.28 (2)
C(6)-O(7)	1.30 (2)	C(12)-O(14)	1.29 (2)	C(18)-O(21)	1.26 (2)
C(1)-C(2)	1.53 (2)	C(7)-C(8)	1.54 (2)	C(13)-C(14)	1.58 (2)
C(1)-C(3)	1.53 (2)	C(7)-C(9)	1.55 (2)	C(13)-C(15)	1.56 (2)
C(1)-C(5)	1.54 (2)	C(7)-C(11)	1.52 (2)	C(13)-C(17)	1.51 (2)
C(3)-C(4)	1.55 (2)	C(9)-C(10)	1.51 (2)	C(15)-C(16)	1.49 (2)
C(5)-C(6)	1.51 (2)	C(11)-C(12)	1.52 (2)	C(17)-C(18)	1.51 (2)
		Nickel(II) Ions			
Ni(1)-O(8)	2.09 (1)	Ni(2)-O(20)	2.13 (1)	Ni(4)-O(8)	2.05 (1)
Ni(1)-O(13)	2.02 (1)	Ni(2)-O(22)	2.03 (1)	Ni(4)-O(9)	1.95 (1)
Ni(1)-O(15)	2.02 (1)	Ni(3)-O(1)	2.08 (1)	Ni(4)-O(23)	2.06 (1)
Ni(1)-O(18)	2.10 (1)	Ni(3)-O(6)	2.03 (1)	Ni(1)-Ni(1)[a]	3.148 (5)
Ni(1)-O(18)[a]	2.09 (1)	Ni(3)-O(8)	2.12 (1)	Ni(1)-Ni(2)	3.041 (4)
Ni(1)-O(22)	2.07 (1)	Ni(3)-O(11)	2.08 (1)	Ni(1)-Ni(3)	2.944 (3)
Ni(2)-O(1)	2.11 (1)	Ni(3)-O(19)[a]	2.08 (1)	Ni(1)-Ni(4)	3.693 (4)
Ni(2)-O(2)	2.01 (1)	Ni(3)-O(22)	2.00 (1)	Ni(2)-Ni(3)	3.169 (3)
Ni(2)-O(15)	2.05 (1)	Ni(4)-O(1)	2.00 (1)	Ni(2)-Ni(4)	3.513 (3)
Ni(2)-O(16)	2.01 (1)	Ni(4)-O(4)	1.99 (1)	Ni(3)-Ni(4)	2.821 (3)
		Tetramethylammonium Ions			
N(1)-Me(11)	1.53 (2)	N(2)-Me(21)	1.52 (2)	N(3)-Me(31)	1.49 (3)
N(1)-Me(12)	1.53 (2)	N(2)-Me(22)	1.53 (2)	N(3)-Me(32)	1.50 (2)
N(1)-Me(13)	1.50 (2)	N(2)-Me(23)	1.52 (2)	N(3)-Me(33)	1.52 (3)
N(1)-Me(14)	1.52 (2)	N(2)-Me(24)	1.53 (2)	N(3)-Me(34)	1.52 (3)
		Hydroxide Group and Coordinated Water Molecule			
O(22)-OH	1.00	O(23)-HOH(1)	1.20	O(23)-HOH(2)	1.21

Angles

Citrate No. 1		Citrate No. 2		Citrate No. 3	
O(1)-C(1)-C(2)	108 (1)	O(8)-C(7)-C(8)	108 (1)	O(15)-C(13)-C(14)	112 (1)
O(1)-C(1)-C(3)	110 (1)	O(8)-C(7)-C(9)	113 (1)	O(15)-C(13)-C(15)	111 (1)
O(1)-C(1)-C(5)	113 (1)	O(8)-C(7)-C(11)	114 (1)	O(15)-C(13)-C(17)	110 (1)
O(2)-C(2)-O(3)	123 (1)	O(9)-C(8)-O(10)	122 (2)	O(16)-C(14)-O(17)	124 (1)
O(2)-C(2)-C(1)	120 (1)	O(9)-C(8)-C(7)	119 (1)	O(16)-C(14)-C(13)	117 (1)
O(3)-C(2)-C(1)	117 (1)	O(10)-C(8)-C(7)	118 (1)	O(17)-C(14)-C(13)	118 (1)
O(4)-C(4)-O(5)	124 (2)	O(11)-C(10)-O(12)	121 (1)	O(18)-C(16)-O(19)	123 (1)
O(4)-C(4)-C(3)	118 (1)	O(11)-C(10)-C(9)	118 (1)	O(18)-C(16)-C(15)	117 (1)
O(5)-C(4)-C(3)	117 (2)	O(12)-C(10)-C(9)	121 (1)	O(19)-C(16)-C(15)	120 (1)
O(6)-C(6)-O(7)	121 (1)	O(13)-C(12)-O(14)	123 (1)	O(20)-C(18)-O(21)	121 (1)
O(6)-C(6)-C(5)	123 (1)	O(13)-C(12)-C(11)	120 (1)	O(20)-C(18)-C(17)	122 (1)
O(7)-C(6)-C(5)	116 (1)	O(14)-C(12)-C(11)	116 (1)	O(21)-C(18)-C(17)	117 (1)
C(1)-C(3)-C(4)	115 (1)	C(7)-C(9)-C(10)	117 (1)	C(13)-C(15)-C(16)	116 (1)
C(1)-C(5)-C(6)	115 (1)	C(7)-C(11)-C(12)	117 (1)	C(13)-C(17)-C(18)	117 (1)
C(2)-C(1)-C(3)	107 (1)	C(8)-C(7)-C(9)	105 (1)	C(14)-C(13)-C(15)	105 (1)
C(2)-C(1)-C(5)	111 (1)	C(8)-C(7)-C(11)	112 (1)	C(14)-C(13)-C(17)	106 (1)
C(3)-C(1)-C(5)	108 (1)	C(9)-C(7)-C(11)	105 (1)	C(15)-C(13)-C(17)	112 (1)
		Nickel(II) Ions			
O(8)-Ni(1)-O(13)	89.8 (4)	O(15)-Ni(2)-O(16)	84.9 (4)	O(1)-N(14)-O(9)	167.8 (4)
O(8)-Ni(1)-O(15)	94.1 (4)	O(15)-Ni(2)-O(20)	83.0 (4)	O(1)-Ni(4)-O(23)	96.7 (4)
O(8)-Ni(1)-O(18)	176.5 (4)	O(15)-Ni(2)-O(22)	83.4 (4)	O(4)-Ni(4)-O(8)	168.5 (4)
O(8)-Ni(1)-O(18)[a]	96.9 (4)	O(16)-Ni(2)-O(20)	87.3 (4)	O(4)-Ni(4)-O(9)	93.0 (5)
O(8)-Ni(1)-O(22)	86.7 (4)	O(16)-Ni(2)-O(22)	92.9 (4)	O(4)-Ni(4)-O(23)	96.0 (4)
O(13)-Ni(1)-O(15)	98.5 (4)	O(20)-Ni(2)-O(22)	166.3 (4)	O(8)-Ni(4)-O(9)	84.3 (4)
O(13)-Ni(1)-O(18)	86.7 (4)	O(1)-Ni(3)-O(6)	91.2 (4)	O(8)-Ni(4)-O(23)	95.3 (4)
O(13)-Ni(1)-O(18)[a]	94.5 (4)	O(1)-Ni(3)-O(8)	82.8 (4)	O(9)-Ni(4)-O(23)	92.2 (4)
O(13)-Ni(1)-O(22)	176.2 (4)	O(1)-Ni(3)-O(11)	88.1 (4)	Ni(1)[a]-Ni(1)-Ni(2)	118.31 (11)
O(15)-Ni(1)-O(18)	87.1 (4)	O(1)-Ni(3)-O(19)[a]	167.5 (4)	Ni(1)[a]-Ni(1)-Ni(3)	111.61 (11)
O(15)-Ni(1)-O(18)[a]	163.0 (4)	O(1)-Ni(3)-O(22)	79.7 (4)	Ni(1)[a]-Ni(1)-Ni(4)	159.56 (11)
O(15)-Ni(1)-O(22)	83.4 (4)	O(6)-Ni(3)-O(8)	166.2 (4)	Ni(2)-Ni(1)-Ni(3)	63.91 (8)
O(18)-Ni(1)-O(18)[a]	82.7 (4)	O(6)-Ni(3)-O(11)	87.3 (4)	Ni(2)-Ni(1)-Ni(4)	61.99 (8)
O(18)-Ni(1)-O(22)	96.7 (4)	O(6)-Ni(3)-O(19)[a]	93.0 (4)	Ni(3)-Ni(1)-Ni(4)	48.71 (7)
O(18)[a]-Ni(1)-O(22)	84.4 (4)	O(6)-Ni(3)-O(22)	103.8 (4)	Ni(1)-Ni(2)-Ni(3)	56.54 (8)
O(1)-Ni(2)-O(2)	79.9 (4)	O(8)-Ni(3)-O(11)	80.2 (4)	Ni(1)-Ni(2)-Ni(4)	68.15 (8)
O(1)-Ni(2)-O(15)	102.1 (4)	O(8)-Ni(3)-O(19)[a]	95.4 (4)	Ni(3)-Ni(2)-Ni(4)	49.62 (7)

Table IV (*Continued*)

Angles					
O(1)-Ni(2)-O(16)	167.9 (4)	O(8)-Ni(3)-O(22)	87.4 (4)	Ni(1)-Ni(3)-Ni(2)	59.55 (8)
O(1)-Ni(2)-O(20)	103.2 (4)	O(11)-Ni(3)-O(19)[a]	103.8 (4)	Ni(1)-Ni(3)-Ni(4)	79.65 (9)
O(1)-Ni(2)-O(22)	78.3 (4)	O(11)-Ni(3)-O(22)	163.6 (4)	Ni(2)-Ni(3)-Ni(4)	71.55 (8)
O(2)-Ni(2)-O(15)	175.6 (4)	O(19)[a]-Ni(3)-O(22)	88.0 (4)	Ni(1)-Ni(4)-Ni(2)	49.86 (7)
O(2)-Ni(2)-O(16)	93.8 (4)	O(1)-Ni(4)-O(4)	94.4 (4)	Ni(1)-Ni(4)-Ni(3)	51.64 (7)
O(2)-Ni(2)-O(20)	92.8 (4)	O(1)-Ni(4)-O(8)	86.5 (4)	Ni(2)-Ni(4)-Ni(3)	58.84 (7)
O(2)-Ni(2)-O(22)	100.9 (4)				
		Bridging Hydroxyl Oxygen Atoms			
Ni(2)-O(1)-Ni(3)	98.4 (4)	Ni(1)-O(8)-Ni(4)	126.2 (5)	Ni(1)-O(22)-Ni(2)	95.7 (4)
Ni(2)-O(1)-Ni(4)	117.6 (5)	Ni(3)-O(8)-Ni(4)	84.9 (4)	Ni(1)-O(22)-Ni(3)	92.6 (4)
Ni(3)-O(1)-Ni(4)	87.5 (4)	Ni(1)-O(15)-Ni(2)	96.6 (4)	Ni(2)-O(22)-Ni(3)	103.5 (5)
Ni(1)-O(8)-Ni(3)	88.7 (4)				
		Tetramethylammonium Ions			
Me(11)-N(1)-Me(12)	109 (1)	Me(21)-N(2)-Me(22)	108 (1)	Me(31)-N(3)-Me(32)	112 (2)
Me(11)-N(1)-Me(13)	112 (1)	Me(21)-N(2)-Me(23)	108 (1)	Me(31)-N(3)-Me(33)	110 (2)
Me(11)-N(1)-Me(14)	108 (1)	Me(21)-N(2)-Me(24)	110 (1)	Me(31)-N(3)-Me(34)	113 (2)
Me(12)-N(1)-Me(13)	111 (1)	Me(22)-N(2)-Me(23)	110 (1)	Me(32)-N(3)-Me(33)	108 (2)
Me(12)-N(1)-Me(14)	108 (1)	Me(22)-N(2)-Me(24)	109 (1)	Me(32)-N(3)-Me(34)	106 (2)
Me(13)-N(1)-Me(14)	109 (1)	Me(23)-N(2)-Me(24)	111 (1)	Me(33)-N(3)-Me(34)	108 (2)

[a] The superscript refers to the symmetry operation 1 − *x*, 1 − *y*, −*z*.

Table V. Comparison of Selected Parameters of Manganous,[20] Ferrous, and Magnesium[20] Complexes with Triionized Citrate from Crystal Structure Determinations[a]

	Mn(II)[20]	Fe(II)	Mg[20]
	Distances (Å)		
C(1)-O(1)	1.278 (2)	1.272 (3)	1.266 (2)
C(3)-O(7)	1.434 (2)	1.442 (3)	1.443 (2)
C(3)-C(6)	1.558 (2)	1.544 (4)	1.555 (2)
O(7)-O(1)	2.756 (3)	2.791 (3)	2.788 (3)
O(7)-O(2)[a]	3.104 (3)	2.976 (3)	2.985 (3)
M(1)-O(9)	2.180 (2)	2.122 (2)	2.081 (1)
M(1)-O(10)	2.181 (2)	2.132 (3)	2.080 (1)
M(1)-O(11)	2.169 (2)	2.115 (3)	2.061 (1)
M(2)-O(1)[a]	2.167 (2)	2.116 (2)	2.072 (1)
M(2)-O(2)[b]	2.123 (2)	2.085 (2)	2.019 (1)
M(2)-O(4)	2.181 (2)	2.116 (2)	2.077 (1)
M(2)-O(5)	2.194 (2)	2.130 (2)	2.081 (1)
M(2)-O(7)	2.224 (2)	2.178 (2)	2.118 (1)
M(2)-O(8)	2.140 (2)	2.090 (2)	2.031 (1)
	Angles (deg)		
C(3)-C(4)-C(5)	115.5 (1)	115.7 (2)	115.3 (1)
O(5)-M(2)-O(7)	73.26	75.0 (1)	76.32 (5)

[a] The superscripts refer to the following symmetry operations: a: 1/2 − *x*, 1/2 + *y*, 3/2 − *z*; b: *x*, *y* + 1, *z*.

The atomic parameters, as determined in the final least-squares cycle, are given in Table III. Included in this table are the individual positions of the tetramethylammonium ions which were treated as rigid bodies along with the thermal parameters of each of the rigid groups and the positions of the hydroxide and water protons which were located in the final difference map. The names of the carbon atoms of the tetramethylammonium ions begin with ME to distinguish them from citrate carbon atoms, C(1)-C(18). The noncoordinated water oxygen atoms are named W(1) through W(18), with the letters A or B to distinguish the two alternate positions of those atoms with fractional occupancies. The hydroxide proton is named OH, the protons of the metal coordinated water molecule are designated as HOH(1) and HOH(2), and the citrate protons are called H(1)-H(12). Interatomic distances and angles with estimated standard deviations calculated from the full inverse matrix are presented in Table IV. Observed and calculated structure factor amplitudes are available.[17]

Results and Discussion

Descriptions of the Structures. $[Fe(II)(H_2O)_6][Fe(II)\text{-}C_6H_5O_7(H_2O)]_2\cdot 2H_2O$. This Fe(II)-triionized citrate complex is isomorphous with the manganese(II) and magnesium complexes of triionized citrate.[8,9,20] A projection of the monoclinic unit cell viewed down the *b* axis is presented in Figure 1.

The triionized citrate ion forms a tridentate chelate in which one terminal carboxyl, the central carboxyl, and the protonated hydroxyl groups are coordinated to a single iron(II) ion, and the other terminal carboxyl group is coordinated to two other iron(II) ions which are related by a screw axis as pictured in Figure 2. The 1:1 complex of ferrous ion:triionized citrate forms an infinite chain of covalently linked units along a 2_1 axis of the unit cell, as presented in Figure 3. The hexaquoiron(II) counterions of this ionic structure which are located on centers of symmetry are linked by extensive hydrogen bonding to the covalent columns of ferrous citrate. Likewise, adjacent columns which are related to one another by a second 2_1 axis are hydrogen bonded together through the water molecule located within the coordination sphere of the citrate chelated ferrous ion and the noncoordinated water of hydration. The water of hydration is additionally hydrogen bonded to the hexaquoiron(II), and thus completes the infinite network of intermolecular bonding.

Glusker and Carrell[20] have made a detailed comparison of the bond distances and angles obtained in the refinements of the manganese and magnesium citrate structures. The long C(3)-C(6) distance observed in the manganese (1.558 (2) Å) and magnesium (1.555 (2) Å) structures is not observed in the ferrous citrate structure where the C(3)-C(6) single bond distance has the more normal value of 1.544 (4) Å. They have observed a slight decrease (3σ) of the C(3)-O(7) distance of the hydroxyl group and a small increase (4σ) of the C(1)-O(1) distance of a terminal carboxyl group in the manganese citrate structure relative to the magnesium citrate structure. In addition, the bifurcation of the hydrogen bond of H(5) between O(1) and O(2) in the magnesium structure[9] is not present in the manganese structure where H(5) is definitely intramolecularly hydrogen bonded to O(1) rather than intermolecularly to O(2). It was suggested that these changes in bond distances reflected a tendency toward increased asymmetry

Table VI. Oxygen–Oxygen and Selected Oxygen–Hydrogen Distances (Å) of Hydrogen Bonded Species of $\{[N(CH_3)_4]_5[Ni(II)_4(C_6H_4O_7)_3(OH)(H_2O)]\cdot 18H_2O\}_2$[a]

		Oxygen–Oxygen			
W(1)–O(12)[a]	2.76 (1)	W(7)–W(12)[h]	2.79 (2)	W(13B)–W(14B)	2.78 (4)
W(1)–O(17)	2.74 (1)	W(8)–W(15A)	2.63 (4)	W(13B)–W(15A)	2.45 (4)
W(1)–W(4)	2.77 (2)	W(8)–W(15B)	2.80 (2)	W(13B)–W(15B)	2.84 (3)
W(2)–O(14)[b]	2.93 (1)	W(8)–W(10)[a]	2.73 (2)	W(13B)–W(16A)	2.48 (5)
W(2)–O(16)	2.72 (1)	W(9)–O(5)	2.83 (2)	W(14A)–O(20)	3.01 (3)
W(2)–W(8)[c]	2.72 (2)	W(9)–W(11)	2.90 (2)	W(14A)–O(21)	2.87 (3)
W(3)–O(10)	2.74 (2)	W(9)–W(12)[g]	2.84 (2)	W(14A)–O(23)	2.95 (3)
W(3)–O(14)	2.76 (1)	W(10)–W(18A)	2.57 (3)	W(14A)–W(16A)	2.67 (5)
W(3)–W(4)[d]	2.72 (2)	W(10)–W(18B)	2.74 (3)	W(14B)–O(21)	2.93 (3)
W(4)–W(8)	2.66 (2)	W(11)–O(7)	2.81 (2)	W(14B)–O(23)	2.70 (3)
W(5)–O(7)[e]	2.79 (2)	W(11)–W(18A)[c]	2.63 (3)	W(15A)–O(21)	2.85 (4)
W(5)–O(10)	2.85 (2)	W(11)–W(18B)[c]	2.88 (3)	W(15B)–O(21)	2.74 (2)
W(5)–W(10)	2.70 (2)	W(12)–W(15B)	2.90 (2)	W(16A)–O(5)[g]	2.66 (4)
W(6)–O(3)[e]	2.72 (2)	W(12)–W(16B)[g]	2.71 (3)	W(16A)–W(16B)[g]	2.47 (5)
W(6)–W(6)[f]	2.75 (2)	W(12)–W(17A)	2.97 (4)	W(16B)–O(5)	2.61 (3)
W(6)–W(9)[g]	2.75 (2)	W(13A)–O(3)[e]	2.67 (4)	W(16B)–W(17B)[g]	2.77 (4)
W(7)–W(17A)[g]	2.93 (3)	W(13A)–W(15A)	2.67 (5)	W(17A)–O(21)	2.70 (3)
W(7)–W(17B)[g]	2.51 (3)	W(13A)–W(15B)	2.66 (4)	W(17B)–O(21)	2.71 (3)
W(7)–W(18A)	2.90 (3)	W(13A)–W(16A)	2.65 (6)	O(15)–O(23)	2.62 (1)
W(7)–W(18B)	2.66 (3)	W(13B)–O(3)[e]	2.80 (2)		
		Oxygen–Hydrogen			
O(15)–HOH(1)	1.46	W(14A)–HOH(2)	1.80	W(14B)–HOH(2)	1.76

[a] The superscripts refer to the following symmetry operations: a: $x, y+1, z$; b: $1-x, 1-y, -z$; c: $x+1, y, z$; d: $-x, 1-y, -z$; e: $x-1, y, z$; f: $-x, 1-y, 1-z$; g: $1-x, 1-y, 1-z$; h: $x, y-1, z$.

of the chemically equivalent C–O bonds of the carboxyls and an attendant increase of "negative charge on O(1) and O(4) and positive charge on O(7) for the manganese salt".[20] Additionally, these effects were correlated with the greater stability[10] of the manganese citrate complex with respect to the magnesium citrate complex. The fourth acid dissociation of manganese citrate was likewise tentatively attributed to the ionization of the hydroxyl group at high pH.

Although the ferrous ion also forms strong complexes with citrate[11,21] and exhibits the fourth acid dissociation with a lowered p*K* of the hydroxyl group,[11,14] the differences between bond distances in the magnesium and manganese citrate structures which were tentatively attributed to the difference between alkaline earth and transition metal complex formation by Glusker and Carrell are not observed in a comparison of the magnesium and ferrous citrate structures (see Table V).

The C(1)–O(1) distance in the ferrous citrate structure is less than 2σ greater than the corresponding distance in the magnesium citrate structure, and the C(3)–O(7) distances are essentially identical in the two cases. The bifurcation of the H(5) hydrogen bond is again present in the iron(II) as in the magnesium structure. A comparison of the metal–oxygen distances clearly indicates that the ionic radii of the metals vary in the order Mn(II) > Fe(II) > Mg(II). Hence, any significant differences among these structures are probably best attributed to the differences in the ionic radii of the metal ions.

$\{[N(CH_3)_4]_5[Ni(II)_4(C_6H_4O_7)_3(OH)(H_2O)]\cdot 18H_2O\}_2$. This Ni(II)-tetraionized citrate complex crystallizes in space group $P\bar{1}$ as confirmed by successful refinement of the structure. The unit cell is composed of one very large, highly charged anionic complex surrounded by tetramethylammonium cations at the surface of the complex. The complex exists as a discrete unit which is surrounded by a hydrogen bonded network of water molecules as shown in Figure 4.

The anionic complex is composed of two highly distorted tetrahedra of nonbonded Ni(II) ions whose coordination sites are occupied primarily by six tetraionized citrate ions. The two tetrahedra are related by a center of symmetry and are bridged by a carboxyl oxygen atom of each of two citrate molecules which are related by the center of symmetry. The union of the two tetrahedra into a single complex is further accomplished by the coordination of the other oxygen atom of the bridging carboxyl group to a Ni(II) ion of the symmetry-related tetrahedron as depicted in Figure 5. A three-dimensional view of the anionic complex is presented in Figure 6 for further clarification of the geometry.

Three of the four independent Ni(II) ions exhibit distorted octahedral coordination. Ni(4) is five-coordinate, approximately square pyramidal, with O(23) at the apex of the pyramid. The angles between the Ni(4)–O(23) vector and the Ni(4)–oxygen vectors of the four other coordinated oxygen atoms vary between 92.2 (4) and 96.7 (4)°. Consequently, the Ni(4) ion is displaced slightly out of the approximate plane of O(1), O(4), O(8), and O(9) toward O(23). The carboxyl oxygen atom O(11), which is coordinated to Ni(3), is located 2.56 (1) Å from Ni(4), a distance considerably longer than a normal nickel–oxygen bond. However, the position of O(11) prevents the coordination of a second molecule of water to produce an octahedral configuration at Ni(4).

The distortion of the tetrahedron of metal ions is evident from the large variation among the nickel–nickel distances and angles as given in Table IV. The tetrahedral face formed by Ni(1), Ni(2), and Ni(3) is capped by a triply-bridging hydroxide ion. The faces formed by Ni(2), Ni(3), and Ni(4), and by Ni(1), Ni(3), and Ni(4) are each capped by triply-bridging citrate hydroxyl oxygen atoms O(1) and O(8), respectively. The fourth face formed by Ni(1), Ni(2), and Ni(4), which contains both of the long nickel–nickel distances, is not triply-bridged. The relatively short Ni(1)–Ni(2) distance is bridged by the citrate hydroxyl oxygen atom, O(15), which is hydrogen bonded to O(23). This water molecule, coordinated to Ni(4), is the only water molecule within a Ni(II) ion coordination sphere.

The configurations of the three independent citrate ions are similar in that all three carboxyl groups of each are coordinated to at least one of the Ni(II) ions of the metal tetrahedra and the hydroxyl oxygen atom of each is coordinated to two or three Ni(II) ions in a bridged configuration. The hydroxyl groups of citrate no. 1 and 2 are necessarily ionized as evidenced by the coordination of each to three different Ni(II) ions. These two citrate ions are coordinated to the Ni(II) cluster in the same manner. Each of the three carboxyl groups is coordinated to a different one of the three Ni(II) ions which are triply bridged by the hydroxyl oxygen atom of that citrate ion. The Ni(II)-coordinated water oxygen atom, O(23), is hydrogen bonded to the hydroxyl oxygen atom of citrate no. 3, O(15), as indicated by the oxygen-oxygen distance of 2.62 (1) Å. The proton of this hydrogen bond, which was located in the final difference map, is 1.2 Å from O(23) and 1.5 Å from O(15). Since O(15) is coordinated to two Ni(II) ions, and on the basis of this evidence for the position of the proton in the hydrogen bond, the hydroxyl group of citrate no. 3 is most probably also ionized.

The coordination of this third citrate ion to the Ni(II) cluster is somewhat different from the other two independent citrate ions. Of the two Ni(II) ions which are bridged by the hydroxyl oxygen atom, O(15), one has oxygen atoms of both the central carboxyl group and one of the terminal carboxyl groups of this citrate ion within its coordination sphere. The other terminal carboxyl group has one of its oxygen atoms coordinated to the other Ni(II) ion bridged by O(15) and to the symmetry related Ni(II) ion of the other nickel tetrahedron. The other oxygen atom of this unique carboxyl group is also coordinated to a different Ni(II) ion of the second tetrahedron.

There are no significant differences between corresponding bond distances and bond angles among the three individual citrate ions. Figure 7 shows that the conformations of the citrate ions are, however, somewhat different. Each of the three pictures of Figure 7 is drawn with the hydroxyl carbon-oxygen bond and the vector between the carbon atoms of the two terminal carboxyl groups parallel to the plane of the paper. In each case, the orientation of the molecule is such that the five-membered ring formed by the central carboxyl group rises above the plane of the paper.

In citrate no. 1, the conformations of the two fused six-membered rings are related by an approximate C_2 axis along the central carbon atom-hydroxyl oxygen atom bond. In the case of citrate no. 2, the conformations of the two fused six-membered rings are related by an approximate mirror plane. The conformations of the six-membered rings of citrate no. 3 are similar to citrate no. 2. However, the terminal carboxyl oxygen atom, O(20), is coordinated to the same Ni(II) ion to which the central carboxyl group is coordinated. This results in the formation of a seven-membered ring composed of O(16), C(14), C(13), C(17), C(18), O(20), and Ni(2).

Table VI contains a tabulation of the oxygen-oxygen distances of all hydrogen-bonded species within the asymmetric unit. Oxygen atoms which were located 2.45-3.01 Å apart were considered to be hydrogen bonded. Among the ordered water molecules, the oxygen-oxygen distances observed fall in the range of 2.66 (2)-2.90 (2) Å, with an average value of 2.76 Å which may be compared with the observed oxygen-oxygen distance of 2.76 Å in ice.[22]

Of the 18 water molecules of the asymmetric unit, six exhibit a complicated disorder. Each of these six has two alternate positions which may be occupied. In all but one case, only one of the two positions may be occupied within a given asymmetric unit, as is evidenced by short distances between the two alternate positions. A listing of all oxygen-oxygen distances which are unreasonably short and therefore represent exclusions of simultaneous occupancy and a table of one of several possible sets of six different models for the asymmetric unit which results in the composite model which is consistent with both the fractional occupancies of the final refinement and the exclusion of pairs of water molecules with unreasonably short oxygen-oxygen distances are included in the supplementary material.[17] The total number of hydrogen bonds varies only slightly from model to model.

Conclusions

Whereas a simple replacement of Mn(II) with Fe(II) in the transition metal-triionized citrate complexes results in the formation of an Fe(II)-citrate(−3) complex that is isomorphous with the Mn(II)-citrate(−3) complex, the ionization of the citrate hydroxyl group results in the formation of a transition metal-citrate complex of substantially different configuration. Since the metal coordination is strongly dependent on the pH of the solutions from which these complexes were crystallized, a comparative study of the structures of transition metal citrate complexes in acidic and alkaline solution was undertaken. The results of these solution studies, which are described in the following paper in this issue, demonstrate that the configurations of ferrous citrate complexes in alkaline solution are substantially different from those observed in acidic solution.

Since a significant fraction of the unit cell of $\{[N(CH_3)_4]_5[Ni(II)_4(C_6H_4O_7)_3(OH)(H_2O)]\cdot 18H_2O\}_2$ is occupied by water, the structure of this Ni(II) complex of tetraionized citrate serves as an excellent model for the structures of transition metal citrate complexes in alkaline solution. ^{13}C NMR spectra of alkaline ferrous citrate are consistent with a structure in which triply-bridging ionized citrate hydroxyl groups occupy the faces of a tetrahedron of Fe(II) ions in a configuration similar to that observed in the Ni(II)-citrate(−4) structure.[14] The fact that all three independent citrate ions observed in the Ni(II)-citrate(−4) structure and the citrate ions of the ferrous citrate complex in alkaline solution exhibit bridged configurations of the ionized citrate hydroxyl oxygen atoms suggests that most stable transition metal-tetraionized citrate complexes have similar bridged configurations. In view of the high pH optimum of aconitase, these transition metal-tetraionized citrate complexes provide additional insight into the nature of the iron(II)-citrate interaction at the active site of aconitase. The possibility of a metal-bridged configuration for an ionized citrate hydroxyl group at the active site of aconitase is discussed in greater detail in the following paper.

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Supplementary Material Available: A listing of the observed and calculated structure factor amplitudes for both the Fe(II) citrate and Ni(II) citrate structures; a table of thermal parameters for the Fe(II) citrate structure; three tables for clarification of the disorder in the Ni(II) citrate structure (22 pages). Ordering information is given on any current masthead page.

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grams written by J. Blount; UCLA versions of ORFLS (Busing, Martin, and Levy), structure factor calculations and full-matrix, least-squares refinement; ORTEP (Johnson), figure plotting; ABSN (Coppens), absorption correction; and ORFFE (Busing, Martin, and Levy), distance, angle, and error computations. The equations used in data reduction are the same as given in A. K. Wilkerson, J. B. Chodak, and C. E. Strouse, *J. Am. Chem. Soc.*, **97**, 3000–3004 (1975). All least-squares refinements computed the agreement factors R and R_w according to $R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$ and $R_w = [\Sigma w_i||F_o| - |F_c||^2/\Sigma w_i|F_o|^2]^{1/2}$, where F_o and F_c are the observed and calculated structure factors, respectively, and $w_i^{1/2} = 1/\sigma(F_o)$. The parameter minimized in all least-squares refinements was $\Sigma w_i||F_o| - |F_c||^2$. All calculations were performed on the IBM 360-91KK computer operated by the UCLA Campus Computing Network.

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^{13}C NMR Studies of Ferrous Citrates in Acidic and Alkaline Solutions. Implications Concerning the Active Site of Aconitase[1]

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Abstract: ^{13}C NMR studies of the paramagnetic 1:1 Fe(II):citrate system in solution were carried out as a function of pH, temperature, and concentration, with the use of natural abundance and [1,2,4,5-^{13}C]citric acid prepared from ethyl bromo[1,2-^{13}C]acetate and ethyl acetoacetate. The temperature and concentration dependence of the large shifts in the positions of the ^{13}C resonances indicated that in acidic samples, chemical exchange is fast on the NMR time scale. The temperature dependence of the spectra of alkaline samples revealed an equilibrium among several forms of the complex which are not exchanging on the NMR time scale. At temperatures near the freezing point of the solution, a single form predominates in which the two halves of the citrate ion are inequivalent. Molecular weight determinations in solutions of high ionic strength indicated that both Fe(II) and Ni(II) complexes of tetraionized citrate are tetrameric, Fe(II)-triionized citrate is monomeric, and Ni(II)-triionized citrate is primarily monomeric with evidence of a small degree of association. The Ni(II) complex of tetraionized citrate is dimeric in solutions of very low ionic strength. The magnetic susceptibilities of Ni(II) complexes of triionized and tetraionized citrate and Fe(II) complexes of tetraionized citrate in solution were measured by an NMR method. No significant magnetic coupling was observed for any of these complexes. A complex of S_4 symmetry based on a tetrahedral cluster of nonbonded Fe(II) ions is proposed as the predominant form of Fe(II)-citrate in cold alkaline solution. In this complex, each face of the tetrahedron is capped with a triply-bridging citrate hydroxyl oxygen atom. The four citrate ions of the cluster have configurations and conformations similar to those observed in a previous crystallographic characterization of a Ni(II) complex of tetraionized citrate. The observation of a metal bridged configuration of the ionized citrate hydroxyl group in the complexes of tetraionized citrate ions with Ni(II) ions and Fe(II) ions characterized by x-ray crystallography and ^{13}C NMR, respectively, suggests that this configuration may be present in most stable transition metal complexes of tetraionized citrate. The implications of these results concerning the nature of the iron citrate complex at the active site of aconitase are discussed.

The Krebs cycle enzyme aconitase catalyzes the interconversions of citrate, *cis*-aconitate, and 2*R*,3*S*-(+)-isocitrate. The presence of Fe(II) and a reducing agent is required for activation of the catalytic function of the enzyme. Although many other metal ions have been tested, only Fe(II) (in the presence of a reducing agent) produces significant activation of the enzyme.[2] A ternary enzyme–Fe(II)–citrate complex at the active site of aconitase has been detected by ^{1}H NMR T_1 measurements.[3,4]

The p*K* of the citrate hydroxyl group is substantially lowered when the citrate ion is coordinated to a transition metal ion.[5–8] When citrate ions are coordinated to Fe(II) ions, the hydroxyl groups are predominantly ionized in neutral or alkaline aqueous solution. X-ray structural studies of an Fe(II)–citrate complex crystallized from acidic solution and a Ni(II)–citrate complex crystallized from alkaline solution revealed that the mode of coordination of the citrate ion to transition metal ions is dependent upon the state of ionization of the citrate hydroxyl group.[9] To examine this phenomenon in solution, a ^{13}C NMR investigation of Fe(II)–citrate complexes in acidic and alkaline solution was undertaken. Since the pH optimum for the enzymatic activity of aconitase from a variety of sources in several different buffers lies in the alkaline range,[2] data obtained from solutions of alkaline ferrous citrate complexes are of special interest as models for the coordination of citrate to the active site of aconitase. ^{13}C NMR of ferrous citrate complexes was utilized because the contact shifts of the citrate resonances are extremely sensitive to the configurations and conformations of the citrate ions and because of the greater number and variety of carbon atoms than protons in citrate. Additionally, the paramagnetic NMR technique is potentially very useful for similar investigations of the configuration and conformation of the citrate ion in the ternary aconitase–Fe(II)–citrate complex. Paramagnetic NMR is particularly valuable in enzyme studies because the resonances of atoms which interact with the metal site are shifted away from the complicated envelope of resonances which arises from the rest of the macromolecule. A synthesis of ^{13}C enriched citric acid was undertaken to enhance the sensitivity of the ^{13}C paramagnetic NMR measurements and to aid in the assignment of the observed