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Preparation and Properties of Fe^{3+}–Amino Acid Complexes

Crystalline Complexes with Aliphatic Amino Acids[1, 2, 3]

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Crystalline complexes of Fe^{3+} and several aliphatic amino acids have been prepared. All have a basic molecular constitution $[Fe[AA]_2H_2O]_3O(ClO_4)_7$, as determined by optical, magnetic, and Mössbauer measurements. The physical properties of these compounds display a marked similarity to those of ferritin.

The study of complexes of transition metals with amino acids as an aid in elucidating the nature of transition metal -protein interaction has a basis which is self-evident. To this end, extensive studies of such complexes have been carried out and are the subject of a number of reviews (1). The study of iron complexes has lagged behind that of other transition metals due, in large part, to the preparative difficulties encountered. We report here the preparation and isolation in crystalline form of complexes of simple aliphatic amino acids with iron(III) perchlorate. The similarities in the spectral and magnetic properties strongly suggest a great similarity in structure among these different complexes.

Here are a group of compounds of known structures with extensive observations of their spectral and magnetic properties. They provide a series of correlations which may have wide application in determining the nature of nonheme iron–protein interactions.

EXPERIMENTAL

Preparation. Crystalline iron(III) glycine, L-alanine, L-valine, L-leucine, L-isoleucine, and L-proline were each prepared as follows: Solutions of the complex were prepared by adding 0.1 M aqueous iron(III) perchlorate to the crystalline, solid amino acid such that the molar ratio was 1($Fe(ClO_4)_3$): 2(amino acid). Crystals of the complexes were obtained by evaporation of these solutions under atmospheric conditions at room temperature. On occasion, complete evaporation would result in the formation of a glass. Repeated redissolution in water and subsequent evaporation would usually result in crystallization. After crystallization was well advanced the material was removed from its mother liquor by vacuum filtration and air dried. The crystals were then stored in tightly stoppered bottles. If left exposed to the atmosphere, desiccation, particularly in the case of leucine, isoleucine, and valine, was observed. Iron(III) salts that have been used other than perchlorate have included the sulfate, nitrate, chloride, tetrafluorborate, hexafluorophosphate, and hexafluoroarsenate. Crystalline complexes have only been obtained when perchlorate was utilized as the counterion.

Amino acids were crystalline A-grade from Calbiochem, used without further purification; iron(III) perchlorate, hydrated yellow, was obtained from G. Frederic Smith Co. This reagent was either used without further purification or was repeatedly extracted with cold (5°C) reagent 70% perchloric acid to yield the pale-violet hexaquoiron(III) perchlorate. No differences in the complexes prepared from the two forms of this reagent could be detected.

The elemental analyses of the complexes are shown in Table I. Carbon, hydrogen, and nitrogen analyses were obtained from standard commercial sources and are shown as the mean of three independent determinations, as are the other values reported. For the glycine, alanine, and proline complexes the analyses

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[3] This paper is the second in a series.

for carbon were consistently low. The formulation shown in Table I is supported, however, through comparison of measured and theoretical densities. The valine complex was formulated in a manner analogous to the glycine, alanine, and proline complexes. This also produced a comparatively low "measured" value for carbon. However, the other elemental determinations produced reasonable values. In the case of the leucine and isoleucine complexes both the carbon and the chlorine percentages were too much at variance to allow the use of the glycine formulation. In order to provide a reasonable fit of the calculated and found it was necessary to add both water and perchloric acid of crystallization. The crystals of both the leucine and *i*-leucine complexes were of insufficient quality to allow either densities or single-crystal X-ray photographs to be obtained. Chlorine was determined both as perchlorate in this laboratory by standard methods (2) and as chloride using standard commercial sources.

The X-ray structure determination of the iron-glycine, iron-alanine, and iron-proline (4) together with the consistency of the carbon and nitrogen analyses, which arise wholly from the amino acid in the preparation, the chloride analyses which arise wholly from the iron perchlorate and which were done in a different laboratory by a method unrelated to the C-N analyses, and by commercial techniques, and the agreement in theoretical and calculated densities, Table II, leads us to believe the formulations presented in Table I are correct.

Physical measurements. All electronic spectra were obtained using a Cary Model 14R spectrophotometer. Polarized and low-temperature spectra were obtained from single crystals as previously described elsewhere (5).

Infrared spectra were obtained on a Perkin-Elmer 621 grating spectrophotometer with the material in the form of either KBr pellets or Nujol mulls between NaCl plates.

Mössbauer spectra were obtained from finely ground samples, held between plastic plates, by means of an Electronics Industries Ltd. Elron Constant Acceleration Transducer Drive coupled to a Nuclear Data Multi-Channel Analyzer. The spectrometer was calibrated with NBS iron foil (standard 1541). Data were evaluated using an unconstrained least-squares fit to an arbitrary number of Lorentzians.

All magnetic susceptibility measurements were made using a Faraday balance containing an Alpha Scientific Lab. Model A17500M Magnet. The balance was calibrated with $CoHg(NCS)_4$.

Empirical molecular weights for complexes of glycine, alanine, and proline were obtained from cell dimensions determined by least-squares fit of the angular position of 12 independent reflections obtained during alignment of a Picker Four-Circle Automated Diffractometer and from densities obtained by flotation in chlorobenzene and 1,2-dibromoethane. The results are shown in Table II together with the molecular weights of the formulations in Table I.

RESULTS

Solid state electronic spectra. Single-crystal electronic spectra for those complexes for which a sufficiently large crystal

TABLE I

ELEMENTAL ANALYSES FOR IRON(III)–AMINO ACID COMPLEXES (with postulated formulations)

Amino acid	Element				
	C	H	N	Cl	Fe
Glycine—found	9.02	2.24	5.55	16.49	10.53
Calcd for $Fe_3O(gly)_6(ClO_4)_7 \cdot 3H_2O$	11.80	2.60	6.06	17.96	12.07
Alanine—found	14.10	3.27	5.27	16.96	11.40
Calcd for $Fe_3O(ala)_6(ClO_4)_7 \cdot 3H_2O$	14.72	3.30	5.72	16.90	11.11
Proline—found	19.67	4.01	4.89	15.05	10.26
Calcd for $Fe_3O(pro)_6(ClO_4)_7 \cdot 3H_2O$	22.19	3.69	5.17	15.30	10.31
Valine—found	20.12	4.23	4.43	14.51	9.36
Calcd for $Fe_3O(val)_6(ClO_4)_7 \cdot 3H_2O$	22.00	4.40	5.13	15.19	10.24
Leucine—found	22.65	4.75	4.43	13.95	9.78
Calcd for $Fe_3O(leu)_6(ClO_4)_7 \cdot 11H_2O \cdot 1/2HClO_4$	22.58	5.29	4.39	13.88	8.75
Isoleucine—found	20.47	4.52	4.07	15.21	8.39
Calcd for $Fe_3O(ileu)_6(ClO_4)_7 \cdot 13H_2O \cdot 2HClO_4$	20.48	5.11	3.98	15.12	7.98

was available are shown in Table III. Spectra were taken at 77°C, and 296°C and in no case was it practical to examine the spectra along three axes. The characteristic bands of these complexes are a broad envelope with a relatively high extinction coefficient at ca. 10,000 cm^{-1} and a single nonpolarizable band in the region 15,000–16,500 cm^{-1}. All of the complexes exhibited a small increase in the molar extinction coefficient of the 10,000 cm^{-1} envelope with decreasing temperature. This was not accompanied by any observable shift of this band. The band at ca. 16,000 cm^{-1} was not noticeably affected by changing temperature.

Infrared spectra. The carboxyl stretch frequency, ν_{asym} (CO_2^-), is observed at ca. 1640 cm^{-1} and the ν_{sym} (CO_2^-) at ca. 1440 cm^{-1} with a difference ($\Delta\nu$) of about 200 cm^{-1}. The carboxylate frequencies are listed in Table IV. Further, a band at 570 cm^{-1} is common for all the complexes.

Magnetic properties. Magnetic parameters for the crystalline solids are listed in Table V. As with the previously reported properties, the magnetic susceptibilites are very similar among the different complexes. They exhibit a value markedly reduced from the expected 5.9 μB for a high-spin iron(III) complex are found to decrease with decreasing temperature.

TABLE II

CELL DIMENSIONS (Å and °) AND MOLECULAR WEIGHTS OF SOME IRON(III)–AMINO ACID COMPLEXES[a]

Complex	a[b]	b[b]	c[b]	β[b]	ρ[c]	Molecular weight	
						Measured	Calcd
Glycine	19.26	15.61	16.69	100.7	1.885	1399[d]	1384
L-alanine	20.11	15.84	17.41	103.8	1.836	1489[d]	1468
L-proline	21.62	17.26	17.19	99.9	1.722	1638[d]	1624

[a] Calculated molecular weights based on the formulations in Table I.

[b] Cell dimensions and angles from X-ray data.

[c] Density determined by flotation in chlorobenzene/1,2-dibromoethane.

[d] Molecular weight obtained from the expression

$$\rho = \frac{1.66(Z)\,(M_r)}{V} \text{ ; in this case } V = abc \sin\beta$$

and Z (the number of molecular units/unit cell) is 4.

TABLE III

SOLID STATE ELECTRONIC SPECTRAL DATA FOR SOME IRON(III)–AMINO ACID COMPLEXES[a]

Complex	Temp. (°K)	Polarization	Absorption bands (cm^{-1})			
L-alanine	296	→	10,500	15,900	—	—
		↑	10,500(13)	16,100	—	—
	77	→	10,300	16,100	—	—
		↑	10,300(16)	16,100	—	—
L-valine	296		10,400	—	—	—
	77		10,300	16,100	—	—
L-leucine	296	→		16,100	20,800	—
		↑	10,400	15,900	—	—
	77	→		15,900	20,800	21,750
		↑	10,300	15,750	—	—
L-proline	296	→	10,250	15,600	—	—
		↑	10,100	15,600	—	—
	77	→	10,250	15,900	—	—
		↑	10,200	15,900	—	—

[a] Because of crystal size and quality it was not possible to obtain spectra along all three axes. Parenthetical values are molar extinction coefficients, —indicates higher energy absorptions obscured by tailing from the uv.

TABLE IV

FREQUENCIES OF INFRARED CARBOXYLATE VIBRATIONS IN SOME IRON(III)-AMINO ACID COMPLEXES

Amino acid	$\nu_{asym}(CO_2^-)$ cm^{-1}	$\nu_{sym}(CO_2^-)$ cm^{-1}	$\Delta\nu$
Glycine	1640	1414	226
Alanine	1640	1432	208
Valine	1639	1441	198
Leucine	1640	1430	210
Isoleucine	1635	1438	197
Proline	1628	1438	190

TABLE V

VALUES OF MAGNETIC PARAMETERS FOR SOME IRON(III)-AMINO ACID COMPLEXES[a]

Complex (crystalline)	Temperature (°K)	$\chi_m \times 10^6$ (cgsu)	μB	$-J$ (cm^{-1})
Glycine	296	4,394	3.24	29.5
	80	6,448	2.04	
L-alanine	296	4,284	3.20	30.7
	80	4,524	1.71	
L-valine	296	4,180	3.16	33.3
	80	6,348	2.02	
L-leucine	296	3,914	3.06	38.5
	80	4,799	1.76	
L-isoleucine	296	4,659	3.34	26.9
	80	8,632	2.36	
L-proline	296	4,510	3.28	28.0
	80	6,175	2.00	

[a] Values calculated assuming the iron(III) L-alanine structure for all complexes, values reported per iron atom. There is an uncertainty of no more than 5% in the iron analyses on which these calculations were based; thus, the calculated χ_m are subject to a similar uncertainty.

The magnetic moments per iron are ca. 3.2 μB at 290°C and decrease to ca. 2.0 μB at 80°C. The claculated spin coupling constant, J, (7) is ca. -30.0 cm^{-1}.

Mössbauer spectra. Mössbauer parameters are listed in Table VI. Again the complexes exhibit very similar behavior. Isomer shifts of ca. 0.68 mm s^{-1} are observed in each case. The quadrapole splitting (ΔEq) is ca. 0.5 mm s^{-1}. The line width (ca. 0.21 mm s^{-1}) is about the same as that of the iron foil used in calibration.

DISCUSSION

Formulation and structure. The crystal structures of the complexes between $Fe(ClO_4)_3$ and the amino acid have been determined by X-ray methods for glycine, alanine, and proline (4). They have a structure which is essentially the same as that of basic iron acetate (7). The amino acids are present as zwitterions and the amino nitrogens are not involved in the iron-ligand bonding but do appear to be extensively hydrogen-bonded to perchlorate anions. The overall structure of these molecules is that of a central oxygen bonded to three iron atoms which are, in turn, bonded to an oxygen of the carboxyl group of each of four amino acid residues (cf. Fig. 1). The sixth coordination site is occupied by water.

The formulation and crystal form of the other complexes suggest that their structures are quite similar. The differences in the numbers of waters and perchloric acids in the formulations could be the result of slightly different packing requirements or of different surface properties. A principal point of the following discussion is that the consistent similarity in spectral and magnetic properties of all of the complexes used in this study make it virtually certain that their structures are very nearly the same and that these properties are consistent with the structures elucidated by X-ray analysis.

Electronic spectra. The spectra of these complexes are similar to those of other iron carboxylate complexes and, also, to those of oxo-bridged iron dimers (8). The relatively low pH (2-3) of the reaction mixture makes the presence of the central oxygen in these complexes rather surprising. However, the data presented here together with

TABLE VI

MÖSSBAUER SPECTRAL DATA FOR SOME IRON(III)-AMINO ACID COMPLEXES[a]

Complex	ΔEq	δ	Line width[b]	
Glycine	0.39	0.67	.20	.22
L-alanine	0.51	0.68	.19	.21
L-valine	0.56	0.68	.19	.21
L-leucine	0.52	0.68	.15	.18
L-isoleucine	0.47	0.69	.12	.22

[a] Values reported in mm s^{-1} relative to sodium nitroprusside, at 298°K.

[b] Full line width at half-maximum line height, lower velocity line reported first.

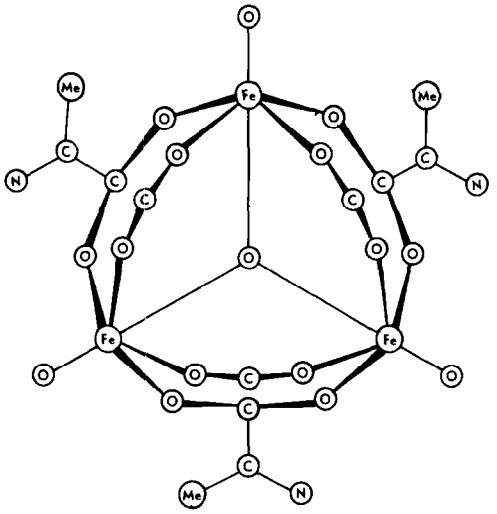

FIG. 1. Schematic representation of the molecular structure of $[Fe(alanine)_2H_2O]_3O(ClO_4)_7$. The ClO_4^- have been omitted from the figure. In addition the α-carbon and residues have been omitted from the forward-projecting carboxylates for clarity.

the X-ray data make this observation certain. The band positions are reasonable for high spin iron(III) in a distorted octahedral environment (9). The molecular extinction coefficients appear superficially to be too large for the formally doubly forbidden transitions of these complexes; but similarly high extinctions have been reported in other polynuclear iron(III) complexes (8). Schugar *et al.* (8) related intensity enhancement in the electronic spectra of oxo-bridged iron(III) dimers to their antiferromagnetic properties. The antiferromagnetism which these complexes exhibit suggests extensive orbital overlap and, hence, increased intensity of the absorption bands especially the broad envelope occurring at 10,000 cm^{-1}.

Infrared spectra. The carboxyl bands of the complexes are found at higher energies than those of the free amino acids. The assigned carboxylate anion vibrations occur at 1560–1600 cm^{-1} and ca. 1410 cm^{-1} for the free amino acids (10). The complexes exhibit bands at ca. 1640 cm^{-1} and 1440 cm^{-1} indicating coordination of the oxygen as is known from the X-ray structure.

A band at 570 cm^{-1} is common to all of the complexes and is not found in any of the parent reagents. We assign this to ν_{asym} (Fe_3O). If this is the case, then ν_{sym} (Fe_3O) would be found at ca. 200 cm^{-1} which is beyond the range of our experimental capability. Long *et al.* (8) have given a band at 520 cm^{-1} the same assignment for iron acetate, predicting the symmetric band would occur at 160 cm^{-1}. The reason for the difference in the position of the bands in Fe–amino acid and Fe–acetate complexes is not apparent but this is a spectral region which calls for more detailed study.

Magnetic properties. The magnetic moments recorded at room temperature are too low for high spin Fe(III), a d^5 system of five unpaired electrons, but they are too high for low spin Fe(III) with one unpaired electron. Further, the magnetic moment is directly related to temperature. These two observations represent strong evidence of antiferromagnetic behavior. This antiferromagnetism results from the extensive orbital overlap occasioned by the Fe–O–Fe bridging (11). This conspicuous and anomalous behavior is one of the most important biological implications of this study since antiferromagnetism is observed in some iron(III)–proteins, indicative of a similar type of bonding (12).

Mössbauer spectral properties. The isomer shift (δ) values of the complexes of ca. 0.68 mm s^{-1} are of the magnitude and direction for high-spin iron(III) complexes (8). The presence of two symmetrically split peaks indicates that there is but one environment for the iron atoms, a result which is consistent with the X-ray structure. The observed values for the isomer shift and the quadropole splitting (ΔEq) are in agreement with reported values for other trimeric iron(III) complexes (8).

GENERAL CONCLUSIONS

A number of general conclusions regarding the solid-state structure of the iron (III)–amino acid complexes may be drawn from the above data. The spectral and magnetic properties are wholly consistent with the basic iron(III) acetate structure (6, 7) which is the general type of structure discovered by X-ray analysis of three of these complexes. The great similarity in properties argues for an extensive similarity in structure and it would seem that differences in the length (isoleucine vs

glycine) and conformation (proline vs valine) of the hydrocarbon chain have but a minor effect on the overall structure. The structure is very different from that reported for crystalline complexes of other transition metals with aliphatic amino acids. These are generally regarded as having both the carboxyl and amino groups coordinated to the metal in a bidentate manner and none appear to adopt a trimeric structure (1). Numerous experiments by ourselves and others have not resulted in any solid reaction product showing simultaneous oxygen and nitrogen coordination with iron(III) (13).

It is important to indicate, at least to some degree, the biological implications of this work. These are threefold. The first is the relative lack of affinity of the amino groups in the amino acids for iron(III). True, these are amino acids and not polypeptides. However, it suggests that the coordination to an oxygen is more strongly favored where iron(III) is involved than the coordination to the nitrogen of the amine group.

Second, it appears that the coordination of the carboxyl groups to a trimeric iron residue is a general feature of the coordinational chemistry of the amino acids. This phenomenon is observed even with di- and tripeptides. Recent work in our laboratories has shown that it is possible to form compounds which appear to be of a similar nature from the amino acids, triglycine and glycyl-leucine.

Third, as has been noted in an earlier publication (4), the trimeric iron species shows a greater similarity to the iron storage protein ferritin than do any of the other oxo or the hydroxo bridge species that have been studied heretofore. It is possible that the trimeric species could exist in ferritin as distinct trimeric units held together by water or hydroxo bonds and that the coupling within the trimer would be of sufficient strength to preclude strong intertrimer coupling and thus long-range ordering. Based upon these data this model must be considered to be the strongest candidate for the subunit until a model which more closely fits the physical parameters of ferritin appears.

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