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Structural Investigation of the Divalent Iron and Manganese Complexes with Citric Acid by Infrared Spectroscopy

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The structures of the complexes of Fe^{2+} and Mn^{2+} with citric acid were investigated by infrared spectroscopy. The compounds used were $[Mn(H_2O)_6][Mn(C_6H_5O_7)H_2O]_2 \cdot 2H_2O$, $Mn(C_6H_6O_7) \cdot H_2O$ and $Na_4[Fe(C_6H_5O_7)_2]$. In the IR spectra of the last complex, the strength of the band assignable to the stretching vibration of the alcoholic OH group of the citrate ion was weaker than in the free molecule. This result suggests that the OH group contributes to the chelate formation. $Na_4[Fe(C_6H_5O_7)_2]$ is considered to be octahedral, with the two trivalent citrate anions binding to the central iron(II) ion. Thus, two of the three carboxyl groups and one OH group within a citrate ion combine with the Fe^{2+} ion, and one carboxyl group of the citrate ion is free. On the other hand, the Mn^{2+} ion within $Mn(C_6H_6O_7) \cdot H_2O$ crystals is considered to take a tetrahedral configuration, and again one of the three carboxyl groups of the coordinated citrate ion remains as nonbonded and undissociated COOH. In $Mn(C_6H_6O_7) \cdot H_2O$, the water molecule seems to bind to the central metal ion. The carboxyl groups of $[Mn(H_2O)_6][Mn(C_6H_5O_7) \cdot H_2O]_2 \cdot 2H_2O$ compound acted as either monodentate or bridging ligand groups.

Keywords——citric acid; Fe^{2+} complex; Mn^{2+} complex; chelate; coordination compound; citrate; aconitase; IR spectra; coordination site; metal complex

Aconitase, one of the enzymes in the Krebs cycle, catalyzes the stereospecific interconversion of citrate, *cis*-aconitate and 2*R*,3*S*-(+)-isocitrate. Aconitase is well known to be activated by Fe^{2+} ion. However, no work has been done to elucidate the mechanism of the reaction of aconitase with metal ions. Villafranca and Mildvan[1,2] have detected the formation of a mixed substrate-Fe^{2+}-enzyme complex, in which the central metal ion is bound to the active site of aconitase, by using a proton nuclear magnetic resonance (NMR) technique. They have also shown that Mn^{2+} ion behaves as a competitive inhibitor of the reaction of aconitase by the formation of a substrate-Mn^{2+}-enzyme mixed complex. Recently, the crystal structure of $[Fe(H_2O)_6][Fe(C_6H_5O_7)H_2O)]_2 \cdot 2H_2O$ has been reported;[3] this complex is known to be isomorphous to the corresponding manganese^{2+} and magnesium^{2+} salts of citric acid.[4-6]

$Na_4[Fe(C_6H_5O_7)_2]$ is used in medical treatments for anemia, but the structure of the compound has not been clarified, although the structure of a similar compound, $[Fe(H_2O)_6][Fe(C_6H_5O_7) \cdot H_2O]_2 \cdot 2H_2O$ has been determined by means of X-ray diffraction. The $[Fe(H_2O)_6]$ moiety participates in the chain structure of the crystal.

In the present work, the structure of $Na_4[Fe(C_6H_5O_7)_2]$ complex is investigated by means of infrared (IR) spectroscopy. The IR spectra of $Mn(C_6H_5O_7) \cdot H_2O$ and $[Mn(H_2O)_6][Mn(C_6H_5O_7) \cdot H_2O]_2 \cdot 2H_2O$ have also been measured and compared with the spectrum of $Na_4[Fe(C_6H_5$-

$O_7)_2]$.

Citric acid has three carboxyl groups, and two of them are equivalent. In the aconitase reaction, the two terminal carboxyl groups of the citric acid unit are not identical in their behavior. In this paper, the antisymmetric property of the two terminal carboxyl groups is discussed on the basis of the IR spectra of the metal complexes of citric acid.

Results and Discussion

Citric Acid and Sodium Citrate

Infrared spectra of anhydrous citric acid and the monohydrate, as well as sodium citrate dihydrate, were measured with a JASCO infrared spectrometer, Model A-302, at room temperature (Table I). In the IR spectrum of citric acid, strong bands were observed at 3500 and 3450 cm^{-1}, and these were also found in the spectra of citric acid monohydrate and sodium citrate. In the 3200—2500 cm^{-1} region of the IR spectra of citric acid and the monohydrate, many absorption bands were observed. In the IR spectra of deuterated citric acid and sodium citrate, the bands originally appearing in the 3600—2500 cm^{-1} region were shifted to the 2500—2000 cm^{-1} region. In the IR spectrum of sodium citrate containing no undissociated carboxyl group, only two bands were observed at 3460 and 3260 cm^{-1}, which were assignable to stretching vibrations of the alcoholic OH groups. The bands weakened and sometimes disappeared on deuteration.7)

In a previous investigation7) the bands observed in the 3600—2500 cm^{-1} region for citric acid were assigned to the stretching vibrations of the alcoholic and carboxylic OH groups, upon which weak bands due to CH stretching are superimposed, and the bands at 3500 and 3450 cm^{-1} were attributed to the alcoholic OH stretching vibration, which is sometimes sensitive to hydrogen bonding. Thus, it was concluded that the bands appearing in the 3600—3400 cm^{-1} region should be assigned as alcoholic OH stretching bands (Fig. 1).

In the IR spectra of citric acid and sodium citrate, strong bands were observed in the 1700—1600 cm^{-1} region. In the spectrum of anhydrous citric acid, two bands were observed at 1745 and 1700 cm^{-1}, while in the spectrum of the monohydrate, we found three bands at 1756, 1724 and 1686 cm^{-1} (Table I). The bands can be assigned to the stretching of the C=O bond of the COOH group within citric acid. In the IR spectrum of sodium citrate, however, only one strong absorption band was recorded at 1591 cm^{-1}, which was attributed to the antisymmetric C=O stretching.7) The strength of the band was intermediate between those of the C=O and C–O bands (Fig. 2).

TABLE I. Infrared Spectral Data for the Ligands and Metal Complexes in KBr Disk (cm^{-1})[a]

Assignment	Citric acid monohydrate	Anhydrous citric acid	Sodium citrate	$[Mn(H_2O)_6][Mn(C_6H_5O_7)\cdot H_2O]_2\cdot 2H_2O$	$Mn(C_6H_6O_7)\cdot H_2O$	Na_4-$[Fe(C_6H_5O_7)_2]$
OH str.	3500 s		3460 s		3490 s	
	3450 m	3360 s		3350 b, m	3400 s	3420 b, m
	3280 s	3220 s	3260 s			
C=O str.	1756 s	1745 s			1728 s	
	1724 s	1700 s				
	1686 s					
COO^- antisym. str.			1591 s	1655 sh	1586 s	1615 s
				1611 sh	1550 sh	1592 s
				1575 s		1560 s
COO^- sym. str. and OH bending	1418 m	1428 m	1441 m	1448 m	1470 w	1485 w
	1392 m	1388 m	1418 m	1421 m	1416 m	1426 m
			1403 m	1402 m	1395 m	1412 m
			1394 m			1395 m

a) s,strong; m, medium; w, weak; sh, shoulder; b, broad.

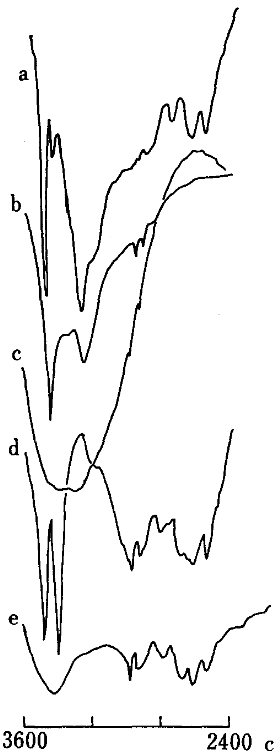

Fig. 1. IR Spectra of (a) Anhydrous Citric Acid, (b) Trisodium Citrate Dihydrate, (c) $[Mn(H_2O)_6][Mn(C_6H_5O_7)H_2O]_2\cdot 2H_2O$, (d) $Mn(C_6H_6O_7)\cdot H_2O$, and (e) $Na_4[Fe(C_6H_5O_7)_2]$

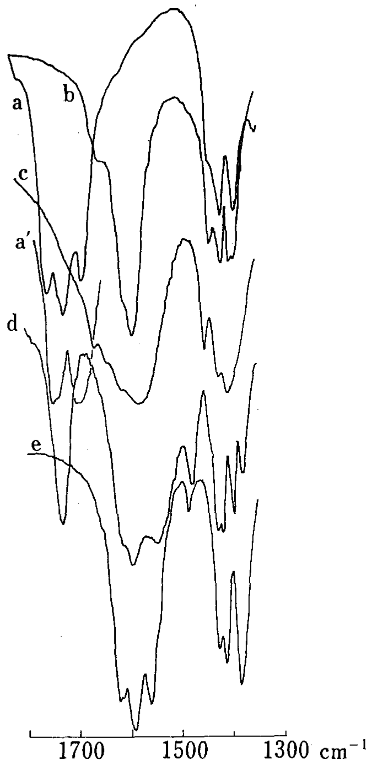

Fig. 2. IR Spectra of (a) Citric Acid Monohydrate, (a') Anhydrous Citric Acid, (b) Trisodium Citrate Dihydrate, (c) $[Mn(H_2O)_6][Mn(C_6H_5O_7)H_2O]_2\cdot 2H_2O$, (d) $Mn(C_6H_6O_7)\cdot H_2O$, and (e) $Na_4[Fe(C_6H_5O_7)_2]$

$Mn(C_6H_6O_7)\cdot H_2O$ and $[Mn(H_2O)_6][Mn(C_6H_5O_7)H_2O]_2\cdot 2H_2O$

In order to interprete the IR spectrum of $Na_4[Fe(C_6H_5O_7)_2]$, the spectra of $Mn(C_6H_6O_7)\cdot H_2O$ and $[Mn(H_2O)_6][Mn(C_6H_5O_7)H_2O]_2\cdot 2H_2O$ were studied to provide a basis for band assignment. Crystals of $Mn(C_6H_6O_7)_2H_2O$ were prepared in this study, but the crystal structure has not been determined yet.

In the IR spectrum of $Mn(C_6H_6O_7)\cdot H_2O$, a strong band was observed at 1586 cm^{-1}, which was attributed to antisymmetric COOH stretching. In the IR spectrum of sodium citrate, the same band has been found at 1591 cm^{-1} (see Table I). Some bands of intermediate strengths at 1441, 1418, 1403 and 1394 cm^{-1} were assigned to symmetric COO$^-$ stretching. A band due to the scissoring vibration of the CH_2 groups adjacent to a carboxyl group may appear in this region.$^{7)}$ The result of the elementary analysis of $Mn(C_6H_6O_7)\cdot H_2O$ is shown in Table II; the ratio of the citrate ion to manganese is 1: 1.

In the IR spectrum of $Mn(C_6H_6O_7)\cdot H_2O$, a band was observed at 1728 cm^{-1} which can be assigned to the symmetric COOH stretching vibration.$^{9)}$ Thus, the complex contains a

Table II. Elementary Analysis Data for $Na_4[Fe^{2+}(C_6H_5O_7)_2]$ (A) and $Mn^{2+}(C_6H_6O_7)\cdot H_2O$ (B)

Compound		C	H	Na	Fe	Mn	Metal/Ligand
A	Calcd	27.40	1.92	17.48	10.62		2.00
	Found	27.46	2.04	17.51	10.69		
B	Calcd	27.39	3.07			20.88	1.00
	Found	27.31	3.11			20.92	

nonbonded and undissociated COOH group. The bands assigned to the stretching vibration of the alcoholic OH group in the 3600—3200 cm^{-1} region were shifted to the lower frequency side, but two bands were still observed at 3490 and 3400 cm^{-1} which were ascribable to the OH stretching of the water molecule contained in the compound and to the OH stretching of the COOH group, respectively. The medium-strength band at 560 cm^{-1} could be assigned to the wagging vibration of the hydrated water molecule.[10] Thus, in $Mn(C_6H_6O_7) \cdot H_2O$ two COO^- groups and one OH group of the citrate ion and a water molecule were expected to coordinate onto the central manganese ion.

In the IR spectrum of $[Mn(H_2O)_6][Mn(C_6H_5O_7)H_2O]_2 \cdot 2H_2O$, a broad absorption band was observed around 3350 cm^{-1}, while the bands appearing in this region which are ascribable to the stretching of the alcoholic OH group were weakened (see Table I). The broad band was mainly attributed to the antisymmetric and symmetric stretchings of the OH groups of the hydrating water molecules. In the crystal structure of the complex,[4] the OH group of the citrate ion binds to the central Mn^{2+} ion. The bands in the 1670—1570 cm^{-1} region were attributed to the antisymmetric stretching of a COO^- group, but a shoulder appearing in the 1660—1600 cm^{-1} region was thus assigned at least partially, to the HOH bending of the hydrating water molecules.[9] The bands assigned to the antisymmetric COO^- stretching at 1655, 1611 and 1575 cm^{-1} were shifted to either the lower or the higher frequency side, depending on the bands, compared with those of sodium citrate (1591 cm^{-1}). This implies that the COO^- groups within $[Mn(H_2O)_6][Mn(C_6H_5O_7)H_2O]_2 \cdot 2H_2O$ coordinate to the central metal ion as either unidentate or bridging ligand groups.[9] The absorption bands which were found in the 1450—1400 cm^{-1} region were mainly due to the symmetric COO^- stretching vibration. A strong band at 1575 cm^{-1}, which was shifted to the lower frequency side compared with that of sodium citrate, may be due to the terminal COO^- groups which have a bridging structure within the compound.[4,9] In the IR spectrum of $Mn(C_6H_6O_7) \cdot H_2O$, a strong band assignable to antisymmetric COO^- stretching was observed at 1586 cm^{-1}, and thus, the COO^- groups of this complex were considered to act as monodentate ligand groups[9] and not as bridging ones.

$Na_4[Fe(C_6H_5O_7)_2]$

Referring to the crystal structures[3-5] and IR spectra[11] of Fe^{2+} complexes with citric acid hitherto reported, we investigated the structure of $Na_4[Fe(C_6H_5O_7)_2]$ by measuring the IR spectrum. In the 3600—3200 cm^{-1} region a very broad absorption band was observed and weak bands were also found in the 3000—2400 cm^{-1} region (see Figs. 1 and 2). Sharp absorption which could be ascribed to the stretching of an alcoholic OH group disappeared, and thus, the OH group of the citrate ion within the complex was considered to coordinate to the central Fe^{2+} ion. It is generally accepted that the hydroxyl groups of citric acid do not easily dissociate. Only one example has been reported, for an Ni^{2+} complex in which a tetravalent citrate anion having a deprotonated hydroxyl group combines with the metal ion,[3] the compound having been prepared in alkaline solution. $Na_4[Fe(C_6H_5O_7)_2]$ crystals were prepared in an acidic solution of $pH \simeq 5.5$ in this study, and the OH group did not dissociate under such conditions. No band which could be assigned to the stretching of COOH groups was observed in the spectrum, and thus, all the carboxyl groups within the complex were deprotonated. Bands assigned to the antisymmetric stretching of a COO^- group were observed at 1615, 1592 and 1560 cm^{-1}. On the other hand, a band attributed to the antisymmetric stretching of a COO^- group was observed at 1591 cm^{-1} in the IR spectrum of sodium citrate. In the spectrum of $Mn(C_6H_6O_7) \cdot H_2O$, two bands were found in this region and the separation between the bands was about 50 cm^{-1}. In the spectrum of $Na_4[Fe(C_6H_5O_7)_2]$, three bands were recorded at 1615, 1592 and 1560 cm^{-1} and the separation between the two bands at the highest and lowest frequencies was 55 cm^{-1}, which corresponded to the band separation found in the Mn^{2+} complex. Accordingly, the strongest band among the three appearing at 1592 cm^{-1} in the IR spectrum of $Na_4[Fe(C_6H_5O_7)_2]$ is probably due to the stretching vibration of an ionized but free COO^- group. A weak band observed at 1485 cm^{-1} was assigned to the symmetric stretching of the COO^- group, which was shifted by 85 cm^{-1} from the original position at about 1400 cm^{-1} in a free citrate anion. The elementary analysis of $Na_4[Fe(C_6H_5O_7)_2]$ showed that the ratio of Fe^{2+} to trivalent citrate anions was 1: 2 and that there was no water molecule within the complex (Table II). Therefore, carboxyl groups except for a free COO^- and the OH group within a citrate anion might coordinate to the Fe^{2+} ion. The effective magnetic moment of this complex was 5.20 B. M. This suggests an octahedral structure of the complex. Since the infrared spectrum of the complex shows the existence of an ionized but free COO^- group within the complex which does not combine with any other Fe^{2+} ion as a bridging ligand, the complex does not have an infinite chain structure such as occurs in the crystal structure of $[Fe(H_2O)_6][Fe(C_6H_5O_7)H_2O]_2 \cdot 2H_2O$.³⁾

When the OH and COO^- groups attached to the central carbon atom of a citrate ion coordinate to the central metal ion, a five-membered chelate ring is formed. Therefore, it may be reasonable to consider that both groups coordinate to the Fe^{2+} ion within the complex. If the COO^- groups at a terminal and the central carbon atoms coordinate to the metal ion, a relatively unstable seven-membered ring would be formed. It is also unlikely that both the terminal COO^- groups combine with the central Fe^{2+} ion, and thus it was concluded that one terminal COO^- group in the citrate ion remains free as an uncombined group.

Experimental

Apparatus——IR spectra were recorded in KBr pellets with a JASCO infrared spectrometer, Model A-302.

Reagents——Anhydrous citric acid, citric acid monohydrate, trisodium citrate dihydrate, and other metal salts were purchased from Wako Pure Chemical Ind. Ltd., Tokyo. They were of reagent grade.

Preparation——Crystals of $[Mn(H_2O)_6][Mn(C_6H_5O_7)H_2O]_2 \cdot 2H_2O$ were prepared by the method described in the literature.⁴⁾

$Na_4[Fe(C_6H_5O_7)_2]$ crystals were prepared in the following way: 2.2 g of sodium citrate and 0.7 g of $FeSO_4 \cdot 7H_2O$ were added to 50 ml of hot water with stirring. Nitrogen gas was bubbled through the solution, which was heated on a water-bath for several hours until pale green polycrystalline precipitates were formed. They were washed with water, ethanol and then acetone. The Fe^{2+} complex thus obtained was dried under reduced pressure.

The method of preparation of $Mn(C_6H_6O_7) \cdot H_2O$ was as follows. A mixture of equimolar amounts of $Mn(OH)_2$ (which had been freshly prepared from $MnCl_2 \cdot 2H_2O$ and NaOH) and citric acid was dissolved in hot water, and heated on a water-bath with stirring until $Mn(OH)_2$ dissolved completely in the solution. Then, the solution was concentrated until precipitates formed. They were collected by filtration and then recrystallized from water. After being washed with water, the precipitates were dried under reduced pressure.

Deuterated compounds were obtained by dissolving the compounds thus prepared in D_2O and drying the solutions over silica gel under reduced pressure.

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