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Selectivity in the Metal-Complex-Catalyzed Decarboxylation of Oxaloacetic Acid and a Role of Metal Ion in an Enzyme System

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The catalytic action of metal ions in the presence of various coordinating agents and a role of metal ions for the activations of an enzyme in a decarboxylation reaction were investigated. Many of the ligands studied decreased the catalytic activity of metal ions. However, α,α'-dipyridyl selectively enhanced the catalytic activities of divalent metal ions, except Cu^{2+}. The activity of Cu^{2+} was specifically enhanced only with histidine and its derivatives. Histidine destroyed Ni^{2+} and Co^{2+} catalysis. The specific enhancement may be due to the increase in the electronegativities of the metal ions with $d\pi$(metal)–$p\pi$(ligand) back donation and to the stereo-configuration of the metal complexes. The catalytic behavior in these metal ion coordinating agent systems was different from that in the metal-enzyme systems. It was assumed that the activation of enzyme resulted from the change in the configuration of enzyme by the coordination of metal ions, and not from the catalytic action of metal ions enhanced by the coordination of enzyme. This was supported by the fact that the catalysis of compounds containing amino groups, active sites of enzyme, remarkably depended on the stereo-configuration. In the enzyme-model system, Mn^{2+} was generally preferable to Cu^{2+} and Ni^{2+}. The catalytic actions of Cu^{2+} and Ni^{2+} were lowered by the complex formation with the active sites. This may solve the question why, in enzymatic reactions, Mn^{2+} having small complex-forming ability is more selective and effective than Cu^{2+} and Ni^{2+} with large complex-forming abilities.

It is well known that metal ions paly an important part in enzymatic reactions, and interesting reports have been published by many authors.[1] Though the role of metal ions is very complicated, it must be noted that a metal ion is specific for the activation of an enzyme, and this specificity should offer a key to the question on the role of metal ion in an enzymatic reaction.

From the concept that the interaction between metal ions and the residual groups of an enzyme is an important factor for enzyme activity, Steinberger and Westheimer[2] have investigated the effect of various coordinating ligands upon copper ion catalysis in the decarboxylation of dimethyloxaloacetic acid, and stated that negative ions, such as citrate and acetate, diminished the catalytic activity of cupric ion, while complexing agents, which did not destroy the charge of the copper ion, did not destroy its catalytic ability. Rund and Plane[3] reported that catalytic activities of manganous and nickel ions for the decaboxylation of dimethyloxaloacetic acid were enhanced in the presence of *o*-phenanthroline, and the enhancement rate was completely comparable to those in the presence of the enzyme.

The present authors also found that α,α'-dipyridyl increased the catalytic activities of divalent cations, such as manganous, cobaltous, nickel and zinc ions, for the decarboxylation of oxaloacetic acid, but both of α,α'-dipyridyl and *o*-phenanthroline selectively destroyed the catalytic ability of cupric ion. Cupric ion catalysis was enhanced only with histidine and its derivatives. The specific enhancement of catalytic activities of metal ions in the presence of such ligands was discussed from a view point of the stereo-configuration of metal complexes and the back donation of the $d\pi$(metal ion)–$p\pi$-(ligand) bonding. The effect of these ligands on the metal ions catalysis appears different from that in the metal-enzyme system, and therefore it seemed unreasonable that the metal-α,α'-dipyridyl or *o*-phenanthroline system was an enzyme-like model.

Many of the works on the role of metal ion in the enzymatic decarboxylation have been carried out mainly from a view-point of catalytic abilities of metal ions, but little attention has been paid

1) See, for example, a) S. Granick, *Chem. Rev.*, **38**, 379 (1946); b) H. S. Mason, *Advan. Enzymol.*, **19**, 79 (1957).

2) R. Steinberger and F. H. Westheimer, *J. Amer. Chem. Soc.*, **73**, 429 (1951).

3) a) J. V. Rund and R. A. Plane, *ibid.*, **86**, 367 (1964); b) J. V. Rund and K. G. Claus, *ibid.*, **89**, 2256 (1967).

to the activation of the enzyme with metal ions. In this paper, a brief discussion on the role of metal ions in the enzymatic reaction was also made from the results obtained in various metal-ligand systems and in some amino compound systems, and an idea that metal ions acted as an enzyme activator, was proposed.

Experimental

Material. A reagent grade oxaloacetic acid was obtained from the Tokyo Kasei Co., Ltd. The aqueous solution was freshly prepared before use. Solutions of metal ions were made up by dissolving the reagent grade chloride salts and the concentrations were gravimetrically determined. Reagent grade coordinating agents were used without further purification, but ethylenediamine, diethylenetriamine and triethylenetetramine were purified by distillation. Buffer solutions were prepared from sodium hydroxide solution and acetic acid or monochloroacetic acid.

Experimental Procedure. The rate of decarboxylation of oxaloacetic acid was determined by measuring the pressure of carbon dioxide gas evolved in Walburg flasks. A freshly prepared solution of oxaloacetic acid was introduced into the main room of a reaction vessel and a solution of a catalyst was put into the sub-chamber of the vessel. All test solutions contained 4×10^{-3}M oxaloacetic acid, 2×10^{-4}M cupric ion or 4×10^{-4}M other divalent metal ions, and 4×10^{-2}M acetic acid or monochloroacetic acid (buffer), in the final concentration. The vessel was immersed in a water bath held at 30.00 ± 0.01°C, and after about five minutes, the catalyst was transferred to the main room and then the solution was stirred. Pressure readings were made at regular intervals. Since decarboxylation reactions follow first order kinetics with respect to the concentration of oxaloacetic acid, the data were treated in the usual manner to determine the observed first-order rate constants.

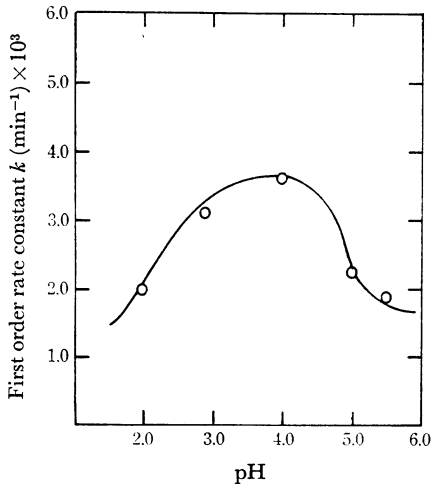


Fig. 1. Effect of pH on the uncatalyzed decorboxylation of oxaloacetic acid.
Oxaloacetic acid: 4×10^{-3}M

Results and Discussion

Effect of pH. As shown in Fig. 1, the first order rate constant of uncatalyzed decarboxylation of oxa-

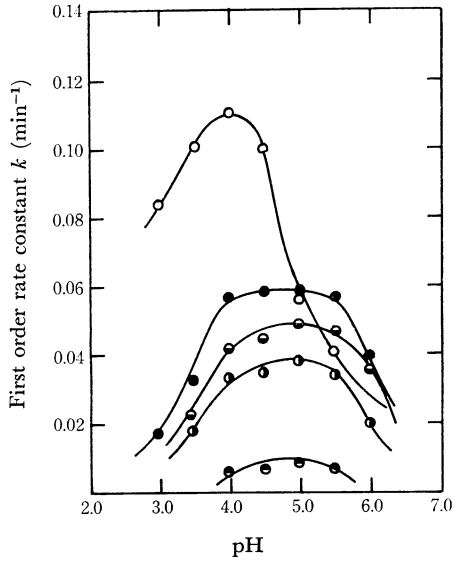


Fig. 2. Effect of pH of the metal-ion-catalyzed decorboxylation of oxaloacetic acid.
○: Cu (2×10^{-4}M); ●: Ni (4×10^{-4}M)
◒: Zn (4×10^{-4}M); ◑: Co (4×10^{-4}M)
◓: Mn (4×10^{-4}M); oxaloacetic acid: 4×10^{-3}M

Table 1. Effect of ligand on catalytic activity of metal ion

Metal ion	Moles of ligand (per mole of metal)	k min^{-1} $\times10^2$	k_0 min^{-1} $\times10^2$ (no ligand)	Relative rate
Ni^{2+}	1 gly	5.84	5.96	0.98
	1 cys	5.95	5.96	~1
	2 cys	5.90	5.96	~1
	1 AA	5.66	5.96	0.95
	2 AA	5.36	5.96	0.90
	2 imid	5.42	5.96	0.91
	2 CN^-	4.41	5.96	0.74
	1 trien	3.53	5.96	0.59
Cu^{2+}	1 gly	5.66	5.78	0.98
	1 cys	5.26	5.78	0.91
	1 AA	5.20	5.78	0.90
	2 AA	4.79	5.78	0.86
	2 imid	5.32	5.78	0.92
	1 en	4.28	5.78	0.74
	1 dien	1.56	5.78	0.27
	1 trien	1.04	5.78	0.18

pH: 5.00; Ni^{2+}: 4×10^{-4}M; Cu^{2+}: 2×10^{-4}M; oxaloacetic acid: 4×10^{-3}M; gly: glycine; cys: cystein; AA: acetylacetone; imid: imidazole; en: ethylenediamine; dien: diethylenetriamine; trien: triethylenetetramine.

TABLE 2. ENHANCEMENT OF THE CATALYTIC ACTIVITY OF METAL ION WITH LIGAND

Moles of ligand (per mole of metal)	Mn^{2+} pH=5.00	Co^{2+} pH=5.00	Ni^{2+} pH=5.00	Cu^{2+} pH=5.00	Cu^{2+} pH=4.00	Zn^{2+} pH=5.00
no ligand	0.815*	3.83	5.96	5.78	11.1	4.90
1 phen	1.10 (1.35)**	6.05 (1.58)	14.4 (2.41)	4.60 (0.80)	8.15 (0.73)	6.66 (1.36)
2 phen	<1.10***	5.29 (1.38)	17.3 (2.90)	2.05 (0.35)	3.69 (0.33)	6.17 (1.26)
3 phen	<1.10***	1.00 (0.26)	1.49 (0.25)	—	—	2.16 (0.44)
1 dipy	1.01 (1.24)	6.01 (1.57)	10.7 (1.80)	5.79 (~1.0)	11.0 (1.0)	7.11 (1.45)
2 dipy	1.07 (1.31)	6.00 (1.57)	13.4 (2.08)	4.90 (0.85)	8.40 (0.76)	7.69 (1.57)
3 dipy	1.07 (1.31)	3.41 (0.89)	1.85 (0.31)	—	—	6.22 (1.27)
1 tpy	—	3.81 (~1.0)	4.11 (0.69)	—	6.66 (6.66)	4.36 (0.89)
1 hid	0.830 (~1.0)	3.64 (0.95)	5.24 (0.88)	9.77 (1.69)	—	5.00 (~1.0)
1 trien	8.90 (10.9)	3.41 (0.89)	3.49 (0.59)	2.11 (0.18)	—	9.09 (1.95)

* (first order rate constant) $\times 10^2$ min^{-1}

** enhancement rate

*** not obey to the first order reaction; metal ion (except of Cu): 4×10^{-4}M; Cu: 2×10^{-4}M; oxaloacetic acid: 4×10^{-4}M; phen: *o*-phenanthroline; dipy: α,α′-dipyridyl; typy: α,α′,α″-terpyridyl; hid: histidine.

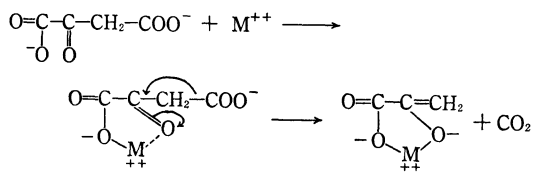
loacetic acid depends largely upon the pH value. This dependency can be caused by the fact that the rate depends on the concentration of oxaloacetic acid anions.[2] The first order rate constants of metal-ion-catalyzed decarboxylation of oxaloacetic acid also depend on pH (Fig. 2). Catalysis with manganous, cobaltous, nickel and zinc ions give maximum near pH 5.00 and that cupric ion, at pH 4.00. The decrease in the catalytic activity above the optimum pH may be due to the formation of metal hydroxide, besides decrease in hydrogen ion concentration. Because copper hydroxide is more stable than the other metal hydroxides, the decrease of the catalysis begins in a lower pH region. The catalytic activities of these metal ions are related to the electronegativities of the metal or the stabilities of the metal-oxaloacetic acid chelates.[4] The order at pH 4.00 is Mn≪Co<Ni<Cu>Zn.

Effect of ligands. The effect of various coordinating agents on the nickel and cupric ion catalysis in decarboxylation of oxaloacetic acid was studied at pH 5.00, and the results are summarized in Table 1. The catalytic activities of nickel and cupric ions are somewhat reduced glycine, cystein and acetylacetone, and strongly hindered with ethylenediamine (en), diethylenetriamine (dien), triethylenetetramine (trien) and cyanide. The phenomena can be explained by the fact that the latter chelates are more stable than the former. Steinberger and Westheimer[2] have reported that the ligands, which do not destroy the charge on the cupric ion, do not destory the catalytic activity. However, in the present experiment, even neutral ligands, such as en, dien, trien and imidazole, destroy the catalytic activities of metal ions. The decrease in the catalytic activities may result from the fact that the electronegativities of metal ions are decreased by the coordination of complexing agents, and the stability of the metal-substrate compounds is lowered with competition with the complexing agents.

Specific Enhancement of Metal Ions with α,α′-Dipyridyl and *o*-Phenanthroline. In contrast to the above complexing agents, there are special cases where the catalytic activities of metal ions are enhanced by the coordination of ligands. Rund and Plane[3] showed that *o*-phenanthroline enhanced the catalytic activities of manganous, nickel and zinc ions. We also found that α,α′-dipyridyl enhanced the catalysis with divalent ions, but both the complexing agents selectively destroyed the cupric ion catalysis. As seen in Table 2, the catalytic activities of the metal ions, except cupric ion, are enhanced in the presence of two moles or less of these agents per mole of the metal ion, and the enhancement is also related to the electronegativity of the metal ion: The order is Mn<Co<Ni>Zn. But the addition of three moles of *o*-phenanthroline decreases the activities of cobaltous, nickel and zinc ions, and the addition of three moles of α,α′-dipyridyl decreases the activities of cobaltous and nickel ions. The decrease may be caused by the fact that all the coordination sites of the metal ions are occupied with the ligands to form the 1 : 3 chelates. In the case of manganous ion with *o*-phenanthroline and α,α′-dipyridyl, and zinc ion with α,α′-dipyridyl, where the decrease in the activity is not observed, the 1 : 3 chelates are not formed even in the presence of three moles of the agents, because of the low stability of the chelates. Generally, the catalysis of metal chelate compounds is stereo-specific, and the characteristics of the atomic orbital (symmetry, orientation, *etc.*) of the metal ions controls the coordination with substrate. Activation of metal ions with α,α′-dipyridyl and *o*-phenanthroline may be due to the specific configuration of the metal chelates

4) J. F. Speck, *J. Biol. Chem.*, **178**, 315 (1949).

and to the increasing electronegativities of the metal ion coordinated with the ligands. From the fact that the catalysis of metal ions is enhanced even in the 1 : 2 chelates, the metal ions must be six-coordinate and octahedral, in order to bind to a substrate which acts as a bidentate ligand, as follows:[2]



Complex (I) Complex (II)

The selective loss of the activity of cupric ion by the chelation can be subsequently explained by the square-planer configuration (dsp^2) of copper complexes.

As described above, the substrate (oxaloacetic acid) more easily combines to metal ion of octahedral configuration, when the metal ion is coordinated with α,α'-dipyridyl or *o*-phenanthroline molecules. However, basides these ligands, many other complexing agents form the octahedral chelate compounds. The specific enhancement with α,α'-dipyridyl and *o*-phenanthroline must, therefore, result from the change in the electronegativities of metal ions, in addition to the stereo-configuration effect. The electronegativities of metal ions coordinated with these ligands are probably increased by the back donation from an occupied $d\pi$ orbital of the metals to an empty $p\pi$ orbital of nitrogen atoms of the ligands. The increasing electronegativity promotes the decomposition of complex (I) to complex (II), as well as the formation of complex (I), in the mechanism described above, and thus enhances the decarboxylation rate. In copper complexes, the effect of the back donation is overcome by the stereo-configuration effect.

Specific Enhancement of Catalytic Activity of Cupric Ion with Histidine and Its Derivatives. The catalytic activity of cupric ion is selectively enhanced with histidine (hid), histidine methyl ester (ϕ-me) and histamine (him), while the activities of manganous and zinc ions are not affected, and the activities of cobaltous and nickel ions are decreased by them. As shown in Table 3, the increasing order of cupric ion catalysis is as follows: ϕ-me > hid > him > *N*-ace-$\phi \simeq H_2O \simeq$ Bu-$NH_2 \leq$ imid $\leq$ DBA $\gg$ gly-ϕ, where *N*-ace-ϕ is *N*-acetylhistidine; Bu-NH_2: butylamine; imd: imidazole; DBA: diamino-butyric acid; and gly-ϕ: glycylhistidine. Histidine is less effective than histidine methyl ester. This may be attributed to the coordination of carboxyl group of the former. The presence of two moles of histidine or histidine methyl ester per mole of copper ion becomes less effective, but this tendency is not observed even in the presence of three moles of histamine. This indicates that histamine complex is less stable, and explains the fact that histamine is less effective than histidine and histidine methyl ester. Diaminobutyric acid, in which imidazol ring of histidine is replaced by amino group, and *N*-acetylhistidine, in which amino group of histidine is blocked, does not influence the activity. The activity is enhanced neither with imidazole nor normal amine. The results indicate that the catalytic activity of cupric ion is enhanced with ligands containing imidazole ring and amino group, but not with ligands containing only imidazole or only amino group. The fact suggests that the stereo-configuration of copper chelate compound is important for the enhancement of the catalysis. It may be considered that the configuration distorted by the Jahn-Taller effect is favorable to be coordinated with substrate. Imidazole ring is indispensable also to the back donation from a $d\pi$ orbital of copper ion to a $p\pi$ orbital of imidazole nitrogen.

Table 3. Enhancement of catalytic activity of copper ion with ligand

Moles of ligand (per mole of metal)	k min^{-1} $\times 10^2$	k_0 min^{-1} $\times 10^2$ (no ligand)	Relative rate
1 hid-me	10.7	5.78	1.85
2 hid-me	8.44	5.78	1.46
1 hid	9.77	5.78	1.69
2 hid	7.69	5.78	1.33
3 hid	7.05	5.78	1.22
1 him	6.13	5.78	1.06
2 him	6.65	5.78	1.15
3 him	6.65	5.78	1.15
1 *N*-acet-hid	5.66	5.78	0.98
2 *N*-acet-hid	5.54	5.78	0.96
2 Bu-NH_2	5.66	5.78	0.98
2 imid	5.32	5.78	0.92
1 DBA	5.43	5.78	0.94
1 gly-hid	3.12	5.78	0.54

pH: 5.00; oxaloacetic acid: 4×10^{-3}M; Cu: 2×10^{-4}M; hid-me: histidine methyl ester; hid: histidine; him: histamine; *N*-acetyl-hid: *N*-acetyl-histidine; Bu-NH_2: butylamine; imid: imidazol; DBA: diaminobutyric acid; gly-hid: glycyl-histidine.

In contrast to the case of α,α'-dipyridyl and *o*-phenanthroline chelate compounds, the catalytic activities of cobaltous and nickel histidine chelate compounds are lower than those of the metal aquo-ions. The reason for this is not yet evident, but it probably results from the difference in the configuration and/or from the weak back donation in the latter chelate compounds.

Consideration on the Role of Metal Ions as Enzyme Activator. It is interesting to reveal the role of metal ions in the enzyme-catalyzed decarboxylation of oxaloacetic acid. Speck[4] has reported

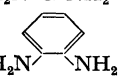
that enzyme of the decarboxylation of oxaloacetic acid required metal ions to have full activity, and in most cases, manganous ion was more effective, while cupric and nickel ions were less effective. The order was Mn>Co>Ni>Cu<Zn. Rund and Plane[3] have described that manganous ion catalysis was enhanced sixteen times or more by the coordination with *o*-phenanthroline, but nickel ion catalysis showed only two-told increase, and they concluded that this activation behavior was almost the same as in the metal-enzyme systems.

In this research, however, the enhancement of manganous ion catalysis is the smallest among metal ions studied in either case of α,α'-dipyridyl or of *o*-phenanthroline (*cf.* Table 2), and the situation is not the same as in the enzyme system. The difference between Rund and Plane's result and the present result might be due to experimental conditions, especially to the metal ion concentration and accordingly, the ligand concentration. They carried out their experiments with 5.78×10^{-2}M manganous ion and with 8.5×10^{-5} or 2.64×10^{-3}M nickel ion, but we kept the metal ion concentration constant, 4×10^{-4}M, because α,α'-dipyridyl and *o*-phenanthroline acted as catalyst, when the concentration exceeded 10^{-3}M. Rund and Plane did not consider the catalysis of the ligands and so they might have overestimated the enhancement of manganous ion catalysis.

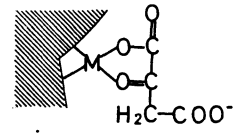
As described above, the enhancement of metal-ligand systems and metal-enzyme systems appears different, that is, manganous ion is not as effective in the former system, but very available in the latter. This may lead to the idea that in enzyme system, metal ions are enzyme activator rather than catalyst and may change the configuration of enzyme protein favorable to interact with substrate.

In order to examine whether enzyme activity is correlated with the configuration or not, the catalysis of some compounds containing amino groups considered to be an active site of enzyme,[5] was studied. Table 4 shows the first-order rate constant of decarboxylation reaction in the absence of any metal ions. As seen in the table, the catalysis is not always positively related with the number of amino groups, but the catalytic activity of diamino compounds remarkably depends upon the apparent distance between two amino groups. Ethylenediamine is most active; urea, in which two amino radicals combine the same carbon atom, and trimethylenediamine and pentamethylenediamine, in which three or five carbon atoms keep away two amino radicals, are less effective. This strongly suggests that catalytic activity is very sensitive to the setero-configuration of enzyme, or to the configuration of active sites.

TABLE 4. CATALYTIC ACTIVITY OF THE COMPOUND CONTAINING AMINE GROUPS

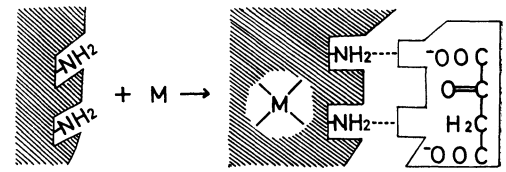
Compound (4×10^{-4}M)		k min^{-1} $\times10^2$
urea	$H_2N-\overset{\overset{O}{\|}}{C}-NH_2$	0.29
PDA		1.58
en	$H_2H-CH_2-CH_2-NH_2$	3.44
TMDA	$H_2N-CH_2-CH_2-CH_2-NH_2$	0.44
PMDA	$H_2N-(CH_2)_5-NH_2$	0.35
Bu-NH_2	$CH_3-(CH_2)_3-NH_2$	0.30
dien	$H_2N-(CH_2)_2-NH-(CH_2)_2-NH_2$	1.56
trien	$(H_2N-(CH_2)_2-NH-CH_2)_2-$	8.66

Oxaloacetic acid: 4×10^{-3}M
pH: 5.00



Enzyme-Metal-Substrate

Model I



Apoenzyme Enzyme Substrate

Model II

Fig. 3. Models for the activation of an enzyme with a metal ion.

From the above results, it seems reasonable to assume that enzyme activation results from the change in the configuration of enzyme caused by the coordination of metal ions (model II in Fig.3), but not from the increase in the metal ion catalysis caused by the coordination of enzyme (model I in Fig. 3). It is not true that electronegativity of manganous ion increases much in the presence of enzyme and becomes greater than that of nickel or cupric ions.

Although model II is no more than imagination at the present time, it is expected to elucidate the role of metal ions in enzymatic reaction.

5) S. Warren, B. Zerner, F. H. Westheimer, *Biochimia*, **5**, 817 (1966).