SOME PROPERTIES OF THE MANGANESE AND COPPER CATALYZED OXIDATION OF CATECHOL AND SOME OTHER *ORTHO*-DIHYDROXYBENZENE DERIVATIVES*

by

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It has been shown by CHAIX *et al.*[1,2,3] that manganese and copper catalyze the oxidation of adrenaline and noradrenaline. In a previous paper, we have demonstrated that these metallic ions catalyze the oxidation of catechol, DOPA, N-methyl-DOPA 3-hydroxytyramine and epinine[4] in slightly alkaline solutions. It was also shown that the metallic ion catalyzed reaction differs from the tyrosinase reaction in a number of ways. The purpose of this investigation was to determine some of the properties of the metallic ion catalyzed reactions.

METHODS AND MATERIALS

The conventional WARBURG technique was used in this investigation to follow the rate of oxidation. All solutions were made with demineralized distilled water. The $MnSO_4$ and $CuSO_4$ solutions were made from C.P. grade metallic salts. The *o*-dihydroxybenzene derivatives were either obtained from commercial houses or were synthesized as were previously described[4].

In order to determine whether chelates were formed between the metallic ions and the *o*-dihydroxybenzene derivatives two methods were used. A Beckman spectrophotometer, Model D.U., was used to determine the shift in the ultra-violet spectrum due to chelate formation. The other method was the manometric method used by CHAIX *et al.*[2]. For this method a 0.03 *N* $NaHCO_3$ solution was equilibrated with a 5 % CO_2–95 % N_2 atmosphere forming a bicarbonate buffer of pH 7.6.

EXPERIMENTAL

The effect of various buffers on the manganese catalyzed oxidation of DOPA and of catechol

Using a phosphate buffer CHAIX *et al.* found that Mn^{++} catalyzed the oxidation of adrenaline only after a lag period of several minutes had elapsed[1]. Since no lag period was apparent during the Mn^{++} catalyzed oxidation of catechol or DOPA in a veronal buffer, the effect of various buffers on the Mn catalyzed reaction was studied. It was found that Mn^{++} had the greatest catalytic effect in either veronal or tris (hydroxymethyl) aminomethane buffers. Glycine, phosphate and citrate buffers inhibited the Mn^{++} catalyzed oxidation of DOPA but had little effect on the autoxidation of DOPA (Table I).

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Consequently, these buffers probably form complexes with the manganese, thereby decreasing the effective concentration of the catalyst. When borate buffers were used both the Mn^{++} catalyzed oxidation and the autoxidation of DOPA were inhibited. Since borate is known to form chelates with ene-diols, it is likely that the borate inhibited reaction could be explained at least in part by the formation of the borate-DOPA chelate thereby decreasing the effective concentration of the substrate.

The Mn^{++} catalyzed oxidation of catechol was also inhibited by glycine and phosphate buffers. On the other hand, when a small amount of threonine (twenty micromoles) was added to the veronal buffer, the rate of the Mn^{++} catalyzed oxidation of catechol was increased about twenty percent.

TABLE I

EFFECT OF VARIOUS BUFFERS ON THE Mn^{++} CATALYZED OXIDATION OF DOPA

Buffer	*pH*	*Mn^{++} (M)*	*O_2 (µl)*	*Inhibition %*
Veronal acetate	7.88	—	71	
	7.88	0.0005	389	—
Tris	7.95	—	63	
	7.95	0.0005	374	2
Sodium citrate	7.92	—	51	
	7.92	0.0005	110	81
Sodium phosphate	7.88	—	69	
	7.88	0.0005	146	76
Glycine·NaCl	7.9	—	51	
	7.9	0.0005	281	28
Sodium borate	7.88	—	0	
	7.88	0.0005	18	94

Conditions: 0.005 *M* (10 µ*M*) DOPA; 0.05 *M* buffer; KOH in center well; temperature, 37° C; time, 1 h.

Effect of various Mn^{++} and Cu^{++} concentrations on the rate of oxidation of 3-hydroxytyramine and of DOPA

Both the rate and the total amount of oxygen absorbed were found to be altered by varying the Mn^{++} concentration. Using ten micromoles of 3-hydroxytyramine ($5 \cdot 10^{-3}$ *M* final concentration) buffered at pH 7.9., the rate of oxygen absorption was increased as the manganese concentration was increased to 0.5 micromole ($2.5 \cdot 10^{-4}$ *M* final concentration). The rate of oxidation was increased only slightly by higher manganese concentrations (Table II). The total amount of oxygen absorbed was inversely proportional to the concentration of Mn^{++}. Similar results were obtained for the Mn^{++} catalyzed oxidation of DOPA.

During the Cu^{++} catalyzed oxidation of 3-hydroxytyramine, it was found that the the rate of oxygen uptake was increased as the Cu^{++} concentration was increased to $2.5 \cdot 10^{-3}$ *M*. Higher Cu^{++} concentrations decreased the total amount of oxygen absorbed during the course of the reaction.

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TABLE II

EFFECT OF VARIOUS Mn^{++} AND Cu^{++} CONCENTRATIONS ON THE OXIDATION OF 3-HYDROXYTYRAMINE

Metal ion Conc. (M)	*O_2 uptake*		*Metal ion Conc. (M)*	*O_2 uptake*	
	Mn^{++} (µl)	*Cu^{++} (µl)*		*Mn^{++} (µl)*	*Cu^{++} (µl)*
—	61	82	$5.0 \cdot 10^{-4}$	429	163
$2.5 \cdot 10^{-5}$	135	—	$1.0 \cdot 10^{-3}$	473	202
$5.0 \cdot 10^{-5}$	182	91	$2.5 \cdot 10^{-2}$	388	293
$1.0 \cdot 10^{-4}$	251	106	$5.0 \cdot 10^{-2}$	—	254
$2.5 \cdot 10^{-4}$	363	127			

Conditions: 0.075 *M* veronal, pH 8.1; 0.005 *M* (10 µ*M*) 3-hydroxytyramine·HCl; KOH in center well; atmosphere, air; temperature, 30° C; time, 90 min.

The formation of chelates

In order to determine if a chelate were formed between DOPA and the metallic ions, the ultra-violet spectrum of a DOPA solution was compared with the ultra-violet spectrum of a solution containing DOPA and the metallic ions. The spectrum of the DOPA solution containing Mn^{++} was identical with the ultra-violet spectrum of a DOPA solution alone. On the other hand there was a marked shift in the spectrum of the DOPA solution containing Cu^{++} (Fig. 1). These data indicate that a chelate was formed between Cu^{++} and DOPA but that no chelate was formed between DOPA and Mn^{++}.

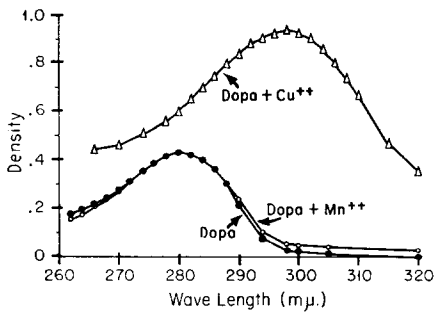

Fig. 1. Absorption spectra of DOPA with metallic ions. Conditions: 0.006 *M* veronal, pH 7.5; $1.6 \cdot 10^{-4}$ *M* DOPA; $1.6 \cdot 10^{-4}$ *M* metallic ions.

Since the spectrum of a chelate is not necessarily different from the spectrum of the ligand, the manometric method used by CHAIX *et al.*[2] was used to corroborate the spectrophotometric method. The metallic ions were equilibrated with the 0.03 *M* $NaHCO_3$ buffer in an atmosphere of 5% CO_2–95% N_2. During this phase carbon dioxide was evolved due to the formation of the metallic hydroxyides and carbonates. Under these conditions the amount of carbon dioxide evolved on the addition of Mn^{++} was negligible indicating that Mn^{++} did not form hydroxides or carbonates to any appreciable extent.

A blue-green precipitate was formed when Cu^{++} was added to the bicarbonate buffer. If this precipitate were $CuCO_3$ the amount of carbon dioxide evolved would be one micromole of carbon dioxide for every micromole of Cu^{++}. Two micromoles of carbon dioxide would have been evolved if the precipitate were $Cu(OH)_2$, while 1.5 micromoles of carbon dioxide would have been evolved if the precipitate were $CuCO_3 \cdot Cu(OH)_2$. CHAIX *et al.* found that one micromole of carbon dioxide was evolved for every micromole of Cu^{++} added. Under the conditions which we used, however, 1.5 micromoles of carbon dioxide were evolved, indicating that the precipitate was probably $CuCO_3 \cdot Cu(OH)_2$.

When catechol, DOPA, N-methyl DOPA or 3-hydroxytyramine·HCl was added to the Mn^{++}-bicarbonate mixture, little or no carbon dioxide was evolved, indicating little if any chelate formation between Mn^{++} and these *o*-dihydroxybenzene derivatives

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(Table III). Using this method, CHAIX *et al.* showed that there was very little chelation between Mn^{++} and adrenaline.

TABLE III

THE AMOUNT OF CO_2 EVOLVED DUE TO THE FORMATION OF METAL CHELATES

Ligand	*Ligand* μmoles	*Metal* μmoles	Mn^{++} μl CO_2	Co^{++} μl CO_2	Cu^{++} μl CO_2
Catechol	4	2	4	3	68
DOPA	4	4	0	30	122
N-methyl-DOPA	4	4	1	11	131
3-hydroxytyramine	5	5	3	13	99

Conditions: 0.03 *M* $NaHCO_3$ (pH 7.6); Atmosphere, 5% CO_2–95% N_2; temperature 30°. Total vol. 2.0 ml.

On the other hand, when catechol was added to $CuCO_3 \cdot Cu(OH)_2$ the precipitate dissolved and a large evolution of carbon dioxide was observed. Similar results were obtained when DOPA, N-methyl DOPA and 3-hydroxytyramine·HCl were added to $CuCO_3 \cdot Cu(OH)_2$ (Table III). This would indicate that chelates were formed between Cu^{++} and these compounds, corroborating the results obtained spectrophotometrically.

In order to determine the catechol/Cu^{++} ratio in the chelate the amount of Cu^{++} was varied. On the addition of catechol to the $CuCO_3 \cdot Cu(OH)_2$ the amount of carbon dioxide evolved due to the formation of the chelate was increased as the $CuCO_3 \cdot Cu(OH)_2$ was increased until the catechol/Cu^{++} ratio equaled two (Table IV). When the catechol was added to higher concentrations of Cu^{++}, the amount of carbon dioxide evolved due to the chelate formation was not affected. Therefore, the chelate contained two molecules of catechol for every Cu^{++} ion. In those vessels containing twice as much catechol as copper, the total amount of carbon dioxide was determined. Since the total carbon dioxide/Cu^{++} ratio was about 3.5, the chelate formed was probably $Cu(Catechol)_2$.

When DOPA or N-methyl DOPA were added to the $CuCO_3 \cdot Cu(OH)_2$ more carbon dioxide was evolved than when 3-hydroxytyramine·HCl was added to it (Table III). This would indicate that Cu^{++} formed a chelate with DOPA through the amino and the carboxyl groups as well as through the phenolic groups.

When 3-hydroxytyramine·HCl was added to various amounts of $CuCO_3 \cdot Cu(OH)_2$, the carbon dioxide evolved was stabilized when the 3-hydroxytyramine·HCl/Cu ratio was two (Table IV). Since the catechol/Cu ratio was also two, this would indicate that Cu^{++} cannot combine with the amino group to any great extent, under these conditions. On the other hand, when DOPA was added to various amounts of $CuCO_3 \cdot Cu(OH)_2$, the carbon dioxide was not stabilized when the DOPA/Cu ratio was two. This would indicate that a chelate was formed between Cu^{++} ion and the carboxyl groups of DOPA.

The effect of manganese dioxide on catechol

Since the manganous ion probably changes valence during the course of the Mn^{++} catalyzed oxidation of catechol, it was considered that a fairly stable chelate might be formed between catechol and Mn^{+3} or Mn^{+4}. In order to determine if such a chelate could be formed, catechol was allowed to react with manganese dioxide in the following manner.

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TABLE IV

THE EFFECT OF VARIOUS Cu^{++} CONCENTRATIONS ON THE CO_2 EVOLVED DURING Cu^{++} CHELATE FORMATION

Cu^{++} conc. (μmoles)	*Catechol 2 μmoles (μl CO_2)*	*DOPA 4 μmoles (μl CO_2)*	*3-Hydroxytyramine 10 μmoles (μl CO_2)*
0.5	24	30.7	37
0.75		33.8	
1.0	33	40.3	
1.5		59	88
2.0	35	72	
2.5		90	
3.0		102	159
4.0	35		201
5.0		133	214
10.0			197
20.0			169

Into vessels containing one micromole of potassium permanganate dissolved in 0.03 *M* sodium bicarbonate buffer under a 5% CO_2–95% N_2 atmosphere at 30° C., various amounts of manganese sulfate were added. A dark precipitate was formed and carbon dioxide was evolved. When 1.5 micromoles of manganese sulfate were added the amount of carbon dioxide evolved indicated that the precipitate was manganese dioxide.

a. $MnO_4^- + 1.5\ Mn^{++} + H_2O \longrightarrow 2.5\ MnO_2 + 2H^+$
b. $2H^+ + 2\ HCO_3^- \longrightarrow 2\ CO_2 + 2\ H_2O$

sum: $MnO_4^- + 1.5\ Mn^{++} + 2\ HCO_3^- \longrightarrow 2.5\ MnO_2 + 2\ CO_2 + H_2O$

When more than 1.5 micromoles of manganese sulfate were added, the amount of carbon dioxide evolved indicated that the following reactions took place.

c. $4\ MnO_4^- + 11\ Mn^{++} + 9\ H_2O \longrightarrow 5\ MnO_2 \cdot Mn_2O_3 + 18\ H^+$
d. $18\ H^+ + 18\ HCO_3^- \longrightarrow 18\ CO_2 + 18\ H_2O$

sum: $4\ MnO_4^- + 11\ Mn^{++} + 18\ HCO_3^- \longrightarrow 5\ MnO_2 \cdot Mn_2O_3 + 18\ CO_2 + 9\ H_2O$

When catechol was added to the insoluble manganese oxides, a rapid absorption of carbon dioxide was observed as the oxides dissolved. After about ten minutes a slow evolution of carbon dioxide was observed and a white precipitate was formed (Fig. 2). The color of the solution changes to a faint yellow when the catechol is added either to the potassium permanganate or to the insoluble manganese oxides.

Although the amount of catechol added was varied from 2.5 to 10 micromoles, neither the rate of carbon dioxide absorbed during the first ten minutes nor the subsequent rate of carbon dioxide evolution was affected. This would indicate that no stable chelate was formed between the catechol and Mn^{+3} or Mn^{+4}.

After the system came to equilibrium the total amount of carbon dioxide evolved was determined.

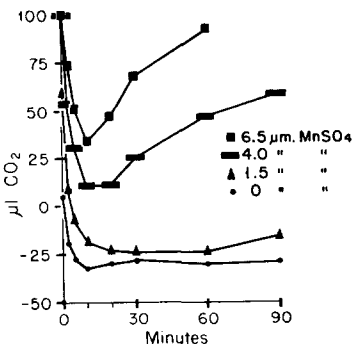

Fig. 2. The effect of $KMnO_4$ and the manganese oxides on catechol. Conditions: 0.03 *M* $NaHCO_3$; Atmosphere. 5% CO_2–95% N_2; pH 7.6; 1 μM. $KMnO_4$; Mn^{++} as indicated; $5 \cdot 10^{-3}$ *M* (10 μM) catechol; temp.. 30° C.

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Although this quantity of carbon dioxide was independent of the catechol concentration, it was dependent on the Mn^{++} concentration according to the following relationship.

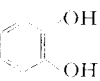
$$\frac{3 \text{ micromoles } CO_2 - \text{total } CO_2 \text{ evolved}}{\text{Total } Mn^{++} \text{ in micromoles}} = 1$$

In this equation the three micromoles of carbon dioxide represents the carbon dioxide absorbed during the oxidation of catechol by either potassium permanganate or the manganese oxides, according to the following equations.

e. $MnO_4^- + 1.5\ Mn^{++} + 2\ HCO_3^- \longrightarrow 2.5\ MnO_2 + 2\ CO_2 + H_2O$

f. $2.5\ MnO_2 + 2.5$  $\longrightarrow 2.5\ Mn^{++} + 2.5$  $+ 5\ OH^-$

g. $5\ OH^- + 5\ CO_2 \longrightarrow 5\ HCO_3^-$

sum: $MnO_4^- + 2.5$  $+ 3\ CO_2 \longrightarrow Mn^{++} + 2.5$  $+ 3\ HCO_3^- + H_2O$

In another experiment, it was found that sodium fluoride, which forms a complex with manganous ions, markedly inhibited the rate of the carbon dioxide evolution which occurred after the first ten minutes. It did not affect the rate of carbon dioxide absorbed during the first ten minutes.

In conclusion, it can be stated that manganese dioxide and probably manganese trioxide oxidize catechol. The evolution of carbon dioxide which follows this oxidation is due to either the formation of insoluble manganese carbonate or to the formation of a chelate between the manganous ion and an oxidation product of catechol.

SUMMARY

The type of buffer used had a profound effect on the Mn^{++} catalyzed oxidation of the *ortho*-dihydroxybenzene compounds used. Veronal or tris (hydroxymethyl) amino methane were found to be the most suitable buffers for the study of these reactions. Glycine, phosphate, citrate and borate buffers inhibited the reaction.

The optimum Mn^{++} concentration for the 3-hydroxytyramine and DOPA oxidation was $2.5 \cdot 10^{-4}$ *M*. Higher Mn^{++} concentrations increased the rate of oxidation only slightly. The optimum Cu^{++} concentration was found to be $2.5 \cdot 10^{-3}$ *M*.

Under the conditions of these experiments chelates were formed between Cu^{++} and catechol, DOPA, N-methyl DOPA and 3-hydroxytyramine. No chelation was detected between Mn^{++} and these compounds.

Catechol was oxidized by MnO_2 and $KMnO_4$.

RÉSUMÉ

Le type de tampon employé a une grande importance dans l'oxydation des orthodiphénols étudiés, catalysée par le Mn^{++}. Les tampons véronal ou tris (hydroxyméthyl) amino méthane sont les tampons les plus convenables pour l'étude de ces réactions. Les tampons glycocolle, phosphate, citrate et borate inhibent la réaction.

La concentration optimum en Mn^{++} pour l'oxydation de la 3-hydroxytyramine et de la DOPA est $2.5 \cdot 10^{-4}$ *M* des concentrations plus élevées en Mn^{++} n'augmentent que faiblement la vitesse d'oxydation. La concentration optimum en Cu^{++} est $2.5 \cdot 10^{-3}$ *M*. Dans les conditions expérimentales employées, des chélates sont formés entre le Cu^{++} et le catéchol, la DOPA, la N-méthyl DOPA et la 3-hydroxytyramine. Il n'y a pas de chélation entre Mn^{++} et ces corps.

Le catéchol est oxydé par MnO_2 et par $KMnO_4$.

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ZUSAMMENFASSUNG

Die Art des verwendeten Puffers beeinflusst die von Mn^{++} katalysierte Oxydation der benutzten *ortho*-Dihydroxybenzol-Verbindungen stark. Veronal oder Tris(hydroxymethyl)aminomethan eigneten sich am besten als Puffer, bei dem Studium dieser Reaktionen. Glykocoll, Phosphat, Citrat und Borat Puffer hemmten die Reaktion.

Das Mn^{++} Konzentrationsoptimum lag für die Oxydation von 3-Hydroxytyramin und DOPA. bei $2.5 \cdot 10^{-4}$ *M*. Höhere Mn^{++} Konzentrationen erhöhten die Oxydationsgeschwindigkeit nur wenig. Das Konzentrationsoptimum für Cu^{++} wurde bei $2.5 \cdot 10^{-3}$ *M* gefunden. Unter den Versuchsbedingungen bildeten sich zwischen Cu^{++} und Katechol, DOPA, N-methyl DOPA und 3-Hydroxytyramin Chelate. Kein Chelation wurde entdeckt zwischen Mn^{++} und diesen Verbindungen.

Katechol wurde oxydiert durch MnO_2 und $KMnO_4$.

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