Studies on a Polyphenolase in *Scopolia japonica* I

On the Oxidation of Catechol*

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鈴木米三： ハシリドコロのポリフェノラーゼの研究 I. カテコールの酸化について

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James and co-workers¹⁾ found a polyphenolase in the leaves of *Atropa belladonna*. In the presence of this polyphenolase, catechol is oxidized and when amino acids are present, an intensively coloured compound, red pigment, is formed by the combination of equimolecular proportions of resulted o-quinone with amino acids. The formation of this pigment was also observed by Trautner and Roberts²⁾ by using a polyphenolase from the leaves of *Duboisia myoporoides*. The quinonoid red pigment has an interesting character; it catalyzes the oxidation of amino acids. A cycle of reactions, which, starting with the combination with one further molecule of amino acid, continues with liberation of ammonia and a keto acid was confirmed by James and co-workers, and Trautner and Roberts.

The mechanism as such was also proposed by James³⁾ as a process in the biosynthesis of 1-hyoscyamine in *Atropa belladonna*. The author has an interest in this problem, so as a first step of the research some characters of a polyphenolase from the subterranean stem of *Scopolia japonica* belongs to same *Solanaceae* are investigated.

Material and Method

The subterranean stems of *Scopolia japonica* were washed and peeled. About 30g. of this material was blended with 150 ml. of cold phosphate buffer (M/30). It was filtered with cheese cloth (gauze), centrifuged for 30 min. at 3500-4000 r.p.m. and a white precipitate, which showed positive iodostarch reaction and gave negative colouration with Millon's reagent, was discarded. The supernatant was used as the crude polyphenolase solution. The activity of the enzyme was determined by measuring oxygen uptake in the Warburg apparatus at 35° C and pH 7.3, taking 1.0 ml. of the enzyme solution, 1.0 ml. of phosphate buffer (M/15), 1.0 ml. of inhibitor and 0.5 ml. or 1.5 ml. of distilled water in the main chamber and 0.5 ml. of substrate solution in the sidearm of a flask, the total volume of liquids being made to 4.0 ml.

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The center well contained 0.3 ml. of 10% KOH. The gas phase was air.

Results and Discussion

1. Oxidation of Phenols.

The activities of the crude polyphenolase to oxidize various phenols are shown in Fig. 1. The highest O_2 uptake was found in the oxidation of catechol. This

Fig. 1. Oxygen-uptake by various polyphenols catalyzed by the crude polyphenolase.
(The final concentration of substrates was 1.25×10^{-3} M. L-tyrosine was used as suspension.)

initial highest rate, however, slows down quite soon. DL-Dihydroxyphenylalanine (DOPA) and pyrogallol were oxidized readily (DOPA was converted into reddish black substance, melanine?), phloroglucinol and hydroquinone at slower rates, while resorcinol, L-tyrosine, phenol and p-cresol were not oxidized at all. A insoluble form of polyphenolase, which was obtained by treating the leaves of *Atropa belladonna* with acetone, is reported to oxidize p-cresol¹¹. By the use of the present material, however, p-cresol could not be oxidized. It may be said that monophenolase is absent from the present material.

2. Effects of Inhibitions.

The effects of various inhibitors on the oxidation of catechol and phloroglucinol were examined by determining the oxygen uptake. The results are listed in Table

Table I. Effects of Inhibitors on the activity of the crude polyphenolase.

Inhibition %			
Inhibitors	Final concentration	Catechol (10min.)	Phloroglucinol (60 min)
Cyanide.....	$0.25 \times 10^{-2}M$	96	81
Azide	$0.25 \times 10^{-1}M$	57 red	17
Dieca	$0.25 \times 10^{-2}M$	59	95
"	$0.25 \times 10^{-3}M$	35 red	
Thiourea	$0.25 \times 10^{-2}M$	0 red	53
8-oxyquinoline	satu/4	0 red	
P-nitrophenol	satu/4	0 red	
Iodide	$0.25 \times 10^{-2}M$	0	0
Ferrocyanide	$0.25 \times 10^{-2}M$	6 red	90
Thiosulfate	$0.25 \times 10^{-2}M$	0	0
Octyl-alcohol	satu/4	0 red	0
Malachite green	$0.25 \times 10^{-2}M$	12 ?	
(oxalate)			
Ferricyanide.....	$0.25 \times 10^{-2}M$	8 red	
Silver nitrate	$0.25 \times 10^{-2}M$	24 red-black	
" "	$0.25 \times 10^{-3}M$	5 red-black	
Monoiodoacetate	$1.25 \times 10^{-2}M$	14 red	
Maleic acid	$0.25 \times 10^{-2}M$	0 red	
aa'-Dipyridyl	$0.25 \times 10^{-2}M$	0 red	
2. 4. Dinitrophenol	$0.25 \times 10^{-3}M$	0 red	0

The final concentration of substrate: $1.25 \times 10^{-3}M$.

(oxygen-uptake in catechol only)-(oxygen-uptake in catechol
plus inhibitor)

$$\% \text{ Inhibition} = 100 \times \frac{\text{(oxygen-uptake in catechol only)}}{\text{(oxygen-uptake in catechol plus inhibitor)}}$$

I. The oxidation of these substrates was strongly inhibited by cyanide. Sodium diethyldithiocarbamate (dieca) inhibited strongly the oxidation of phloroglucinol, but it was less effective upon catechol. Thiourea inhibited 50% of the oxidation of phloroglucinol, but it did not inhibit the oxidation of catechol.

In the poisoning effect of azide, an agent also sensitive to copper enzymes was, however, rather weak even at a higher concentration. This might be due to the medium being neutral⁴⁾. 8-Oxyquinoline and p-nitrophenol⁵⁾ were found not to be effective upon the oxidation of catechol. Iodide, ferrocyanide and thiosulphate are known to combine with copper ions in neutral solutions. Among these, ferrocyanide inhibited considerably the oxidation of phloroglucinol, but it inhibited the catechol oxidation quite a little. Iodide and thiosulphate, on the other hand, were not effective upon the oxidation of these substrates.

Ferricyanide, silver nitrate, monoiodoacetate and maleic acid are used generally for the purpose of detecting SH groups in enzyme protein, but in this experiment

their effect on the oxidation of catechol was not remarkable.

The results obtained in the present work as well as those of Barron and Singer⁶⁾ may suggest that SH groups are not indispensable for the activity of polyphenoloxidase.

Fig 2. Effects of thiosulphate, dieca and cyanide on the oxidation of catechol.

(The final concentration of catechol was 1.25×10^{-3} M; the final concentration of thiosulphate, dieca and cyanide was 0.25×10^{-2} M)

Fig 3. Oxygen-uptake by the crude polyphenolase system containing catechol, ornithine and octyl-alcohol as compared with controls.

(Final concentrations; catechol 1.25×10^{-3} M, octyl-alcohol saturate/4, DL-ornithine-HCL 1.25×10^{-2} M)

As a result of studying various inhibitors it was found that the inhibition of oxygen uptake is accompanied by the failure of the production of the red pigment. Among the inhibitors investigated, thiosulphate did not inhibit the oxygen uptake by catechol, but it hindered the production of the red pigment. The curves of oxygen uptake are shown in Fig. 2.

3. Oxidation of amino acid by the red pigment, in the absence of dehydrogenase.

It is generally said today that the first step in the oxidation of an amino acid is dependent on the dehydrogenases⁷⁾.

As shown in Fig. 3 and Table II, the reaction system constituted of ornithine, catechol, inhibitor of dehydrogenase and crude polyphenolase consumed much more oxygen than that of catechol and crude polyphenolase. These results support the conclusion of James and co-workers¹⁾.

Summary

1. A crude polyphenolase

Table II. Oxidation of ornithine in the presence of red pigment.

Exp. No.	Substrate & others	Oxygen-uptake (μ l) in	
		10 min.	60 min.
I.	catechol	54.0	58.5
	catechol+KCN+ornithine	2.6	10.8
	KCN+ornithine	1.1	4.5
	ornithine	1.5	4.0
II.	catechol	39.6	46.3
	catechol+NaN ₃ +ornithine	31.5	56.5
	NaN ₃ +ornithine	1.9	6.3
	ornithine	2.0	5.5
III.	catechol	35.0	43.5
	catechol+M. G.+ornithine	43.1	61.7
	M. G.+ornithine	1.6	3.7
	ornithine	2.0	4.7
IV.	catechol	34.0	41.8
	catechol+AgNO ₃ +ornithine	29.7	51.5
	AgNO ₃ +ornithine	2.0	5.0
	ornithine	2.0	2.5
V.	catechol	30.7	40.7
	catechol+ferricyanide+ornithine	24.2	54.3
	ferricyanide+ornithine	2.4	5.0
	ornithine	2.4	5.1
VI.	catechol	64.3	72.5
	catechol+Na ₂ S ₂ O ₃ +ornithine	63.4	97.1
	catechol+ornithine	67.7	103.1
	ornithine	2.6	5.0

Final concentrations of substrate and other substances were;

catechol, $0.25 \times 10^{-2}\text{M}$; DL-ornithine-HCL, $1.25 \times 10^{-2}\text{M}$; cyanide, $0.25 \times 10^{-2}\text{M}$ azide, $0.25 \times 10^{-1}\text{M}$; malachite green (oxalate) (M. G.), $0.25 \times 10^{-2}\text{M}$; AgNO₃, $0.25 \times 10^{-3}\text{M}$; ferricyanide, $0.25 \times 10^{-2}\text{M}$; Na₂S₂O₃, $0.25 \times 10^{-2}\text{M}$.

has been extracted from the subterranean stem of *Scopolia japonica*, into the phosphate buffer.

2. In the presence of this enzyme catechol takes up oxygen at a high rate, DL-dihydroxyphenyl-alanine and pyrogallol are oxidized rapidly, phloroglucinol and hydroquinone rather slowly, while resorcinol, L-tyrosine, phenol and p-cresol are not oxidized at all.

3. Among the inhibitors of the oxidation of catechol, cyanide is very effective, but sodium diethyldithiocarbamate is less effective.

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Abnormal Eggs from *Coccophora*, with Special Interests in the Origin of Half Embryos.

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中沢信午: スギモクの異常卵と半胚の起原について

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There are six different types concerning the course of ovogenesis in Fucaceae, which are in accordance with the systematic arrangement of those⁹. The genus *Coccophora* is separated from *Sargassum*, *Turbinaria*, *Hizikia*, and species of *Cysiosiphylum*. In the latter a single cell bears eight haploid nuclei in consequence of one hetero- and two successive homoeotypic divisions of a diploid mother nucleus^{1, 8, 9}. While in *Coccophora*, seven of the eight nuclei formed in the same way are destined to degenerate with maturation of the oospore remaining one central nucleus¹¹. The former and the latter are also different in the type of cleavage after fertilization. In *Coccophora*, fertilized eggs cleave directly into two daughter cells¹³, while in *Sargassum* etc., when any one of the eight nuclei is fertilized, the other seven degenerate and disappear sooner or later, and the remaining one takes part in development^{1, 12}. In other words, the difference is attributed to the stage of occurrence of the nuclear degeneration, which takes place before maturation in *Coccophora*, but after fertilization in *Sargassum* etc. However, as was studied

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