On the Respiratory System of Lysichiton Spadix

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桑山弥寿男：ミズバショウの肉穂花序の呼吸系について

Introduction

Since the discovery by James et al. (1950)[1,2] that the rapid oxidation processes characteristic of *Arum* spadix tissues are not significantly influenced by cyanide, there have been furnished a series of evidences to demonstrate that the respirations in spadices of *Symplocarpus*[13,21], *Philodendron* and *Paltandra*[11] are also insensitive to cyanide. But the respiratory characteristics of an aroid species *Zantedeschia* spadix are similar to those of most other plant tissues, and such characteristics are in marked contrast to the respiratory pattern reported on other aroid spadices[15]. The respiration of spadix tissues of *Lysichiton* (Mizubasho in Japanese) growing wild in Hokkaido, Japan is not only uninhibited by cyanide but positively markedly stimulated[24].

With a view to making clear an atypical respiratory mechanism of aroid spadices, there have been a number of investigation conducted[4,5,7–13,17,19,21,22] and reviews published [3,14,16]. As an explanation for the mechanism of such cyanide insensitive respiration observed in most aroid spadices, "alternate pathway" and "excess oxidase" theories have been postulated, but, these theories can not explain the mechanism of cyanide stimulative respiration of *Lysichiton* spadix.

The present paper is concerned with the respiratory properties of *Lysichiton* spadix tissues. Atypical respiratory characteristics of these tissues are discussed in connection with the mechanism of stimulation by cyanide addition, and are explained in terms of the specific natures of terminal oxidative systems.

Materials and Methods

Materials were collected from *Lysichiton camtschatcense* Schott (Mizubasho in Japanese) growing wild in Hokkaido, Japan, during April, May and June in 1959, 1960 and 1961 respectively, and were stored at about -4°C. The classification of growing stages of the spadix is as follows. The stage of "young spadix" (the spadix is wrapped tightly in the spath), the stage of "prior flowering" (just prior to the opening of the spath), the stage of "in flowering" (anthers and pistils protrude) and the stage of "after flowering" (pollens were scattered).

Spadix slices (approx. 0.2 mm thick) were cut from fresh spadix tissues, central rachis

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being separated. Leaf slices (approx. 1 mm × 10 mm) used for comparison were chopped from leaves of other plants.

Mitochondrial preparation was obtained from spadix without central rachis at a stage of "prior flowering". Spadix tissues (100g) were hand-ground with sand in a medium (100 ml) containing 250 mM sucrose, 20 mM phosphate buffer (pH 7.0), 1 mM sodium floride and 1 mg/1 ml ovalbumin. All manipulations were carried out at about 0° C. The homogenates were strained through cheesecloth and centrifuged at 3,500 r. p. m. for 5 minutes to remove the cell debris and the sand. The remaining supernatant was centrifuged at 10,000 r. p. m. for 15 minutes and the pellet was washed by centrifugation and finally suspended in a small volume of isolating medium. These suspended particles were referred to as the mitochondria.

To prepare acetone powder of mitochondrial fraction, the particles were prepared by modified procedures described above, because of a large amount of spadix tissues being treated. Mitochondrial suspensions were droped in about 10-fold volumes cold acetone (approx. -10° C) which was stirring strongly. From sediments obtained by filtration, the acetone was evaporated by use of a vacuum pump. The color of the acetone powder thus obtained was white.

Boiled extracts of plant tissues were prepared as follows. Plant tissues were first boiled with the same volume water at 90° C for 30 minutes, then, were ground and the procedures described above were repeated. The boiled homogenates were strained through cheesecloth and centrifuged at 3,000 r. p. m. for 10 minutes. The supernatants thus obtained were referred to as the boiled extracts of plant tissues.

Ash of boiled extracts was prepared by use of a porcelaneous melting-pot. Ash was suspended in water of the same volume as boiled extracts. To avoid production of chlorides, hydrogen chloride was not used for resolubling ash.

Crude succinoxidase was prepared from beef heart muscle by the method of Kusunose[27] and was stored frozen. When used, fresh weight 2 g of the preparations was homogenized in 6 ml of 100 mM phosphate buffer (pH 7.0) by use of ice-cold glass homogenizer.

Cytochrome c of 76 % purity prepared from horse heart muscle, was obtained from Sigma Chem. Co., U. S. A.

The rates of oxygen uptake were determined by the conventional techniques of Warburg manometer. Spectroscopic observations were made by using Zeiss microspectroscope. Cytochrome c oxidation activities were measured by use of Shimazu's spectrophotometer (Type QB-50).

Results

1. *On the Existence of Cyanide Stimulative Respiration*

High activities of endogenous respiration were noticed in sliced spadices at the growing stages of "young spadix", "prior flowering" and "in flowering", compared with those in the spadix at a stage of "after flowering", as shown in Table I. It is reported that Qo_2 (μl O_2-uptake/mg dry weight/hr) value of *Arum* spadix tissues was more than 30[2] and

Table I The effects of cyanide upon the oxygen uptake of sliced *Lysichiton* spadix in the various growing stages. Reaction medium contained 50 mM phosphate buffer (pH 7.0) and sliced tissues of 100 mg fresh weight. Temperature, 30° C. Gas exchange measured for one hour.

Growing stage	Endogenous O_2-uptake	+1 mM HCN		+2 mM HCN	
		O_2-uptake	Stimulation	O_2-uptake	Stimulation
Young spadix	108 μl	124 μl	15 %	121 μl	12 %
Prior flowering	107	159	49	155	45
In flowering	110	145	32	144	31
After flowering	95	105	11	113	19

that the value of *Symplocarpus* was also very high[13]. Qo_2 value of *Lysichiton* spadix tissues was 8 at a stage of "prior flowering". Although this value is low compared with the values of *Arum* and *Symplocarpus*, it is higher than those of tissues of other higher plants. Inhibitive effects of cyanide addition upon sliced tissues were not observed at any of the growing stages, but on the contrary, it was noticed that cyanide even stimulated the respiration. No difference was found in cyanide concentrations between 1 and 2 mM. This stimulative effect was most remarkable in spadix tissues at a stage of "prior flowering".

Table II shows that the activties of endogenous respiration of central rachis tissues were low compared with spadix tissuess, and that cyanide stimulative respiration occurred only in spadix tissues. In similar experiments on anthers removed from spadix (contained pollens) and other portions of spadix, it was found that endogenous respiration in each portion was stimulated by cyanide addition as shown in Table III.

Table II The effects of cyanide upon the oxygen uptake of sliced *Lysichiton* spadix and the central rachis in prior-flowering stage.
Reaction medium contained 50 mM phosphate buffer (pH 7.0) and sliced tissues of 100 mg fresh weight. Temperature, 30° C. Gas exchange measured for one hour.

Tissue	Endogenous O_2-uptake	+1 mM HCN		+2 mM HCN	
		O_2-uptake	Stimulation (+) or inhibition (−)	O_2-uptake	Stimulation (+) or inhibition (−)
Spadix	107 μl	159 μl	+49 %	155 μl	+45 %
Central rachis	25	21	−16	20	−20

Table III The effects of cyanide upon the oxygen uptake of the anthers and other portion of *Lysichiton* spadix in prior-flowering stage. Reaction medium contained 50 mM phosphate buffer (pH 7.0) and tissues of 100 mg fresh weight. Temperature, 30° C. Gas exchange measured for one hour.

Tissue	Endogenous O_2-uptake	+1 mM HCN	
		O_2-uptake	Stimulation
Anthers	130 μl	156 μl	20 %
Other portion	87	117	34

Table IV The effects of various respiratory inhibitors upon the oxygen uptake of sliced *Lysichiton* spadix in prior-flowering stage. Reaction medium contained 50 mM phosphate buffer (pH 7.0) and sliced tissues of 100 mg fresh weight. Temperature, 30° C. Gas exchange measured for one hour.

Inhibitor	Concentration	Stiumlation (+) or inhibition (−)
HCN	1 mM	+49%
HCN	2	+45
NaN_3	1	+12
NaN_3	2	+17
DIECA*	2	+ 4
Acriflavin	0.1	0
Acriflavin	1	− 8
CO**	95%(in dark)	+17
CO**	95%(in light)	+11

*Diethyldithiocarbamate.
**Control ; 95% N_2+5% O_2.

The natures of the respiratory systems were examined with the aid of specific inhibitors, and the results obtained were summarized in Table IV. Oxygen uptake was stimulated by 1 or 2 mM sodium azide, which is a powerful inhibiting agent as cyanide of enzymes containing heavy metals, although its stimulative effect was less than cyanide. In the case of the addition of diethyldithiocarbamate, an inhibitor of copper enzymes (phenolases, ascorbic oxidase etc.), oxygen uptake was not reduced. Gas mixture of 95% carbon monoxide and 5% oxygen, which is a specific inhibitor of cytochrome oxidase, also reduced no oxygen uptake. Acriflavin, used as an inhibitor of flavin enzymes, inhibited slightly the respiration only in high concentration (1 mM).

Since these findings suggested a possibility of flavoprotein functioning as a terminal oxidase in respiration of *Lysichiton* spadix, pO_2^{50} (O_2 partial pressure required for half the maximal respiratory rate) value was measured. It is known that flavin oxidases have generally low affinity to oxygen. As shown in Fig. 1, pO_2^{50} value was approximately 0.01 atmosphere (1% O_2+99% N_2). Since this value indicates relatively high affinity to oxygen in sliced tissues, it is probable that flavin oxidase does not play a significant role, even if it functions in the respiration.

Cytochrome components of mitochondria prepared from the spadix tissues were observed spectroscopically, as shown in

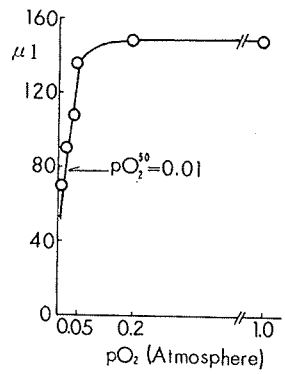

Fig 1. The effects of oxygen partial pressure, pO_2, upon the oxygen uptake of sliced *Lysichiton* spadix in prior-flowering stage. The curve was determined manometrically. Reaction medium contained 50 mM phosphate buffer (pH 7.0) and sliced tissues of 100mg fresh weight. Temperature, 30° C. Gas exchange measured for one hour.

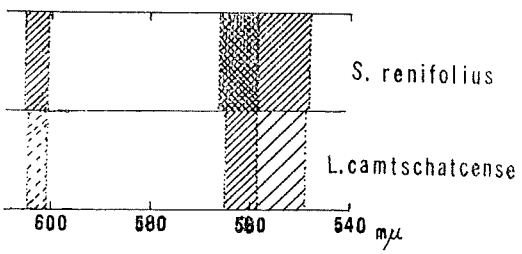

Fig 2 Absorption bands (α-bands) observed in *Lysichiton* spadix mitochondria compared with skunk cabbage (*Symplocarpus renifolius*) spadix mitochondria.
Preparative procedures of mitochondria were same on two spadices. Reduction by dithionite.

Fig. 2. Cytochromes of a, b and c were found, but only in small amounts compared with those of *Symplocarpus* (skunk cabbage, Zazenso in Japanese). The band of a-type cytochrome in particular was very faint, and the absorption of b-type cytochromes was strong compared with other cytochrome components. From these findings it is presumed that cytochrome b_7, which is known to be present in spadices of *Arum*[10] or *Symplocarpus*[21], is also functioning in the respiration of *Lysichiton* spadix.

2. *On the Mechanism of Cyanide Stimulative Respiration*

The mitochondria prepared from *Lysichiton* spadix was very unstable. Oxygen uptake of the mitochondria decreased to half in 2 hours after it was prepared, though it was stored at low temperature. Addition of adenosine tri-phosphate or Mg^{++} for protecting the activities of mitochondria, produced no effect. The effect of boiled extracts of spadix tissues upon endogenous respiration of the spadix mitochondria was evidently manifested as an inhibition, as shown in Table V. Addition of cyanide alone in the reaction medium produced no effect on the oxygen uptake. In the case of the addition of both boiled extracts and cyanide, the oxygen uptake was stimulated evidently compared with the case of boiled extracts alone, and the activity was recovered completely to a level of the endogenous respiration. This fact shows that an inhibitor or inhibitors of the respiration may exist in the boiled extracts and that its inhibitory effect has been removed by cyanide. Since this boiled extrrcts were prepared by heating, the inhibitory effect seems not to be enzymes action.

Table V The effects of boiled extracts of *Lysichiton* spadix and of cyanide upon the oxygen uptake of the spadix mitochondria.
Reaction medium contained 40 mM phosphate buffer (pH7.0) and 0.7 ml mitochondrial suspension (dry weight, 76.4 mg). 1 mM HCN and/or 0.5 ml boiled extracts were added as indicated. Final volume, 2.0 ml. Temperature, 30° C. Gas exchange measured for 40 minutes.

Addition	O_2-uptake	Stimulation (+) or inhibitlon (−)
		%
None	24.4 μl	
HCN	25.4	+ 4
Extract	14.1	−42
Extract, HCN	26.3	+ 8

It was reported that the mitochondria prepared from *Arum* oxidized thoroughly the members of Tri-carboxylic cycle[4]. But the mitochondria of *Lysichiton* had only weak oxidative activities on succinate or malate. Weak succinate oxidation of the mitochondria was inhibited by either cyanide or the boiled extracts, and both inhibitors scarcely competed with each other. Since it has been noticed that the effect of the cyanide additon markedly differs between endogenous respiration and succinate oxidation, it is presumed that main substrate in endogenous respiration of *Lysichiton* mitochonaria is not succinate.

Since the mitochondria had only weak oxidative activity on succinate, a crude typical succinoxidase was prepared from beef heart muscle, and the effect of boiled extracts of the spadix was examined on this oxidase. Table VI shows that the succinoxidase activities were reduced markedly by the addition of either boiled extracts or cyanide, and both inhibitors competed slightly with each other. In the case of succinate oxidation of

Table VI The effects of boiled extracts of *Lysichiton* spadix and of cyanide upon the activities of succinoxidase of beef heart. Reaction medium contained 40 mM phosphate buffer (pH 7.0), 50 mM potassium succinate and 0.3 ml enzyme suspension. 1 mM HCN and/or boiled extracts were added as indicated. Final volume, 2.0 ml. Temperature, 30° C. Gas exchange measured for one hour.

Extract	Succinate oxidation		Inhibition	
	−−	+HCN	by extract	by extract plus HCN
ml	O_2 μl	O_2 μl	%	%
0	144	0		100
0.0002	143	0	0	100
0.002	131	0	10	100
0.005	85	0	40	100
0.01	71	0	50	100
0.02	30	0	80	100
0.30	16	1	90	100
0.45	23	17	80	90
0.60	22	16	90	90
0.75	21	13	90	90
1.20*	26	0	80	100
2.40*	0	0	100	100

* Concentrated extracts used.

sonicated cell-free extracts of *Proteus vulgaris*, a rotting bacteria, the inhibition by cyanide was reduced likewise by the addition of boiled extracts of the spadix tissues.

In order to determine whether or not such an effect of boiled extracts is specific in *Lysichiton* spadix tissues, the leaves of two species of higher plants, which showed remarkable characteristics in the effects of cyanide upon their endogenous respirations, were used for preparation of boiled extracts (Table VII). The boiled extracts of tobacco and sugar-beet leaves also inhibited respectively the succinate oxidation of heart succinoxidase, although their inhibitions were weak compared with that of the spadix as shown in Table VIII. However, it is regarded as a remarkable difference between them that those boileld extracts did not compete with cyanide. The competition between cyanide and boiled extracts is specific in boiled extracts of *Lysichiton* spadix tissues. And it seems probable that the boiled extracts contain unknown compound or compounds, which are able to depoison the cyanide.

It is evident that boiled extracts of those higher plant tissues contain an inhibitory factor or factors of succinate oxidation.

In order to make examination as to whether

Table VII The effects of cyanide upon the oxygen uptake of sliced tissues of various plants. Reaction medium contained 40 mM phosphate buffer (pH 7.0) and sliced plant tissues (fresh weight, 100 mg). 1 mM HCN was added as indicated. Final volume, 2.0 ml. Temperature, 30° C. Gas exchange measured for one honr.

Tissue	Endogenous O_2-uptake	+HCN	Stimulation (+) or inhibition (−)
Lysichiton spadix	107 μl	157 μl	+50%
Tobacco leaf	34	35	0
Sugar beet leaf	51	27	−50

Table VIII The effects of boiled extracts of plant tissues and cyanide upon the activities of succinoxidase of beef heart.
Reaction medium contained 40 mM phosphate buffer (pH 7.0), 50 mM potassium succinate and 0.3 ml enzyme suspension. 1 mM HCN and/or 0.6 ml boiled extracts were added as indicated. Final volume, 2.0 ml. Temperature, 30° C. Gas exchange measured for one hour.

Addition	Succinate oxidation	Inhibition	
		by extract	by extract plus HCN
	O_2 μl	%	%
None	125		
HCN	0		100
Lysichiton spadix extract	20	80	
Lysichiton ext.+HCN	14		90
Tobacco leaf extract	53	60	
Tobacco ext.+HCN	0		100
Sugar beet leaf extract	56	60	
Sugar beet ext.+HCN	0		100

Table IX The effects of ash prepared from extracts of plant tissues and of cyanide upon the activities of succinoxidase of beef heart.
Reaction medium contained 40 mM phosphate buffer (pH 7.0), 50 mM potassium succinate and 0.3 ml enzyme suspension. 1 mM HCN and/or ash from 0.6 ml boiled plant extracts were added as indicated. Final volume, 2.0 ml. Temperature, 30°C. Gas exchange measured for one hour.

Addition	Succinate oxidation	Inhibition	
		by ash	by ash plus HCN
	O_2 μl	%	%
None	131		
HCN	0		100
Lysichiton spadix ash	90	30	
Lysichiton ash+HCN	0		100
Tobacco leaf ash	88	30	
Sugar beet leaf ash	76	40	

the factor or factors are organic or inorganic, the extracts were reduced to ash, which contained only inorganic compounds. The inhibitory effects were evidently reduced in ash when compared with the effects of original boiled extracts as shown in Table IX. In the case of *Lysichiton* ash, the inhibitory effect was markedly reduced, and the effect did not compete with the inhibition of cyanide. These findings suggest that higher plant tissues contain an inorganic compound or com pounds, which inhibit succinoxidase and do not react with cyanide.

From the results described above, it is presumed that the added cyanide removes the unknown factor or factors (pobably organic) which exists in the tissues and bring on the inhibition of mitochondrial respiration. This may be a mechanism of cyanide stimulative respiration in *Lysichiton* spadix[26].

3. *On the Cytochrome c Oxidation of Acetone Powder of Mitochondria*

Large amount of acetone powder of spadix mitochondria was prepared during flowering season of *Lysichiton*. Although fresh mitochondria was unstable, this acetone powder was

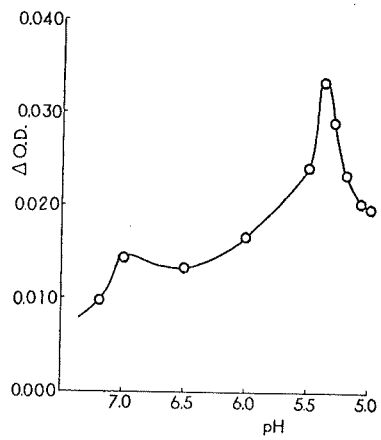

Fig 3 The effects of pH upon cytochrome c oxidation activities of acetone powder of *Lysichiton* spadix mitochondria. Reaction medium contained 25 mM phosphate buffer, 13 μM reduced cytochrome c and 10 mg powder. Absorbancy changes of cytochrome c were measured at 550 mμ with reaference to 570 mμ, for 2 minutes. Room temperature.

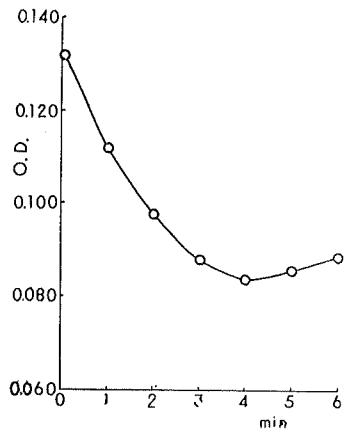

Fig 4 Cytochrome c oxidation activities of acetone powder of *Lysichiton* spadix mitochondria. Reaction medium contained 25 mM phosphate buffer (pH 5.4), 13 μM reduced cytochrome c and 10 mg powder. Absorbancy change of cytochrome c was measured at 550 mμ with reference to 570 mμ. Room temperature.

stable when stored in a desiccator, and showed weak activities of malic, β-hydroxybutylic, succinic, alcoholic and glucose dehydrogenases. But the acetone powder did not show the activities of pyruvic, citric, α-ketoglutaric and lactic dehydrogenases. The acetone powder possessed a weak oxidative activity of malate or succinate, and the activities increased by the addition of cytochrome c. For the investigation of terminal oxidases in respiration of the spadix, this acetone powder was used for experiments on cytochrome c oxidation. Fig. 3 shows pH dependence of cytochrome c oxidation of the acetone powder determined spectrophotometrically. Optima lay at pH 5.4 and 7.0, and the activity was greater at pH 5.4. The rate of cytochrome c oxidation by the acetone powder decreased in 2 minutes after beginning of the reaction as shown in Fig. 4.

With the aid of various inhibitors, oxidases functioning these two optima pH of the cytochrome c oxidation were investigated as shown in Table X. The effects of cyanide addition or of sodium azide in high concentration (5 mM) showed marked differences between pH 5.4 and 7.0. The effects of 8-hydroxyquinoline and salicylaldoxime, which are known as inhibitors of copper enzymes, were also tested and salicylaldoxime only in a high concentration (10 mM) at pH 7.0 showed a significant inhibition. Chlorpromazine, an effective inhibitor of flavoproteins, inhibited cytochrone c oxidation of the acetone powder only at pH 5.4. Hydroxylamine as an inhibitor of peroxidase caused marked inhibition also at pH 5.4, though cyanide or sodium azide did not inhibit the oxidation at this pH. These findings suggest that cytochrome c oxidation at pH 5.4 and 7.0 is catalyzed by different oxidases.

Teble X The effects of the inhibitors upon cytochrome c oxidation activities of acetone powder of *Lysichiton* spadix mitochondria. Reaction medium contained 25 mM phosphate buffer, 13 μM reduced cytochrome c and 10 mg powder. Absorbancy changes of cytochrome c were measured at 550 mμ with reference to 570 mμ, for 2 minutes. Room temperature.

Inhibitor		Stimulation (+) or inhibition (−)	
		at pH 5.4	at pH 7.0
mM		%	%
1	HCN	+25	−70
2	HCN	+15	−70
5	HCN	+18	
1	NaN_3	+ 4	− 9
2	NaN_3	− 6	−13
5	NaN_3	− 4	−42
2.5	8-Hydroxyquinoline	+ 8	+ 6
2.5	Salicylaldoxime	+13	− 3
10	Salicylaldoxime		−10
0.01	Chlorpromazine	− 6	+ 2
0.1	Chlorpromazine	−27	+18
1	Chlorpromazine	−24	
1	Hydroxylamine	−25	− 7
2	Hydroxylamine	−37	

With a view to making clear the nature of the oxidase functional mainly at pH 7.0, the effect of carbon monoxide was examined with manometric method. Ascorbate was applied as an electron donor to cytochrome c. The activity of ascorbate oxidation by the acetone powder in the absence of additional cytochrome c was, at first, determined. In this case, ascorbate oxidation proceeded as shown in Table XI. In this oxidation, it could not be decided whether functional enzyme is ascorbic oxidase or cytochrome c oxidase. However, since it was observed that the rate of ascorbate oxidation increased by cytochrome c addition, it is probable that cytochrome c oxidase functions in the process of the oxidation.

In the case of cytochrome c oxidation when the ascorbate was used as an elec-

Table XI Ascorbate oxidation activities of acetone powder of *Lysichiton* spadix mitochondria at pH 7.0.
Reaction medium contained 25 mM phosphate buffer and 300 mg powder. 20 mM Sodium ascorbate and/or 10 μM cytochrome c were added as indicated. Final volume, 2.0 ml. Temperature, 30° C. Gas exchange measured for 20 minutes.

Addition	O_2-uptake	Increase by cytochrome c
None	0 μl	%
Cytochrome c	Trace	—
Ascorbate	31	
Ascorbate, cytochrome c	37	19

Table XII The effects of carbon monoxide upon cytochrome c oxidation activities of acetone powder of *Lysichiton* spadix mitochondria at pH 7.0.
Reaction medium contained 25 mM phosphate buffer, 10 μM cytochrome c, 300 mg powder and 20 mM sodium ascorbate as an electron donor. Final volume, 2.0 ml. Temperature, 30° C. Gas exchange measured for 20 minutes.

Gas phase	O_2-uptake in dark		O_2-uptake in light (Inhibition)		Recovery by light
	μl	(%)	μl	(%)	%
95% N_2+5% O_2 (control)	40		41		
95% CO+5% O_2	29	(27)	37	(10)	17

tron donor, the oxygen uptake was inhibited by carbon monoxide in the dark, and its inhibition was recovered by light as shown in Table XII. This fact suggests that cytochrome c oxidase functions in cytochrome c oxidation of the acetone powder at pH 7.0. On the other hand, the possibillity could not be neglected that ascorbic oxidase functions partially at pH 7.0.

Table XIII The effects of boiled extracts of *Lysichiton* spadix and of cyanide upon cytochrome c oxidation activities of acetone powder of the spadix mitochondria.
Reaction medium contained 25 mM phosphate buffer, 13 μM reduced cytochrome c and 10 mg powder. 2 mM HCN and/or boiled extracts were added as indicated. Final volume, 3.0 ml. Absorbancy changes of cytochrome c were measured at 550 mμ with reference to 570 mμ, for 2 minutes. Room temperature.

Addition	Stimulation (+) or inhibition (+) at pH 5.4	at pH 7.0
HCN	+15%	−70%
0.05 ml Extract	+ 5	0
〃 Extract, HCN	+10	−30
0.5 ml Extract	−20	+ 4
〃 Extract, HCN	−25	−32

The effects of the boiled extracts of spadix tissues (described in 2.) upon the cytochrome c oxidation of the acetone powder were examined with spectrophometric method. As shown in Table XIII, in the case of pH 5.4, the larger amount of boiled extracts decreased the rate of cytochrome c oxidation of the acetone powder, and the inhibition was not reduced by cyanide addition. On the other hand, in the case of pH 7.0, the boiled extracts exerted no influence on the rate of cytochrome c oxidation, and it brought on evident decrease of cyanide inhibition. Since the decreasing effect of cyanide inhibition of the boiled extracts at pH 7.0, is similar to the effect in the case of succinoxidase obtained from beef heart muscle (Table VI), it seems probable that an unknown factor or factors, which react with cyanide only when cytochrome c oxidase functions, may be contained in *Lysichiton* spadix tissues.

Discussion

The respiration in tissues of higher plants in general is not completely inhibition by cyanide. It is an interesting problem what kind of terminal oxidase participates in cyanide insensitive parts of plant respirations. It was reported by Hackett et al.[23] that

cytochrome b_7, which was found first in *Arum* spadix[10], participates in the respiration of potato tubers in which the cyanide insensitive respiration develops when the tubers are incubated in water. Cytochrome b_3 also is autoxidizable and cyanide insensitive, and it exists in the microsomes of plant tissues[6]. Since it is found spectroscopically that the evident absorption bands of b-type cytochromes exist in *Lysichiton* spadix mitochondria, it seems probable that cytochrome b_7 or b_3 participates in respiratory systems of *Lysichiton* spadix. On the other hand, two peroxidases, which have absorption bands at 556 and 566 mμ respectively, were purified from wehat embryos[18]. This fact suggests a possibility that a part of absorption at 560 mμ region may be due to the peroxidases in *Lysichiton* spadix mitochondria. In fact, experimental results on acetone powder of the mitochondria supported a view that a kind of peroxidase functions on cytochrome c oxidation at pH 5.4.

Cyanide stimulation in respiration of higher plants has been reported in mature leaves, and as for its mechanism it was presumed that cyanide would remove accumulated heavy metals which brought on the inhibition of respiration in plant tissues[20]. In the case of *Lysichiton* spadix, it seems probable that some unknown organic compound or compounds involved in the spadix, which have an inhibitory effct against spadix respiration, do play a significant role in decreasing cyanide inhibition in the respiration. Some inorganic compound or compounds in the spadix tissues were found to inhibit partially the respiration, but did not play an important role in decreasing cyanide inhibition (Table IX). In the case of cytochrome c oxidation by acetone powder of the mitochondria at pH 7.0, boiled extracts of spadix tissues produced no effect on the oxidation, and it brought on evident decrease of cyanide inhibition (Table XIII). This fact suggests a possibility that boiled extracts may contain both the respiratory inhibiting factor or factors and the decreasing factor or factors of cyanide inhibition.

From the investigations on cytochrome c oxidation by acetone powder of the mitochondria, it seems probable that there are at least two oxidases functioning on cytochrome c oxidation in the spadix. One of the two oxidases is presumed to be a cytochrome c oxidase which is inhibited by cyanide and its inhibition is evidently decreased by boiled extracts of *Lysichiton* spadix. The other oxidase is presumed to be a cytochrome c peroxidase[25] which is relatively insensitive to cyanide.

Summary

1. Endogenous respiration of sliced spadix of *Lysichiton* caused a marked stimulation by cyanide addition, especially at a growing stage prior to flowering. Central rachis tissues had low activities of endogenous respiration, and the respiration was inhibited by cyanide addition. The anthers with pollens and the other portions in spadix tissues had the cyanide stimulative respiration respectively. Similarly by sodium azide and carbon monoxide, the respiration of spadix tissues was stimulated, although in a less degree than in the case of cyanide.

2. The endngenous respiration of mitochondria prepared from the spadix was inhibited

by boiled extracts of the tissues, and the inhibition was greatly removed by the addition of cyanide. In the case of succionxidase prepared from beef heart, the oxygen uptake was inhibited by either cyanide or boiled extracts of spadix, and both inhibitors competed slightly with each other. This competition between cyanide and boiled extracts was lost when ash prepared from the extracts was used. From these finding the mechanism of cyanide stimulation of the spadix respiration was discussed.

3. Acetone powder of mitochondria had two optima pH 5.4 and 7.0 on cytochrome c oxidation, and oxidative activity was greater at pH 5.4. In the case of cytochrome c oxidation at pH 5.4, the oxidative rate was not decreased by cyanide and azide, although it was decreased by chlorpromazine and hydroxylamine. The activity at pH 7.0, on the contrary, was not decreased by chlorpromazine and hydroxylamine, and it was decreased by cyanide, azide and carbon monoxide in the dark. From these findings the natures of oxidases which participate in cytochrome c oxidation of *Lysichiton* spadix tissues were discussed.

Acknowledgement

To Professor Shoichiro Usami of Hokkaido University, the writer is greatly indebted for his valuable advice during the course of this work.

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