THE VARIATION OF THE RESPIRATION WITH AGING IN SWEET POTATO SLICES

Saburo OKAMOTO, Yoshikiyo OJI and Goro IZAWA

Although the gaseous exchange of storage tissues and influences of potassium nutrition on the metabolic changes induced in the tissues have been the subjects of intensive investigation by many workers, only a small accomplishment has been made in the recognition of the enzymatic processes. The previous works (1,2) revealed that a higher application of potassium enhanced significantly the transformation and the translocation of organic constituents in sweet potato plants and in taro plants during their growth, and that the amount of potassium required for a complete prevention of blackening of taro corms or sweet potatoes might be relatively higher than that for good yields of these plants. This paper describes the respiratory responses of sweet potato slices to various inhibitors, indoleacetic acid, and substrates, particularly in relation to the influence of aging on the respiration in the slices.

Materials and Methods

The sweet potato variety "Kohkei-14" was used. Shoots, each 30 cm long and with 5 nodes, were planted in the field on May 24 in 1963 or on June 2 in 1964. The amounts of fertilizers applied per ha. were 5.67 tons of urea, 5,67 tons of superphosphate, and 18 tons of potassium chloride. The tuberous roots harvested were stored at 15-20°C. Slices in size 1×3×20mm were cut out of the inner section of the tuberous roots. The aging procedure used consisted of the incubation with deionized water on a plastic screen at 25°C and continuously aerated. They were rinsed with running tap water for a few minutes, drained with filter paper, and divided into two groups. One group of the rinsed slices was analysed for 80% ethanol insoluble solids, total nitrogen, and sugars on the basis of dry matter. The analytical methods used have been described elsewhere (1). Another group of slices was used for the determination of the respiration by means of the standard Warburg manometric technique (3). Each vessel contained in the main reservoir 300-400mg of slices and phosphate- or TRIS-EDTA buffer. Total volume of reaction mixture was 3ml, and the temperature was 25°C. The oxygen uptake was expressed as a value per hour during the period from 30 to 90 minutes after tipping of test compounds from the side arm.

Results and Discussion

1. *Effects of rinsing on the respiration and various constituents in slices.* The slices were rinsed with running tap water in order to remove the cell debris which occurred in cutting slices. The incubation procedure used also served to remove contents of broken cells from the cut surface of slices. Table 1 shows effects of rinsing on the respiration and contents of various constituents in the slices. The data reveal that the rinsing had not an important effect upon the respiration. The respiratory increment in rinsed slices may result from a development of sucrose synthesis in the slices, since there is some evidence which a rapid sucrose synthesis begins when potato tubers are cut (4).

2. *Variation in the respiration and contents of constituents in slices during aging.* The upper four figures in Fig. 1 illustrate variations in $QO_2(N)$, dry matter percentages, total-N, ethanol insoluble solids, sugars in slices during 7 days incubation with aerated water at 25°C, and the lower two show variations in $QO_2(N)$, dry matter percentages, and total-N during

Table 1. Effects of Rinsing on the Respiration and Constituents in Sweet Potato Slices

	Rinsing before	Rinsing after
$QO_2(F)$	62.5	68.1
QO_2 (pH 6.0 in assay)	0.193	0.218
$QO_2(N)$	65.2	71.9
Dry matter, % fr. wt.	32.40	31.25
EtOH insol. solids, % dry wt.	77.90	83.40
Total nitrogen , "	0.296	0.303
Total sugars , "	17.44	13.28
Nonreducing sug., "	7.42	4.76
Reducing sugars , "	10.02	8.52

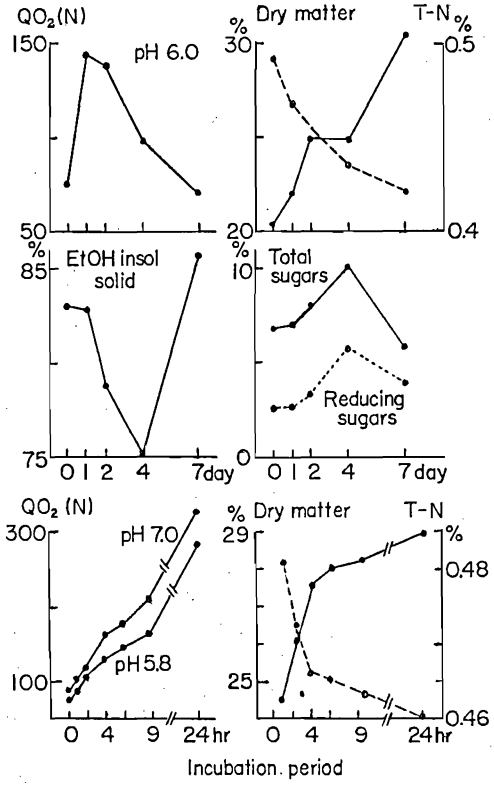

Fig. 1. Variation in the respiration and contents of constituents in sweet potato slices during the incubation with aerated water at 25°C.

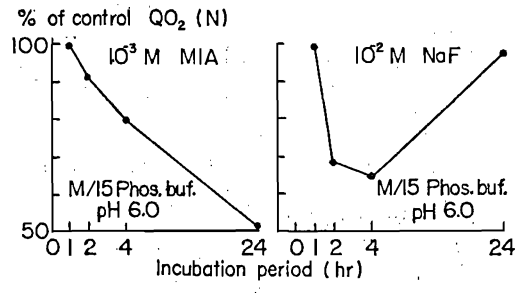

Fig. 2. Effects of MIA and NaF on the respiration in sweet potato slices.

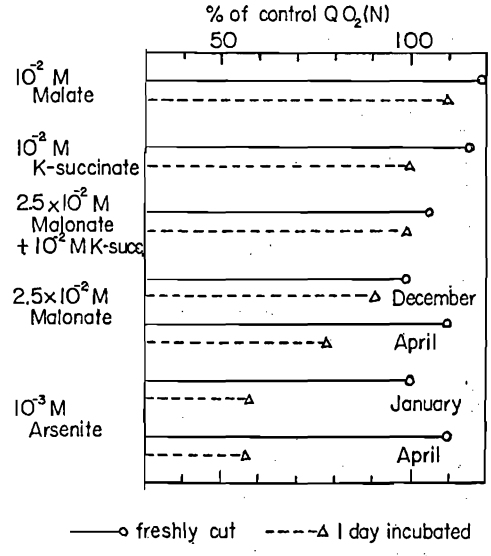

Fig. 3. Effects of malate, succinate, malonate, and arsenite on the respiration in sweet potato slices.

24 hours incubation. The respiratory rate increased with time and attained its maximum at 24 hours after cutting, approximately 2.5-3 folds of levels in the freshly cut slices. In one day aging, the slices turned slightly brown, and further aging resulted in their deeper coloration. The chromogens accumulated during the incubation may be largely associated with the respiratory decrease in further aging. The data obtained suggest that after 4 days aging ethanol insoluble solids could hardly be transformed to sugars and then contents of sugars (respiratory substrates) decreased sharply.

3. *Effects of glycolytic inhibitors on the respiration in slices.* Fig. 2 shows effects of 10^{-3}M monoiodoacetic acid (MIA) or 10^{-2}M sodium fluoride on the respiration. The data are expressed as percentages of $QO_2(N)$ in control. The respiration in freshly cut slices was not inhibited by these inhibitors, but the MIA-or NaF-sensitive respiration developed in a few hours after cutting slices. These poisons might block a larger part of the respiratory increment induced during aging, leaving the initial or basal respiration of the freshly cut slices unaffected. A failure of NaF-effect on the respiration in one day aged slices may be caused by the following, a) some factors other than enolase activity play a limiting role for the oxygen uptake, and b) excess respiratory substrates for TCA-cycle are accumulated during aging.

4. *Effects of TCA-cycle members and its inhibitors on the respiration in slices.* The data are summarized in Fig. 3. Malate and succinate increased the respiratory rate in freshly cut slices, but affected no effect on 1 day aged slices. Malonate and arsenite inhibited significantly the respiration in the aged slices, whereas they had no inhibitory effects on the freshly cut slices. The malonate solution used was adjusted to pH 5.0 with KOH, and KCl was added to the control to balance the potassium ion concentration as well as in the case of the assay of succinate-effect. However, the concentration of malonate used

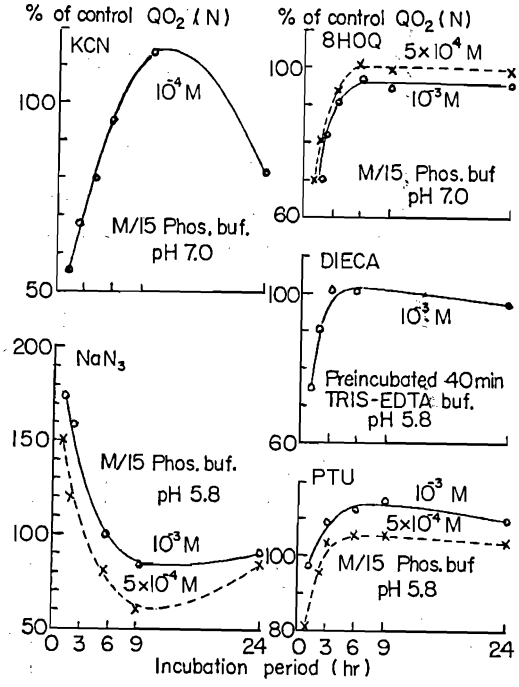

Fig. 4. Effects of terminal oxidase inhibitors on the respiration in sweet potato slices.

might be rather lower compared with that required for it to affect a definite inhibition (5). The results obtained coincide well with LATIES's work which has shown that the TCA-cycle in fresh slices of potatoes is blocked between the steps of citrate and α-ketoglutarate oxidation, and that the rotation of TCA-cycle is set out with aging (6).

5. *Effects of terminal oxidase inhibitors on the respiration in slices.* Fig. 4. shows the effects of potassium cyanide (KCN), sodium azide (NaN_3), 8-hydroxy quinoline (8-HOQ), Na-diethyldithiocarbamate (DIECA), and phenyl thiourea (PTU) at the concentration and the assay pH as indicated. The pretreatment of slices with DIECA was made according to HONDA's procedure (7). NaN_3 resembles cyanide in its capacity of forming complexes with metals, combining with cytochrome oxidase as undissociated acid and with cytochrome c as N_3^- ion (8). However, both inhibitory patterns were reverse to each other. Since NaN_3 can often stimulate the oxygen uptake of plant tissues as seen in the case of DNP-treatment (9), the respiratory stimulation by NaN_3 during the earlier incubation period may be attributed to its uncoupling function. 8-HOQ, DIECA, and PTU which have been used chiefly as inhibitors of copper-enzyme became less inhibitory with aging and had no effect on one day aged slices.

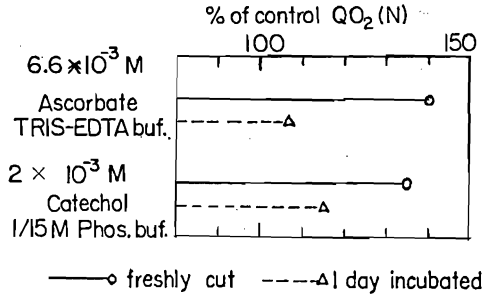

Fig. 5. Effects of ascorbate and catechol on the respiration in sweet potato slices.

The data obtained suggest that iron-enzyme coexisting with copper-enzyme in freshly cut slices may become predominant with aging, though non-metalic enzyme may also develop their activities.

Effects of additional ascorbate or catechol on the respiration are shown in Fig. 5. The ascorbate treatment was made by HONDA's procedure (7). The oxygen uptake was determined for 30 minutes after tipping a test compound, and the data were expressed as percentages of control $QO_2(N)$. The pH in assay with ascorbate was 5.8 and 6.0 in the case of catechol. The results revealed that activities of ascorbic acid oxidase and catechol (or possibly polyphenol) oxidase were higher in freshly cut slices than in one day aged slices, which agreed with the results obtained on the inhibition of copper-enzyme above mentioned. In addition, the slices turned brownish black within 1 hour after catechol addition, and the respiratory rate decreased to minor level compared with that of the control. This may have resulted from an inhibition of dehydrogenase activity due to o-quinone produced by the oxidation of catechol as well as chlorogenic acid (10, 11). However, the black color induced by catechol addition to the freshly cut slices was returned to their original color when ascorbate was added, which indicates that o-quinone can be reduced immediately by ascorbate. Therefore, these results may suggest the presence of such a respiratory chain as substrate-ascorbate-polyphenol-oxygen, especially in freshly cut slices (12).

6. *Effects of indoleacetic acid on the respiration in slices.* As shown in Table 2, IAA had no effect on the respiration in freshly cut slices independently of its treatment, while it stimulated slightly the respiration in aged slices, especially in the case of its preincubation. Effects of aging on the respiration, mentioned earlier, and some evidence which IAA appears to participate in some part of the respiratory process (13) may account acceptably for the respiratory increment induced by IAA in aged slices.

7. *Effects of 2.4-dinitrophenol of the respiration*

Table 2. Effects of Indoleacetic Acid on the Respiration in Sweet Potato Slices

Incubation		$QO_2(N)$	
medium	period	Control	10^{-4}M IAA
H_2O	0	88.9 (100)	89.9 (101)
	24	137.6 (155)	149.8 (169)
10^{-5}M IAA	0	106.8 (100)	108.0 (101)
	24	216.3 (203)	227.4 (213)

in slices. Fig. 6 summarizes effects of DNP on the respiration in slices at varying pH in assay. The respiratory stimulation attained its maximum with $5x10^{-5}$M DNP, especially at pH 5.8. Hence, further experiments were conducted with 5×10^{-5}M DNP and at pH 6.0. Table 3 show DNP-effects on the respiration at various periods, media, and temperatures of the incubation, and with or without aeration. The respiration in slices incubated at 25°C for 24 hours

Table 3. Effects of 2,4-DNP on the Respiration in Sweet Potato Slices

Incubation			$QO_2(N)$	
period	medium	temp. (aeration)	Control	5×10^{-5}M DNP (% of control)
0 hr			79.9	133.3 (167)
24	H_2O	1°C(+)	77.2	135.5 (176)
		25°C(−)	142.6	175.9 (123)
		25°C(+)	177.8	243.4 (137)
0 hr	H_2O	25°C(+)	85.2	107.1 (126)
24			180.7	259.9 (144)
0	$5x10^{-5}$M		78.8	99.8 (127)
24	DNP		212.6	254.5 (120)
0day	H_2O	25°C(+)	68.6	78.9 (115)
1			144.5	159.9 (111)
2			138.1	150.6 (109)
3			93.1	115.1 (124)
4			98.5	125.4 (127)
7			69.5	81.1 (117)

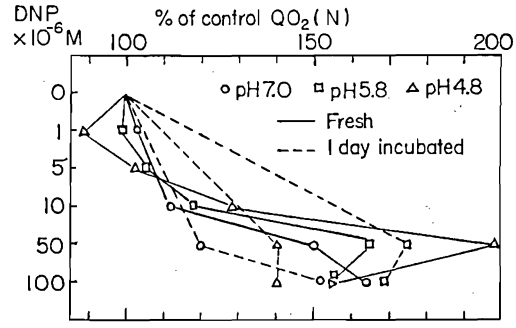

Fig. 6. Effects of DNP on the respiration in sweet potato slices at varying pH of reaction mixture in assay.

under anaerobic condition was lower by 33% compared with that under aerobic condition, while the respiratory rate in the slices incubated at 1°C with aeration for 24 hours was not varied from that of freshly cut slices. Interestingly, the extent of respiratory stimulation induced by an additional DNP alone to aged slices compared with that induced by a preincubation with DNP plus an addition of DNP. The data on a variation of DNP-effect during 7 days aging suggest that DNP-sensitive respiration may run parallel with the endogenous respiration.

Influences of KCN on the DNP-induced respiration are shown in Table 4. The respiration resistant to 10^{-4}M KCN in the slices treated with DNP was at the same level as that of control, and in other words the increased respiration by DNP was completely reduced by KCN both in freshly cut slices and in aged ones. Therefore, DNP may affect effectively the activity of cytochrom oxidase system even though indirectly.

Finally, it has been reported that the respiratory rate in freshly cut slices of potatoes is independent of the thickness of slices, whereas the developing respiration with aging is commonly controlled by the thickness (14), From this, a variety of the respiratory rate in aged sweet potato slices may be attributed to the thickness of slices prepared.

Table 4. Effects of Cyanide on the DNP-induced Respiration in Sweet Potato Slices

Sample	Freshly cut		1 day incubated	
Pretreatment	Control	10^{-4}M KCN	Control	10^{-4}M KCN
	$QO_2(N)$			
H_2O	76.3	39.8 (52)	171.0	128.0 (75)
$5x10^{-5}$M DNP	108.0	40.3 (37)	209.2	134.5 (64)

Figures in paren heses represent percentages of control.

Acknowledgement

Sincere thanks are due to Mr. Takaya Shibano and Mr. Hiroyuki Okamoto for their kind assistance in experiments.

Summary

The variation of the respiration with aging in sweet potato slices (1 mm in thickness) was investigated. A short term aging (within one day) under favourable conditions in aeration and temperature resulted in a significant activation of the enzymes involved in glycolysis and TCA-cycle, and of the terminal oxidase containing iron as active center in the storage tissues. A lower temperature (1°C) in aging prevented such a development of respiration as observed at a higher temperature (25°C). The respiratory increment induced by 2,4-DNP was completely suppressed by KCN. IAA was effective only on the respiration in aged slices. (Laboratory of Plant Nutrition, Received Aug. 31, 1966)

Literature Cited

1) G. Izawa and S. Okamoto, *Soil and Plant Food*, **4**, 163 (1959).
2) G. Izawa and S. Okamoto, *ibid.*, **6**, 127 (1961).
3) S. Okamoto and G. Izawa, *Sci. Rep. Hyogo Univ. Agric., Ser. Agr. Chem.*, **7**, 1 (1965).
4) E. F. Hopkin, *Botan. Gaz.*, **84**, 75 (1927).
5) H. B. Vickery and J. K. Palmer, *J. Biol. Chem.*, **225**, 629 (1957).
6) G. G. Laties, *Plant Physiol.*, **39**, 654 (1964).
7) S. I. Honda, *ibid.*, **32**, 23 (1957).
8) B. L. Horecker and J. N. Standard, *J. Biol. Chem.*, **172**, 599 (1948).
9) E. M. Case and H. McIlwain, *Biochem. J.*, **48**, 1 (1951).
10) N. Suzuki and S. Toyoda, *Bull. Nat. Inst. Agr. Sci., Ser. C.*, No. 8, 131 (1957).
11) I. Uritani and T. Akazawa, *J. Agr. Chem. Soc., Japan*, **29**, 381(1955).
12) L. W. Mapson and E. M. Moustafa, *Biochem. J.*, **62**, 248 (1956).
13) B. S. Meyer and D. B. Anderson, Plant Physiology, D. Van Nostrand Co., Inc., Princeton, p. 568 (1959).
14) G. G. Laties, *Plant Physiol.*, **37**, 679 (1962).