Pergamon

PII: S0031-9422(96)00824-2

Phytochemistry, Vol. 45, No. 2, pp. 225–227, 1997

0031-9422/97 $17.00+0.00

EFFECTS OF CITRATE ON RESPIRATORY GAS EXCHANGE AND METABOLISM IN CARROT ROOT TISSUES

HISASHI KATO-NOGUCHI

Department of Bioresource Science, Faculty of Agriculture, Kagawa University, Miki-cho, Kagawa 761-07, Japan

(*Received in revised form* 8 *November* 1996)

Key Word Index—*Daucus carota*; Umbelliferae; carrot; citrate; phosphofructokinase; glycolytic intermediate; regulation of glycolysis; respiration.

Abstract—This study was undertaken to determine the effects of citrate on respiratory gas exchanges and glycolytic metabolism in carrot root tissues (*Daucus carota* L.). Carrot root discs were incubated on media with citrate, and the resulting changes in the rates of CO_2 production and O_2 consumption, and levels of glycolytic intermediates were monitored. Citrate inhibited the rates of CO_2 production and O_2 consumption of carrot root tissues at concentrations higher than 1 mM. Fructose 6-phosphate accumulated and fructose 1,6-bisphosphate decreased in response to elevated concentration of citrate applied. These results suggest that citrate may inhibit the respiratory gas exchanges in carrot root tissues by inhibiting the phosphorylation of fructose 6-phosphate to fructose 1,6-bisphosphate.

INTRODUCTION

Phosphofructokinase (ATP-PFK; ATP: D-fructose 6-phosphate 1-phosphotransferase, EC 2.7.1.11) catalyses the phosphorylation of fructose 6-phosphate (Fru-6-P) to fructose 1,6-bisphosphate (Fru-1,6-P_2) in the glycolic pathway. This enzyme is regulated by a large number of allosteric activators and inhibitors [1–3], and it has been suggested that its allosteric properties may contribute to the regulation of glycolytic activity in mammalian, fungal, bacterial and plant tissues [4–7].

The activity of plant ATP-PFK is inhibited by citrate *in vitro* and this inhibition is highly cooperative [3, 8, 9]. To our knowledge, however, very little information is available about the effect of citrate on respiration of plant tissues *in vivo*. The object of this research was to investigate the effects of citrate on respiratory gas exchanges and glycolytic metabolism. Thus, CO_2 production and O_2 consumption rates and levels of glycolytic intermediates were determined in carrot root tissues incubated with citrate.

RESULTS AND DISCUSSION

Effect of citrate on respiratory gas exchange

Changes in rates of CO_2 production and O_2 consumption in carrot root tissues on application of citrate were shown (Fig. 1). The rates of CO_2 production and O_2 consumption of the control carrot tissues were stable at about 80 ml kg^{-1} fr. wt hr^{-1} for the duration of the experiments, a value comparable to those reported in similar plant material [10]. At concentrations higher than 1 mM, citrate inhibited the rates of CO_2 production and O_2 consumption. Both rates of CO_2 production and O_2 consumption decreased rapidly and reached plateaus after 24 hr. These plateau values were dependent on the concentrations of citrate applied. The stable rates of CO_2 production were 65, 53, 39 and 32 ml kg^{-1} fr. wt hr^{-1} and those of O_2 were 69, 58, 46 and 39 ml kg^{-1} fr. wt hr^{-1} for concentrations of 1, 3, 10 and 30 mM citrate after 36 hr, respectively. Citrate is a substrate for respiration in mitochondria, however, exogenously applied citrate probably did not serve as a substrate for respiration because the permeability of citrate to mitochondria was very low [11]. Thus, respiratory gas exchanges of carrot root tissues may be inhibited by exogenously applied citrate.

Effect of citrate on glycolytic intermediates

Table 1 shows the levels of the glycolytic intermediates in the carrot root tissues after 36 hr of incubation. Citrate affected the levels of the intermediates at concentrations higher than 1 mM. Significant changes were an increase of Fru-6-P level and a decrease of Fru-1,6-P_2 level in response to the elevated concentration of citrate. The level of Fru-6-P in the tissues at concentration of 30 mM citrate was 177% of that of control tissues (0 mM), and the level of Fru-1,6-P_2 in the tissues at the same concentration of citrate was 19% of that of the control.

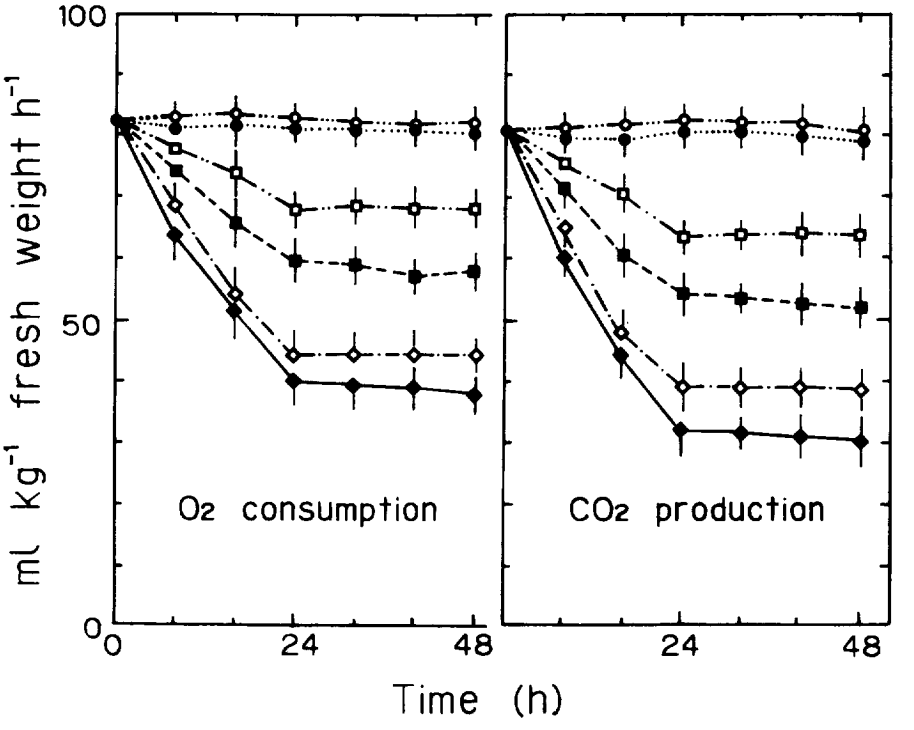

Fig. 1. Effects of citrate on CO_2 production and O_2 consumption by carrot root tissues. The carrot root disks were incubated on media with citrate of 0 (○), 0.3 (●), 1 (□), 3 (■), 10 (◇) and 30 (◆) mM, and CO_2 production and O_2 consumption were measured at indicated times. Means ± s.e. from three replicate experiments with three incubations for each determination are shown.

The phosphorylation of Fru-6-P to Fru-1,6-P_2 are catalysed by two enzymes [12], ATP-PFK and PPi:D-fructose 6-phosphate 1-phosphotransferase (PPi-PFK; EC 2.7.1.90). The activity of ATP-PFK was inhibited by citrate *in vitro* [5, 8, 12, 13], however, PPi-PFK was not [14]. The inhibition of ATP-PFK activity by citrate is highly cooperative with respect to its substrate Fru-6-P [9]. Thus, the increase of Fru-6-P level and decrease of Fru-1,6-P_2 level in carrot root tissues in response to citrate may be caused by inhibition of ATP-PFK activity *in vivo*.

In summary, citrate inhibited the rates of CO_2 production and O_2 consumption of carrot root tissues at concentrations higher than 1 mM (Fig. 1). The accumulation of Fru-6-P and decrease of Fru-1,6-P_2 were found in carrot root tissues in response to the elevated concentration of citrate (Table 1). These findings suggest that citrate may inhibit the respiratory gas exchanges in carrot root tissues by inhibiting the activity of ATP-PFK, which catalyses the reaction from of Fru-6-P to Fru-1,6-P_2 in the glycolytic pathway, and this reaction may be one of the target points in the activity of respiration process affected by exogenously applied citrate.

EXPERIMENTAL

Plant materials. Mature roots of carrot (*Daucus carota* L.), which were certified free of postharvest

Table 1. Effects of citrate on the concentration of glycolytic intermediates in carrot root tissues

Intermediate	Intermediate concentration (μmol kg^{-1} fresh weight)					
	Citrate concentration (mM)					
	0	0.3	1	3	10	30
Glucose 6-phosphate	257 ± 19	254 ± 16	259 ± 19	264 ± 19	275 ± 18	287 ± 14
Fructose 6-phosphate	69 ± 8	70 ± 8	76 ± 10	88 ± 5	97 ± 8	122 ± 10
Fructose 1,6-bisphosphate	26 ± 3	25 ± 2	20 ± 2	16 ± 2	8 ± 1	5 ± 1
Dihydroxyacetone phosphate	24 ± 3	26 ± 3	21 ± 2	20 ± 2	20 ± 2	17 ± 2
3 Phosphoglycerate	27 ± 3	27 ± 3	25 ± 2	23 ± 2	18 ± 2	18 ± 2
Phosphoenolpyruvate	24 ± 2	24 ± 2	22 ± 3	20 ± 2	18 ± 2	17 ± 1
Pyruvate	39 ± 3	39 ± 3	38 ± 3	35 ± 3	33 ± 3	31 ± 3

The carrot root discs were incubated on media with citrate for 36 hr, and the intermediates were determined by enzymatic analysis. Means ± s.e. from three replicate experiments with at least three assays for each determination are shown.

chemicals, were obtained from a local wholesaler. The roots were washed and sliced transversely into discs (5 mm thick). The uniform discs (25–30 mm i.d.) were sterilized in a 0.02% NaOCl soln, rinsed thoroughly in H_2O and blotted dry. 10 disks (*ca* 30 g fr. wt) were placed on a basal medium (100 ml) of Murashige and Skoog [15] with or without citrate (monohydrate, Sigma) containing 3% sucrose and 0.9% agar in a 1.5-l flask. The pH of the media was adjusted to 6.5 before autoclaving with KOH. The flask was sealed with a rubber cap which has inlet and outlet glass tubes, and a stream of air was passed through the flask at a rate of 10 ml min^{-1}, which was sufficient to keep CO_2 accumulation below 0.3%. The flasks were ventilated for 2 hr to allow dissipation of wound responses of carrots [10]. All experiments were performed at 20°.

For quantification of glycolytic intermediates, 3 flasks for each treatment were removed after 36 hr. Then, the carrot disks were washed in H_2O, immediately frozen in liquid N_2 and stored at −80° until extraction.

Measurement of respiratory gas exchanges. O_2 and CO_2 conc of the effluent stream from the flasks was measured by analysis of a 10-ml gas sample using GC equipped with TCD detector. O_2 was analysed on a Molecular sieve 13X 30/60 column (3 mm × 4 m; GL Sciences, Tokyo) at 40°. CO_2 was analysed on a Unibeads C 80/100 column (molecular sieve, 3 mm × 3 m; GL Sciences). The oven temp started at 40° (6 min) and increased at 20° min^{-1} to 220°. The flow rate of carrier gas was 30 ml min^{-1} of He in both cases. Pure O_2 and CO_2 were first injected for determination of the correct retention time and the conc of O_2 and CO_2 of sample was calculated from the peak area.

Quantification of glycolytic intermediates. Frozen carrot disks (*ca* 5 g fr. wt) were powdered by homogenization in a mortar containing liquid N_2 using a pestle. The powder was extracted with 10 ml of ice-cooled 0.45 M $HClO_4$, and the homogenate was kept for 30 min at 4° with occasional shaking and centrifuged at 30 000 *g* for 15 min at 4°. The supernatant was neutralized to pH 7.0–7.5 with 2.5 M K_2CO_3 and left on ice for 10 min. The precipitated $KClO_4$ was removed by centrifuged (3000 *g*, 5 min) and the resulting supernatant was used for analysis of the intermediates.

The quantification of the glycolytic intermediates was carried out spectrophotometrically by monitoring the oxidation/reduction of NADH/NADP at 340 nm for 10–15 min at 30° in 1-ml of TEA buffer (pH 7.6) according to the procedure described in ref. [16]. The recovery of the glycolytic intermediates through the quantification process was 93 ± 3, 89 ± 3, 90 ± 2, 88 ± 4, 91 ± 4, 87 ± 4 and 88 ± 3% for glucose 6-phosphate, Fru-6-P, Fru-1, 6-P_2, dihydroxyacetone phosphate, 3 phosphoglycerate, phosphoenolpyruvate and pyruvate, respectively, as calculated from 6 replications of the test run with pure glycolytic intermediates in the extracts of carrot disks incubated with 30 mM citrate or without citrate (3 × each).

REFERENCES

1. Hers, H.-G. and Schaftingen, Van E., *Biochemistry Journal*, 1982, **206**, 1.
2. Solomos, T., in *Senescence and Aging in Plants*, eds L. D. Noodén and A. C. Leopold, Academic Press, San Diego, CA, 1988, p. 111.
3. Turner, J. F. and Turner, D. H., *Annual Review of Plant Physiology*, 1975, **26**, 159.
4. Ap Rees, T., in *Higher Plant Cell Respiration*, ed. R. Douce and D. A. Day. Springer, Berlin, 1985, p. 391.
5. Preiss, J. and Kosuge, T., *Annual Review of Plant Physiology*, 1970, **21**, 433.
6. Turner, J. F. and Turner, D. H., in *The Biochemistry of Plants*, Vol. 2, ed. D. D. Davies. Academic Press, NY, 1980, p. 281.
7. Uyeda, K., in *Advances in Enzymology*, ed. A. Meister, Academic Press, NY, 1979, p. 193.
8. Dennis, D. T. and Coultate, T. P., *Biochemistry and Biophysics Research Communications*, 1966, **25**, 187.
9. Kelly, G. J. and Turner, J. F., *Biochemistry Journal*, 1969, **115**, 481.
10. Leshuk, J. A. and Saltveit, M. E. Jr, *Physiologia Plantarum*, 1991, **82**, 559.
11. Wiskich, J. T. and Dry, I. B., in *Higher Plant Cell Respiration*, ed. R. Douce and D. A. Day. Springer, Berlin, 1985, p. 281.
12. Black, C. C., Mustardy, L., Sung, S. S., Kormanik, P. P., Xu, D.-P. and Paz, N., *Physiologia Plantarum*, 1987, **69**, 387.
13. Ashihara, H., Komamine, A. and M. Shimokoriyama, M., *Phytochemistry*, 1972, **11**, 2717.
14. Ashihara, H. and Stupavska, S., *Journal of Plant Physiology*, 1984, **116**, 241.
15. Murashige, T. and Skoog, F., *Physiologia Plantarum*, 1962, **15**, 473.
16. Bergmeyer, H. U., *Methods of Enzymatic Analysis*. Chemie, Weniheim, FL, 1983.