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SHORT COMMUNICATION

INHIBITION OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE FROM TOBACCO TISSUE CULTURE BY SCOPOLETIN AND SCOPOLIN

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Abstract-The pH optimum for glucose-6-phosphate dehydrogenase from tobacco tissue culture was determined to be between 8 and 9. The plot of the reaction velocity vs. glucose-(i-phosphate concentration showed a non-sigmoidal, hyperbolic saturation curve. A similar curve was obtained for the $NADP^+$ saturation function. Compounds that may be related to glucose-6-phosphate metabolism were tested for their effects on the enzyme. It was found that the phenolic compounds, scopoletin, scopolin, esculin and ferulic acid, inhibited the enzyme. Similar inhibitions by scopolin and scopoletin were observed for three other NADPH producing dehydrogenases from the same plant cells; namely, 6-phosphogluconate dehydrogenase, $NADP^+$ specific isocitrate dehydrogenase and $NADP^+$ specific malate dehydrogenase.

INTRODUCTION

GLUCOSE-6-phosphate dehydrogenase catalyzes the initiating reaction for the pentose phosphate pathway. This pathway contributes to the formation not only of NADPH, but also of 3-deoxy-D-arabinoheptulosonic acid-7-phosphate thus initiating the shikimic acid pathway. Shikimic acid is an important precursor of aromatic amino acids and phenolic compounds in plants. Therefore, we have examined the possibility for a feedback type of metabolic control by certain phenolic and related compounds for glucose-6-phosphate dehydrogenase.

RESULTS

The pH optimum for glucosed-phosphate dehydrogenase from tobacco tissue culture WR-132 was between 8 and 9. The enzyme displayed non-sigmoidal rectangular hyperbola saturation kinetics with both glucose-6-phosphate and $NADP^+$. K_m's determined from Lineweaver-Burk plots' were 3×10^{-4} M and $4{\cdot}5 \times 10^{-5}$ M for glucose-6-phosphate and $NADP^+$, respectively. These two K_m's are very similar to those for crystalline glucose-6-phosphate dehydrogenase from yeast:[2] $2{\cdot}3 \times 10^{-4}$ M for glucosed-phosphate and $6{\cdot}7 \times 10^{-5}$ M for $NADP^+$.

Since glucosed-phosphate dehydrogenase may be considered to be involved in phenolic compound biosynthesis, the effects of scopoletin, scopolin and some other phenolic compounds on glucosed-phosphate dehydrogenase from tobacco tissue were investigated. The results of these studies on scopoletin and scopolin are included in Table 1. Scopolin, the 7-*O*-glucoside of scopoletin (6-methoxy-7-hydroxycoumarin), inhibited glucose-6-phosphate dehydrogenase less than the aglycone. Double reciprocal plots of reaction velocity vs. glucosed-phosphate concentration at 0·1 mM of scopoletin and scopolin for glucose-6-phosphate, showed that both compounds inhibit the enzyme non-competitively. The effects

[1] H. LINEWEAVER and D. BURK, *J. Am. Chem. Soc. 56,658* (1934).
[2] H. J. ENGEL, W. DOMSCHKE, M. ALBERTI and G. F. DOMAGK, *Biochim. Biophys. Acta* 191,509 (1969).

of 0·1 mM esculin and 0·1 mM ferulic acid on the enzyme were tested to illustrate the effect of other phenolic compounds on glucose-6-phosphate dehydrogenase. Esculin gave a 30 % and ferulic acid a 15 % inhibition. A concentration higher than 0·1 mM of these compounds was not used because the high absorption by these compounds at 330 nm made accurate assay impossible.

TABLE 1. PERCENTAGE OF INHIBITION OF NADPH PRODUCING DEHYDROGENASES FROM TOBACCO TISSUE BY SCOPOLETIN AND SCOPOLIN

Enzyme		% Inhibition		
		0·1 mM	0·05 mM	0·01 mM
Glucose-6-phosphate dehydrogenase	Scopoletin	25	12	0
	Scopolin	9	6	0
6-Phosphogluconate dehydrogenase	Scopoletin	22	13	0
	Scopolin	12	7	0
$NADP^+$ specific isocitrate dehydrogenase	Scopoletin	19	8	0
	Scopolin	7	3	0
$NADP^+$ specific malate dehydrogenase (or malic enzyme)	Scopoletin	31	18	2
	Scopolin	A	0	0

Einhellig *et al.*[3] determined the effects of scopoletin on the growth of tobacco seedlings A threshold level of inhibition was found to be between 1×10^{-4} and 1×10^{-3} M, with 1×10^{-4} M scopoletin showing no visible inhibition, 1×10^{-3} M great inhibition and 5×10^{-4} M having an intermediate effect on growth. The higher levels of scopoletin inhibited photosynthesis, but did not affect respiration.

Scopoletin and scopolin also were found to have similar effects on three other $NADP^+$ requiring enzymes from tobacco tissue: 6-phosphogluconate dehydrogenase, $NADP^+$-specific isocitrate dehydrogenase and $NADP^+$-specific malate dehydrogenase (or malic enzyme). These results are shown in Table 1. They illustrate that scopolin consistently inhibited all the NADPH producing enzymes less than scopoletin.

In order to establish the mode of binding of the phenolic compounds to the dehydrogenases, glucose-6-phosphate dehydrogenase was preincubated with 0·1 mM scopoletin for different periods of time and then assayed. The percentage of inhibition was independent of preincubation time at least for 12 hr. Further the enzyme solution that had been preincubated with 0·1 mM scopoletin for 60 min was dialyzed overnight and assayed. The enzyme activity was all regained after the dialysis, indicating that scopoletin binds to the enzyme reversibly.

To investigate the significance of glucose-6-phosphate dehydrogenase in the metabolism of tobacco tissue, several metabolic compounds that may be related to glucose-6-phosphate metabolism were tested for their effect on the enzyme. Each of the following compounds was tested at a concentration of 2 mM: ATP, ADP, AMP, phosphate, glyoxylic acid, ribose-5-phosphate, L-phenylalanine, L-tryptophan and glyceraldehyde-3-phosphate. None of the compounds above had any effect on the enzyme.

[3] F. A. EINHELLIG, E. L. RICE, P. G. RISSER and S. H. WENDER, *Bull, Torrey Botan. Club 97.22* (1970).

DISCUSSION

The results show that the phenolic compounds tested inhibited glucose-6-phosphate dehydrogenase. Thus, the possibility for a feedback type of metabolic control is suggested by the results.

It is interesting that scopolin showed much less inhibition of all four dehydrogenases than did scopoletin. Similarly, Schaeffer and coworkers[4] have shown that scopoletin is more than 10 times as effective as scopolin in the inhibition of indoleacetic acid oxidase. Scopolin is the glucoside of scopoletin and it is quite possible that these two compounds are enzymatically interconvertible within the cell. The metabolic significance of glucosylation is not clear, but the fact that scopolin inhibited NADPH producing dehydrogenase and indoleacetic acid oxidase much less than scopoletin, suggests glucosylation may be permitting the reduction of the inhibitory effect of the compound by detoxification.

EXPERIMENTAL

Growth ***of cultures.*** A suspension tobacco tissue culture line WR-132 (*Nicotiana tabacum* L., var. Xanthi) obtained from Dr. A. C. Olson (USDA, Albany, California) was used in this study. The cells (1·6–2 g) were aseptically transferred to 50 ml of medium (Linsmaier and Skoog, 1965) in a 125 ml flask and then grown in constant agitation on a reciprocal shaker (Eberbach; 95-105 reciprocations/min) for 10 days at room temp. After growth, the cells were collected by suction filtration and thoroughly washed with 0·1 M imidazole–0·03 M β-mercaptoethanol buffer (pH 6.5).

Enzyme ***preparation. 40 g*** of the washed cells were mixed with 40 g glass beads, 25 g of polyclar AT (which had been thoroughly washed (H_2O) and hydrated overnight), 0·8 ml of 200 mM EDTA and 80 ml of cold 0·1 M imidazole-0.03 M β-mercaptoethanol buffer (pH 6.5). This mixture was mixed for 10 min in a blender (Sorvall Omnimixer) at 5000 rev/min. This crude enzyme solution was centrifuged at 34,800 ***g*** for 10 min to remove the glass beads, polyclar AT and cell debris. All operations concerned with the preparation of the enzyme were performed at approximately 4".

Solid $(NH_4)_2SO_4$ was added slowly to the supernatant obtained after centrifugation. The protein which precipitated between 17 g $(NH_4)_2SO_4$ per 100 ml of preparation and 46.8 g per 100 ml of preparation was collected by centrifugation at 34,800 ***g*** and resuspended in 5 ml of 0·1 M imidazole-0.03 M β-mercaptoethanol buffer (pH 6.5). After dialysis overnight, the solution was diluted with an appropriate volume of buffer to a final concentration of 1·5 mg of protein per ml of preparation.

Protein concentration was determined by the micro-biuret method of Itzhaki and Gill.[5]

Enzyme assays. The standard assays of Brown and Wray,[6] Yamamoto[7] and Hsu, Lardy and Cleland[8] were used to assay for glucose-6-phosphate dehydrogenase, 6-NADP+-specific isocitrate dehydrogenase and the $NADP^+$-specific malate dehydrogenase (or malic enzyme), respectively. The assay method would not distinguish the malic enzyme and malate dehyrogenase. The standard assay solution for 6-phosphogluconate dehydrogenase contained: 5 mM 6-phosphogluconic acid; 5 mM $MgCl_2$; 1 mM $NADP^+$; 100 mM Tris-HCl (pH 8·0) and the enzyme solution. All assays had a total volume of 3 ml and were initiated with $NADP^+$ after 5 min preincubation of the enzyme with the substrate (glucose-6-phosphate, 6-phosphogluconic acid, isocitric acid or malic acid).

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[5] R. F. ITZHAKI and D. M. GILL, *Anal. Biochem.* **9,** 401 (1964).
[6] A. P. BROWN and J. L. WRAY. *Biochem. J.* **108,437** (1968).
[7] Y. YAMAMOTO, *Plant Physiol.* **44,** *262 (1969).*
[8] R. Y. Hsu, H. A. LARDY and W. W. CLELAND, *J. Biol.* Chem. **242,** 5315 (1967).