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EFFECT OF PHENOLIC COMPOUNDS ON GLUCOSE-6-PHOSPHATE DEHYDROGENASE ISOENZYMES

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Abstract—Effector studies with two isoenzymes (I and IV) of glucose-6-phosphate dehydrogenase (G6PDH) from tobacco suspension culture WR-132 revealed that chlorogenic acid, at 0.4 mM, inhibited both isoenzymes almost 100%, with the inhibition decreasing as the concentration of the acid was reduced. At 0.3 and 0.4 mM, the coumarin glucosides scopolin and esculin were inhibitory, whereas their aglucones scopoletin and esculetin were less inhibitory, and at low concentrations of glucose-6-phosphate (G6P), the latter two were actually stimulatory for G6PDH I. Of the possible effectors studied, only scopoletin and esculetin exhibited a significant activation of G6PDH I under these conditions. However, with G6PDH IV these two effectors do not show the same marked activation at the low G6P concentrations. The phenolic acids, caffeic and ferulic, were less inhibitory than the coumarins tested. The activation of G6PDH I by scopoletin, a compound which accumulates in tobacco under certain stress conditions, gives a possible clue as to the resulting enhanced activity of the hexose monophosphate pathway that has been reported for some plants subjected to stress conditions.

INTRODUCTION

Glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate: $NADP^+$ 1-oxidoreductase, EC 1.1.1.49), (G6PDH), is the first enzyme of the hexose monophosphate pathway. This enzyme and phosphogluconate dehydrogenase have been suggested as the controlling enzymes of the pathway [1]. Erythrose-4-phosphate (E4P), an intermediate of the pathway, can combine with phosphoenolpyruvic acid to initiate the shikimic acid pathway, which is used by many plants in the biosynthesis of their phenolic compounds. The possible role of the hexose monophosphate pathway as a supplier of building blocks in phenolic compound biosynthesis has been suggested by Ashihara and Komamine [2], and these workers have also reported an inhibition of G6PDH by 0.7 and 1.4 mM concentrations of E4P. Bonsignore and De Flora [3] have recently reviewed the regulatory properties of G6PDH. The possible effect of a major concentration change in certain phenolic compounds on the activity of G6PDH, however, has not been adequately investigated.

The literature contains numerous reports which show that under various stress conditions in higher plants, the concentrations of many phenolic compounds change. For example, in tobacco plants infected with *Pseudomonas solanacearum*, there is an increase in the concentration of scopoletin (6-methoxy-7-hydroxycoumarin) and scopolin (7-glucoside of scopoletin) around the infected regions [4]. Other reported conditions which lead to an accumulation of scopolin and/or scopoletin include: boron deficiency in tobacco plants [5]; treatment of tobacco plants with 2,4-D [6, 7]; or with 4-amino-3,5,6-trichloropicolinic acid [8], maleic hydrazide [9], UV [10] or X-ray irradiation [11]. In grapefruit peel, gamma irradiation caused the accumulation of scopoletin and scopolin [12]. Nitrogen deficient tobacco plants showed an increase in chlorogenic acid [13]. Godin [14] reported that it is an enhancement of the hexose monophosphate pathway that leads to an accumulation of phenolic compounds. Farkas and Kiraly [15] have discussed possible relations between pentose phosphate pathway activity and enhanced synthesis of phenolic compounds. In tobacco tissue surrounding the lesions produced by tobacco mosaic virus, the activity of G6PDH was found to increase 300% over the control [16]. In tobacco leaves infected with potato virus [17], and in potato tuber cells after slicing [18], G6PDH also increased in activity.

Since there appears to be some relationship between the accumulation of the phenolic compounds scopolin, scopoletin, and/or chlorogenic acid in some plant tissue and the activity of G6PDH and the hexose monophosphate pathway during certain stress conditions, we undertook an investigation of the effect of these and other individual phenolics on the activity of two G6PDH isoenzymes, G6PDH I and G6PDH IV. The separation and characterization of these two isozymes were described in the previous paper [19]. Kajinami *et al.* [20], in their initial studies with G6PDH, found that scopoletin and scopolin affected the activity of a crude G6PDH preparation from tobacco tissue in culture.

RESULTS AND DISCUSSION

Results of the studies on the effect of the tested pheno-

lic compounds on the activity of G6PDH I and 6GPDH IV at varying concentrations of effector and at varying saturation levels of G6P are summarized in Table 1 (saturating: $S_{1.0}$ = 5 mM G6P; half saturating: $S_{0.5} = K_m$; and one-fourth saturating: $S_{0.25} = 1/3K_m$). Other compounds tested, although not listed in this table are glucose, sucrose, shikimic acid, ferulic acid β-D-glucoside, and coumarin. None of these, however, had any significant effect upon the reaction velocities of either G6PDH I or G6PDH IV.

Of those compounds tested, the one which had the most pronounced effect on the activity of G6PDH I and G6PDH IV was chlorogenic acid. Chlorogenic acid, at 0.4 mM, inhibited G6PDH almost 100%, with the inhibition decreasing as the concentration of the acid was reduced. Recently, in our laboratory, chlorogenic acid has been found to inhibit markedly several other enzymes: isoperoxidases A_1, A_2, and A_3 from tobacco tissue culture W-38 [21–23], isoperoxidases C_3 and C_4 from tobacco suspension cultures WR-132 [22, 23], and glutathione reductase as well as G6PDH from rat lung [24]. Scopolin and esculin, in the experiments performed, were found to be much more inhibitory than their aglucones, scopoletin and esculetin. The phenolic acids, caffeic and ferulic, though inhibitory at low G6P concentrations, were not as potent inhibitors as the coumarins tested.

G6PDH I behaved quite differently from G6PDH IV in one respect. At low G6P concentrations, scopoletin and esculetin accelerate the G6PDH I reaction (151% of the control for scopoletin, and 158% for esculetin). Of the compounds studied, only scopoletin and euscletin exhibited this activation. They do not, however, significantly activate the G6PDH IV reaction at low concentrations of G6P. The activation of G6PDH I by scopoletin appears to have a threshold effect. At high scopoletin and high G6P concentrations, this reaction is inhibited; however, at the low G6P concentration (one-fourth saturating G6P levels), the reaction with G6PDH I is activated by scopoletin. This situation occurs at 0.4, 0.3 and 0.2 mM concentrations of scopoletin. At concentrations lower than these, namely 0.1 and 0.04 mM, scopoletin did not activate the reaction. This finding appears to agree with previous results obtained by Einhellig *et al.* [25], who reported that scopoletin also has a threshold effect on the growth of tobacco. Scopoletin was supplied to the plants in a nutrient culture. At 10^{-3} M, or greater, scopoletin was inhibitory to growth. A scopoletin concentration of 10^{-4} M to 10^{-3} M produced no major growth effects, whereas scopoletin concentrations of 5×10^{-5} M to 10^{-4} M appeared to stimulate growth of the tobacco.

E4P, which has been shown by Ashihara and

Table 1. Effect of compounds on activity of G6PDH isoenzymes I and IV at varying concentrations

	% of control activity											
Conc. of phenolic compound (mM)	$S_{1.0}$G6P		$S_{0.5}$G6P		$S_{0.25}$G6P		$S_{1.0}$G6P		$S_{0.5}$G6P		$S_{0.25}$G6P	
	G6PDH I	G6PDH IV	G6PDH I	G6PDH IV	G6PDH I	G6PDH IV	G6PDH I	G6PDH IV	G6PDH I	G6PDH IV	G6PDH I	G6PDH IV
	Scopolin						Esculin					
0.4	21	14	17	14	24	18	30	21	34	30	50	16
0.3	50	50	49	52	64	50	64	63	38	72	75	68
0.2	82	85	74	84	76	79	90	90	91	94	101	92
0.1	101	101	88	93	90	89	98	98	94	95	94	86
0.04	103	95	96	93	94	85	100	93	92	93	97	86
	Scopoletin						Esculetin					
0.4	34	27	42	56	110	45	79	68	78	73	158	91
0.3	76	85	93	103	151	105	80	97	77	96	125	105
0.2	90	101	96	105	140	112	90	98	87	100	107	104
0.1	96	105	97	99	100	103	96	102	89	95	100	96
0.04	98	102	91	94	97	89	98	99	91	92	99	92
	Ferulic acid						Caffeic acid					
0.4	86	89	78	86	67	94	86	90	85	89	80	82
0.3	88	95	82	88	70	96	88	98	96	89	87	83
0.2	91	98	88	87	83	92	93	97	93	90	89	82
0.1	95	95	96	86	91	86	101	97	91	88	92	79
0.04	97	99	100	88	95	92	101	94	93	87	93	87
	Trans-cinnamic acid						*Para*-coumaric acid					
0.4	106	94	108	91	97	100	102	90	100	94	94	98
0.3	114	99	110	96	101	100	106	97	100	95	94	95
0.2	113	86	119	84	103	96	106	96	100	94	96	92
0.1	110	102	106	90	100	94	108	101	100	91	101	90
0.04	111	94	103	96	102	92	112	94	100	91	99	90
	Chlorogenic acid						Indole-3-acetic acid					
0.4	1	7	0	4	10	8	93	86	91	90	94	96
0.3	25	28	0	24	23	23	95	92	96	92	100	95
0.2	75	76	60	74	66	69	95	92	91	92	97	90
0.1	90	94	100	85	84	84	98	93	94	92	93	92
0.04	93	95	100	92	85	91	98	92	100	91	99	92
	Erythrose-4-phosphate											
0.4	89	111	91	91	98	79						
0.3	90	110	89	91	96	86						
0.2	89	107	93	92	97	100						
0.1	93	107	93	104	99	100						
0.04	93	112	97	109	100	92						

Komamine to inhibit G6PDH in *Phaseolus mungo* at concentrations of 0.7 and 1.4 mM [26], had practically no effect, at concentrations of 0.2 mM or less in our studies, on G6PDH I or G6PDH IV obtained from tobacco tissue in culture. However, at low G6P concentration and 0.4 mM E4P concentration (the highest tested), we did find that the activity of G6PDH was inhibited by *ca* 20%.

EXPERIMENTAL

Growth of the WR-132 tobacco tissue culture, and the procedure for isolation of G6PDH I and G6PDH IV were carried out as previously described in ref. [19]. Routine assays for G6PDH were performed with 100 mM Tris–HCl buffer (pH 8.1), 5 mM Mg^{2+}, plus enzyme. The concns of G6P, $NADP^+$, and potential effector compounds were varied, depending on the situation. Saturating levels of G6P and $NADP^+$ were 5 mM and 1 mM, respectively. All reactions were initiated by the addition of $NADP^+$, and the production of NADPH was followed.

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