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GLUCOSE-6-PHOSPHATE DEHYDROGENASE AND 6-PHOSPHOGLUCONIC ACID DEHYDROGENASE OF WILD OAT SEEDS

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Abstract—The presence of the initial enzymes of the pentose phosphate pathway, namely glucose-6-phosphate dehydrogenase and 6-phosphogluconic acid dehydrogenase, has been demonstrated in dormant seed of wild oat. Before a partial characterization of these enzymes was made, an inherent NADP-reducing activity and an enzyme deactivating component, both present in the crude extract, were removed by ammonium sulphate precipitation and subsequent desalting. Both enzymes were then shown to be NADP-specific. Typical Michaelis–Menten kinetics were shown by each enzyme towards NADP and their respective substrates. Soluble cytoplasmic dehydrogenase enzymes were present in both embryo and endosperm extracts.

INTRODUCTION

The increased rate of breakdown of glucose-6-phosphate (G6P) by the pentose phosphate pathway (PPP) at the expense of glycolysis has been postulated as a possible biochemical basis of a common dormancy-breakage mechanism in seeds [1–3]. Evidence for this comes from several sources, the most direct being derived from studies related to the calculation of C_6/C_1 ratios during the utilization of ^{14}C-labelled glucose [4–6]. A decrease in this ratio is assumed to reflect an increased contribution of PPP compared to glycolysis. This method has been used to demonstrate increased PPP activity in non-dormant as compared to dormant wild oat caryopses during the early hours of imbibition [7]. Conclusions drawn from this indicate that a shift in the C_6/C_1 ratio, in favour of PPP, is an essential step in the preparation of these caryopses for germination.

An alternative and possibly more direct estimation of the relative participation of these two pathways in G6P breakdown in wild oat was made by Kovacs and Simpson [8]. The presence of both the first and second enzymes of the PPP, namely glucose-6-phosphate dehydrogenase [(D-glucose-6-phosphate NAD(P) oxidoreductase; EC 1.1.1.49) G6PDH] and 6-phosphogluconic acid dehydrogenase [(6-phosphogluconic acid NAD(P) oxidoreductase; EC 1.1.1.44) 6PGDH] in both dormant and non-dormant caryopses was demonstrated. During the first few hours of steeping, a large increase in activity of both enzymes was observed in non-dormant caryopses while a steady decrease in activity was found with dormant caryopses.

In a more recent study [9] the activities of cytosolic and organellar enzymes of PPP in hazel cotyledonary tissue were assayed throughout stratification and over a similar period at 20°. The results showed an increase in the activities of the enzymes during stratification with the major increases coinciding with dormancy breakage but prior to the initiation of germination.

The present paper describes the extraction, partial purification and characterization of G6PDH and 6PGDH from the caryopses of wild oat.

RESULTS AND DISCUSSION

In an initial experiment using the supernatant of the 45000 *g* spin, the cytoplasmic enzyme fraction from the endosperm tissue was shown to exhibit a small amount of NADP reduction prior to the addition of dehydrogenase substrate. The problems of inherent NADP reduction have been previously pointed out [10] and possible reasons put forward to explain this phenomenon. To avoid similar complications all the samples used in this study were purified by the passage through Sephadex G-25 which removed the inherent NADP reduction. Samples prepared solely by this method did not retain a constant dehydrogenase activity for more than 12 hr, activity decreasing to half after 24 hr. This deactivation was prevented by precipitation of the crude extracts with ammonium sulphate prior to the passage through the Sephadex G-25 column. This method of partial purification was the only satisfactory one found for the elimination of both phenomena.

The effect of varying G6P concentration on the activity of G6PDH from the embryo and endosperm extracts has been studied and similar observations for the effect of NADP concentration made. From Lineweaver–Burk plots, apparent K_m and V_{max} values have been calculated (Table 1); similar parameters for 6PGDH are given (Table 2). Correlation coefficients are shown for the double reciprocal plots. In both extracts the enzymes were shown to be specific to NADP with no reduction of NAD occurring.

Table 1. Kinetic parameters for G6PDH from wild oat embryos and endosperm regions

	Embryo		Endosperm	
	NADP	Substrate	NADP	Substrate
*V_{max}	18.8	16.8	9.3	9.9
†Km	2.3	13.3	1.0	14.8
Correlation coefficient	0.9917	0.9983	0.9946	0.9959

* V_{max}: nmol NADP reduced/min/mg protein/ml.
† K_m: M($\times 10^{-5}$).

Table 2. Kinetic parameters for 6PGDH from wild oat embryos and endosperm regions

	Embryo		Endosperm	
	NADP	Substrate	NADP	Substrate
*V_{max}	75.60	89.8	80.8	54.5
† K_m	3.2	7.1	0.6	40.2
Correlation coefficient	0.9925	0.9984	0.9914	0.9968

* V_{max}: nmol NADP reduced/min/mg protein/ml.
† K_m: M($\times 10^{-5}$).

The presence of G6PDH in animal and plant tissues is well documented; however, the physiological control of the pathway remains poorly understood. Studies have been carried out on tissues from hazel [10], black gram [11], sweet potato [12] and wild oat [8]; several of these reports show how the extent of purification can effect kinetic studies. The present characterization of G6PDH from the embryo of the wild oat reveals that the V_{max} values for both substrate and coenzyme are approximately twice those for the endosperm enzyme. However, with the knowledge that the apparent K_ms are similar in both cases, this is tentatively interpreted as indicating a higher proportion of enzyme to total soluble protein in the embryo.

The possible complicating effect of 6PGDH on the assay of G6PDH has been reported [10, 13, 14]. Using a previously described technique [10], it has shown that in the assay of G6PDH for every mol of G6P utilized 2 mol of NADP were reduced due to the inability to prevent the second reaction occurring. Consequently all the G6PDH activities reported in this paper are presented with the appropriate correction.

The characterizations carried out with 6PGDH from the embryo of wild oat reveal the V_{max} for both substrate and coenzyme to be approximately the same; however the apparent K_ms show some variation. It is not clear if this is due to differing isozyme variations between the two tissues or to some other factors.

This study demonstrates that the 6PGDH enzyme complex, found in both the seed tissues of wild oat, reduced NADP at an apparent rate of five times greater than that of G6PDH at equivalent substrate and coenzyme concentrations. This is in agreement with Kovacs and Simpson [8] who show a similar activity ratio. It is unknown at present if this difference is due to the presence of a higher proportion of 6PGDH to G6PDH in both the embryo and endosperm region or a higher catalytic centre activity of the former enzyme. The results, however, are in agreement with the widely accepted view that G6PDH is the primary regulatory enzyme of the PPP. The results reported in this paper provide the basis for a study of PPP dehydrogenase activities throughout the period of after-ripening in wild oat and this will be the topic of a subsequent report.

EXPERIMENTAL

Seed stocks. Fully mature dormant wild oat seeds (*Avena fatua* L.) were collected from plants grown as an established line at the Weed Research Organization (WRO), Oxfordshire. The batches were stored dry at 5° in the dark until used 6 months later; during this period the germination potential of the seed batch did not change. At the time of use the husks were removed and the caryopses imbibed for 48 hr in petri dishes, placed at 15 in a darkened incubator. Subsequently any visibly damaged or germinated caryopses were removed. Following sterilization in 1% NaClO for 5 min and 10 washes in distilled water, the dormant caryopses were dissected under aseptic conditions into embryo and endosperm regions.

Extraction procedure. Three replications of approximately 250 caryopses were initially separated and the parts ground in a chilled pestle and mortar with a small vol. of extraction medium (0.02 M phosphate buffer, pH 7.5 + 0.48 M mannitol) at 5 . The resulting macerates were then extracted and the filtrate purified according to ref. [10]. The protein fractions collected were stored at 5 and all activities assayed within 24 hr. From a partial characterization of the crude extract it was calculated that approx. 10% of the original activity was lost by the partial purification technique used.

Assay of dehydrogenase enzymes. The activity of the dehydrogenases in the samples were measured spectrophotometrically after Gosling and Ross [10]. The conditions of assay are similar to those previously described for these enzymes and were chosen so that a comparative study could be made.

Assay of protein. Protein was measured after the method of Sedmak and Grossberg [15] using Coomassie Brilliant Blue G250 dye.

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