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Metabolon formation in dhurrin biosynthesis

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Abstract

Synthesis of the tyrosine derived cyanogenic glucoside dhurrin in *Sorghum bicolor* is catalyzed by two multifunctional, membrane bound cytochromes P450, CYP79A1 and CYP71E1, and a soluble UDPG-glucosyltransferase, UGT85B1 (Tattersall, D.B., Bak, S., Jones, P.R., Olsen, C.E., Nielsen, J.K., Hansen, M.L., Høj, P.B., Møller, B.L., 2001. Resistance to an herbivore through engineered cyanogenic glucoside synthesis. Science 293, 1826–1828). All three enzymes retained enzymatic activity when expressed as fluorescent fusion proteins *in planta*. Transgenic *Arabidopsis thaliana* plants that produced dhurrin were obtained by co-expression of CYP79A1/CYP71E1-CFP/UGT85B1-YFP and of CYP79A1/CYP71E1/UGT85B1-YFP but not by co-expression of CYP79A1-YFP/CYP71E-CFP/UGT85B1. The lack of dhurrin formation upon co-expression of the two cytochromes P450 as fusion proteins indicated that tight interaction was necessary for efficient substrate channelling. Transient expression in *S. bicolor* epidermal cells as monitored by confocal laser scanning microscopy showed that UGT85B1-YFP accumulated in the cytoplasm in the absence of CYP79A1 or CYP71E1. In the presence of CYP79A1 and CYP71E1, the localization of UGT85B1 shifted towards the surface of the ER membrane in the periphery of biosynthetic active cells, demonstrating *in planta* dhurrin metabolon formation.
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1. Introduction

Cyanogenic glucosides are amino acid-derived natural products (Conn, 1981; Zagrobelny et al., 2004; Bak et al., 2006; Morant et al., 2007). The ability to synthesize these glucosides is common across many plant genera, including several plant species that are important crop plants like sorghum (*Sorghum bicolor*), cassava (*Manihot esculenta*), flax (*Linum usitatissimum*) and almonds (*Prunus dulcis*). Degradation of cyanogenic glucosides is catalyzed by β-glucosidases and α-hydroxynitrilases and results in release of hydrogen cyanide. Accordingly, cyanogenic glucosides are classified as phytoanticipins that may play a role in plant defense (Tattersall et al., 2001). The biosynthetic pathway for the cyanogenic glucoside dhurrin has been

Abbreviations: CaMV, cauliflower mosaic virus; CFP, cyano fluorescent protein; CLSM, confocal laser scanning microscopy; YFP, yellow fluorescent protein; 3D, three dimensional.

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elucidated using a microsomal system from sorghum that catalyses the conversion of the parent amino acid L-tyrosine to the aglycone *p*-hydroxymandelonitrile (Morant et al., 2003; Møller and Seigler, 1999). The pathway involves a number of unusual and labile intermediates like an *N*-hydroxyamino acid, an *N,N*-dihydroxyamino acid, *E*- and *Z*-oximes and a cyanohydrin (Fig. 1). Except for the *Z*-oxime, these intermediates are efficiently channelled for dhurrin production and difficult to trap during biosynthesis (Møller and Conn, 1980; Kahn et al., 1997; Sibbesen et al., 1995; Kristensen et al., 2005). At the genetic level, the pathway is surprisingly simple because the conversion from tyrosine to the aglycone is catalysed by two multifunctional cytochromes P450 (CYPs) each encoded by a single structural gene. CYP79A1 catalyzes conversion of L-tyrosine into *Z*-*p*-hydroxyphenylacetaldoxime (Koch et al., 1995; Sibbesen et al., 1995) which is subsequently converted by CYP71E1 to the cyanohydrin *p*-hydroxymandelonitrile (Bak et al., 1998; Kahn et al., 1997). Finally, the labile *p*-hydroxymandelonitrile is stabilized by glucosylation *via* a soluble UDP-Glc glucosyltransferase UGT85B1 to produce dhurrin (Jones et al., 1999; Kahn et al., 1999; Thorsøe et al., 2005). The entire pathway for dhurrin synthesis has been transferred to *Arabidopsis thaliana* and *Lotus japonicus* using genetic engineering (Tattersall et al., 2001; Morant et al., 2003,2007; Kristensen et al., 2005). The dhurrin content of these transgenic *A. thaliana* plants is high and accounts for 4% (w/w) of leaf dry-weight. As with *S. bicolor*, pathway intermediates were hardly detectable.

Glycosyltransferases involved in plant natural product synthesis primarily belong to family 1 and are designated UDP-glycose-glycosyltransferases (UGTs) (Mackenzie et al., 1997). The fully sequenced *A. thaliana* genome (The Arabidopsis Genome Initiative, 2000) has provided annotation of 112 predicted full-length family 1 UGTs and eight apparent pseudo-genes (Paquette et al., 2003). All three biosynthetic enzymes including sorghum UGT85B1 are required for dhurrin production in transgenic *A. thaliana* plants (Tattersall et al., 2001). Thus none of the endogenous *A. thaliana* glucosyltransferases available are able to mediate α-carbon specific glucosylation of *p*-hydroxymandelonitrile. Like other cyanohydrins, *p*-hydroxymandelonitrile is labile at physiological pH dissociating into *p*-hydroxybenzaldehyde and hydrogen cyanide (Fig. 1). To avoid constant generation of toxic hydrogen cyanide in those parts of the sorghum plant where dhurrin synthesis takes place, UGT85B1 is envisioned to glucosylate the labile cyanohydrin before it dissociates. The early

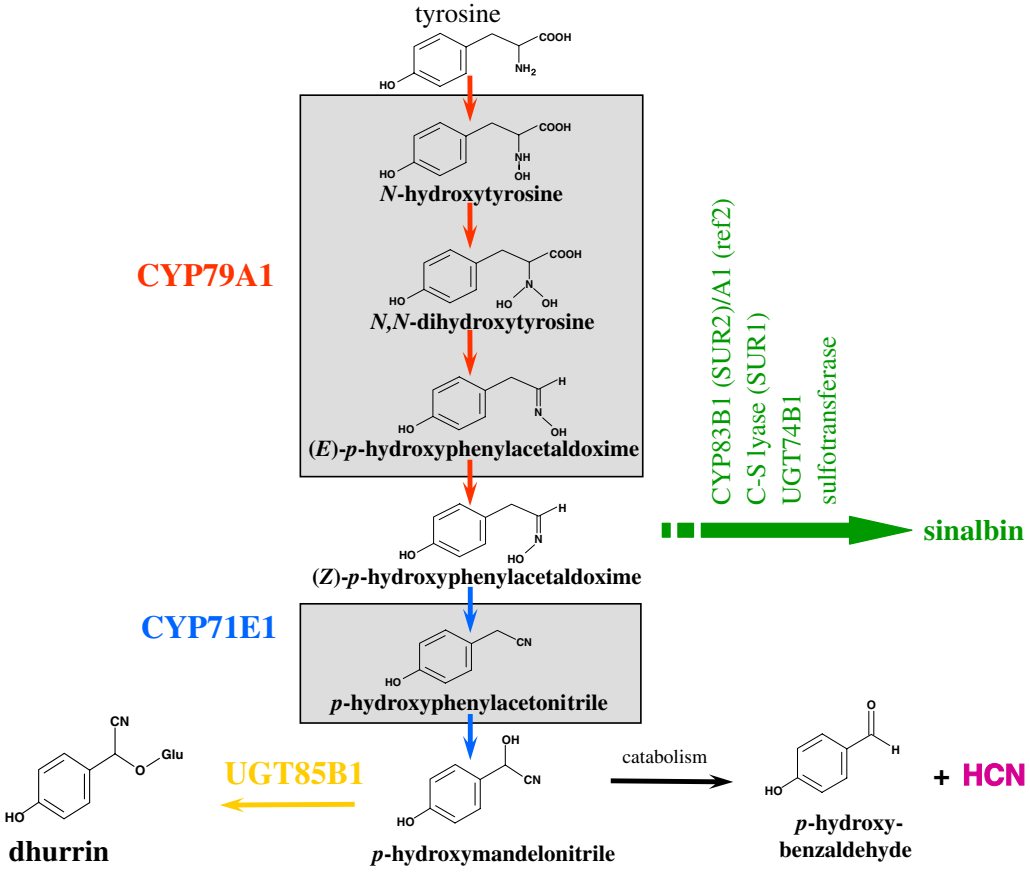

Fig. 1. Biosynthetic pathway for the cyanogenic glucoside dhurrin. The scheme shows the enzymatic reactions catalysed by each of the three biosynthetic enzymes CYP79A1, CYP71E1 and UGT85B1 isolated from *Sorghum bicolor*. Intermediates entrapped in the active sites of the two multifunctional cytochrome P450s are shown on a gray background.

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