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Molecules of Interest

Saponins in cereals

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Abstract

Saponins are a diverse family of secondary metabolites that are produced by many plant species, particularly dicots. These molecules commonly have potent antifungal activity and their natural role in plants is likely to be in protection against attack by pathogenic microbes. They also have a variety of commercial applications including use as drugs and medicines. The enzymes, genes and biochemical pathways involved in the synthesis of these complex molecules are largely uncharacterized for any plant species. Cereals and grasses appear to be generally deficient in saponins with the exception of oats, which produce both steroidal and triterpenoid saponins. The isolation of genes for saponin biosynthesis from oats is now providing tools for the analysis of the evolution and regulation of saponin biosynthesis in monocots. These genes may also have potential for the development of improved disease resistance in cultivated cereals.

Keywords: Saponins, Triterpenoids; Cereals; *Avena*; Oats; Disease resistance; β-Amyrin; Avenacins

1. Introduction

Saponins are a major family of secondary metabolites that occur in a wide range of plant species (Hostettmann and Marston, 1995). These molecules are synthesised from mevalonic acid via the isoprenoid pathway and are derived from triterpenoid or steroid cyclisation products of 2,3-oxidosqualene. Saponins are characterised by their surfactant properties (the name saponin is derived from *sapo*, the Latin word for soap) and give stable, soap-like foams in aqueous solution. Various members of this important family of plant secondary metabolites are exploited commercially for a variety of purposes including as drugs and medicines, precursors for hormone synthesis, adjuvants, foaming agents, sweeteners, taste modifiers and cosmetics. Since many saponins have potent antimicrobial activity the natural role of these molecules in plants is likely to be in conferring protection against attack by potential pathogens (Morrissey and Osbourn, 1999). The process of saponin biosynthesis is not well understood despite the considerable interest in this important group of natural products (Haralampidis et al., 2001a). This is due in part to the complexity of the molecules and also to the lack of pathway intermediates for biochemical studies. A more detailed understanding of these secondary metabolite pathways and of the genes that are involved will facilitate the development of plants with altered or novel saponin content, either by classical plant breeding or by transformation-mediated genetic modification.

Triterpenoid saponins are found principally in dicotyledonous species, while many of the major steroidal saponins are synthesised by monocots such as members of the Liliaceae, Dioscoraceae and Agavaceae families (Hostettmann and Marston, 1995). Cereals and grasses appear to be generally deficient in these secondary metabolites with the exception of oats (*Avena* spp.). Members of the genus *Avena* synthesise two different families of saponins, the steroidal avenacosides (Tschesche et al., 1969; Tschesche and Lauven, 1971) and the triterpenoid avenacins (Crombie and Crombie, 1986; Crombie et al., 1986a). The distribution of these two classes of saponin is mutually exclusive, avenacosides accumulating in the leaves and avenacins in the roots. The major oat root saponin avenacin A-1 is esterified with *N*-methyl anthranilic acid (Fig. 1) and so fluoresces under ultra-violet light (Crombie and Crombie, 1986; Crombie et al., 1986a). This property is

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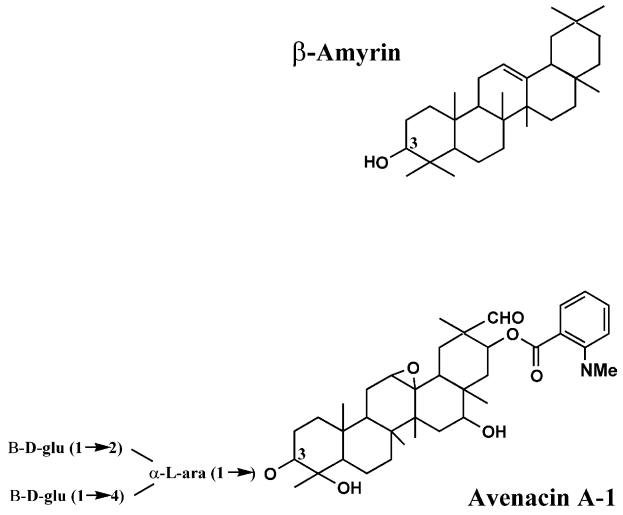

Fig. 1. The 2,3-oxidosqualene cyclisation product β-amyrin and the major oat root saponin avenacin A-1.

extremely unusual amongst saponins and has proved to be a valuable tool for the dissection of triterpenoid saponin biosynthesis (Papadopoulou et al., 1999). Progress that has been made in understanding avenacin biosynthesis is the focus of this article.

2. Structure

The avenacins are a family of four structurally related molecules, avenacins A-1, B-1, A-2 and B-2 (Crombie and Crombie, 1986; Crombie et al., 1986a). Avenacin A-1 is the most abundant of the four in extracts from young oat roots, comprising around 70% of the total avenacin content (Crombie and Crombie, 1986). This family of saponins is derived from the triterpenoid β-amyrin (Trojanowska et al., 2000, 2001; Haralampidis et al., 2001b) (Fig. 1). All four avenacins have a common trisaccharide moiety consisting of β,1–2- and β,1–4-linked D-glucose molecules attached via L-arabinose to the aglycone at the C-3 carbon. Avenacins A-1 and B-1 are esterified with *N*-methyl anthranilic acid and so are fluorescent under ultra-violet light while avenacins A-2 and B-2 are esterified with benzoic acid and have little or no UV-fluorescence. The A-1 and A-2 compounds each contain one extra oxygen atom at C-4 in comparison to their B-1 and B-2 counterparts.

3. Biological activity

Avenacins, like many other saponins, have potent antifungal activity (Turner, 1953; Crombie et al., 1986b). The antifungal properties of saponins are generally ascribed to the ability of these molecules to complex with sterols in fungal membranes, so causing pore formation and loss of membrane integrity (reviewed in Morrissey and Osbourn, 1999). Experiments with planar lipid bilayers have confirmed that avenacin A-1 induces permeabilisation in a sterol-dependent manner and that it also affects membrane fluidity (Armah et al., 1999). The presence of an intact sugar chain attached to the C-3 position is critical for these effects on artificial membranes (Armah et al., 1999) and also for effective antifungal activity (Turner, 1961; Crombie et al., 1986b; Osbourn et al., 1991). The sugar chains may mediate the aggregation of saponin–sterol complexes in the membrane, so facilitating membrane disruption (reviewed in Morrissey and Osbourn, 1999). Removal of a single D-glucose molecule from the trisaccharide chain results in a substantial reduction in biological activity (Turner, 1961; Crombie et al, 1986b; Osbourn et al., 1991; Armah et al., 1999). It is not clear how oats protect themselves from the membrane-permeabilising effects of the saponins that they produce. Avenacins, like many other plant secondary metabolites, are likely to be sequestered in the vacuoles of plant cells. The vacuolar membranes of saponin-containing cells may have low sterol content or contain a high proportion of substituted sterols that do not favour the formation of saponin–sterol complexes.

4. Role in disease resistance

The major avenacin, avenacin A-1, is localised in the epidermal cell layer of oat root tips and also in the emerging lateral root initials (Turner, 1960; Osbourn et al., 1994) (Fig. 2a,b), and so is ideally positioned to represent a chemical barrier to invading soil-borne microbes that damage plant tissue. Evidence for a role for avenacins in disease resistance has come from investigation of oat variants that differ in saponin content. There is very little natural variation in avenacin content within *Avena* spp. although at least one diploid oat species (*Avena longiglumis*) has been shown to lack avenacin A-1 and significantly is more susceptible to fungal disease than its avenacin-producing relatives (Osbourn et al., 1994). Unfortunately *A. longiglumis* does not hybridise readily with other oat species, making genetic analysis of the association between avenacin content and disease resistance difficult. However the fluorescence associated with avenacin A-1 can be readily visualised in roots of young oat seedlings and has been exploited in a screen to isolate mutants of a diploid avenacin-producing oat species *Avena strigosa* that are deficient in their ability to synthesise avenacins (Papadopoulou et al., 1999) (Fig. 2b). These saponin-deficient (*sad*) mutants are compromised in their resistance to a range of pathogens, providing strong evidence to indicate that avenacins do indeed act as preformed chemical defenses against pathogen attack (Papadopoulou et al., 1999).

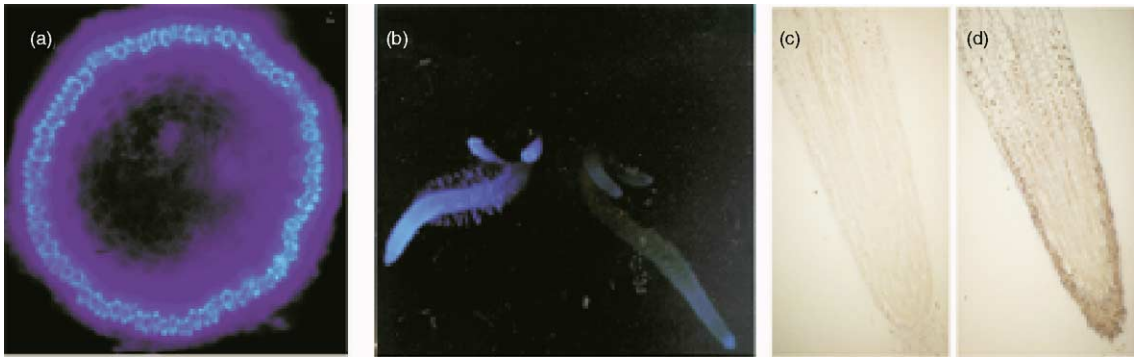

Fig. 2. (a) Cross-section of a young oat root showing localisation of fluorescence associated with avenacin A-1 in the epidermal cell layer. (b) Isolation of saponin-deficient oat mutants using a simple screen for reduced fluorescence in the roots of young seedlings (left, wild type *A. strigosa* parent; right, a saponin-deficient mutant). (c,d) In situ mRNA hybridisation showing expression of the oat β-amyrin synthase gene *AsbAS1* in the epidermal cells in longitudinal root sections (c, sense control; d, *AsbAS1* probe).

5. Synthesis—cyclisation of 2,3-oxidosqualene to β-amyrin

The first committed step in avenacin biosynthesis is the cyclisation of 2,3-oxidosqualene to β-amyrin, which is catalysed by the oxidosqualene cyclase enzyme β-amyrin synthase (Haralampidis et al., 2001a,b). Two lines of evidence indicate that the root tip is the main site of synthesis of β-amyrin. Firstly, incorporation of radioactivity from *R*[2-^{14}C]MVA into β-amyrin and avenacins occurs primarily in the growing tips of oat roots (Trojanowska et al., 2000). Secondly, β-amyrin synthase activity is high in the root tips and low or undetectable in older parts of the root (Trojanowska et al., 2001). Oat β-amyrin synthase (AsbAS1) has been cloned by expressed sequence tag (EST)-analysis of cDNA clones derived from oat root tips (Haralampidis et al., 2001b) and shown to correspond to *Sad1*, one of the genes defined by mutagenesis as being required for avenacin biosynthesis (Papadopoulou et al., 1999; Haralampidis et al., 2001b). In situ hybridisation indicates that the gene is expressed primarily in the epidermal cells of the root tip, consistent with the localisation of the fluorescent avenacins (Osbourn et al., 1994; Haralampidis et al., 2001b) (Fig. 2c,d). These observations suggest that the complete biosynthetic process is likely to take place in the root epidermis.

AsbAS1 is the first triterpene synthase to be cloned from monocots. Although it shares overall amino acid sequence similarity with other members of the oxidosqualene cyclase superfamily (triterpene synthases and cycloartenol synthases from plants and lanosterol synthases from animals and fungi), it is substantially different from these enzymes (Haralampidis et al., 2001b). Thus AsbAS1 defines a new class of oxidosqualene cyclases.

6. Elaboration of β-amyrin into antifungal saponins

The conversion of β-amyrin into antifungal avenacins will require a series of modifications involving cytochrome P450-dependent monooxygenases, acyltransferases and glycosyltransferases (Haralampidis et al., 2001a,b). The relevant genes have not yet been cloned although the *sad* mutant collection has defined at least six additional loci that are required for avenacin biosynthesis (Papadopoulou et al., 1999). Given the complexity of the avenacins it is likely that further mutant screening will identify more loci in addition to the original seven. The trisaccharide chain attached to the C-3 carbon is likely to be synthesized by sequential addition of single sugar molecules to the aglycone by glycosyltransferases (Haralampidis et al., 2001a). Two *sad* mutants that accumulate monodeglucosyl avenacins have been identified (Papadopoulou et al., 1999) although it is not yet known whether these mutants are specifically defective in glycosyltransferases required for avenacin biosynthesis. A combination of classical genetics, ESTs, biochemistry and other complementary approaches is proving powerful in the isolation of further genes for saponin biosynthesis from *A. strigosa*.

7. Evolution of triterpenoid saponin biosynthesis in the Gramineae

Avenacins are restricted to the genus *Avena* and the closely related species *Arrhenatherum elatius*. Other cultivated cereals appear to be generally deficient in antifungal saponins of any kind. Orthologs of *AsbAS1* are absent from modern cereals and may have been lost during selection, raising the possibility that this gene could be exploited to enhance disease resistance in crop plants (Haralampidis et al., 2001b). However it is not yet known whether other biosynthetic steps are also missing in these other species. The isolation of additional genes for saponin biosynthesis from *A. strigosa* will help to shed light on this.

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