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Birch PR-10c interacts with several biologically important ligands

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Abstract

PR-10c is a unique member of PR-10 proteins in birch, since it is the only one known to be post-translationally modified by glutathione and is not constitutively expressed in pollen. Both reduced and S-glutathiolated forms of PR-10c show low ribonuclease activity. However, the major function of the protein is apparently not yet resolved. Our protein–ligand interaction studies with saturation transfer difference (STD) NMR revealed that PR-10c interacts with several biologically important molecules, including cytokinin, flavonoid glycosides, sterols and emodin. Competition study with deoxycholate and kinetin revealed no statistically significant binding interference, indicating that these ligands have different binding sites in PR-10c. Ligand docking studies with a molecular model of PR-10c support the STD NMR results of ligand binding and binding epitopes, suggesting that there are three potential binding sites in PR-10c: two in the hydrophobic cavity and one in the glycine-rich loop. Our docking calculations suggested that only kinetin interacts with the glycine-rich loop, the binding occurring through its adenine moiety. Clear ligand specificity could be observed in the binding of nucleotide derivatives. S-glutathiolation of PR-10c did not affect kinetin binding. The present results suggest that birch PR-10c is a multifunctional protein, which has diverse roles in plant stress responses.

Keywords: Saturation transfer difference NMR; Molecular modelling; Pathogenesis-related proteins; Glutathiolation; Cytokinins; Phytosterols; Flavonoids; Emodin

1. Introduction

The PR-10 (pathogenesis-related class 10) protein family consists of a large group of homologous proteins found from several plant species. The expression of PR-10 proteins is up-regulated as a response to various biotic and abiotic factors. Several food and pollen allergens, e.g., Bet v 1 (Breiteneder et al., 1989), Api g 1 (Breiteneder et al., 1995), Mal d 1 (Vanek-Krebitz et al., 1995; Pühringer et al., 2000), Pru av 1 (Scheurer et al., 1997) and hazelnut major allergen Cor a 1.04 (Lüttkopf et al., 2002) are included in the PR-10 family. Furthermore, the sequence and structural homology of PR-10 proteins with major latex proteins (MLP) suggest that these proteins are related and might share a similar function (Osmark et al., 1998).

Although several PR-10 proteins have been reported to possess RNase activity, including birch Bet v 1 (Swoboda et al., 1996; Bufe et al., 1996), birch PR-10c (Koistinen et al., 2002a), lupin root LaPR-10 (Bantignies et al., 2000), cotton GaPR-10 (Zhou et al., 2002), PR-10 protein from *Pachyrrhizus erosus* seeds (Wu et al., 2002) and pepper CaPR-10 (Park et al., 2004), also other functions have been ascribed to PR-10 proteins. Structural homology of Bet v 1 with the START domain of MLN64 (Tsujishita and Hurley, 2000) suggested a similar function for Bet v 1 as a steroid binding protein; until now, Pru av 1 (Neudecker et al., 2001), Bet v 1

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(Mogensen et al., 2002) and Bet v 1l (Marković-Housley et al., 2003) have been shown to interact with phytosteroids. Furthermore, Bet v 1 interacts with fatty acids, cytokinins and flavonoids (Mogensen et al., 2002). It has been reported recently that Hyp-1 protein from *Hypericum perforatum*, which shows high sequence similarity with PR-10 proteins, is able to convert emodin to hypericin in vitro (Bais et al., 2003).

Although PR-10c shares high sequence similarity with Bet v 1 and other PR-10 proteins in birch, it is a unique member of PR-10 proteins, and appears to belong to a different subfamily. It has much higher sequence identity with Cor a 1.04 proteins from hazelnut than with any other birch PR-10 protein. Furthermore, unlike the *Bet v* 1 genes, *PR-10c* is not constitutively expressed in pollen (Swoboda et al., 1995). The most interesting similarity between PR-10c and four Cor a 1.04 proteins is the presence of a cysteine residue in position 82-83, which is absent from the other PR-10 proteins in birch. This cysteine in PR-10c can be post-translationally modified by glutathione both in vitro and in vivo (Koistinen et al., 2002a). Even though the PR-10 proteins share a high sequence similarity, the different subfamilies may differ in their structure. Pasternak et al. (2005) reported that LlPR-10.2A, a member of a novel subclass of lupin PR-10, deviates structurally from LlPR-10.1A and LlPR-10.1B of another subclass of lupin PR-10 family by having a different shape and volume in the hydrophobic ligand-binding cavity.

Furthermore, different expression profiles have been described for *PR-10c* and *PR-10a* genes in birch in response to wounding (Poupard et al., 1998) and auxin treatment (Poupard et al., 2001). The different PR-10 proteins also respond differently to copper exposure (Koistinen et al., 2002b). Even though at least three possible functions have been proposed for PR-10 proteins, the exact role of these proteins in plant stress responses, defence, stress tolerance and pollen development is still unclear.

The aim of the present study was to examine the possible binding of various biologically important ligands to PR-10c, and to determine the binding epitope of the ligands using saturation transfer difference (STD) NMR spectroscopy (Mayer and Meyer, 1999) and molecular modelling in order to shed more light to the role of PR-10 proteins in stress response and plant defence.

2. Results

2.1. Binding of PR-10c to nucleotide derivatives

The structure, biological function and binding epitope of ligands interacting with PR-10c-His are presented in Table 1. Binding of cytokinin (kinetin) was studied both on the reduced and glutathiolated form of PR-10-His protein. The STD NMR results indicated that both forms interact with kinetin (Fig. 1). The calculated STD factors show that adenine rather than furan moiety of the molecule interacts with the protein (Fig. 2(a)). The interaction of kinetin was also tested with a higher concentration of another PR-10c-His protein construct (containing thrombin protease cleavage site) with similar results. Binding of another adenine derivative, i.e., ATP, to PR-10c-His protein could not be detected with STD NMR. The method has been used recently to characterize the binding of ATP to protein kinases (McCoy et al., 2005).

2.2. Birch PR-10c binds flavonoid glycosides

The interaction of PR-10c-His with flavonoids was studied with two quercetin glycosides, i.e., quercetin-3-*O*-galactoside (hyperoside) and quercetin-3-*O*-rutinoside (rutin). Both hyperoside and rutin interact with PR-10c-His. The results clearly indicated that the interaction occurs through the B ring of quercetin moiety since no saturation transfer was observed for the glycosidic and A ring protons (Fig. 3).

2.3. Interaction of PR-10c-His with other biologically important molecules

PR-10 homologue Hyp-1 protein has been reported to form hypericin from emodin in vitro (Bais et al., 2003). The present results indicate that emodin clearly interacts with PR-10c-His protein (Fig. 4). However, formation of hypericin from emodin could not be demonstrated within 72 h with NMR experiments. Neither was any significant difference observed in STD factors for aromatic protons.

The PR-10c-His protein interacted also with deoxycholate. The CH_3-19 appears to have the largest relative STD-effect. STD-effects were also calculated for some other CH_3-groups; those were all smaller than the STD-% of CH_3-19. No significant effects were seen for aliphatic chain hydrogens. This could indicate, together with the fact that the molecule is roughly planar, that the steroid nucleus of the molecule is closer to the protein. Competition experiment with kinetin showed that these two molecules do not interfere with each other statistically significantly (Fig. 2), suggesting that deoxycholate and kinetin have different binding sites in the protein.

Mogensen et al. (2002) have reported that birch Bet v 1 does not interact with indole-3-acetic acid or gibberellic acid, and these molecules were thus used as negative controls for the STD NMR measurements. The present results indicated that, even though PR-10c bound several biologically important molecules, no interaction occurs between PR-10c-His and indole-3-acetic acid or gibberellic acid.

2.4. Molecular modelling of PR-10c and ligand docking

Docking of kinetin, emodin, gibberellic acid and deoxycholate revealed two separate theoretical binding sites in the hydrophobic binding cavity of PR-10c (Fig. 5). The binding sites locate at the opposite corners of the cavity so that two molecules may bind to the cavity at the same