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Specific interaction of jacalin with phycocyanin, a fluorescent phycobiliprotein

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ABSTRACT

Recent research has shown that, like porphyrins, phycocyanin (PC) too can produce singlet oxygen upon excitation with the appropriate radiation and hence could be useful in photodynamic therapy (PDT) for cancer. Unlike porphyrins, PC has the advantage of being a non-toxic, non-carcinogenic, soluble protein. However, the challenge would be to target the fluorescent phycobiliprotein to malignant cells. We report here that the tumor-specific lectin, jacalin, binds PC specifically in a carbohydrate-independent manner and with affinities better than that for porphyrins. Hence the lectin could prove to be a useful carrier for targeted delivery of PC. The interaction involves both ionic and hydrophobic interactions and more than one contact site.

1. Introduction

Lectins are broadly defined as multivalent carbohydrate-binding proteins that recognize diverse sugar structures with high specificity. They exert their biological effects in cell–cell recognition, host pathogen interactions, malignancy, cellular signaling and differentiation and immune responses through binding to appropriate carbohydrates [1,2]. Most thoroughly studied are those lectins extracted from plants, whose abundant availability and ease of isolation make them attractive candidates from the point of view of applications. Plant lectins are now being increasingly used in various applications requiring identification of protein glycosylation patterns and are now an integral part of glycomic approaches to understanding cellular processes [3–6].

Of late, it has been shown that carbohydrate binding may be only one of the several aspects of the endogenous function of lectins. Thus several lectins have been shown to be multifunctional. In the case of animal lectins this has been more the rule than the exception (see for example, [7,8]). The significance of non-carbohydrate binding by plant lectins have been more difficult to elucidate although some progress has been made in recent times [9–11]. It has also been shown *in vitro* that plant lectins may bind carbohydrate–mimetic peptides as well as hydrophobic ligands such as 1,8-anilinonaphthalene sulphonic acid (ANS), 2,6-toludinylnaphthalene sulphonic acid (TNS), adenine or other phytohormones and porphyrins (for review see [12]).

Of the plant lectins that have been studied for carbohydrate-independent protein–protein interactions is *Artocarpus integrifolia* agglutinin (jacalin) that triggers CD4-mediated lymphocyte signaling via protein–protein interactions. Indeed, jacalin is being used in the research of acquired immunodeficiency syndrome (AIDS) due to its ability to specifically stimulate CD^{4+} cells in comparison to primary T cells [13–18]. Jacalin also inhibits *in vitro* HIV-1 infection of various lymphoid cells [19]. The lectin has also excited a great deal of interest, given its unique ability to recognize the T-antigenic determinant Galβ-1–3-GalNAc [20] and thereby act as a potential candidate for targeted drug delivery. Its high specificity for O-linked glycosylation has seen its extensive use in the purification of glycoproteins such as mucin and IgA1 [21]. Using a series of O-linked glycopeptides, it was further shown that jacalin preferentially recognizes GalNAcα-1-peptides whose C6 hydroxyl is free but cannot recognize those with substitutions at this position [22]. Jacalin is also capable of recognizing additional ligands besides galactose (Gal) and its derivatives. For example, it has been shown that the carbohydrate recognition domain (CRD) of the lectin has the plasticity to recognize mannose also, albeit with lower affinity than Gal [23–25].

Additionally, it has been shown that jacalin can bind porphyrins with high affinity and in a carbohydrate-independent manner in solution [26]. It has been hypothesized that hydrophobic interactions with the tetrapyrrole ring are most likely responsible for the lectin–porphyrin interaction that is observed. These novel interactions throw up additional questions on the kinds of interactions that jacalin is capable of in solution and suggests the potential use of jacalin as a drug delivery agent in photodynamic therapy (PDT) [26,27].

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Like porphyrins, phycocyanin (PC) too is a cytotoxic photosensitizer capable of killing cells via singlet oxygen production upon excitation with the appropriate radiation and could be a useful candidate for PDT [28,29]. Yet, unlike porphyrins, PC is a soluble protein, making it a far more attractive candidate for PDT. Also, it is non-carcinogenic, non-toxic and is currently used as a coloring agent in food and cosmetics. PC has been shown to possess significant antioxidant, anti inflammatory, hepatoprotective and radical scavenging properties [30,31]. It has also been shown to provide protection against oxidative damage to DNA *in vitro* [32]. Further, PC could protect rats from kainic acid induced neuronal damage without any accompanying toxicity, suggesting that it could have potential applications in treatment of neurodegenerative diseases such as Alzheimer's and Parkinson's [33]. Thus, PC appears to be an attractive candidate to link up to jacalin for targeted drug delivery, provided PC does not interfere with the lectin's carbohydrate-specificity.

Unlike porphyrins that have a tetrapyrrole ring, PC has a covalently attached open chain tetrapyrrole called 'bilin' as its prosthetic group. So, the first question we chose to address was whether jacalin could specifically interact with PC. If so, we also wanted to study the strength of this interaction and whether it would affect the affinity of the lectin for its carbohydrate ligand. We present results here that suggest that jacalin does indeed exhibit specific, carbohydrate-independent, protein–protein interactions with PC. We also show that these interactions are predominantly hydrophobic in nature, although repulsive ionic interactions between the two proteins may play an important role in allowing carbohydrate binding by jacalin in the presence of PC. Further, PC interacts with jacalin with affinities better than those observed for porphyrins with jacalins.

2. Material and methods

Jackfruit seeds were obtained from local sources. Guar gum was obtained from SRL. Sodium phosphate dibasic and monobasic, sodium chloride, sodium citrate, citric acid and galactose (Gal) were purchased from Merck. Phycocyanin, acrylamide and bis-acrylamide were purchased from Sigma, U.S.A. All other reagents were of analytical grade.

2.1. Purification of jacalin

Jacalin was purified by affinity chromatography on cross-linked guar gum by established protocols [20]. The purified protein was thoroughly dialyzed against 20 mM sodium phosphate buffer, pH 7.4, containing 150 mM NaCl (PBS) in order to free it from the eluting sugar (galactose). Authenticity of the affinity purified protein was verified by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE) [34]. Jacalin concentration was estimated using its absorbance at 280 nm ($A_{1\%,1cm}$ = 11.4) [35].

2.2. Agglutination assay

Hemagglutination assays were carried out using serial dilution of the lectin using a 4% suspension of human A+ erythrocytes. Hemagglutination inhibition assays were performed in duplicates by serial dilution of the protein in phosphate buffered saline.

2.3. Interaction of PC with jacalin

The interaction of phycocyanin to jacalin was investigated at room temperature (~25 °C) using fluorescence spectroscopy. Fluorescence spectroscopy was performed on a Cary Varian spectrofluorimeter equipped with a constant temperature cell holder. The fluorescence emission spectra were measured using a 1 cm path length cell. When monitoring the fluorescence emission of PC, the sample was excited at 580 nm and emission was observed at 610–700 nm using slit widths of 5 nm for excitation and 10 nm for emission. When monitoring the fluorescence emission of jacalin, the sample was excited at 295 nm and emission was observed at 310–400 nm. Each spectrum was an average of 10 scans. The binding data was analyzed using double log plots as described by Sharon and co-workers [36].

2.4. Modification of Lys residues

Modification of lysine residues were done by treating both jacalin and PC separately with *N*-succinimidyl 3-(2-pyridyldithio)propionate (SPDP) as reported in literature [37]. The extent of primary amino group (lysine or *N*-terminal-NH_2) modified was calculated by measuring the liberation of pyridine-2-thione group following treatment with DTT. From the absorption of pyridine-2-thione at 343 nm, the concentration of the liberated group was calculated by using the extinction coefficient $\varepsilon_{343} = 8.08 \times 10^3\ M^{-1}$ [38]. In the case of jacalin, we estimated that 2.66 ± 0.22 primary amines per subunit were modified. In the case of PC, under similar conditions, 3.41 ± 0.07 residues were modified per monomer.

2.5. Chemical cross-linking with glutaraldehyde

Cross-linking of jacalin with PC was carried out by a single-step conjugation in the presence of 220 μl glutaraldehyde (0.5%) for one hour at room temperature. The reaction was stopped by addition of excess 1 M glycine, dialyzed, centrifuged and supernatant analyzed by SDS–PAGE.

3. Results

Lectins have been popularly known as molecules that help decipher the glycocode. The common understanding is that since lectins can be used in the identification of glycoproteins, their interaction with proteins must necessarily imply the occurrence of specific glycosylation patterns on the latter. Thus these interactions are presumably generally mediated via the carbohydrate recognition domain (CRD) of the lectin. Indeed, lectins are routinely used in the identification and purification of glycoproteins [39]. However, in recent times, it has been shown that carbohydrate binding, although significant, is not necessarily the only kind of recognition that lectins are capable of [12]. Using fluorescence spectroscopy, we present results to show that jacalin is capable of carbohydrate-independent protein–protein interaction with PC, an unglycosylated, phycobiliprotein.

When excited specifically at 295 nm, Trp residues of proteins give a typical emission spectrum that is very sensitive to solvent polarity and hence to the environment around Trp residues. Jacalin gives a Trp emission spectrum that is sensitive to the interaction of the lectin with PC. It may also be noted that PC itself does not show any significant fluorescence emission when excited at this wavelength. However, it is possible to excite PC within its Soret band, an absorption characteristic of the tetrapyrrole moiety of PC. Hence the interaction could also be studied by specific excitation at 580 nm.

3.1. Interaction of jacalin with PC can be monitored using Trp fluorescence

Upon titration with PC, the fluorescence emission intensity of jacalin is specifically enhanced (Fig. 1A) in a manner similar to that observed for lectin–carbohydrate interactions. This enhancement