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Title of PhD- Spectroscopic Characterization of Lectin-Phycobiliprotein Interaction

ABSTRACT

Phycobiliproteins are highly water-soluble and shows no change in spectroscopic or other physical properties on storage in aqueous solution for long periods of time. Three main classes of phycobiliproteins exist: allophycocyanin (APC, bluish green), phycocyanin (PC, blue) and phycoerythrin (PE, red) having A_{max} of 650-655nm, 615-640nm and 565-575nm respectively. These can be readily coupled to a variety of small molecules (e.g. biotin, digoxigenin) or proteins (e.g. monoclonal antibodies, avidin, streptavidin. The fluorescent applications of phycobiliproteins (PBPs) have been taken into account for our protein-protein interaction studies.

Cyanobacterium *Spirulina platensis -S5* grown under CFTRI medium (pH-9.5); 12:12 light & dark at 30$\pm$1°C from harvested cell mass. Absorption and fluorescence spectroscopy showed peaks at 620nm & 643nm respectively, which confirmed that purified phycocyanin is C-Phycocyanin. Conformational stability of Phycocyanin was investigated by fluorescence, fluorescence quenching and CD spectroscopy. Both Urea & Gd-HCL were used as denaturants. Unfolding of PC was found to be a two state process showing sigmoidal curve. The values of ΔG^{o} (denaturant = 0) by both CD and fluorescence spectroscopy were roughly comparable in both cases. Thermal unfolding of the protein was also studied. The melting point or temperature of unfolding of the C-PC (T_m) corresponded to 329 Kelvin. Lectins are broadly defined as multivalent carbohydrate-binding proteins that recognize diverse sugar structures with high specificity. Jacalin and PNA were purified by affinity chromatography on cross linked guar gum.

Interaction studies had been performed between PC and lectins (Jacalin, PNA and ConA). Jacalin-PC interaction occurs *via* two independent sites on the jacalin in a carbohydrate independent manner (using intrinsic fluorescence of the two proteins). The legume lectins ConA and PNA, shared some commonalities in their interaction with PC.

Similar to jacalin, ConA and PNA too appeared to interact with at least two sites. Like jacalin, the legume lectins continue to exhibit hemagglutination activity in the presence of PC. However, due to differences in their quaternary associations as well as their ligand binding sites, the two legume lectins also show several differences with respect to each other. The extent of ionic interactions at *site1*, as judged by the effect of high ionic strengths on the association, is higher in PNA as compared to Con A.

The interaction between the jacalin and the PC is predominantly hydrophobic in nature. Legume lectins on the other hand appeared to have a significant amount of ionic interactions at *site1* and predominantly hydrophobic interactions at *site2*. Modification of Lys residues with SPDP on PC leads to enhanced interaction at both sites in case of jacalin. For ConA modification of Lys residues on PC leads to marginal enhancement in interaction at *site1* and a significant enhancement at *site2*. PNA on the other hand shows ten-fold weaker interactions at *site1* and an almost unaltered interaction at *site2* under similar condition.

For conjugation studies, clinically useful molecules (lectins) have been used with PBP. In case of PC-Jacalin it is possible to envisage covalently linking PC to jacalin to generate lectin-PC conjugates that could be far more robust for clinical applications. PC is a cytotoxic photosensitizer which, upon excitation with the appropriate radiation, kills cells via singlet oxygen production and hence could be a useful candidate for PDT. Thus, PC could also be a candidate for targeted drug delivery. Further, it may be possible to engineer PC molecules with higher specificity for jacalin if the Lys residues involved in the interaction could be identified and mutated.

Both ConA and PNA also have several clinical applications and have the potential of acting as the carrier molecule in targeted drug delivery. As it is known that protein-protein interactions could also be deliberately used in clinical applications to improve targeting. In our study, the use of SPDP was deliberate since this allows us a handle for cross-linking PC to PNA *via* the chemically introduced thiol moeities. Although in this case the length of the linker and the extent of Lys modification by SPDP were perhaps insufficient for the cross-linking to happen with high efficiency, it does provide us with clues for a probable strategy for generating lectin-PC conjugates. Covalent cross-linking of SPDP-modified PC with the SPDP-modified legume lectins yielded conjugates of the expected molecular weights. These lectin-PBP conjugates could be used as fluorescent marker to malignant tissues and many more clinical applications. Alternatively, these conjugates could also be used for easy identification of tumour tissue or for identifying specific kinds of/ aberrant glycosylation patterns in pathology labs.