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Energetics of carbohydrate binding to *Momordica charantia* (bitter gourd) lectin: An isothermal titration calorimetric study

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Abstract

Physico-chemical and carbohydrate binding studies have been carried out on the *Momordica charantia* (bitter gourd) seed lectin (MCL). The lectin activity is maximal in the pH range 7.4–11.0, but decreases steeply below pH 7.0. The lectin activity is mostly unaffected in the temperature range 4–50 °C, but a sharp decrease is seen between 50 and 60 °C, which could be correlated to changes in the structure of the protein as seen by circular dichroism and fluorescence spectroscopy. Isothermal titration calorimetric studies show that the tetrameric MCL binds two sugar molecules and the binding constants (K_b), determined at 288.15 K, for various saccharides were found to vary between 7.3×10^3 and 1.52×10^4 M^{-1}. The binding reactions for all the saccharides investigated were essentially enthalpy driven, with the binding entropy (ΔH_b) at 288.15 K being in the range of −50.99 and −43.39 kJ mol^{-1}, whereas the contribution to the binding reaction from the entropy of binding was negative, with values of binding entropy (ΔS_b) ranging between −99.2 and −72.0 J mol^{-1} K^{-1} at 288.15 K. Changes in heat capacity (ΔC_p) for the binding of disaccharides, lactose and lactulose, were significantly larger in magnitude than those obtained for the monosaccharides, methyl-β-D-galactopyranoside, and methyl-α-D-galactopyranoside, and could be correlated reasonably well with the surface areas of these ligands. Enthalpy–entropy compensation was observed for all the sugars studied, suggesting that water structure plays an important role in the overall binding reaction. CD spectroscopy indicates that carbohydrate binding does not lead to significant changes in the secondary and tertiary structures of MCL, suggesting that the carbohydrate binding sites on this lectin are mostly preformed.

Keywords: Agglutinin; pH dependence; Thermal inactivation; Saccharide specificity; Heat capacity; Enthalpy–entropy compensation

Lectins, the carbohydrate binding proteins of non-immune origin, are ubiquitous in nature, their presence being demonstrated in a variety of plants, animals including human beings as well as microbes and fungi [1,2]. Because of the various interesting biological properties exhibited by lectins such as their ability to distinguish between normal and malignant cells and to specifically recognize different types of human blood groups, they are being investigated with considerable interest [2,3]. The ability of lectins to specifically recognize unique carbohydrate structures is exploited in the purification and characterization of glycoconjugates and in the study of cell-surface architecture [4]. Most interestingly, lectins are also found to be useful in the fractionation of cells for their use in bone marrow transplantation [2].

In all the above processes involving biological recognition, binding of the lectins to carbohydrate ligands plays a crucial role. Therefore, the study of lectin–sugar interactions is very important in the characterization of any lectin. In particular, isothermal titration calorimetry (ITC) directly yields accurate and valuable information on the thermodynamic forces that govern the binding of ligands to macromolecules and is being used currently for the study of carbohydrate–lectin interaction [5,6].

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Affinity purification and macromolecular characterization of *Momordica charantia* lectin (MCL)[1]—a galactose-specific protein present in the seeds of bitter gourd—were reported about two decades ago. MCL is a tetrameric glycoprotein with $\alpha_2\beta_2$-type subunit architecture and exhibits strong type-1 and weak type-2 ribosome inactivating protein activities as well as insulinomimetic activity [7–10]. Chemical modification studies have shown that the side chains of tryptophan and tyrosine residues are important for the hemagglutinating and carbohydrate-binding activity of this lectin [9]. Steady-state and time-resolved fluorescence studies suggested that the tryptophan residues of MCL are in a heterogeneous environment, with at least two populations of tryptophan residues with different degrees of exposure being present in the protein [11]. Circular dichroism studies indicate that this protein contains 13% α-helix, 36% β-sheet, 21% β-turns, and rest unordered structure [12]. MCL interacts with various free-base and metallo-porphyrins and the binding is governed primarily by enthalpic forces [12]. In addition to hemagglutination-inhibition studies, the binding of a few saccharides has been investigated by monitoring saccharide-induced changes in the intrinsic fluorescence emission properties of the protein [13]. These studies yielded the association constants, but information on the thermodynamic forces that govern the binding of different carbohydrate ligands to MCL is still lacking.

Although the thermodynamic parameters associated with the binding of the fluorescently labeled saccharides, 4-methylumbelliferyl-α-D-galactopyranoside and 4-methylumbelliferyl-β-D-galactopyranoside to MCL were determined by fluorescence titrations [14,15], so far there have been no reports on the determination of thermodynamic parameters associated with the binding of underivatized sugars to MCL. In this study, we have characterised MCL further with respect to its physicochemical and carbohydrate-binding properties. The effect of pH and incubation at different temperatures on the hemagglutination activity of the lectin has been investigated and thermodynamic parameters associated with the binding of a number of carbohydrate ligands to MCL have been elucidated by isothermal titration calorimetry. The results indicate that carbohydrate binding to MCL is governed primarily by enthalpic forces, with negative contribution from the entropy of binding. The ΔC_p values for the interaction of mono- and disaccharides were found to be negative and their magnitudes could be correlated with their size. Enthalpy–entropy compensation was observed for all the sugars investigated, emphasizing the role of water structure in the binding interaction.

[1] *Abbreviations used:* MCL, *Momordica charantia* lectin; SGSL, snake gourd (*Trichosanthes anguina*) seed lectin; TCSL, *Trichosanthes cucumerina* seed lectin; PBS, 20 mM phosphate buffer, pH 7.4, containing 0.15 M NaCl and 0.02% sodium azide; Gal, galactose; Glc, glucose; Fru, fructose; MeαGal, methyl-α-D-galactopyranoside; MeβGal, methyl-β-D-galactopyranoside; *p*NPβGal, *p*-nitrophenyl-β-D-galactopyranoside; *p*NPβ thioGal, *p*-nitrophenyl-β-D-thiogalactopyranoside.

Materials and methods

Materials

Bitter gourd seeds were obtained from local seed shops. Guar gum, bovine serum albumin, lactose, lactulose, melibiose, methyl-α-D-galactopyranoside, methyl-β-D-galactopyranoside, *p*-nitrophenyl-β-D-galactopyranoside, *p*-nitrophenyl-β-D-thiogalactopyranoside, and the reagents used in polyacrylamide gel electrophoresis were obtained from Sigma (St. Louis, MO, USA). All other reagents and chemicals used were obtained from local suppliers and were of the highest purity available.

Purification of *M. charantia* seed lectin

MCL was purified by a combination of ammonium sulfate precipitation and affinity chromatography on cross-linked guar gum [16], essentially as described earlier [11]. All purification steps were carried out in 20 mM phosphate buffer, pH 7.4, containing 150 mM sodium chloride and 0.02% sodium azide (PBS) and the purified MCL was used in the same buffer for all further experiments unless otherwise indicated. The affinity-purified protein yielded a single band on polyacrylamide gel electrophoresis [17], consistent with earlier reports [9,11]. Concentration of purified MCL was determined by using a ε_{280} value of 140,590 $M^{-1}\,cm^{-1}$, calculated by the method of Edelhoch [18], as described in [19]. For this purpose, the number of tryptophan, tyrosine, and cystine residues was taken from [9]. It was assumed that all cysteine residues exist in the disulfide bridged form; however, because the extinction coefficient of cystine is very small compared to tryptophan and tyrosine, even if cystine contribution is ignored, the molar extinction coefficient of the protein would be affected by $<0.5\%$, which is negligible.

Absorption spectroscopy

Absorption measurements were made on a Shimadzu UV-3101PC UV-Vis-NIR spectrometer (Shimadzu, Kyoto, Japan, website: www.shimadzu.com) using 1 cm pathlength cells.

Agglutination assay

Cell-agglutination activity of MCL was assayed by the hemagglutination technique as described previously for *T. cucumerina* seed lectin [20]. A 4% suspension of human O (+) erythrocytes in PBS was mixed with seri-