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Binding of 4-methyl umbelliferyl-*α*-D-glucopyranoside to *Vicia faba* lectin: Fluorescence-quenching studies

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Abstract. On binding to *Vicia faba* lectin, the fluorescence of 4-methylumbelliferyl-*α*-D-glucopyranoside was quantitatively quenched showing that the interaction of 4-methylumbelliferyl-*α*-D-glucopyranoside took place in a binding environment. The binding of the fluorescent sugar was saccharide specific as evidenced by the reversal of 4-methyl-umbelliferyl-*α*-D-glucopyranoside fluorescence quenching by D-fructose. The association constant, K_a, values for the 4-methylumbelliferyl-*α*-D-glucopyranoside was determined by competition study employing reversal of fluorescence quenching of 4-methylumbelliferyl-*α*-D-glucopyranoside by D-fructose. The K_a value obtained for D-fructose was $1{\cdot}07 \pm 0{\cdot}03 \times 10^4$ M^{-1} and for 4-methylumbelliferyl-*α*-D-glucopyranoside was $1{\cdot}60 \pm 0{\cdot}05 \times 10^4$ M^{-1} at 15°C. The K_a values of $2{\cdot}51 \pm 0{\cdot}06 \times 10^4$ M^{-1}, $1{\cdot}26 \pm 0{\cdot}02 \times 10^4$ M^{-1} and $0{\cdot}56 \pm 0{\cdot}01 \times 10^4$ M^{-1}, respectively at 10°, 20° and 30°C were obtained from the Chipman equation. The relative fluorescence quenching, ΔF_a, at infinite concentration of the free saccharide sites of *Vicia faba* lectin [*P'*] was 93·5% at 30°C and the binding constant for 4-methylumbelliferyl-*α*-D-glucopyranoside lectin interaction as derived by Yank and Hanaguchi equation was $0{\cdot}63 \pm 0{\cdot}01 \times 10^4$ M^{-1}.

Keywords. *Vicia faba* lectin; lectin-saccharide binding; fluorescence quenching; binding constants.

Introduction

Sugars, such as methyl-umbelliferyl glycosides, have been used as probes for the investigation of binding of lectins to their specific sugars. The fluorescence of methyl-umbelliferyl glycosides was considerably quenched upon binding to lectin (Dean and Homer, 1973; Privat *et al*., 1974; Loontiens *et al*., 1977; van Landschoot *et al*., 1977; De Boeck *et al*., 1981; Thompson and Lakowicz, 1984). This has provided a sensitive determination of association constants and other binding characteristics for the lectin.

Lectin from *Vicia faba* seed had been isolated and carbohydrate specificity has been reported (Goldstein and Hayes, 1978; Debray *et al*., 1981). The structural requirements for binding of oligosaccharides and glycopeptides to immobilized *V. faba* agglutinin were investigated (Katagiri *et al*., 1984). The spectral properties of 4-methyl-*α*-D-umbelliferyl glycosides were investigated in order to assess their usefulness as probes of microenvironment of sugar binding sites on lectin molecules (Monique *et al*., 1984). The present communication deals with the physico-chemical aspects of 4-methyl-umbelliferyl-*α*-D-galactopyranoside (4-Met-umb-Glu)-*V. faba* lectin interaction.

Abbreviations used: 4-Met-umb-Glu, 4-Methylumbelliferyl-*α*-D-glucopyranoside; 4-Met-umb-Gal, 4-methyl umbelliferyl-*α*-D-galactopyranoside.

Materials and methods

4-Met-umb-Glu, 4-methyl umbelliferyl-α-D-galactopyranoside (4-Met-umb-Gal) and D-fructose were from Sigma Chemical Co., St. Louis, Missouri, USA.

Sugars

The umbelliferyl sugar solutions were analysed according to Loontiens *et al*. (1977) and were found to be free from 7-hydroxy-4-methyl coumarin. The 4-Met-umb-Glu and 4-Met-umb-Gal concentrations were determined at 318 nm using E=1·36 × 10^{-4} M^{-1} cm^{-1}.

Lectins

V. faba lectin was purified (Datta *et al*., 1984a) and was dissolved in 0·15 M phosphate buffered saline pH 7·2 containing 0·1 mM $CaCl_2$, $MnCl_2$ and $MgCl_2$. Protein concentrations were determined using $E\,\big|^{\%}_{cm}$ = 7·22 at 280 nm (Datta *et al*., 1984a).

Fluorescence titrations

The binding of 4-Met-umb-Glu to *V. faba* lectin was monitored by fluorescence quenching in Aminco-Bowman Spectrophotofluorimeter, sensitivity upto 10^{-4}. The excitation was done at 318 nm, and the emission spectra were recorded above 330 nm. Fluorescence quenching studies were done by titrating a definite concentration of lectin solution (225 μM) against varying amounts (0–200 μl) of 4 μM 4-Met-umb-Glu solution at 20°C. Competitive binding study with D-fructose was carried out as follows. Lectin was preincubated with 4-Met-umb-Glu (4 μM) and then titrated with aliquots of 0·1 M D-fructose solution at 15°C. The binding of the competitive sugar was deduced by observing the increase in fluorescence resulting from the dissociation of 4-Met-umb-Glu-*V. faba* lectin complex. The binding studies were done by titrating a fixed concentration of lectin (200 μM) against varying concentrations, 20–100 μM, of 4-Met-umb-Glu at 10°, 20° and 30°C. The resulting fluorescence in the presence and absence of lectin gave the amount of bound and total glucoside respectively. Corrections were made for inner filter effect (Martens and Kagi, 1979).

Results and discussion

The fluorescence of the sugar was completely quenched on binding to the *V. faba* lectin. It indicated that there was a change in the environment of the umbelliferyl moiety when 4-Met-umb-Glu specifically bound to *V. faba* lectin. The decrease in fluorescence was probably due to methyl-umbelliferyl group anchorage at or near the binding region through carbohydrate specific binding suggesting great reduction in the polarity of the fluorophor environment. The binding of a 4-methylumbelliferyl glycoside to a specific protein leads to a total fluorescence quenching for wheat germ agglutinin (Privat *et al*., 1974; van Landshoot *et al*., 1977), and for Con A (Dean and Homer 1973; Loontiens *et al* 1977).

The index of homogeneity or heterogeneity of lectin-sugar binding was determined by Sips (1948) equation. The index of heterogeneity is '*a*' and it ranges from 0 to 1. The value of $a = 1$ indicates a homogenous protein. The value of '*a*' was calculated as 1·0007 ± 0·0003 from the slope in figure 1 which indicated the homogenous binding between *V. faba* lectin and 4-Met-umb-Glu.

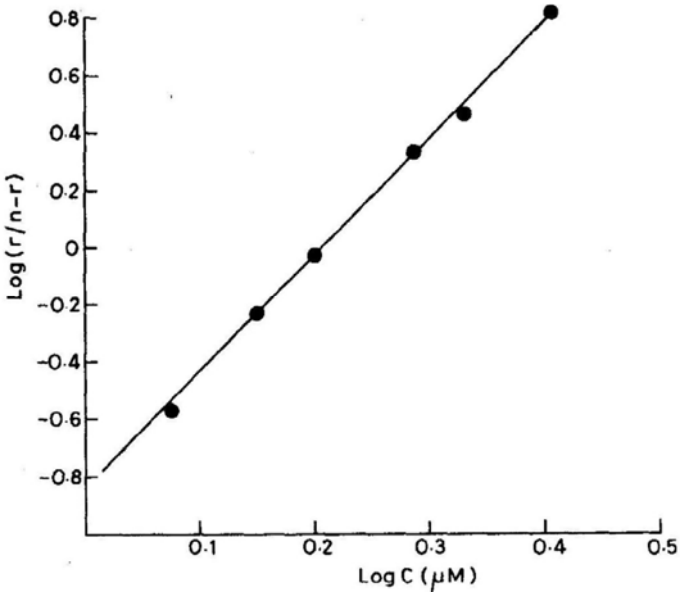

Figure 1. The index of homogeneity or heterogeneity of the purified *V. faba* lectin. Procedural details are described in the text.

The fluorescence quantum yield of 4-Met-umb-Glu bound to *V. faba* lectin was obtained by extrapolating the plot of $F_0/(F_0 - F)$ *vs* $1/[P]$ to $1/[P] = 0$ of the equation given by Dean and Hormer (1973) where F_0 and F were the measured fluorescence of sugar alone and in the presence of lectin respectively at protein concentration $[P]$. The plot gave straight line with an intercept on the ordinate $F_0/(F_0 - F) = 0{\cdot}97$, demonstrating that the fluorescence quantum yield of 4-Met-umb-Glu bound to *V. faba* lectin was zero (figure 2), and indicating that the fluorescence quantum yield could be used as a measure of ligand binding to *V. faba* lectin in titration. The number of sugar binding sites of *V. faba* lectin (n = 4) was reported earlier (Datta *et al*., 1984b; Datta *et al*., 1984c; Datta *et al*., 1986).

The quenching of 4-Met-umb-Glu on binding to *V. faba* lectin was completely reversed by addition of 0·1 M D-fructose, showing that quenching was due to sugar specific binding (figure 3). The fluorescence of 4-Met-umb-Gal was not quenched when titrated with *V. faba* lectin, thus excluding the presence of any other binding site with which 4-methyl umbelliferyl moiety of the galactoside could interact. The lectin pretreated with D-fructose did not quench the fluorescence of 4-Met-umb-Glu and demonstrated that both the sugars competed for the same binding site of the *V. faba* lectin. The binding of D-fructose (*I*) by *V. faba* lectin (*P*) in the presence of 4-Met-umb-Glu (*M*) was determined by the reaction scheme of Bessler *et al*. (1974).

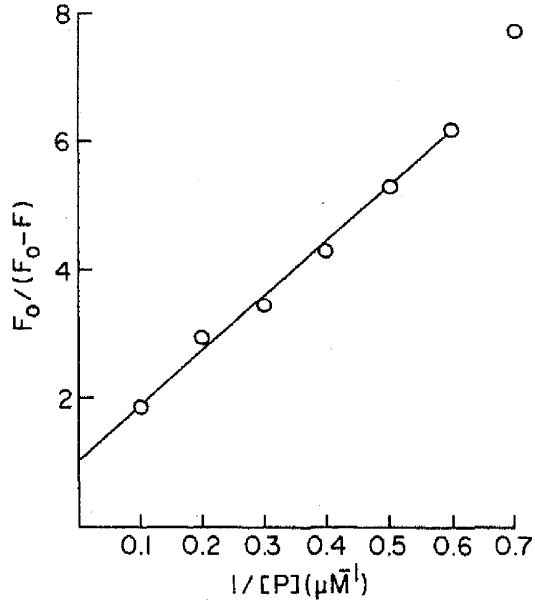

Figure 2. Plot of the fluorescence intensity changes of ($F_o/\Delta F$) of Met-umb-Glu upon addition of *V. faba* lectin at 20°C. Aliquots of a solution of *V. faba* lectin, 225 μM, were added to 1 ml of Met-umb-Glu (4 μM) solution. The experimental procedure is given in the text.

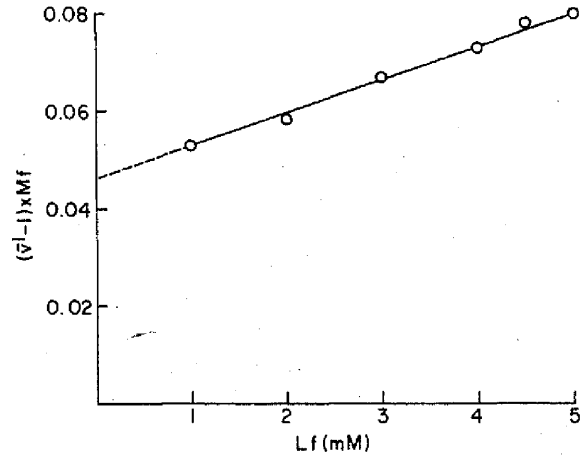

Figure 3. Competitive binding of saccharides with *V.faba* lectin at 15°C. $[L]_f$ is the free concentration of the competing sugar (fructose); $[M]_f$ is the free concentration of Met-umb-Glu. The fraction of M bound per total protein, $[PM]/[P]_t$, is represented by *v. V.faba* lectin 160 μM, was incubated with Met-umb-Glu (4 μM) and then titrated with aliquots of 0·1 M D-fructose solution. The rest of the procedure is described in the text

The association constant K_a for the competing sugar ligand could be determined from the slope and intercept of the slope obtained from a straight line which was drawn by plotting $(V^{-1} - 1)\ (M)_f$ against $[L]_f$ where V is the fraction of 4-Met-umb-Glu per total lectin. $[L]_f$ is the free concentration of the D-fructose and $[M]_f$ is the

free concentration of Met-umb-Glu. The K_a values at 15°C for 4-Met-umb-Glu was $1{\cdot}60 \pm 0{\cdot}05 \times 10^4$ M^{-1} and that for D-fructose was $1{\cdot}07 \pm 0{\cdot}03 \times 10^4$ M^{-1}.

The values of association constant K_a of the lectin-sugar interaction was estimated using the quenching values of 4-Met-umb-Glu fluorescence by varying concentrate-ions of *V. faba* lectin. A plot of log $(F_0 - F)/(F - F_\alpha)$ against log $[P]_f$ from Chipman (1967) equation gave a straight line where F_0, F and F_α were the values of the fluorescence maxima of 4-Met-umb-Glu in the absence of protein, in the presence of protein and at infinite protein concentration (figure 4) respectively and $[P]_f$ was the free protein concentration. The values of the association constants $2{\cdot}52 \pm 0{\cdot}06 \times 10^4$ M^{-1} at 10°C, $1{\cdot}26 \pm 0{\cdot}02 \times 10^4$ M^{-1} at 20°C and $0{\cdot}56 \pm 0{\cdot}01 \times 10^4$ M^{-1} at 30°C were obtained from the intercepts on the abcissa.

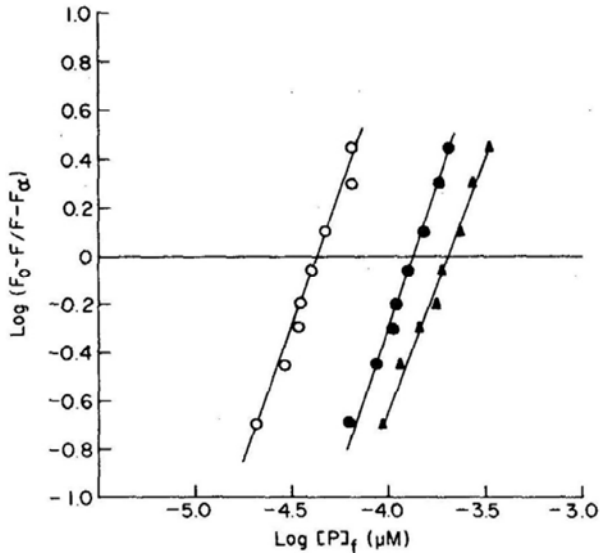

Figure 4. A quenching of the fluorescence spectra of Met-umb-Glu by *V. faba* lectin.

The values of the association constants were obtained from the intercept on the x-axis. (O), 10°C; (●), 20°C and (▲), 30°C. The experimental details are given in the text.

The association constant K_a was determined at 30°C in another set of experiment by the method of Yank and Hamaguchi (1980) as modified by De Boeck *et al*. (1981) (figure 5). The association constant $0{\cdot}63 \pm 0{\cdot}01 \times 10^4$ M^{-1} at 30°C was calculated from the slope of a straight line obtained by plotting $\Delta F/[P']$ *vs* ΔF where ΔF is the relative fluorescence quenching and $[P']$ was the concentration of the free saccharide sites of *V. faba* lectin. ΔF_α at infinite $[P']$ was 93·5% as calculated from the intercept. The fluorescence of 4-Met-umb-Glu was quantitatively quenched on binding to *V. faba* lectin. The binding of the fluorescent sugar was saccharide specific as evidenced by the reversal of 4-Met-umb-Glu fluorescence quenching by D-fructose. The plot of fluorescence quantum yield of 4-Met-umb-Glu bound to *V. faba* lectin was a sensitive measurement of the equilibrium parameters at difference temperatures.

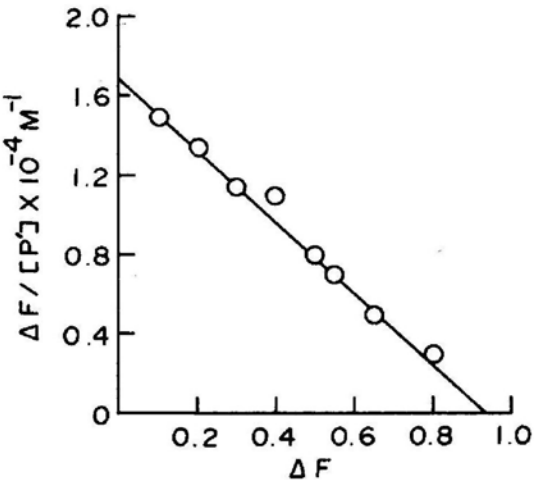

Figure 5. Association constant of purified *V. faba* lectin from $\Delta F/[P']$ *vs* ΔF plot.

ΔF is the relative fluorescence quenching and $[P']$ is the concentration of free sites; both are corrected for dilution. The values of K (in M^{-1}) and ΔF_{α}, at infinite $[P']$ were calculated from the slope, and intercept in the ordinate respectively.

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