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Note

Interaction of *Vicia faba* lectin with methyl α-D-mannopyranoside, investigated by ultraviolet difference spectroscopy

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The capacity of lectins to bind specifically to various carbohydrates makes them useful for the isolation and structural analysis of such glycoconjugates as membrane receptors[1,2]. *Vicia faba* lectin, like other agglutinins, reacts with the surface of cytoplasmic membranes and causes cell agglutination. The association constants and other thermodynamic parameters of *V. faba* lectin–glycoconjugate interactions have been determined[3,4] by spectroscopy and by binding studies of *V. faba* lectin to umbelliferyl sugars by fluorescence quenching. The structural requirements for binding of oligosaccharides and glycopeptides to immobilized *V. faba* lectin have been studied by Katagiri *et al.*[5]. Ultraviolet difference spectroscopy is a valuable tool for the investigation of saccharide binding to lectins[5–10]. We describe here the thermodynamics of *V. faba* lectin–sugar interaction as determined by u.v. difference spectroscopy.

RESULTS AND DISCUSSION

The lectin of *V. faba* showed low absorption between 265 and 290 nm at pH 7.2. Absorption in the aromatic region increased gradually with increase in the pH to 9.6, 10.6, 11.6, and 14, suggesting that aromatic amino acids hidden at neutral pH become exposed at higher pH. Ionization of the hydroxyl group of tyrosine in a protein might contribute towards the increase of u.v. absorption, although the content of tyrosine in *V. faba* lectin[11] is low. Concanavalin A (Con A) was used as a control for u.v. scanning procedures. The u.v. spectra of Con A at pH 7.2 and above showed peaks at 280 nm, in agreement with the results of Agarwal and Goldstein[12]. It was thus presumed that *V. faba* lectin underwent denaturation at higher pH.

The u.v. difference spectrum obtained by the addition of different concentrations of methyl α-D-mannopyranoside (**1**) to *V. faba* lectin is displayed in Fig. 1. A low hump is observed in the region 265–290 nm. Identical shapes for the u.v.-differ-

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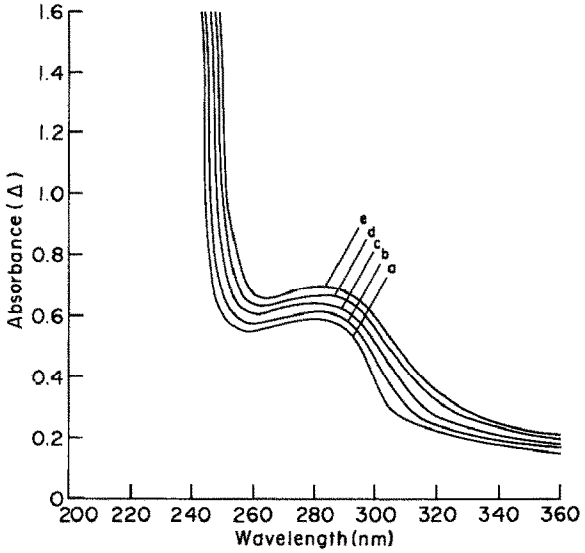

Fig. 1. Ultraviolet difference spectra of the *V. faba* lectin–methyl α-D-mannopyranoside interaction: (*a*) with *V. faba* lectin (0.25 mg/mL) alone; (*b*) with 0.25mM sugar, corresponding to 50% sugar saturation; (*c*) with 0.5mM sugar, corresponding to 75% sugar saturation; (*d*) with 0.75mM sugar, corresponding to 90% sugar saturation; and (*e*) with mM sugar, corresponding to 95% sugar saturation. Reactions were performed at 25°.

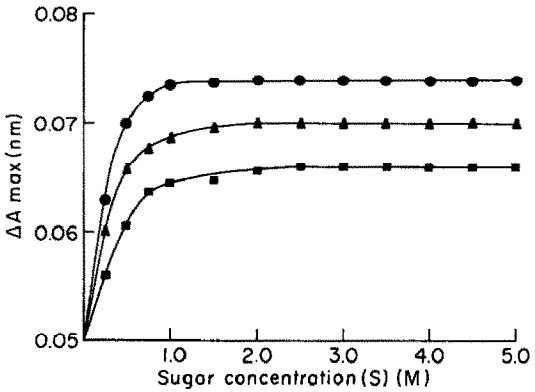

Fig. 2. Spectrophotometric titrations of *V. faba* lectin with methyl α-D-mannopyranoside. The variation in magnitude of the absorbance difference at 280 nm with various concentrations of methyl α-D-mannopyranoside at a constant concentration of lectin (0.25 mg/mL) is a function of the total sugar concentration, [*S*], at 15° (—●—), 25° (—▲—), and 35° (—■—).

ence spectrum of *V. faba* lectin–**1** interaction at various degrees of site saturation demonstrated good signal-to-noise ratios of the difference spectra, and indicates highly specific binding of **1** with lectin. Control experiments showed that the lectin–methyl α-D-galactopyranoside interaction did not produce any difference in the u.v.-spectrum. The maximum u.v. difference spectra at 280 nm (Fig. 2) for titration of a constant concentration (0.25 mg/mL) of *V. faba* lectin with various concentrations of **1** at 15, 25, and 35°, respectively exhibited a plateau at high sugar con-

TABLE I

THERMODYNAMIC PARAMETERS FOR BINDING OF METHYL α-D-MANNOPYRANOSIDE BY THE PURIFIED *V. faba* LECTIN

Temperature (°)	*Association*[a] *constant* (K_a) $10^4\ M^{-1}$	*Dissociation*[b] *constant* $K_d \times 10^4\ M^{-1}$	*Free energy* $(-\Delta G^0)$ $KJ\ mol^{-1}$	*Enthalpy* $(-\Delta H^0)$ $J\ deg^{-1}\ mol^{-1}$	*Entropy* $(-\Delta S^0)$
15	2.9 ±0.09	4.0 ±0.1	24.8 ±0.42		62.71 ±4.2
25	1.6 ±0.05	7.0 ±0.25	24.2 ±0.42	42.92 ±0.82	61.72 ±4.2
35	0.9 ±0.02	10.0 ±0.4	23.5 ±0.42		62.87 ±4.2

[a]Association constants were obtained from the intercept on the ordinate of Fig. 3. [b]Dissociation constants were obtained from the intercept on the abscissa of Fig. 4.

centrations that was assumed to correspond to 100% saturation of the lectin binding-sites with the sugar. No change in overall shape of the difference spectral curve was found for this lectin–sugar binding at three different temperatures. However, in all instances, the absorption at 280 nm decreased with increase in temperature, demonstrating that the sugar-binding affinity of the lectin decreased with rise in temperature.

The association constants (K_a) and dissociation constants (K_d) for the *V. faba* lectin–1 interaction were determined from the intercepts on the ordinate of the Scatchard plot $\Delta A/\Delta A_{max} \times 1/[S]_f$ *vs.* $\Delta A/\Delta A_{max}$ (Fig. 3) and the intercepts on the abscissa of the plot $[S]/\Delta A_{max}$ *vs.* $[S]$ (Fig. 4), respectively, by the equation of Matsumoto[6]; where ΔA = u.v. difference spectral value; ΔA_{max} = maximum u.v. difference spectral value at 280 nm; $[S]_f$ = free concentration of **1** after binding to lectin; and $[S]$ = total concentration of **1**. The values of K_a and K_d at three different temperatures are given in Table I, and the thermodynamic parameters, ΔG^0, ΔH^0, and ΔS^0, were calculated (Table I) by the van't Hoff equation from the K_a values.

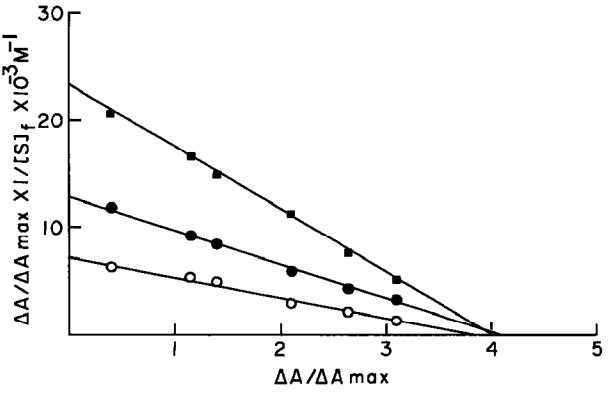

Fig. 3. Determination of association constants by means of a Scatchard plot. A constant concentration of lectin (0.25 mg/mL) was titrated with various concentrations of methyl α-D-mannopyranoside (0.25–3.0mM). Experiments were performed at 35° (—○—), 25° (—●—), and 15° (—■—) in PBS.

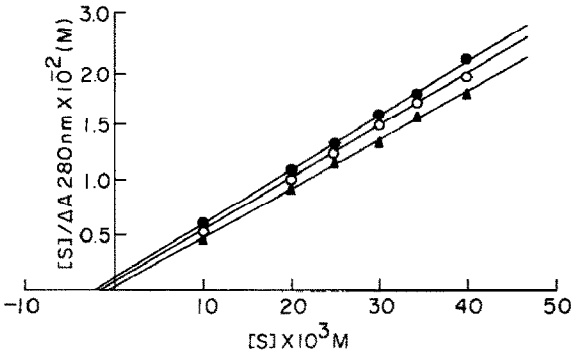

Fig. 4. The binding of methyl α-D-mannopyranoside to a fixed amount of *V. faba* lectin (2.5 mg/mL) at 15° (—▲—), 25° (—○—), and 35° (—●—). Experimental details are given in the text.

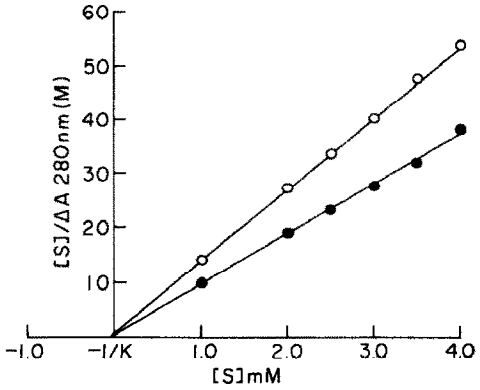

Fig. 5. The pattern of methyl α-D-mannopyranoside binding by a fixed amount of *V. faba* lectin at 15°; ——●——●——, 0.5 mg lectin/mL; and ——○——○—— 1.0 mg lectin/mL.

The van't Hoff plot for the *V. faba* lectin–**1** interaction was linear over the temperature range employed, indicating an exothermic reaction between **1** and the lectin. The K_a values for lectin–sugar binding decreased with the rise in temperature, whereas the K_d values for the same binding increased with increase in temperature. the interaction of **1** with *V. faba* lectin was associated with an enthalpy change (ΔH^0). The ΔG^0 values decreased with rise in temperature, whereas the ΔS^0 values remained almost the same.

The two straight lines for two different concentrations of *V. faba* lectin obtained from the plot of $[S]/\Delta A_{max}$ *vs.* $[S]$ at 15° (Fig. 5) converged at the same point on the abscissa, suggesting that the affinity for **1** was not dependent on the concentration of *V. faba* lectin. Caron *et al.*[10] have shown that the affinity of lactose for peanut agglutinin is independent of lectin concentration.

Our results using u.v. difference spectroscopy indicate the binding of sugar to be highly specific and independent of lectin concentration. There was a pH-dependent denaturation of the *V. faba* lectin. The lectin–saccharide interaction is exothermic. Exothermic reactions are best performed at low temperature, and this

might explain the agglutinin-like behaviour of *V. faba* lectin[3] in the cold. The increased association constant for binding of *V. faba* lectin with the sugar, and the thermodynamics of the reaction system, have important consequences in the interpretation of experiments that attempt to correlate lectin binding with temperature-dependent variation in agglutination. The involvement of tyrosyl residues, free amino groups, and free carboxyl groups in the binding of sugars has been reported earlier[4]. The specific role of these residues in the saccharide-binding activity of *V. faba* lectin remains unknown.

EXPERIMENTAL

Materials. — Methyl α-D-mannopyranoside (**1**) and methyl α-D-galactopyranoside were obtained from Sigma Chemicals Co., U.S.A. Lectin of *V. faba* was purified to homogeneity by affinity chromatography on chitin[11]. Protein concentrations were determined according to the method of Lowry *et al.*[13], using bovine serum albumin as standard.

Spectra. — Ultraviolet difference spectra were recorded with a Gilford spectrophotometer. Aliquots (1 mL) of lectin solution, 0.25 mg/mL, in 0.15M phosphate-buffered saline, pH 7.2 containing 0.1mM $MgCl_2$, $MnCl_2$, and $CaCl_2$ (PBS) were added to both sample and reference cuvettes. The base line was recorded into the instruments memory unit to be automatically subtracted from subsequent spectra. Small aliquots (1–2 μL) of a solution of **1** were added to the sample cuvette, while the same amount of PBS was added to the reference cuvette. The difference spectrum was recorded after thermal equilibration had been established. This was first performed to establish the lectin–sugar interaction. The total volume of ligand solution varied from 10 to 20 μL, rendering concentration corrections for dilution unnecessary. Control experiments were conducted with methyl α-D-galactoside, which is not an inhibitor of *V. faba* lectin. Measurements of *V. faba* lectin–**1** interaction at various concentrations of **1** gave the u.v. difference spectral values, ΔA.

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