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BINDING OF *p*-SUBSTITUTED-PHENYL GLYCOSIDES TO THE LECTINS FROM THE PEA AND THE LENTIL

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ABSTRACT

The effect of *p*-substitution on the behaviour of phenyl α-D-mannopyranosides as specific ligands for the lectins from *Pisum sativum* (pea) and *Lens culinaris* (lentil) is reported. The binding affinities are related to the electronic properties of the substituents, expressed as the Hammett substituent constant ($\rho = -0.4$). Inductive and mesomeric contributions are best considered separately. Neither steric hindrance nor hydrophobic binding of the substituents is observed. For the binding of the corresponding α-D-glucopyranosides to the *Pisum sativum* lectin, the same effect is maintained ($\rho = -0.4$). In a series of *p*-substituted-phenyl β-D-glucopyranosides, the nature of the substituent has almost no effect on the binding to the *Pisum sativum* lectin.

INTRODUCTION

It has been reported[1] for concanavalin A, the lectin[2] from *Canavalia ensiformis*, that Hammett plots with $\rho = -0.5$ are valid for the specific binding of *p*-substituted-phenyl α-D-mannopyranosides ($\rho = -0.513 \pm 0.074$) and α-D-glucopyranosides, whereas any influence of the hydrophobic character of the substituent is absent. This dependence on both substituent parameters is inverted[1,3] for the corresponding β-D-glucopyranosides, which are poor ligands. These observations with concanavalin A indicate that the anomeric binding-specificity may involve an advantageous interaction with a protein electrophile, together with the absence of non-specific interaction by the aglycon group. We have applied this approach to examine the α-anomeric binding-specificity of the lectins from the pea, *Pisum sativum* (PsL), and from the lentil[4], *Lens culinaris* (LcL). These lectins have striking similarities[2] to concanavalin A. PsL[5] and LcL[4,6] also agglutinate trypsinated erythrocytes of the ABO(H)-group and are inhibited by sugars of the Mäkelä group III (D-mannose, D-glucose, and derivatives); PsL[7] and LcL[8] require Mn^{2+} and Ca^{2+} ions; PsL[9] and LcL[4,10] also stimulate the transformation and mitosis of lymphocytes.

EXPERIMENTAL

The characteristics of the glycosides used in this study have been reported[1]. *Saccharomyces cerevisiae* mannan[11] was isolated as described. The isolation of *Pichia pinus* phosphomannan was analogous to the procedure[12] for the phosphomannan from *Hansenula hostii* NRRL Y-2448. PsL[13] and LcL[14] were isolated by selective binding on Sephadex G-75, followed by elution with 0.15M D-glucose. They were stored in M sodium chloride at 4°. Protein concentrations were determined in a Zeiss PMQII-M4QIII spectrophotometer at 280 nm, using $\varepsilon = 1.2\ cm^2/mg$ of protein. The binding parameters N (number of independent carbohydrate binding-sites) and K (intrinsic association constant) for the binding of *p*-nitrophenyl α-D-mannopyranoside to PsL (3 mg/ml, molecular weight[13] 54,000) were obtained from equilibrium dialysis[1] data (4°) and plotted[15] according to the equation $\bar{v}/L_f = NK - \bar{v}K$. The amount of *p*-nitrophenyl α-D-mannopyranoside free at equilibrium (L_f) was determined photometrically at 313 nm. The binding ratios $\bar{v}$ were in the range 0.15–0.75 mole of ligand per mole of protein. Buffers (0.1M) contained M sodium chloride, 0.1mM manganese(II) chloride, and 0.1mM calcium chloride.

The turbidimetric assay, for interaction of the lectin with the appropriate mannan yielding the highest sensitivity, was performed either without glycoside, or in the presence of various amounts of each glycoside, to determine the molarity giving 50% inhibition (M_{50}). The test solutions (3 ml, 25°) were contained in cylindrical Klett tubes that were read at 405 nm in an Eppendorf photometer against air as a reference. The assay for PsL was performed in 3 ml of M sodium chloride–17mM Tris-HCl (final pH 8.1) containing 156 μg of yeast mannan, 1.75 mg of PsL, and various amounts of glycoside. The assay for LcL was performed in 3 ml of M sodium chloride–17mM sodium phosphate (final pH 7.0) containing 1.0 mg of LcL and 150 μg of *Pichia pinus* phosphomannan and various amounts of glycoside. The tests were started by addition of the lectin; the contact time was 15 min. After measurement at 405 nm, 0.2 ml of 2% aqueous methyl α-D-mannopyranoside was added to each tube to clarify the turbid solution. Thus, a combined blank for tube and polysaccharide was obtained.

The Hammett substituent constant[16] (σ_H), its inductive[17] (σ_I) and resonance ($\sigma_R = \sigma_{H,para} - \sigma_I$) contributions, and the Hansch hydrophobic parameter[18] were used in multi-parameter regression analysis of $-\log M_{50}$. In these analyses, n is the number of substituents used in the regression, s is the standard error of the estimated $-\log M_{50}$, and r is the regression coefficient; values between brackets are the partial correlation products. Values for the unsubstituted phenyl glycopyranosides were omitted from the regressions.

RESULTS

Pisum sativum *lectin.* — As obtained from equilibrium dialysis experiments at 4°, K for *p*-nitrophenyl α-D-mannopyranoside is maximal around pH 8.2: $(4.5 \pm 0.9) \times$

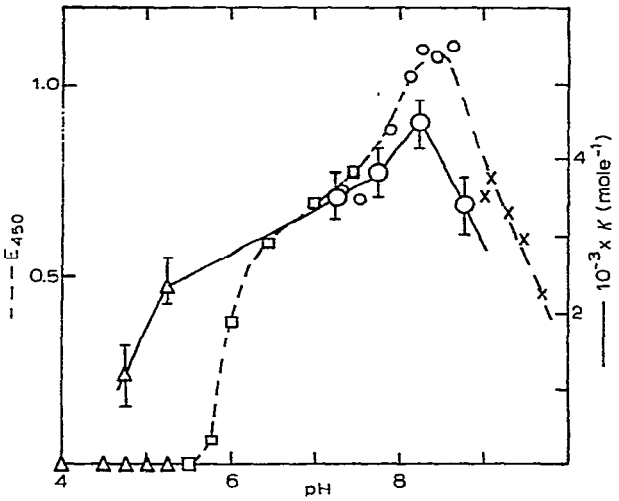

Fig.* 1. Dependence of PsL binding on pH, estimated turbidimetrically with yeast mannan in the absence of any glycoside (– – –), or expressed as the association constant (K) for p-nitrophenyl α-D-mannopyranoside (———); buffers (0.17mM) were 0.1mM sodium acetate–acetic acid (△), sodium phosphate (□), Tris-HCl (○) and $Na_2B_4O_7$–NaOH (×) in M sodium chloride.

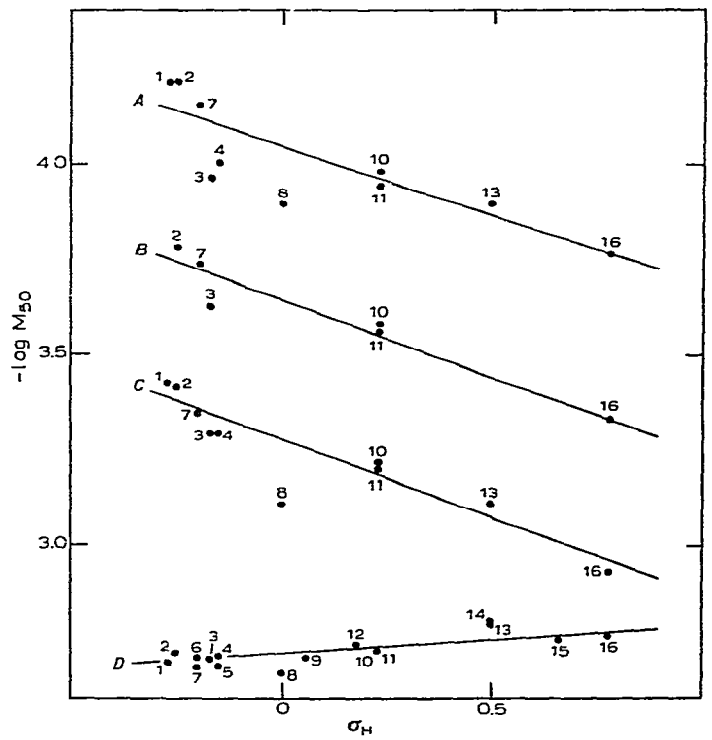

Fig. 2. Correlations of σ_H for p-substituted-phenyl α-D-mannopyranosides with PsL (A), α-D-glucopyranosides with PsL (B), α-D-mannopyranosides with LcL (C), and β-D-glucopyranosides with PsL (D).

*For mole^{-1} please read M^{-1} i.e. the reciprocal of molarity.

10^3 M^{-1} (Fig. 1). In the pH range 4.75–8.75, the mean value of N, obtained at six pH values, is 2.1 $\pm$0.15. At the higher pH values, a parallelism is noted between the variation of K for *p*-nitrophenyl α-D-mannopyranoside and the turbidimetric assay with yeast mannan (Fig. 1). Below pH 5.5, however, no precipitation occurs with the polysaccharide, although *p*-nitrophenyl α-D-mannopyranoside shows $K = 1.2 \times 10^3$ M^{-1} at pH 4.75. A similar situation was observed[19] for concanavalin A.

M_{50} values for the interaction of *p*-substituted-phenyl α-D-mannopyranosides and α-D-glucopyranosides are given in Table I, and their dependence on σ_H is illustrated in Fig. 2.

TABLE I

M_{50} VALUES FOR *p*-SUBSTITUTED-PHENYL α-D-MANNOPYRANOSIDES AND α-D-GLUCOPYRANOSIDES

Compound	*Para substituent*	$10^4 \times M_{50}$ *in PsL-yeast mannan test*		$10^4 \times M_{50}$ *in LcL-*Pichia *phosphomannan test*
		α-D-*Mannosides*	α-D-*Glucosides*	α-D-*Mannosides*
1	Methoxy	0.62	—	3.78
2	Ethoxy	0.61	1.69	3.84
3	Methyl	1.12	2.40	5.29
4	Ethyl	0.94	—	5.1
7	*tert.*-Butyl	0.70	1.86	4.5
8	None	1.30	—	7.05
10	Chloro	1.05	2.66	6.33
11	Bromo	1.15	2.77	6.20
13	Acetyl	1.30	—	7.81
16	Nitro	1.77	4.80	11.85

p-*Substituted-phenyl* α-D-*mannopyranosides.* — The correlation with σ_H is according to equation **1**, in which derivative **8** was not included. This equation can be improved by introducing σ_I and σ_R (Table II), yielding equation **2**; a correlation including π (Table II), as additional parameter, produces a negative and artificial correlation with π (equation **3**).

$$-\log M_{50} = 4.045 - (0.363 \pm 0.065)\sigma_H \qquad (1)$$
$$n = 9, \qquad r = -0.884, \qquad s = 0.073.$$

$$-\log M_{50} = 3.998 - 0.272\sigma_I - 0.494\sigma_R \qquad (2)$$
$$(0.266) \quad (0.578) \qquad r^2 = 0.844$$
$$n = 9, \qquad r = -0.919, \qquad s = 0.066.$$

$$-\log M_{50} = 4.005 - 0.278\sigma_I - 0.498\sigma_R - 0.01\pi \qquad (3)$$
$$r^2 = 0.846 \quad (0.270) \quad (0.585) \quad (-0.01)$$
$$n = 9, \qquad r = -0.920, \qquad s = 0.072.$$

TABLE II

SUBSTITUENT PARAMETERS AND M_{50} VALUES FOR THE BINDING OF *p*-SUBSTITUTED-PHENYL β-D-GLUCOPYRANOSIDES TO PsL

Compound	*Para substituent*	σ_I	σ_R	π	$10^4 \times M_{50}$
1	Methoxy	0.25	−0.518	−0.04	20.6
2	Ethoxy	0.27	−0.51	0.46	19.5
3	Methyl	−0.05	−0.120	0.52	20.1
4	Ethyl	−0.05	−0.101	0.97	19.9
5	Propyl	−0.03	—	1.47	21.0
6	*sec*-Butyl	−0.03	—	1.82	20.0
7	*tert*-Butyl	−0.07	−0.127	1.68	21.2
8	None	0	0	0	21.8
9	Fluoro	0.52	−0.458	0.15	20.1
10	Chloro	0.47	−0.243	0.70	19.2
11	Bromo	0.45	−0.218	1.02	19.1
12	Iodo	0.38	−0.21	1.26	18.6
13	Acetyl	0.29	0.212	−0.37	16.5
14	Propionyl	—	—	0.13	16.1
15	Cyano	0.58	0.080	−0.32	18.0
16	Nitro	0.76	0.018	0.24	17.6

p-*Substituted-phenyl* α-D-*glucopyranosides*. — The correlation with σ_H is according to equation **4**, whereas the three-parameter equation **5** shows an apparent contribution for π of ~10%. This should be interpreted with care, as only six substituents were considered in the regression.

$$-\log M_{50} = 3.635 - (0.388 \pm 0.059)\sigma_H \qquad (4)$$
$$n = 6, \qquad r = -0.960, \qquad s = 0.045.$$

$$-\log M_{50} = 3.379 - 0.275\sigma_I - 0.580\sigma_R + 0.067\pi \qquad (5)$$
$$r^2 = 0.992 \quad (0.427) \quad (0.461) \quad (0.104)$$
$$n = 6, \qquad r = -0.996, \qquad s = 0.021.$$

It is clear that the binding of *p*-substituted-phenyl α-D-mannopyranosides and α-D-glucopyranosides to PsL is dominated by the polar nature of the substituent to a mutually comparable extent. In the Hammett equations **1** and **4**, $\rho = -0.4$; electron-releasing substituents favour the binding.

p-*Substituted-phenyl* β-D-*glucopyranosides*. — The M_{50} values for binding to PsL are given in Table II. Here, para substitution in the phenyl ring has almost no influence on the binding. The Hammett plot (Fig. 2), according to equation **6**, is in striking contrast with those for the α-D-glycosides. A three-parameter relation with σ_I, σ_R, and π (equation **7**) is not entirely satisfactory and shows a minor influence of π in comparison with polar effects. Substituents **5**, **6**, **8**, and **14** were not used in the regressions.

$$-\log M_{50} = 2.708 + (0.073 \pm 0.011) \times \sigma_H \quad (6)$$
$$n = 12, \qquad r = 0.855, \qquad s = 0.016.$$

$$-\log M_{50} = 2.716 + 0.051\sigma_I + 0.078\sigma_R - 0.014\pi \quad (7)$$
$$r^2 = 0.768 \quad (0.251) \quad (0.367) \quad (0.150)$$
$$n = 12, \qquad r = 0.876, \qquad s = 0.017.$$

Lens culinaris *lectin.* — At a protein concentration of 3 mg/ml, no dependable estimate of N and K could be obtained by equilibrium dialysis with *p*-nitrophenyl α-D-mannopyranoside. Because of the lower association constants of glycosides for LcL, reliable turbidimetric determinations of M_{50} values could be performed only for the α-D-mannosides.

p-*Substituted phenyl* α-D-*mannopyranosides.* — The M_{50} inhibition values for LcL are given in Table I. As with PsL, $-\log M_{50}$ can be correlated fairly well with σ_H (equation **8**, Fig. 2). Moreover, the ρ values (−0.4) are comparable for both proteins. According to equation **9**, the correlation product of σ_R is larger than for σ_I. Introduction of π (equation **10**) yields an artifact, showing the inadequacy of the hydrophobic parameter in relation to the binding phenomenon. Derivative **8** was not used in the regressions.

$$-\log M_{50} = 3.279 - (0.409 \pm 0.037) \times \sigma_H \quad (8)$$
$$n = 9, \qquad r = -0.972, \qquad s = 0.039.$$

$$-\log M_{50} = 3.256 - 0.364\sigma_I - 0.474\sigma_R \quad (9)$$
$$r^2 = 0.960 \quad (0.446) \quad (0.514)$$
$$n = 9, \qquad r = -0.978, \qquad s = 0.034.$$

$$-\log M_{50} = 3.270 - 0.381\sigma_I - 0.479\sigma_R - 0.019\pi \quad (10)$$
$$r^2 = 0.965 \quad (0.466) \quad (0.519) \quad (-0.021)$$
$$n = 9, \qquad r = -0.983, \qquad s = 0.035.$$

DISCUSSION

The effect of para substitution in phenyl glycopyranosides on their binding to PsL and LcL, as described above, can be compared with their binding[1,3] to concanavalin A. As only the interaction of the α-D-mannopyranosides with LcL was noticeable in our turbidimetric test, the results for α-D-glucopyranosides and β-D-glucopyranosides are lacking for the latter protein.

For these three lectins isolated from leguminosae, the binding specificity decreases in the order α-D-mannose, α-D-glucose, β-D-glucose, as can be observed with the simple sugars and with the corresponding methyl or phenyl glycopyranosides.

For these three lectins, the binding of *p*-substituted-phenyl α-D-mannopyranosides, expressed as $-\log M_{50}$, correlates fairly well with the Hammett sub-

stituent constant σ_H, yielding ρ values equal to -0.5 or -0.4. The contribution of the hydrophobic character of the substituent in multi-parameter relations is either small or artificial, when compared with the predominating polar character expressed as σ_H or, preferably, as σ_I and σ_R. Thus, electron-releasing substituents favour the binding of *p*-substituted-phenyl α-D-mannopyranosides by an increase in electron density, either at the anomeric oxygen atom or in the phenyl ring. The interaction of the α-anomeric oxygen atom itself with a protein electrophile seemed[1] more plausible for concanavalin A. In addition, binding of the α-D-mannosides and α-D-glucosides appears to be identical for concanavalin A and PsL, as far as para substitution in the phenyl ring is concerned; Hammett plots are parallel for PsL ($\rho = -0.4$, Fig. 2) and for concanavalin A ($\rho = -0.5$)[1]. Binding of the anomeric oxygen atom *via*, for example, a hydrogen bridge as the electrophile can explain the binding preferences for α-D-mannosides over α-D-glucosides; disregarding any specific interaction of HO-2, partial protonation of the α-anomeric oxygen atom of the glycopyranosidic ring in the *CI*(D) conformation would be less hindered by an axial HO-2, as in α-D-mannose, than by an equatorial group as in α-D-glucose.

Both PsL and concanavalin A weakly bind β-D-glucosides. The interaction of *p*-substituted-phenyl β-D-glucopyranosides with LcL is not detectable in our turbidimetric test. The binding data of these derivatives for PsL yield a Hammett plot with $\rho = 0$ or nearly so (Fig. 2). For concanavalin A, they correlate[3] extremely badly with σ_H.

For both PsL and concanavalin A, the binding of the two anomeric glycoside types seems totally different. From crystallographic[20] data obtained with concanavalin A, it has been deduced that α and β anomers show a dystopic binding: the α-D-glucoside oxygen atom points towards the surface, whereas the β-D-glucosidic ring is inverted with the aglycon group located in the interior of the protein. Whether such a different binding orientation for both anomeric hexopyranosidic rings also occurs with PsL is questionable. With concanavalin A, hydrophobic interactions[1,3] occur in, for example, a series of *p*-alkylphenyl β-D-glucopyranosides, which bind with the alkyl group located in the interior of the protein. Such a dependence on π is not observed with PsL; with the five *p*-alkyl substituents used, π varies from 0.52 to 1.82. As seen from Table II, the M_{50} values for these *p*-substituted-phenyl β-D-glucopyranosides are constant within 3%, showing their total lack of hydrophobic interaction with PsL. Thus, for the binding of *p*-substituted-phenyl β-D-glucopyranosides to PsL, the para substituent either points into the protein, without making any hydrophobic contact (which is improbable), or points towards the solution. It should be noted, however, that no β-D-linked polysaccharides are known to precipitate with PsL.

In conclusion, it can be stated that the binding of *p*-substituted-phenyl α-D-mannopyranosides and α-D-glucopyranosides to concanavalin A, PsL, and LcL show a common, favourable dependence on the electron-releasing potency of the substituent. The Hammett ρ values are mutually comparable (-0.5 and -0.4) for five substituent series obtained with these three lectins. This observation may be related

to the α-anomeric binding-specificity of these lectins. *p*-Substituted-phenyl β-D-glucopyranosides bind to PsL, independently of the polar and hydrophobic nature of the substituent, and to concanavalin A, dependent on the hydrophobic character.

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