Carbohydrate Research, 44 (1975) 150–153

Note

Concanavalin A: Relation between hapten inhibition indexes and association constants for different glycosides

FRANK G. LOONTIENS, JEAN P. VAN WAUWE, AND CLEMENS K. DE BRUYNE
Laboratorium voor Algemene en Biologische Scheikunde, Rijksuniversiteit te Gent, Ledeganckstraat 35, B-9000 Gent (Belgium)

(Received January 20th, 1975; accepted for publication in revised form, May 19th, 1975)

In studies on carbohydrate specificity with the lectin[1] concanavalin A (con A), carbohydrates of low molecular weight have been extensively[2] used as hapten inhibitors of turbidity formed between the lectin and polysaccharides.

These results, expressed as inhibition indexes, have been interpreted in terms of association constants[2], and have been used[3,4] in linear free-energy relations. The evidence supporting this approach is either restricted to carbohydrates with low affinity[5], or is not entirely consistent when methyl[6] or *p*-nitrophenyl glycopyranosides[7] of α-D-glucose or α-D-mannose are compared. We now report that inhibition indexes of several glycosides show a simple relationship with their association constants at 25°, ranging from 70 to at least 6×10^4 M^{-1}. This clearly illustrates the value of the

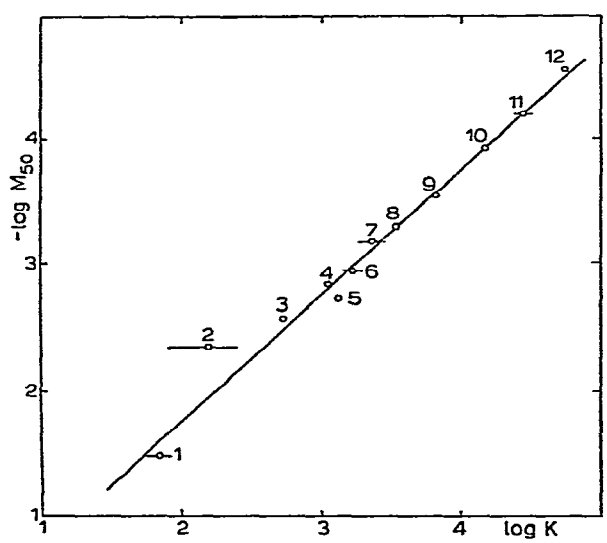

Fig. 1. Correlation between inhibition indexes (M_{50}) and association constants for glycosides interacting with con A. The glycosides are numbered according to Table I.

TABLE I

COMPARISON OF M_{50} VALUES OF SEVERAL TYPES OF GLYCOSIDES WITH THEIR ASSOCIATION CONSTANTS OBTAINED BY THREE DIFFERENT METHODS

Glycopyranosides	$10^{-3} \times K$	*Temp. (degrees)*	*pH*	*Method*[a]	*Ref.*	$10^3 \times M_{50}$	*Ref. of data used for calculation*
Methyl β-D-glucopyranoside (1)	0.070 ±0.015	25	5.6	1	12	33.9	—
	0.070	27	7	2	5		
p-Nitrophenyl β-D-glucopyranoside (2)	0.2 ±0.1	25	5.4	3	—	4.53	—
Methyl β-D-fructopyranoside (3)	0.53	27	7	2	5	2.7	13
o-Iodophenyl β-D-glucopyranoside (4)	1.14	27	7	2	5	1.45	3
Maltose (5)	1.33	27	7	2	5	1.89	14
Methyl α-D-glucopyranoside (6)	1.70 ±0.30	25	5.6	1	12	1.15	—
p-Nitrophenyl α-D-glucopyranoside (7)	2.34 ±0.6	25	5.4	3	—	0.652	—
Methyl α-D-sophoroside (8)	3.3	27	7	2	5	0.506	6
Methyl α-D-mannopyranoside (9)	8.3	27	7	2	5	0.304	—
p-Nitrophenyl α-D-mannopyranoside (10)	14.94 ±0.08	25	5.4	3	4	0.121	—
	15.0	27	7	2	5		
p-Chlorophenyl α-D-mannopyranoside (11)	28.5 ±3.8	25	5.4	3	—	0.0644	—
p-Ethoxyphenyl α-D-mannopyranoside (12)	57.3 ±10	25	5.4	3	—	0.028	—

[a]Methods: 1, ^{13}C-N.m.r. spectroscopy; 2, difference spectrum with *p*-nitrophenyl α-D-mannopyranoside and competition with nonchromophoric glycosides; 3, equilibrium dialysis.

turbidimetric inhibition test, even for glycosides with high affinity, and indicates that all glycosides compete with the polysaccharide, at a single binding-region.

Experimental and literature values of the association constants for glycosides are compared with their M_{50} values in Table I. Their relationship is illustrated in Fig. 1. These values were determined with con A samples, potentially containing slightly different amounts of intact polypeptide chain; it is known that con A, entirely composed of intact chains, shows affinities that are higher[8] by 10–15% (Ref. 9) than for naturally occurring con A. Nevertheless, there is excellent agreement between the association constants, determined by three different methods in the pH range 5.4–7, and the M_{50} values.

The graph in Fig. 1 corresponds to the equation $-\log M_{50} = \log K - 0.245$, the experimental parameter of which is determined by the concentration and the affinity of the polysaccharide. The deviation for *p*-nitrophenyl β-D-glucopyranoside (2) is disregarded as the low affinity precludes measurements of significant differences by photometric assay, even at concentrations of con A of 20 mg/ml.

The observed linear relationship corresponds to the equation valid for competitive enzyme kinetics, namely,

$$\frac{I_{50}}{K_i} = 1 + \frac{S}{K_m},$$

in which I_{50} is the inhibitor concentration giving 50% inhibition at constant substrate concentrations S, and K_m and K_i are the Michaelis constant and the inhibitor (dissociation) constant. A similar equation can be valid for hapten inhibition of con A-glycogen turbidity, provided that (*a*) the turbidity is proportional to the concentration of the lectin–polysaccharide complex, and (*b*) in this mutual, depletion binding system, the affinity of the polysaccharide is considerably higher than the affinity of the glycoside in order to substitute the total concentration of the latter for its concentration free at equilibrium.

These conditions seem to be met with in the con A-glycogen system, as the linear relationship extends to the α-D-mannosides with highest affinities. Thus, all M_{50} values obtained are a dependable measure of affinity and may be used in linear free-energy relationships. It can be anticipated that this holds *a fortiori* for affinity studies using polysaccharides or glycoproteins having nonreducing end-groups identical to the monosaccharide for which the protein has the highest affinity.

An important conclusion about the carbohydrate binding-site of con A is that all methyl or aryl glycosides (α or β) compete for glycogen at the carbohydrate binding-site with affinities that show a simple relationship with their association constants. This conclusion applies to other results[3,5] for *o*-iodophenyl β-D-glucopyranoside and strongly suggests that all glycosides bind at a single, and carbohydrate, specific binding-site in con A, adding additional and quantitative support to the conclusion of Bessler *et al.*[5].

EXPERIMENTAL

Con A was isolated using[10] Sephadex G-75. Glycosides were obtained as reported[4]. Inhibition indexes are expressed as the molarity of a given glycoside causing 50% inhibition (M_{50}) of the turbidity formed between 1.5 mg of glycogen (Merck) and 0.37 mg of con A (3 ml, 25°, pH 7). Some literature values for inhibition indexes were calculated[4] as M_{50} values. Association constants were determined[4] by equilibrium dialysis at pH 5.4 and 25°, by assay of the ligand free at equilibrium. *p*-Nitrophenyl glycosides were determined spectrophotometrically at 313 nm, and D-mannose was determined[11] with *p*-hydroxybenzoic acid hydrazide at 410 nm (ε_M 1.64 10^4 M^{-1}) in neutralized acid hydrolysates.

REFERENCES

1 H. Lis and N. Sharon, *Ann. Rev. Biochem.*, 42 (1973) 541–574.
2 R. D. Poretz and I. J. Goldstein, *Biochemistry*, 9 (1970) 2890–2896.
3 R. D. Poretz and I. J. Goldstein, *Biochem. Pharmacol.*, 20 (1971) 2727–2739.
4 F. G. Loontiens, J. P. Van Wauwe, R. De Gussem, and C. K. De Bruyne, *Carbohyd. Res.*, 30 (1973) 51–62.
5 W. Bessler, J. A. Shafer, and I. J. Goldstein, *J. Biol. Chem.*, 24 (1974) 2819–2822.
6 L.L. So and I. J. Goldstein, *Biochim. Biophys. Acta*, 165 (1968) 398–404.
7 G. S. Hassing and I. J. Goldstein, *Eur. J. Biochem.*, 16 (1970) 549–556.
8 B. A. Cunnningham, J. L. Wang, M. N. Pflumm, and G. M. Edelman, *Biochemistry*, 11 (1972) 3233–3239.
9 J. P. Van Wauwe, Ph.D. Thesis, Rijksuniversiteit te Gent, 1975.
10 B. B. L. Agrawal and I. J. Goldstein, *Biochim. Biophys. Acta*, 147 (1967) 262–271.
11 M. Lever, *Anal. Biochem.*, 47 (1972) 273–279.
12 C. F. Brewer, H. Sternlicht, D. M. Marcus, and A. P. Grollman, *Biochemistry*, 12 (1973) 4448–4457.
13 L. L. So and I. J. Goldstein, *Carbohyd. Res.*, 10 (1969) 231–244.
14 I. J. Goldstein, R. N. Iyer, E. E. Smith, and L. L. So, *Biochemistry*, 6 (1967) 2373–2377.