Fluorimetric studies of the binding of *Momordica charantia* (bitter gourd) lectin with ligands

Manoj K. DAS, M. I. KHAN and Avadhesh SUROLIA*

Departments of Enzyme and Biochemical Engineering, Indian Institute of Experimental Medicine,
Calcutta 700 032, India

(Received 20 January 1981/Accepted 28 January 1981)

The association constants for the binding of a series of ligands with a galactose-specific lectin from *Momordica charantia* (bitter gourd) has been determined through the ligand-induced quenching of protein fluorescence. Analysis of the iodide quenching suggested that there is a slight increase in the accessibility of tryptophan residues of the lectin on binding lactose.

Lectins, with their specificity for sugar residues, have been a subject of intensive current investigation because of their use in various aspects of membrane and cellular studies (Nicolson, 1974; Lis & Sharon, 1977). Before the analysis of their interactions with the cell-bound carbohydrate structures, the binding parameters of lectins with the free ligands should be determined. A qualitative determination of association constants of a series of closely related ligands also offers knowledge about the combining sites of the lectins. The lectin from *Momordica charantia* [bitter gourd (Cucurbitaceae)] seeds has been purified and found by haemagglutination to have specificity for galactose residues (Majumdar *et al.*, 1981). This lectin is tetrametric in nature, containing two binding sites per molecule. In the present paper we have determined the binding parameters for the association of some ligands with *Momordica* lectin through the ligand-induced quenching of fluorescence. The accessibility of tryptophan residues in the presence and absence of a ligand (lactose) was studied by KI quenching.

Materials and methods

Momordica lectin was purified from bitter-gourd seeds as described previously (Majumdar *et al.*, 1981). The carbohydrate ligands were obtained either from Sigma Chemical Co. (St. Louis, MO, U.S.A.) or from P-L Biochemicals (Milwaukee, WI, U.S.A.). An Aminco–Bowman spectrofluorimeter was used for fluorescence measurements. The association constants (K_a) were determined by the

method of Jolley & Glaudemans (1974), which has been previously used for studying the binding of saccharides to anti-(carbohydrate myeloma) immunoglobulins (Glaudemans, 1975). To the lectin solution (A_{280} 0.14) in 0.02 M-Tris/HCl, pH 7.5, containing 0.15 M-NaCl, the ligand solution of appropriate concentration was added through a Hamilton micro-syringe and the change in fluorescence intensity was recorded. The excitation wavelength was 290 nm and emission was measured at 335 nm. All experiments were performed at 25°C. The free energies of binding were calculated by using the equation:

$$\Delta G^0 = -RT \ln K_a$$

KI quenching was performed by the addition of 5 M-KI in small portions. The iodide solution contained sodium thiosulphate (200 μ M) to suppress tri-iodide formation. The non-specific changes in the fluorescence intensity due to ionic-strength variation was checked with 5 M-NaCl. The emission intensities were corrected for the dilution effect. The data were plotted as:

$$\frac{F_0}{F} = 1 + K_q [KI]$$

where F and F_0 are the quenched and unquenched fluorescence intensities and K_q is the quenching constant.

Results and discussion

Table 1 lists the association constants (K_a), free energy of binding ($-\Delta G^0$) and the total maximal quenching of fluorescence ($\Delta F_{\max}$) of *Momordica* lectin with the ligands. The $\Delta F_{\max}$ observed is quite

* To whom correspondence and requests for reprints should be directed.

Table 1. Binding constants (K_a), maximal fluorescence changes (F_{max}) and free energies of binding ($-\Delta G^0$) for a number of ligands with *Momordica* lectin

Ligands	K_a (M^{-1})	$-\Delta G^0$ (kJ/mol)	$-\Delta F_{max}$ (%)	Concentration (mM) needed for 50% inhibition of haemagglutination*
D-Galactose	4.30×10^2	15.00	3.5	3.2
L-Arabinose	4.00×10^2	14.93	3.0	1.56
2-Deoxy-D-galactose	75	10.75	5.5	N.d.†
N-Acetyl-D-galactosamine	5.30×10^2	15.60	3.0	25.6
D-Galactosamine	2.50×10^2	13.59	5.5	N.d.†
Methyl α -D-galactoside	6.30×10^2	16.06	3.5	1.8
Methyl β -D-galactoside	2.80×10^3	19.82	4.0	1.2
Lactose	8.1×10^3	22.5	6	0.6
Lactulose	2.88×10^3	19.83	3.0	N.d.†
Stachyose	1.97×10^3	18.91	6	1.8
Melibiose	3.2×10^3	20.12	4	1.6

* Data taken from Majumder *et al.* (1981).

† N.d., not done.

low, varying from 3 to 6%. This lectin binds both methyl α - and methyl β -linked galactosides, but shows a preference towards the β -anomer. L-Arabinose binds as strongly as D-galactose, suggesting that the C-5 hydroxymethyl group is not essential for the binding. In this respect it resembles *Ricinus communis* (castor-bean) agglutinin (van Waave *et al.*, 1973). The importance of the 2-hydroxy group is evident from the poor binding of 2-deoxy-D-galactose. Substitution at this position by an $-\text{NHCOCH}_3$ or an $-\text{NH}_2$ group favours binding slightly over the deoxy derivative. Lactose is found to be the most active ligand in this series; it binds nearly three times as much as does methyl β -D-galactoside to the *Momordica* lectin. It is noteworthy that the affinities of these ligands are in good agreement with the haemagglutination-inhibition studies with trypsin-treated rabbit erythrocytes (Majumdar *et al.*, 1981). The glucose moiety in lactose must be a contributing factor for the enhanced binding of lactose to the lectin. If we subtract the free energy of binding of lactose (-22.5 kJ/mol) from that of methyl β -D-galactose (-19.82 kJ/mol), the difference obtained (-2.28 kJ/mol) could be attributed to the glucose residue of lactose. Stachyose and melibiose, possessing terminal methyl α -D-galactose residues, however, have higher K_a values than that of methyl α -D-galactose.

Addition of the contribution of glucose residue (-2.68 kJ/mol) obtained from lactose binding to the binding energy of methyl α -D-galactose (-16.06 kJ/mol) interestingly gives -18.74 kJ/mol, a value quite comparable with the binding energy of stachyose (-18.91 kJ/mol). The slightly stronger binding of melibiose (-20.12 kJ/mol) compared with stachyose might be due to the 'better fit' that the glucose moiety in this ligand (Gal α 1 $\rightarrow$ 6Glc) has with

the combining site of *Momordica* lectin. It might be suggested on this basis that the lectin has another subsite, contiguous to the one specific for galactose, that could accommodate glucose (or other pyranosides). That this subsite is specific for pyranosides is supported by the observation that fructofuranose residue in lactulose (Gal β 1 $\rightarrow$ 4Fru f) does not contribute much to the binding. The free energy of binding of lactulose (-19.83 kJ/mol) is same as that of methyl β -D-galactose (-19.82 kJ/mol).

The quenching of protein fluorescence by iodide ion has been extensively used to probe the accessibility of tryptophan residues in proteins and the change in accessibility induced by ligand binding (Galley & Milton, 1979). We have used this approach for studying the complex of *Momordica* lectin and lactose. The results are shown in Figs. 1 and 2. The emission maximum of this protein is at 335 nm, which broadens on adding either KI or lactose. Quenching data were subjected to the Stern–Volmer plot (Fig. 2) (Stern & Volmer, 1919; Lehrer, 1971). The points could not be made to pass through a single line, indicating heterogeneity of tryptophan exposure in the lectin.

A low degree of quenching was noticed; the slope (K_q) decreases with increasing concentration of iodide ion. The low accessibility of tryptophan residues in *Momordica* lectin is consistent with its emission maximum at 335 nm. Proteins that have fully surface-exposed tryptophan exhibit emission at 346 nm (Burstin *et al.*, 1973).

In the presence of saturating amounts ($\sim 90\%$) of lactose, the heterogeneous nature of tryptophan does not change, but an increased accessibility towards iodide ion is observed. The lactose-induced exposure of tryptophan residues of *Momordica* lectin is in contrast with that found in other lectins. For

Fig. 1. KI quenching of *Momordica* lectin in the presence of lactose

Spectrum 1, lectin alone; 2, lectin + lactose (2.10 mM); 3, lectin + KI (0.53 M); and 4, lectin + lactose (2.10 mM) + KI (0.51 M). The absorbance of protein was 0.14 at 280 nm. The excitation wavelength was 290 nm for recording the spectra.

Fig. 2. Stern-Volmer (1919) plot of *Momordica* lectin in the absence (Δ) and presence ($\circ$) of lactose (2.10 mM). The other conditions were the same as those in Fig. 1.

example, binding of galactose decreased the accessibility of these residues in *Abrus precatorius* seed agglutinin (Herrmann & Behnke, 1980). A similar effect was observed when tri-*N*-acetylchitotriose bound to wheat-germ agglutinin (Privat & Monsigny, 1975). However, binding of galactose to its receptor protein from the bacterium *Salmonella typhimurium* increases the availability of the 5-iodoacetamidofluorescein reporter group (Zukin *et al.*, 1977).

The results presented here quantitatively describe the binding parameters of a galactose-specific lectin with some ligands. From these values a less specific subsite capable of accommodating glucose or other hexopyranosides, in addition to the galactose-specific one, has been proposed. Binding of lactose possibly, brings out changes in the environment of tryptophan residues as studied by KI quenching of lectin fluorescence.

We thank Professor B. K. Bachhawat for encouragement, and the Department of Science and Technology, India, and the Indian National Science Academy for financial support. M. I. K. is a Junior Research Fellow of the Council of Scientific and Industrial Research.

References

- Burstein, E. A., Vedenkina, N. S. & Ivkova, M. N. (1973) *Photochem. Photobiol.* **18**, 263–279
- Glaudemans, C. P. J. (1975) *Adv. Carbohydr. Chem. Biochem.* **31**, 313–346
- Galley, W. C. & Milton, J. G. (1979) *Photochem. Photobiol.* **29**, 179–184
- Herrmann, M. S. & Behnke, W. D. (1980) *Biochim. Biophys. Acta* **621**, 43–52
- Jolley, M. E. & Glaudemans, C. P. J. (1975) *Carbohydr. Res.* **33**, 377–382
- Lehrer, S. S. (1971) *Biochemistry* **10**, 3254–3263
- Lis, H. & Sharon, N. (1977) in *The Antigens* (Sela, M., ed.), vol. 4, pp. 429–529, Academic Press, New York
- Majumdar, T., Gaur, N. & Surolia, A. (1981) *Eur. J. Biochem.* in the press
- Nicolson, G. L. (1974) *Int. Rev. Cytol.* **39**, 89–190
- Privat, J. J. & Monsigny, M. M. (1975) *Eur. J. Biochem.* **60**, 555–567
- Stern, O. & Volmer, M. (1919) *Phys. Z.* **20**, 183
- van Wauve, J. P., Loontjens, F. G. & De Bruyne, C. K. (1973) *Biochim. Biophys. Acta* **313**, 99–105
- Zukin, R. S., Hartig, P. R. & Koshland, D. E., Jr. (1977) *Proc. Natl. Acad. Sci. U.S.A.* **74**, 1932–1936