22. Deb, S. and Chatterjee, A., *Mutagenesis*, 1998, **13**, 243–248.
23. Dev-Giri, S. and Chatterjee, A., *Mutat. Res.*, 1998, **413**, 227–234.
24. Sarma, L, Devasagayam, T. P. A., Mohans, H., Mittal, J. P. and Kesavan, P. C., *Int. J. Radiat. Biol.*, 1996, **69**, 633–643.
25. Goto, K., Akematsu, T., Shimagu, H. and Sugiyama, T., *Chromosoma*, 1975, **53**, 223–230.
26. Meister A., in *Metabolic Pathways* (ed. Greenberg, D. M.), Academic Press, New York, 1975.
27. Beutler, E., Duron, O. and Kelly, B. M., *J. Lab. Clin. Med.*, 1974, **61**, 882–888.
28. Uma Devi, P. and Prasanna, P. G. S., *Radiat. Res.*, 1990, **124**, 165–170.
29. Fujimoto, J., *Cancer Res.*, 1974, **34**, 2969–2974.
30. Dethmers, J. K. and Meister, A., *Proc. Natl. Acad. Sci. USA*, 1981, **78**, 7492–7496.
31. Edgren, M. and Revesz, L., *Br. J. Radiol.*, 1987, **60**, 723–724.
32. Edgren, M., Nishida, T., Scott, O. C. A. and Revesz, L., *Int. J. Radiat. Biol.*, 1985, **47**, 463–474.
33. Bump, E. A., Ning, Y. Y. and Brown, J. M., *Science*, 1982, **217**, 544–545.
34. Koch, C. J., Stobbe, C. C. and Bump, E. A., *Radiat. Res.*, 1984, **98**, 141–144.
35. Mitchell, F. B., Russo, A., Biaglow, J. E. and McPhersons, S., *Radiat. Res.*, 1983, **96**, 422–428.
36. Kihlman, B A., *Caffeine and Chromosomes*, Elsevier, Amsterdam, 1977.
37. Revesz, L., Edgren, M. and Nishidai, T., in *Modification of Radiosensitivity in Cancer Treatment*, Academic Press, Japan, 1984, pp. 13–29.
38. Roots, R. and Okada, S., *Int. J. Radiat. Biol.*, 1972, **21**, 329–342.
39. Ward, J. F., *Int. J. Radiat. Biol.*, 1994, **66**, 427–432.
40. Nygren, J., Ljungman, M. and Ahnstrom, G., *Int. J. Radiat. Biol.*, 1995, **68**, 11–18.
41. Modig, H. G., *Biochem. Pharmacol.*, 1973, **22**, 1623.
42. Brown, P. E., *Nature*, 1976, **213**, 363–364.
43. Speit, G., Wolf, M. and Vogel, W., *Mutat. Res.*, 1980, **78**, 267–272.
44. Chatterjee, A., Chattopadhyay, A. and Lawlor, C. J. Z., *Mutat. Res.*, 1995, **327**, 171–177.
45. Xue, L. Y., Friedman, L. R. and Oleinick, N. L., *Radiat. Res.*, 1988, **114**, 11–27.
46. Glover, D., Fox, K. R., Weiler, C., Kligerman, M. M., Turrisi, A. and Glock, J. H., *Pharmacol. Ther.*, 1988, **39**, 3–7.
47. Wolf, C. R., Lewis, A. D., Carmichael, J., Adam, D. J., Allan, S. G. and Anjell, D. J., *Biochem. Soc. Trans.*, 620th meeting, Dublin, 1987.
48. Littlefield, L. G. and Hoffmann, G. R., *Environ. Mol. Mutagenesis*, 1993, **22**, 225–230.

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Further characterization of the saccharide specificity of snake gourd (*Trichosanthes anguina*) seed lectin

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The galactose-specific lectin present in the seeds of *Trichosanthes anguina* (snake gourd) has been further characterized by isoelectrofocusing experiments to identify the number of isolectins. Its carbohydrate-binding specificity has also been characterized in greater detail by the haemagglutination-inhibition technique using several mono-, di- and oligosaccharides. The results suggest that affinity purified snake gourd seed lectin (SGSL) is made up of a single isolectin with a pI of 5.0. The lectin exhibits a strong preference for the β-anomer of galactose over the α-anomer and ligands with a hydrophobic moiety attached to the β-anomer of galactose such as *p*-nitrophenyl-β-D-galactopyranoside and 4-methylumbelliferyl-β-D-galactopyranoside, inhibit the lectin activity better than the simple methyl glycosides of galactose, suggesting that additional hydrophobic interactions stabilize the binding of these ligands bearing nonpolar moieties. The disaccharides lactulose and thiodigalactoside exhibit considerably higher affinity than lactose and MeβGal, indicating that their reducing sugar moieties may have additional favourable interactions with the lectin combining site.

WHILE the exact biological functions of lectins, particularly those from plant sources, continue to intrigue and challenge biochemists, there has been growing evidence that implicates lectins in a large number of biological processes involving their extraordinary ability to recognize specific and unique saccharide structures[1]. Glycosylation is the key step in a number of processes at the cellular level. Cell-surface oligosaccharides are known to get altered in various kinds of pathological conditions including malignant transformations[2]. It is now universally acknowledged that a variety of such phenomena involving glycoconjugates are mediated via lectin–saccharide interactions. Among the roles attributed to lectins are such varied ones as cell recognition and adhesion, transport of specific ligands to target cells and recognition of pathogenic organisms[3]. Their selectivity towards specific monosaccharides as well as their fine-tuned distinction of more complex carbohydrates make them very versatile tools in biotechnology and glycobiology[4].

In view of the foregoing, it is important to characterize lectins at the molecular level and get an understand-

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ing of their saccharide specificity with respect to mono-, di- and oligosaccharides. Such an understanding is essential for their use in isolating glycoconjugates or in clinical applications. An intimate knowledge of the combining site is also necessary for protein engineering techniques that are used to alter the binding specificity of a protein or to fine-tune it. We have earlier reported the purification in high yield of the snake gourd (*Trichosanthes anguina*) seed lectin (SGSL) using affinity chromatography[5]. This lectin was found to be galactose-specific with no major blood group specificity. Using chemical modification studies, we have also reported the involvement of histidine residues in the carbohydrate-binding site of the lectin[6]. In this paper we present the results of isoelectrofocusing (IEF) experiments aimed at determining the isolectin profile of this lectin, and haemagglutination-inhibition experiments aimed at further characterizing the carbohydrate-binding specificity and understanding the nature of the combining site of the lectin.

Snake gourd seed lectin was purified by affinity chromatography on cross-linked guar gum[7] as reported earlier[5]. The protein concentration was estimated by the method of Lowry *et al.*[8] using bovine serum albumin as the standard. The ampholytes and most of the sugars were obtained from Sigma Chemical Company, USA. IEF experiments were performed using a Bio-Rad Rotofor system with a mini-rotofor chamber at 10 W constant power. Experiments in the pH range of 3–10 as well as 3–8 were performed following the instructions given in the manual provided by Bio-Rad using appropriate ampholytes/ampholyte mixtures. Haemagglutination and haemagglutination-inhibition experiments were carried out in 96-well ELISA plates using a 4% suspension of human B(+) erythrocytes as described earlier[5,6].

Results of isoelectrofocusing experiments indicated that SGSL has a single isoform. Both in the broader pH range 3–10 as well as in the narrower range 3–8, only a single peak could be seen in the elution pattern of the lectin from the isoelectrofocusing chamber. A representative IEF profile in the pH range 3–8 is given in Figure 1. The pI determined from this profile is about 5.0. Of the other cucurbit seed lectins, the *Trichosanthes kirilowii* lectin is also reported to have a single isoform in the acidic range (pI ≈ 5.2) (ref. 9).

The results of agglutination-inhibition studies using various sugars in the present study are summarized in Table 1. Additionally, the inhibition data for the sugars investigated in our earlier study[5] have also been listed in this table for the sake of comparison. Our earlier study established that SGSL is a galactose-specific lectin which has a greater affinity for its β-anomer compared to the α-anomers. Glucose, mannose and their derivatives were inactive[5] (see Table 1 for the inhibition data). Thus the inhibitory potency of Meβgal is greater than that of Meαgal while $GalNH_2$, GalNAc,

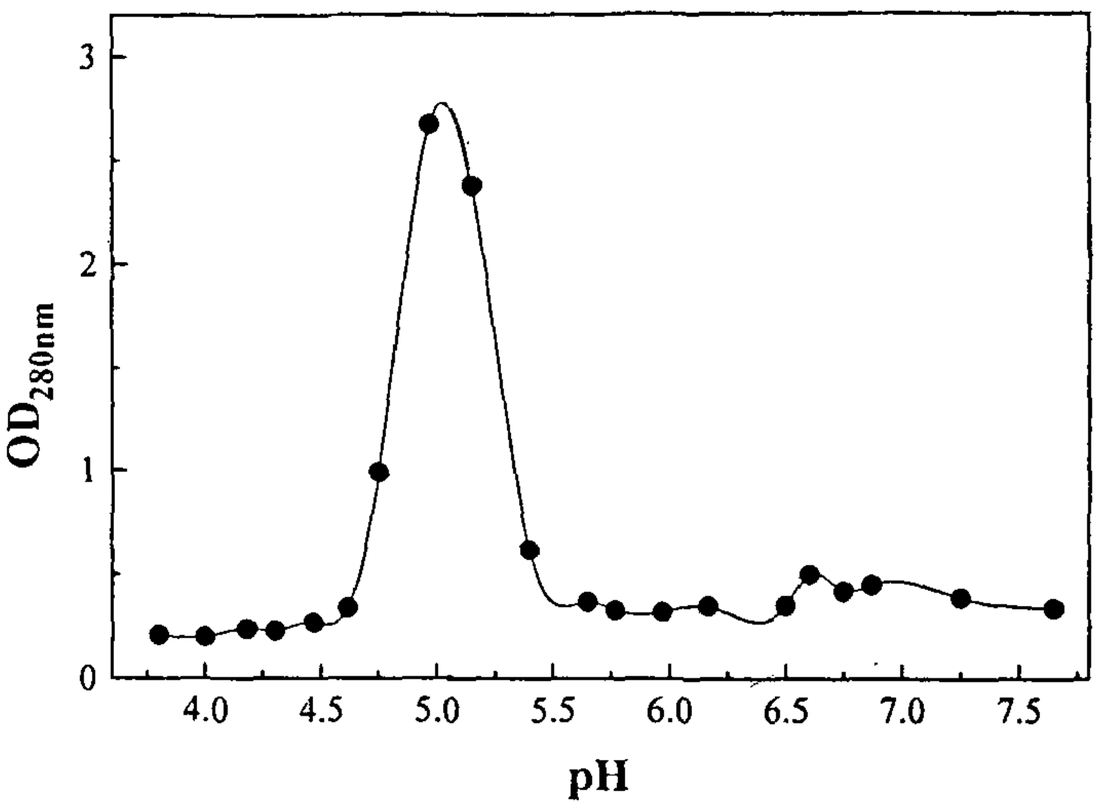

Figure 1. Isoelectrofocusing of snake gourd (*T. anguina*) seed lectin in the pH range 3–8. See text for details.

2-deoxygalactose, fucose and melibiose are comparable to the latter in their affinity for SGSL. The interaction of raffinose with SGSL is about as strong as that of galactose while stachyose and the T-antigenic disaccharide, Galβ13GalNAc, were slightly weaker. Arabinose was nearly eight times less effective as galactose in inhibiting agglutination while lactose was twice as efficient. Both *p*-nitrophenyl-α-D-galactopyranoside (PNPαGal) and its β-anomer had greater inhibitory powers than galactose, although the β-anomer was 4 times better than the α-anomer. Thiodigalactoside (TDG) was nearly as effective as PNPβGal, while lactulose was only slightly weaker. The most potent inhibitor proved to be 4-methylumbelliferyl-β-D-galactopyranoside (MUβGal), which is 15 times stronger than galactose in its ability to inhibit the lectin activity.

The data presented in Table 1 clearly show that the primary requirement for carbohydrate interaction with SGSL is the presence of an axial hydroxyl group at the C-4 position. This is borne out by the fact that all the sugars that are recognized by this lectin such as galactose and its derivatives ($GalNH_2$, GalNAc, 2-deoxy Gal), as well as other sugars like fucose and L-arabinose, have an axial hydroxyl group at the C-4 position. Other, non-inhibitory monosaccharides such as glucose, mannose and GlcNAc have an equatorial hydroxyl at C-4. This not only strongly suggests that the axial C-4 hydroxyl forms favourable interactions with the lectin combining site, but also that the equatorial hydroxyl at this position may interact unfavourably with the lectin. SGSL clearly prefers the β-anomer of galactose since it binds MeβGal with 4 times greater affinity than MeαGal. The presence of a hydrophobic group at the C-1 position appears to improve the interaction between the lectin and the carbohydrate. Here again the β-anomer seems to promote favourable hydrophobic interactions and/or fulfil the correct steric requirements for

Table 1. Minimum concentration required for inhibition of the haemagglutinating activity of snake gourd (*Trichosanthes anguina*) seed lectin by various sugars and their relative inhibitory potencies compared to D-galactose

Sugar*	Concentration for 50% inhibition (mM)	Relative inhibitory potency#	Approximate K_a (M^{-1})
Galactose†	0.8	1.0	1.25×10^3
MeαGal†	1.6	0.5	0.625×10^3
MeβGal†	0.4	2.0	2.5×10^3
GalNAc†	1.6	0.5	0.625×10^3
$GalNH_2$†	1.6	0.5	0.625×10^3
2dGal	1.6	0.5	0.625×10^3
Fucose†	1.6	0.5	0.625×10^3
L-Arabinose†	6.4	0.125	0.156×10^3
Lactose†	0.4	2.0	2.5×10^3
Melibiose††	0.8	1.0	1.25×10^3
Thiodigalactoside	0.165	4.85	6.06×10^3
Raffinose	0.8	1.0	1.25×10^3
Stachyose	1.28	0.625	0.781×10^3
MUβGal	0.053	15.0	18.75×10^3
PNPβGal	0.162	4.95	6.2×10^3
PNPαGal	0.64	1.25	1.56×10^3
Galβ13GalNAc	1.33	0.6	0.75×10^3
Lactulose	0.20	4.0	5.0×10^3
Glucose†	>100	–	–
MeαGlc†	>100	–	–
MeβGlc†	>100	–	–
GlcNAc†	>100	–	–
Man†	>100	–	–
MeαMan†	>100	–	–
Fructose	>100	–	–
Cellobiose†	>100	–	–
Maltose	>100	–	–
LacNAc	>2.5	–	–

*All sugars are D-sugars unless otherwise specified.
† Inhibition data for these sugars were taken from ref. 5.
††The relative inhibition data for this sugar differed slightly from that reported by us earlier[5].
#Relative inhibitory potencies for various saccharides are calculated with reference to galactose which was assigned an arbitrary value of 1.0.

binding. Thus MUβGal and PNPβGal encounter favourable interactions at the binding site. The binding affinity of SGSL for PNPαGal is 4 times weaker than the corresponding β-anomer, yet it is better than that for MeαGal, indicating that a larger hydrophobic substituent in the α-position may have some favourable interaction with the lectin.

Among the disaccharides and oligosaccharides too, those with β-conformation for the terminal non-reducing galactose residues are the better ligands. Lactose binds with approximately the same affinity as MeβGal and 4 times better than melibiose (Galα16Glc). Lactulose (Galβ14Fru) and TDG bind to SGSL with twice and 2.5 times higher affinity, respectively, as compared to lactose. The trisaccharide and tetrasaccharide, raffinose and stachyose, respectively, are only as good as or slightly weaker than galactose. This is consistent with SGSL showing higher preference for the β-anomer of galactose, because the galactose moiety at the non-reducing terminus in these two sugars is in the α-configuration. However, among these two sugars, raffinose (Galα16Glcβ12Fru) is approximately 1.6 times better a ligand than stachyose, presumably due to favourable interactions with the other carbohydrate residues in the chain compared to stachyose (Galα16Galα16Glcβ12Fru), or because of steric constraints imposed by the larger oligosaccharide moiety attached to the non-reducing terminal galactose residue in the latter.

The C-2 position only weakly affects binding to the lectin. The replacement of the hydroxyl with acetamido/amine group (as in GalNAc or $GalNH_2$) or with a H (as in 2dGal) at this position reduces the binding affinity by half, suggesting that the C-2 hydroxyl may be involved in a stabilizing hydrogen-bonding interaction that is lost upon substitution with any of the aforementioned groups. Unlike galactose, which has a $-CH_2OH$ moiety attached at the C-5 position, arabinose and fucose have a –H and a methyl group, respectively, in the corresponding position. This implies that arabinose and fucose are denied any favourable hydrogen-bonding interactions involving the hydroxyl of the primary alcohol moiety that might exist in SGSL–Gal complexes. This is borne out by the fact that both these sugars are weaker ligands for SGSL than Gal itself. The poorer binding of L-arabinose as compared to D-fucose appears to be due to the better steric fit of the methyl group in the latter *vis-à-vis* the –H in the former.

Though the saccharide-binding specificity of SGSL appears to be broadly similar to that of other Cucurbitaceae seed lectins, there are finer differences which might be found useful in studying glycoconjugates and/or cell-surface saccharides. The *Momordica charantia* (MCL) seed lectin is also a galactose-specific lectin with preference for the β-anomer of galactopyranosides[10]. While the binding of mono- and disaccharides by this lectin is generally similar to that of SGSL, binding affinity of MCL to MUβGal and PNPβGal is comparable to that of lactose[11], whereas SGSL binds these two ligands with 7.5 and 2.5-fold higher affinity than lactose. MCL exhibits a 3-fold higher affinity for lactose than lactulose, whereas SGSL exhibits a two-fold higher affinity for lactulose over lactose (Table 1). Further, MCL shows no influence of substitution at C-5 position since it has the same binding constant for arabinose as it does for Gal[10], while SGSL shows a much weaker affinity for arabinose (Table 1). The *T. kirilowii* seed lectin is also a β-Gal specific lectin[9] which exhibits reduced affinity for sugars in which the C-2 hydroxyl is replaced by H, acetamido or amino group. Specificity of this lectin for most other sugars is also rather similar to that exhibited by SGSL. However, the *T. kirilowii* lectin activity is not inhibited by melibiose and lactulose at concentrations

up to 100 mM, whereas SGSL is strongly inhibited by both these sugars. Comparison with the *Trichosanthes cucumerina* seed lectin (TCSL), which exhibits antigenic similarity with SGSL, again shows that the relative trends remain the same[12]. Its binding to thiodigalactoside is however nearly 4 times weaker than to Gal and the lectin does not show a significantly higher affinity for sugars with hydrophobic substituents in the β-anomeric position. For example, the inhibition of lectin-activity by PNPβGal is only 1.33 times that by Gal, while the inhibitory potency of MUβGal is comparable to that shown by Gal.

Carbohydrate binding, particularly of mono- and disaccharides, is the defining feature of lectins and the basis of many methods of classifications of plant lectins[13]. Further, a good understanding of the primary binding site of lectins is critical in understanding the evolutionary patterns that members of the same lectin family might share. The haemagglutination-inhibition studies on the snake-gourd seed lectin suggest that like other cucurbit seed lectins, the lectin recognizes galactopyranosides, i.e. the axial hydroxyl at C-4 position is crucial for recognition by the lectin. The binding site of the lectin prefers the β-anomer of galactopyranosides compared to the α-anomer. Hydrophobic substituents at C-1 greatly improve the inhibitory potency of the ligands in these experiments, possibly due to favourable hydrophobic interactions at or close to the sugar-binding site of the lectin. The C-2 and the C-5 positions are not critical for binding, although reducing possible hydrogen bonding interactions by substituting the hydroxyls with –H, $-CH_3$, CH_3CONH- or $-NH_2$, can reduce the binding affinity. Comparison with reported agglutination-inhibition trends of various sugars for three other cucurbit seed lectins, *M. charantia* lectin, *T. kirilowii* seed lectin and *T. cucumerina* seed lectin, indicates that despite gross similarities in sugar binding displayed by them, fine variations exist among these four lectins, which could be found useful in investigations on glycoconjugates and in the study of cell-surface architecture.

1. Sharon, N. and Lis, H., *Lectins*, Chapman and Hall, London, 1989.
2. Dwek, R. A., *Chem. Rev.*, 1996, **96**, 683–720.
3. Sharon, N. and Lis, H., *Essays Biochem.*, 1995, **30**, 59–75.
4. Lis, H. and Sharon, N., *Annu. Rev. Biochem.*, 1986, **55**, 35–67.
5. Komath, S. S., Nadimpalli, S. K. and Swamy, M. J., *Biochem. Mol. Biol. Int.*, 1996, **39**, 243–252.
6. Komath, S. S., Nadimpalli, S. K. and Swamy, M. J., *Biochem. Mol. Biol. Int.*, 1998, **44**, 107–116.
7. Appukuttan, P. S., Surolia, A. and Bachhawat, B. K., *Indian J. Biochem. Biophys.*, 1977, **14**, 382–384.
8. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., *J. Biol. Chem.*, 1951, **193**, 265–273.
9. Falasca, A. I., Abbondanza, A., Barbieri, A., Bolognesi, A., Rossi, C. A. and Stirpe, F., *FEBS Lett.*, 1989, **246**, 159–162.
10. Das, M. K., Khan, M. I. and Surolia, A., *Biochem. J.*, 1981, **195**, 341–343.
11. Khan, M. I., Mazumder, T., Pain, D., Gaur, N. and Surolia, A., *Eur. J. Biochem.*, 1981, **113**, 471–476.
12. Padma, P., Komath, S. S., Nadimpalli, S. K. and Swamy, M. J., *Phytochemistry*, 1998, accepted for publication.
13. Sharma, V. and Surolia, A., *J. Mol. Biol.*, 1997, **267**, 433–445.

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Effect of zinc deficiency on boundary layers of seminiferous tubules of testes of Wistar albino rat

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Dialogue between Sertoli cell and peritubular cell appeared to be disturbed, leading to various lesions in boundary layers of seminiferous tubules of zinc-deficient Wistar rats. Structural deformities included thickening of collagen fibres, accumulation of oedematous fluid, increased vacuolization in endothelial cells, several infoldings in lamina propria and extensive damage of boundary layers. Thus the arrest of spermatogenesis, a common feature of zinc deficiency, might be because of alternations in the microenvironment of seminiferous tubules owing to pertuberations in the boundary layers of seminiferous tubules.

ZINC deficiency leads to gonadal dysfunction, decreases testicular weight, causes shrinkages of seminiferous tubules, alters testicular steroidogenesis and defective capacitation of sperm[1–6]. The importance of the dialogue between peritubular myoid cells and Sertoli cells has been demonstrated in the fetal testis, the prepubertal testis and the adult testis[7–13]. Peritubular cells insure the structural cohesion, contraction of seminiferous tubule and are known to be one of the constituents of blood testis barrier[14–18]. In cooperation with Sertoli cells they

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