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# Entropy Driven Binding of *O*-Glycan and Glycoproteins to *Artocarpus hirsuta* Lectin: An SPR Study

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**Abstract:** In this study, thermodynamics of binding of *O*-glycan (Galβ1→3GalNAcα1→*O*Ser) and the glycoproteins possessing it, viz., fetuin and mucin to *A. hirsuta* lectin was studied using Surface Plasmon Resonance (SPR). The binding affinities were in the order of asialomucin >mucin>asialofetuin>fetuin>*O*-glycan and found to increase with increase in valency of the ligand. Unusual for a lectin-ligand interaction, the binding was endothermic and entropically driven and the higher affinity was associated with a large favorable entropy term. The native fetuin and mucin showed lower affinity than their desialylated counterpart. Kinetic analysis of the binding revealed that the difference in the affinity of different ligands was due to different rates of their association, whereas the dissociation rates were similar and showed decrease with temperature. The activation energy of the association process was lower with desialylated glycoproteins than that of sialylated one resulting in their faster association and higher affinity.

**Key words:** Artocarpus, lectin, *O*-glycan, thermodynamics, kinetics

## INTRODUCTION

Lectins, a group of carbohydrate binding proteins, could be utilized as excellent macromolecular tools for the study of carbohydrate architecture and dynamics at the cell surface during cell division, differentiation, malignancy and in isolation and characterization of glycoconjugates (Sharon and Lis, 1989), whereas their potential as an anti-tumor agent (Liu *et al*., 2009; Sharon, 2007) renders them as molecules of biomedical interest with a hope to open new ways in cancer treatment in near future. However, their utilization in such a way is subjected to exploration of their specificity and thermodynamic behavior towards glycoproteins of human and animal origin. This communication presents a classical and interesting study of a plant lectin with few glycoproteins of animal origin.

*Artocarpus hirsute* is an α-galactoside binding tetrameric lectin which was purified from its seeds (Gurjar *et al*., 1998). The lectin has been crystallized in the presence of Me-α-Gal (Rao *et al*., 1999) and the structure has been solved (Rao *et al*., 2004). Differential modes of denaturation and aggregation have been observed in the presence of chemical denaturant and temperature (Gaikwad *et al*., 2002; Gaikwad and Khan, 2003). The binding of *A. hirsuta* lectin to galactose and its derivatives was explored employing spectrofluorimetry and it was found to be enthalpically driven with monosaccharides but entropically driven with

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disaccharides (Gaikwad *et al*., 1998; Gaikwad and Khan, 2006). Although, the *A. hirsuta* lectin shows resemblance in structure and carbohydrate specificity with jacalin (*Artocarpus integrifolia* agglutinin) (Jeyaprakash *et al*., 2002, 2003; Mahanta *et al*., 1990; Sastry *et al*., 1986) the binding with T-antigen disaccharide was found to be energetically different (Gaikwad and Khan, 2006). Jacalin showed enthalpically driven binding with T-antigen disaccharide, while *A. hirsuta* lectin exhibited entropically driven binding with the same. The different nature of the binding was presumably attributed to the difference in the terminal residues of jacalin and *A. hirsuta* lectin. The electron density corresponding to the three N-terminal and two C-terminal residues of the β-chain of the jacalin is absent from the map of *A. hirsuta* lectin. No electron density beyond the eighteen residues of the β-chain was present in Form I, whereas only alanine could be fitted in the poorly defined electron density area at the position of the nineteenth residue in Form II (Gaikwad and Khan, 2006).

In the present study, interaction of *A. hirsuta* lectin with *O*-glycan was confirmed and further explored using SPR. We have also reported the thermodynamic and kinetic parameters of binding of Galβ1→3GalNAcα1→*O*Ser and the glycoproteins (fetuin and mucin) possessing it to the lectin.

## MATERIALS AND METHODS

### Materials

Fetuin, mucin (Sigma Chemical Co. St. Louis, USA) Galβ1→3GalNAcα1→*O*Ser (Dextra Lab., London, UK); Certified grade CM5 sensor chip and amine coupling kit (*N*-ethyl-*N*'-(dimethylaminopropyl-) carbodiimide hydrochloride and *N*-hydroxysuccinamide) (Pharmacia Biosensor AB, Uppsala, Sweden) were used. All other chemicals used were of analytical grade.

### Purification of *A. hirsuta* Lectin

Purification of the lectin was carried out as described by Gurjar *et al*. (1998) and protein concentrations were determined according to Bradford (1976) using BSA as standard.

### Biacore Biosensor Assays

Biospecific interactions studies were performed on a BIAcore 2000 (Pharmacia Biosensor AB, Uppsala, Sweden) biosensor system based on the principle of surface plasmon resonance. *Artocarpus hirsuta* lectin (100 μg $mL^{-1}$) in 10 mM sodium acetate buffer, pH 4.0 was coupled (2000 response units), to a certified grade CM5 chip at a flow rate of 5 μL $min^{-1}$ for 50 min using the amine coupling kit. The unreacted groups, on the surface of the chip, were blocked with ethanolamine. A blank channel as a control was created in the analogous way using ethanolamine in place of the lectin. The sensorgram obtained from blank channel was subtracted from the sensorgram of the lectin immobilized channel to eliminate the bulk effect of the buffer change and non-specific binding of the ligand to the sensor chip. All measurements were done using 10 mM phosphate buffer, pH 6.0, (containing 100 mM NaCl, 0.02% w/v sodium azide and 0.05% v/v Tween-20). Prior to injection, glycan and protein samples were dissolved and diluted in the above buffer to avoid buffer mismatch. The association rate constants, at different temperatures, were determined by passing the *O*-glycan solution (0.15-9.6 μM) over the chip at a flow rate of 5 μL $min^{-1}$ for 300 sec. The dissociation rate constants, on the other hand, were determined in a similar manner by passing plain buffer at a flow rate of 5 μL $min^{-1}$ for 300 sec. The glycoproteins asialomucin

(0.39-25 nM), mucin (0.78-50 nM), asialofetuin (0.78-52.6 nM) and fetuin (0.08-5.26 μM) were passed at a high flow rate 50 μL $min^{-1}$ for 120 sec to reduce the mass transport effect and the dissociation was followed by passing buffer at a flow rate of 50 μL $min^{-1}$ for 300 sec. After every cycle, the chip was regenerated by treating with 200 mM sodium carbonate, pH 9.5, for 2 min. Each data point was an average of three independent sets of experiments with SD less than 5%.

Association and dissociation rate constants were obtained by nonlinear fitting of the primary sensorgram using the BIAevaluation software version 3.1. The dissociation rate constants were derived using the Eq. 1:

$$R_t = R_{t0} e^{-k-1} (t - t_0) \quad (1)$$

where, $R_t$ is the response at time t and $R_{t0}$ is the amplitude of the initial response. The association rate constant $k_1$ was derived by Eq. 2 using the measured $k_{-1}$ values:

$$R_t = R_{max} [1 - e^{-(k1C + k-1) (t - t0)}] \quad (2)$$

where, $R_{max}$ is the maximum response and C is the concentration of the analyte (ligand) in the solution. $K_a$ ($k_1/k_{-1}$) is the association constant.

Free energy changes of association (ΔG) were determined by the Eq. 3:

$$\Delta G = - RT \ln K_a \quad (3)$$

Temperature dependence of the association constants was used to determine the thermodynamic parameters. Changes in enthalpy (ΔH) were determined from Van't Hoff plots by using Eq. 4:

$$\ln K_a = (-\Delta H/RT) + \Delta S/R \quad (4)$$

where, ΔH is enthalpy change, R is gas constant, ΔS is entropy change and T is the absolute temperature. The entropy change was obtained from the Eq. 5:

$$\Delta G = \Delta H - T\Delta S \quad (5)$$

Activation enthalpies ($\Delta H^{\ddagger}$), entropies ($\Delta S^{\ddagger}$) and energies were calculated using the following Eq. 6-8:

$$\Delta H^{\ddagger} = E_A - RT \quad (6)$$

$$\ln (k^{\ddagger}/T) = -\Delta H^{\ddagger}/RT + \Delta S^{\ddagger}/R + \ln (k'/h) \quad (7)$$

$$\Delta G^{\ddagger} = \Delta H^{\ddagger} - T\Delta S^{\ddagger} \quad (8)$$

where, $k^{\ddagger}$ is the appropriate rate constant, k' is Boltzman's constant and h is Planck's constant. The overall change in enthalpy, entropy and free energy were determined by the Eq. 9:

$$\Delta H^0 = \Delta H^{\ddagger}_1 - \Delta H^{\ddagger}_{-1}, \Delta S^0 = \Delta S^{\ddagger}_1 - \Delta S^{\ddagger}_{-1} \text{ and } \Delta G^0 = \Delta G^{\ddagger}_1 - \Delta G^{\ddagger}_{-1} \quad (9)$$

## RESULTS

We have reported earlier that the intrinsic fluorescence of *A. hirsuta* lectin is enhanced upon binding with α-galactosides, which has been exploited to determine affinity constants with monosaccharides Me-α-Gal ($K_a$ = 2.5×10$^4$ M$^{-1}$) and Me-umbelliferyl-α-galactoside ($K_a$= 3.3 × 10$^5$ M$^{-1}$) in an enthalpically driven reaction (Gaikwad *et al*., 1998). Further studies revealed, the binding of lectin to disaccharide such as Galβ1→3GalNAcα→*O*Me, Galβ1→3GalNAc and Galα1→6Glc is entropically driven (Gaikwad and Khan, 2006). In the present investigation Galβ1→3GalNAcα1→*O*Ser and the glycoproteins possessing it, viz., fetuin and mucin were used to understand the binding mechanism of the lectin.

The SPR studies showed that the interaction of *A. hirsuta* lectin with ligands resulted in significant changes in RUs (Fig. 1). The non-linear fitting of primary sensorgram to yield rate constants resulted in a $\chi^2 < 2$. $K_a$ for the lectin-ligand interactions was determined at different temperatures, which increased with increasing temperature for all the ligands (Table 1). Van't Hoff plots for the association of different ligands to the *A. hirsuta* lectin were linear (r>0.9) in the temperature range studied (Fig. 2). The positive ΔH value indicated endothermic nature of the binding and the positive value of ΔS indicated entropically driven nature of the binding (Table 1).

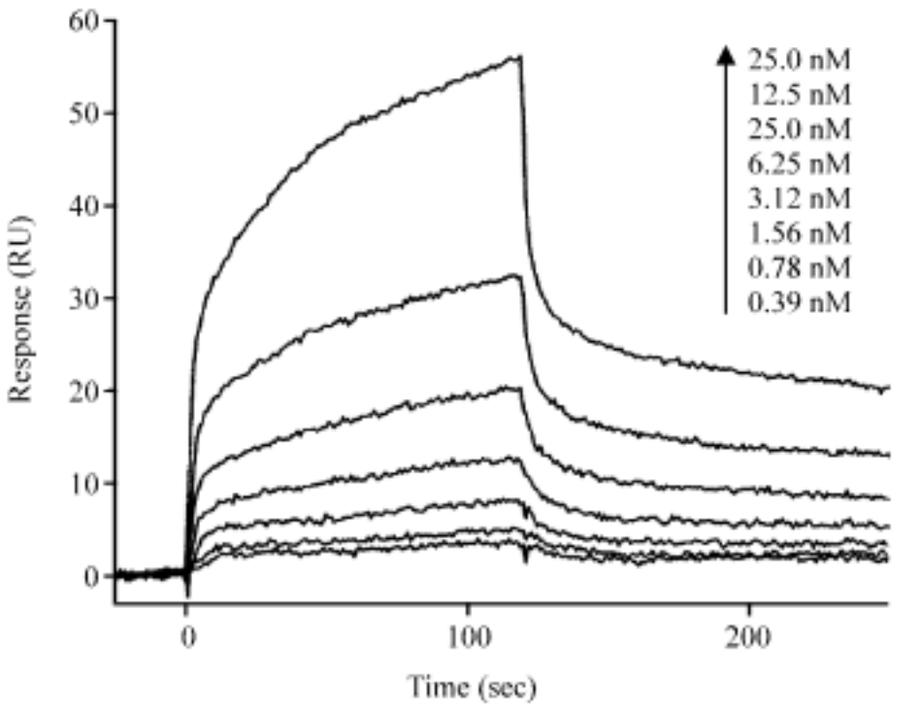


Fig. 1: The representative sensorgrams depicting interactions of increasing amounts of asialomucin to the immobilized *A. hirsuta* lectin at 25°C. The glycoprotein, ranging in concentration from 0.39 nM (bottom trace) to 25 nM (top trace), was injected for 120 sec at a flow rate of 50 μL min$^{-1}$. The dissociation reaction was recorded by flowing buffer at 50 μL min$^{-1}$ for 300 sec. The surface of the chip was regenerated by treating with 200 mM sodium carbonate, pH 9.5, for 120 sec

Table 1: Association constants and thermodynamic parameters for the binding of different ligands to immobilized *A. hirsuta* lectin at different temperatures

| | $10^{-5}\times K_a$ ($M^{-1}$) Temp. (°C) | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | | ΔH | Δg[a] | ΔS |
| Sugar | 20 | 25 | 30 | 35 | ------(kJ mol$^{-1}$)------ | | (J mol$^{-1}$ K$^{-1}$) |
| Galβ1→3GalNAcα1→OSer | 0.97 | 1.35 | 1.83 | -- | 46.85 | -29.26 | 256.3 |
| Asialomucin | -- | 2070 | 3770 | 6470 | 89.54 | -47.44 | 459.6 |
| Mucin | -- | 820 | 1600 | 3010 | 98.58 | -45.16 | 482.3 |
| Asialofetuin | -- | 19.2 | 21.4 | 24.0 | 17.30 | -35.81 | 178.3 |
| Fetuin | -- | 5.91 | 7.74 | 10.1 | 40.71 | -33.02 | 247.4 |

[a]At 25°C

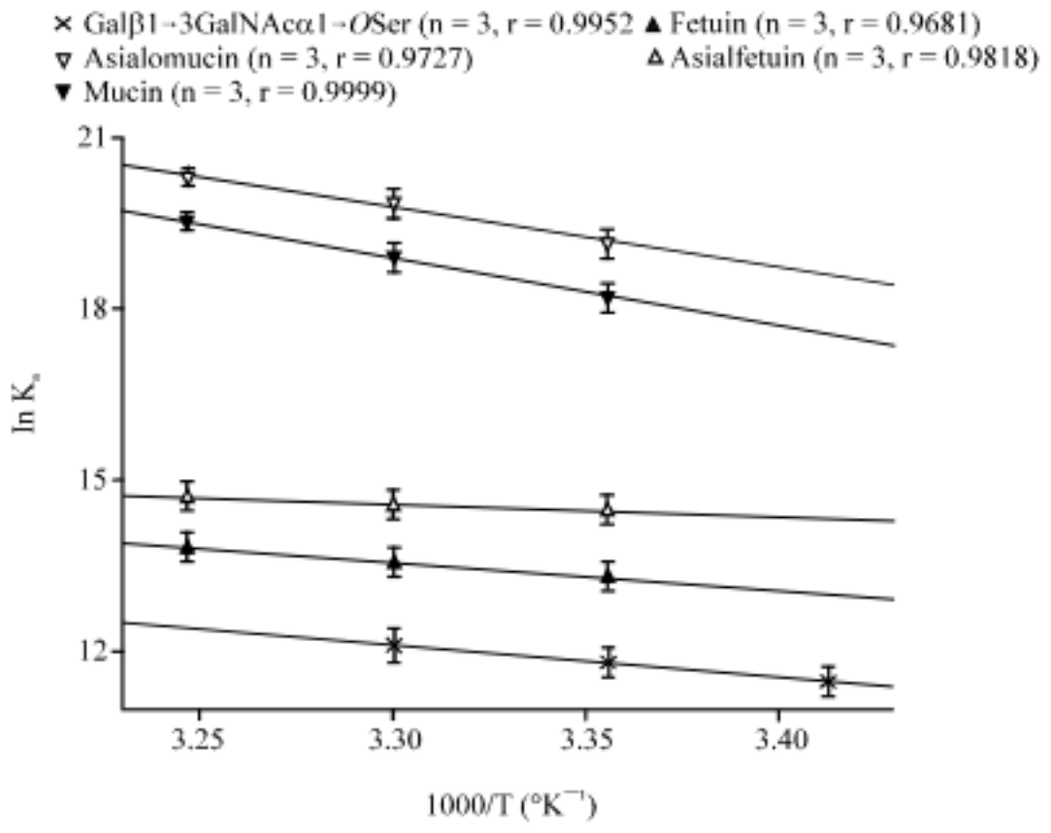


Fig. 2: Van't Hoff plots for the association of various ligands to *A. hirsuta* lectin are drawn according to the regression equation

The affinity of the Galβ1→3GalNAcα1→*O*Ser ($K_a$ = 1.35×10$^5$ M$^{-1}$) (Table 1) was comparable with Galβ1→3GalNAcα→*O*Me, ($K_a$ = 5.72×10$^5$ M$^{-1}$) (Gaikwad and Khan, 2006) where, the more hydrophobic methyl group (than serine) led to increase in affinity. Both the ligands showed increase in the entropy (+ve ΔS) of the system during binding, indicating the enhanced steric hindrance leading to the conformational adjustment to allow the binding.

Binding studies of the *A. hirsuta* lectin was also carried out with glycoproteins possessing *O*-glycan (Galβ1→3GalNAcα1→*O*Ser) viz., asialofetuin and asialomucin and their sialylated forms. Mucin contains several *O*-linked Galβ1→3GalNAcα1→*O*Ser glycans, whereas, fetuin has only three *O*-linked structures and three N-linked triantennary structures with ultimate Galβ1→4GlcNAc (Spiro and Bhoyroo, 1974; Townsend *et al*., 1986). No interaction was observed between the lectin and purified N-linked glycan or Galβ1→4GlcNAc in another set of experiments, indicating only *O*-linked glycan of the fetuin participate in the binding. Affinity of asialomucin ($K_a$ = 2.07×10$^8$ M$^{-1}$) and asialofetuin ($K_a$ = 1.92×10$^6$ M$^{-1}$) was 172 and 16 times higher than *O*-glycan, respectively. Thermodynamic analysis revealed entropic driven nature of the binding. Asialomucin showed higher value of the entropy change (ΔS = 459.6 J mol$^{-1}$ K$^{-1}$) than *O*-glycan (ΔS = 256.3 J mol$^{-1}$ K$^{-1}$) indicating increased non-polar protein-protein interaction between the lectin and asialo-mucin. On the other hand, asialo-fetuin showed lower value of entropy (ΔS = 178.3 J mol$^{-1}$ K$^{-1}$) and enthalpy (ΔH = 17.3 kJ mol$^{-1}$) than *O*-glycan (ΔS = 256.3 J mol$^{-1}$ K$^{-1}$, ΔH = 46.85 kJ mol$^{-1}$) (Table 1). A positive enthalpy value in ligand-protein interactions indicates that the energy released during new bond formation is relatively lower than that required to break existing bonds. The existing bonds refer to hydrogen bonds prevalent between water molecules and lectin/ligand. Although, binding of the *A. hirsuta* lectin with asialo-fetuin results in loss of favorable entropy but also increase in favorable enthalpy (+ΔH is low) leading to higher affinity than *O*-glycan. Hence, higher affinity of the lectin towards asialomucin than *O*-glycan is due to the positive entropy contribution, whereas that of asialofetuin is due to favorable enthalpic as well as entropic contribution. Moreover, the difference in the affinity of *O*-glycan, asialo-fetuin and asialomucin, can be explained on the basis of the valency, as

the asialofetuin is a trivalent whereas asialomucin is a multivalent structure. Increase in valency related $K_a$ is attributed to increase in entropy of binding. Multivalency, due to clustering effect, often results in several fold increase in the affinity. Similar observations were made in *Dioclea grandiflora* lectin and concanavalin A, where increase in $K_a$ values of the multivalent carbohydrates relative to monovalent analogs are shown to be due to more positive entropy ($T\Delta S$) (Dam *et al*., 2000).

The sialylated glycoproteins fetuin ($K_a = 5.91\times10^5\ M^{-1}$) and mucin ($K_a = 8.2\times10^7\ M^{-1}$) also bind to *A. hirsuta* lectin but with 2.5-3 fold less affinity than their desialylated forms. The presence of neuraminic acid seems to create some unfavorable interactions caused by steric hindrance or due to the negative charge. The presence of neuraminic acid results in increase in unfavorable positive enthalpy leading to comparatively weaker binding of sialylated glycoproteins, which could be utilized overcoming some steric constraints.

**Kinetic Analysis**

The association ($k_1$) and dissociation rate constants ($k_{-1}$) determined at different temperatures are given in Table 2. The Arrhenius plots for all the ligands were linear ($r>0.9$) in the temperature range studied (Fig. 3). The glycoproteins showed significantly higher association rate constants than *O*-glycan. Differences in $K_a$ for the ligands studied were found primarily due to differences in their association rate constants, whereas their dissociation rate constants were found to be similar. The association rate constants were observed in the range of $10^2$-$10^5\ M^{-1}\ sec^{-1}$. These second order rate constants are slower than a diffusion-controlled process and are similar to those for the binding of fluorogenic/chromogenic ligands to *Ricinus communis* agglutinin (Podder *et al*., 1978), soybean lectin (De Boeck *et al*., 1984) and *Fusarium solani* lectin (Khan *et al*., 2007). The kinetics of ligand binding to *A. hirsuta* lectins is qualitatively consistent with a single-step binding mechanism and can be depicted by the equation:

$$P + L \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} PL\ ;\ K_a = k_1/k_{-1}$$

Table 2: Rate constants and activation parameters for the interaction of different ligands to *A. hirsuta* lectin

| | Association | | | | |
|---|---|---|---|---|---|
| Ligand | $k_1\times10^{-3}$ ($M^{-1}\ sec^{-1}$) | $E^{\ddagger}_1$ | $\Delta H^{\ddagger}_1$ | $\Delta G^{\ddagger}_1$ | $\Delta S^{\ddagger}_1$ ($J\ mol^{-1}\ K^{-1}$) |
| | | ----------(kJ $mol^{-1}$)---------- | | | |
| Galβ1→3GalNAcα1→*O*Ser | 0.14 | 6.46 | 3.99 | 30.81 | -90.00 |
| Asialomucin | 294 | 22.24 | 19.77 | 41.81 | -73.99 |
| Mucin | 62.8 | 59.75 | 57.27 | 68.91 | -39.07 |
| Asialofetuin | 2.53 | 6.85 | 4.39 | 53.47 | -165.03 |
| Fetuin | 0.856 | 29.44 | 26.97 | 56.25 | -98.27 |
| | Dissociation | | | | |
| Ligand | $k_1\times10^{-3}$ ($sec^{-1}$) | $E^{\ddagger}_1$ | $\Delta H^{\ddagger}_1$ | $\Delta G^{\ddagger}_1$ | $\Delta S^{\ddagger}_1$ ($J\ mol^{-1}\ K^{-1}$) |
| | | ----------(kJ $mol^{-1}$)---------- | | | |
| Galβ1→3GalNAcα1→*O*Ser | 1.47 | -40.44 | -42.92 | 60.12 | -345.50 |
| Asialomucin | 1.42 | -64.57 | -67.05 | 89.25 | -524.61 |
| Mucin | 0.76 | -39.56 | -42.04 | 114.04 | -484.70 |
| Asialofetuin | 1.31 | -9.97 | -12.45 | 89.42 | -341.87 |
| Fetuin | 1.45 | -11.35 | -13.83 | 89.15 | -345.83 |

Values of $k_1$, $k_{-1}$, $\Delta G^{\ddagger}_1$ and $\Delta G^{\ddagger}_{-1}$ are determined at 25°C.$\Delta H^0$: 46.91 kJ $mol^{-1}$, $\Delta G^0$: -29.31 kJ $mol^{-1}$ and $\Delta S^0$: 255.5 J $mol^{-1}\ K^{-1}$ for Galβ1→3GalNAcα1→OSer. $\Delta H^0$: 86.82 kJ $mol^{-1}$, $\Delta G^0$: -47.44 kJ $mol^{-1}$ and $\Delta S^0$: 450.6 J $mol^{-1}K^{-1}$ for asialomucin. $\Delta H^0$: 99.31 kJ $mol^{-1}$, $\Delta G^0$: -45.13 kJ $mol^{-1}$ and $\Delta S^0$: 445.6 J $mol^{-1}\ K^{-1}$ for mucin. $\Delta H^0$:16.84 kJ $mol^{-1}$, $\Delta G^0$: -35.95 kJ $mol^{-1}$ and $\Delta S^0$ = 176.8 J $mol^{-1}\ K^{-1}$ for asialofetuin. $\Delta H^0$: 40.80 kJ $mol^{-1}$, $\Delta G^0$: -32.90 kJ $mol^{-1}$ and $\Delta S^0$: 247.5 J $mol^{-1}\ K^{-1}$ for fetuin

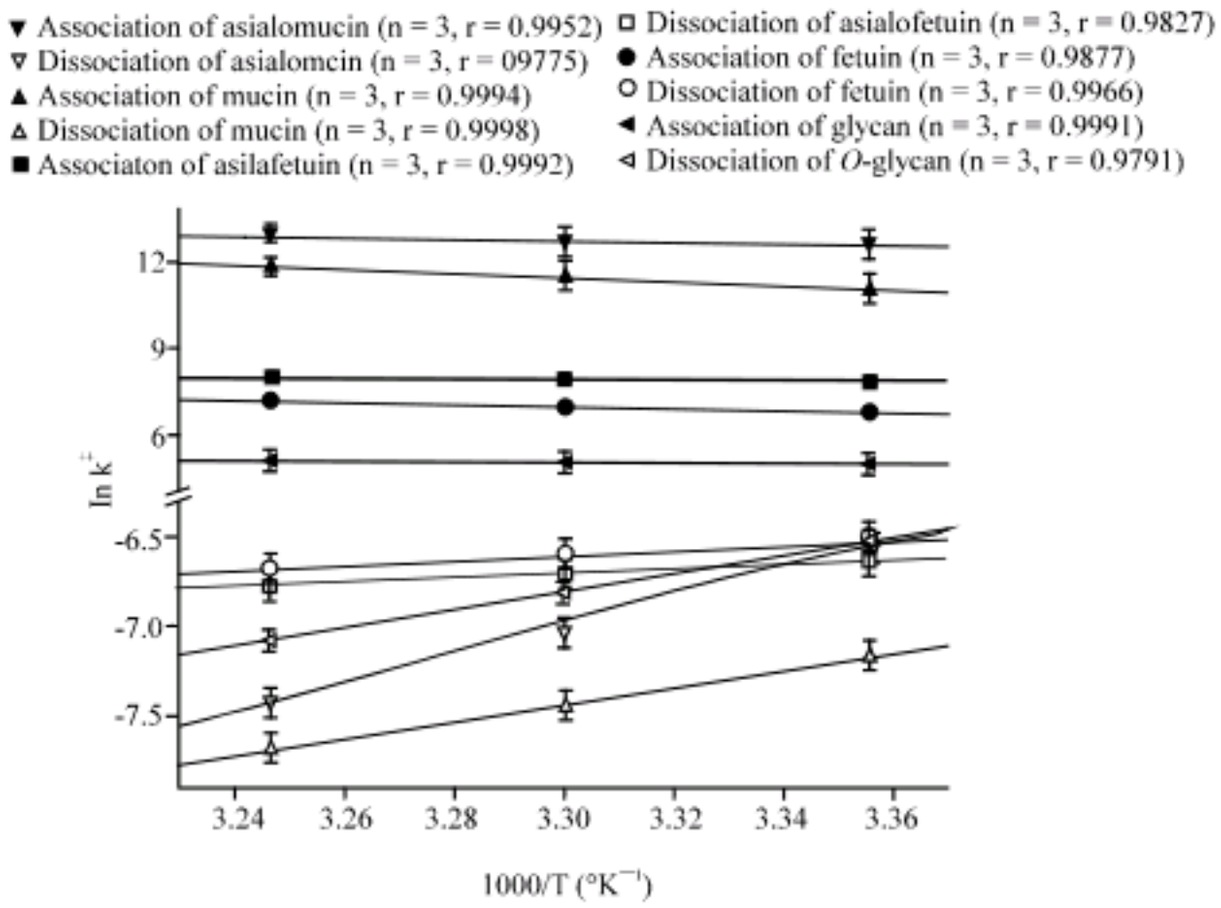


Fig. 3: Arrhenius plots for the association and dissociation kinetics of various ligands to *A. hirsuta* lectin

Generally, when the second order rate constants for the ligand (L) binding to a protein (P) are slower by several orders of magnitude than those seen in diffusion controlled reactions, the binding is presumed to involve a putative intermediate complex PL*, which then isomerizes into a final complex PL.

$$P + L \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} PL^* \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} PL$$

where, $K_1 = k_1/k_{-1}$ and $K_2 = k_2/k_{-2}$. In the SPR, the response depends only on the amount of the ligand bound to the chip and not on the stereochemistry of the ligand-lectin complex. Hence, the complex $PL^*$ will not be able to generate distinct SPR response and the first step will not be observable by SPR. It is possible that we are observing only the second step. The agreement between entropy-enthalpy changes determined by Van't Hoff plots (Eq. 4) and Arrhenius plots (Eq. 7), indicates that these changes are related to the total binding process and not due to any intermediate that contributes appreciably to these parameters for the saccharide binding (Table 1, 2). Linearity of Arrhenius plots, however, also rules out to a great extent, the formation of such an intermediate(s) and the occurrence of dramatic conformational changes in the lectin molecule in the temperature range studied. Thus it can be concluded that the binding of glycans and glycoproteins to the *A. hirsuta* lectin is consistent with a single step bimolecular association reaction which is of several orders of magnitude slower than diffusion controlled reaction.

Dissociation rate constants showed a decrease with increasing temperature, which indicates that temperature slows down the dissociation process leading to increase in the affinity of *A. hirsuta* with increasing temperature. The activation energy ($E^{\ddagger}_1$) of association for asialo-fetuin ($E^{\ddagger}_1$ = 6.85 kJ mol$^{-1}$) and asialomucin ($E^{\ddagger}_1$ = 22.24 kJ mol$^{-1}$) is higher than *O*-glycan (Galβ1→3GalNAcα1→*O*Ser) ($E^{\ddagger}_1$ = 6.46 kJ mol$^{-1}$), indicating relatively high amount

of the energy has to be expended for the binding of asialo-glycoproteins than *O*-glycan. The sialylated glycoproteins fetuin ($E^{\ddagger}_1$ = 29.44 kJ mol$^{-1}$) and mucin ($E^{\ddagger}_1$ = 59.75 kJ mol$^{-1}$) showed 4 and 2.6 times, respectively, higher activation energy of association than their desialylated counterpart. This energetic barrier could be utilized in overcoming some steric constraints, caused by the presence of unfavorable group neuraminic acid (NeuAcα2→3/6Galβ1→). The considerably high difference between the activation energy of asialomucin ($E^{\ddagger}_1$ = 22.24 kJ mol$^{-1}$) and asialo-fetuin ($E^{\ddagger}_1$ = 6.86 kJ mol$^{-1}$) could be correlated to the breaking of different levels of hydrogen bonds existing between these glycoproteins and solvent.

A comparison between entropy of association for different ligands provides an insight to the mechanism of binding of these ligands to the *A. hirsuta* lectin (Table 2). Asialofetuin showed highest negative entropy of association ($\Delta S^{\ddagger}_1$ = -165.03 J mol$^{-1}$ K$^{-1}$) indicating involvement of a highly ordered transition state of the ligand. The slowest ligand, *O*-glycan, showed higher negative entropy of the association ($\Delta S^{\ddagger}_1$ = -90.0 J mol$^{-1}$ K$^{-1}$) than asialomucin ($\Delta S^{\ddagger}_1$ = -73.99 J mol$^{-1}$ K$^{-1}$) indicating involvement of comparatively high ordered transition state of the ligand than asialomucin. Relatively positive entropy of association suggests that the ligand can approach the binding sites in several ways often leading to its faster association. Involvement of a comparatively more ordered transition state of asialo-fetuin than asialomucin might be one of the factors responsible for its slow association than asialomucin. In the case of sialylated glycoproteins viz. mucin ($\Delta S^{\ddagger}_1$ = -39.07 J mol$^{-1}$ K$^{-1}$) and fetuin ($\Delta S^{\ddagger}_1$ = -98.27 J mol$^{-1}$ K$^{-1}$), the entropy of association is lower than their desialylated counterparts but associated with a comparatively large activation energy ($E^{\ddagger}_1$) (Table 2), which seems to be responsible for slowing down their association.

The enthalpy of the association ($\Delta H^{\ddagger}_1$) is lower for *O*-glycan ($\Delta H^{\ddagger}_1$ = 3.99 kJ mol$^{-1}$) and asialo-fetuin ($\Delta H^{\ddagger}_1$ = 4.39 kJ mol$^{-1}$) than other ligands. Higher enthalpy of the association indicates requirement of considerable reorientation of water molecules around the ligand and/or the corresponding *loci* of the lectin. Relatively higher enthalpy of the association of sialylated glycoproteins than desialylated glycoproteins indicates energetically costly disruption of the hydrogen bonding network of water molecules in the binding cleft of the lectin by the sialylated glycoprotein presumably results in their relatively low rate of association.

## DISCUSSION

Usually lectins show very high affinity for multivalent ligands viz., glycoproteins (Kobayashi *et al*., 2004; Wu *et al*., 2001; Khan *et al*., 2007) Millimolar affinity is observed for lectins binding to monosaccharides whereas, with longer oligosaccharides, increased affinity (up to micromolar range) can be observed due to an extended binding site (Imberty *et al*., 2005). These type of interactions, brought about by branched oligosaccharides, are typified by a favorable enthalpy resulting from large number of hydrogen bonds. Such interactions are compensated by an unfavorable entropy contribution, attributed either to solvent rearrangement (Lemieux *et al*., 1991) or to loss of ligand conformation (Carver, 1993). Moreover, such interactions typified by a negative enthalpy, also seems to be related to a much stronger prevalence of polar-polar interactions and a higher interaction cooperativity (Garcia-Hernandez *et al*., 2000). However, contrary to this generally observed phenomenon, in the present investigation the binding of glycans to *A. hirsuta* lectin is entropically driven. The lectin-carbohydrate interactions optimize in such a way that their packing is significantly tighter than the packing of protein-protein interfaces

or protein interiors (Garcia-Hernandez *et al*., 2000). The lectin-carbohydrate and protein-protein systems differ from each other not only in their stereochemical metrics, but also rely on different energetic bases (Garcia-Hernandez and Hernandez-Arana, 1999; Xu *et al*., 1997).

The ligand-protein interactions involve removal of water molecules near the binding surface of the protein and ligand. These water molecules become more mobile and cause an increase in the entropy of the system. Whereas, a ligand molecule bound to the protein stays in a more ordered state compared to the free mobile state in the solvent resulting in decrease in entropy of the system. Summation of these two opposite effects of the entropy change determines the overall change in the entropy of system. Such an overall change in the entropy of the ligand-protein interaction will depend on the magnitude of these changes. In the present situation, the binding of the *O*-linked glycan results in an overall increase in the entropy of the system.

In conclusion, the binding of *A. hirsuta* lectin to *O*-glycan and glycoproteins, contrary to other lectin-glycan interactions, is entropically driven and the increase in $K_a$ with temperature is due to increase in association rates and decrease in dissociation rates. Moreover, the effect of ligand valency is also prominent as the increase in valency of the ligand results in increased affinity.

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