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Changes in Biological Activities of Anti-LH Lectin— *Erythrina lithosperma*

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ABSTRACT The present paper deals with the biological properties of the *Erythrina lithosperma* lectin *viz* sugar inhibition, effect of pH, concentration, thermostability, lectins extracted from different parts of the plant, apart from seeds.

INTRODUCTION

A lectin is a sugar-binding glycoprotein of non-immune origin (Goldstein et al., 1980). Like other proteins, the properties of lectins are altered by various physical and chemical factors. A new red blood cell membrane specificity in man called LH, has been extracted in the seeds of a legume plant-*Erythrina lithosperma* (Shrivastava et al., 1979).

The agglutination activity of Con-A with human erythrocytes is found to be affected by temperature, concentration and various chemical and mechanical factors (Schnebi and Bacht, 1975). Vlodavsky et al. (1975) reported that lectins from *Phaseolus vulgaris* and *Glycine max* are temperature sensitive for agglutination reaction with human erythrocytes. Both temperature and pH were also reported to be responsible for the reduced haemagglutination reaction in rice lectin (Indravathamma and Seshadri, 1984). Inactivation of lectins with chemicals like urea, sodium decylbenzene sulphate have been reported by Liener and Wada (1956) and Liener (1985). Paulova et al. (1971) found that ions like Mn^{2+} and Ca^{2+} did affect the haemagglutinating activity of lectins.

The anti-LH lectin is now being successfully used for population discriminations. Various biological properties of this lectin play a key role in its haemagglutination reaction with human red cell surface receptors. Keeping this view in mind, various biological properties of the anti-LH lectin *viz.* sugar inhibition, change in pH, concentration, thermostability, and lectins prepared from different parts of the plant other than seeds, have been studied in the present paper.

MATERIAL AND METHODS

A total of 125 human blood samples (25 individuals from each of five types *viz.* A/LH^+ A/LH^-, B/LH^+, B/LH^- and O/LH^+) were collected from Varni Pathology Clinic, Sagar, Madhya Pradesh. The anti-LH lectin was prepared from the seeds of *Erythrina lithosperma*, collected from Botanical Survey of India, Calcutta, following the procedure of Shrivastava et al. (1979). Sugars like L-arbinose, D-glucose, maltose, N-acetyl-D-glucosamine, salicin, sucrose, D-galactose, lactose, raffinose and melibiose were used for the lectin inhibition experiments.

The pH of the anti-LH lectin was estimated by digital pH meter. The haemagglutination activity of the lectin was studied at different pH levels ranging from 1.0 to 7.0.

Lectin was prepared in different concentrations ranging from 1:5 to 1:18. For sugar inhibition tests, lectin preparation in different concentrations, thermostability tests and lectin preparation from different plant parts other than seeds, procedures as described by Shrivastava et al. (1979) were followed.

RESULTS AND DISCUSSION

Table 1 shows the inhibition of the anti-

LH lectin with different sugars. The haemagglutinating activity of anti-LH lectin is inhibited by D-galactose, lactose, raffinose and melibiose, but not by L-arabinose, D-glucose, maltose, N-acetyl-D-glucosamine, sucrose and salicin.

Table 2 shows the effect of change in pH on the reactivity of *E. lithosperma* lectin. The pH of the native anti-LH lectin was estimated to be 5.20. When the pH was raised to 7.0, the lectin lost its haemagglutination activity. The lectin reacted with different LH-positive red blood cells at pH 5.50, 5.75, 6.25 and 6.95. Similarly we conducted haemagglutination experiments at different levels of pH *viz.* 5.00, 4.75, 4.25, 3.75, 3.25, 2.75, 2.00, 1.75, 1.30. 1.25, 1.00, respectively. Upto pH 1.30 the lectin retained its reactivity but at pH 1.25 lost it comletely.

The reactivity of the anti-LH lectin at different concentrations is shown in table 3. To find out the best concentration at which the

Table 1 : Inhibition of the anti-LH lectin with different sugars

Name of sugar	*Erythrocytes*				
	A/LH⁺ (n=25)	*A/LH⁻ (n=25)*	*B/LH⁺ (n=25)*	*B/LH⁻ (n=25)*	*O/LH⁺ (n=25)*
L-arabinose	+	+	+	+	+
D-glucose	+	+	+	+	+
Maltose	+	+	+	+	+
N-acetyl-D-glucosamine	+	+	+	+	+
Salicin	+	+	+	+	+
Sucrose	–	–	–	–	–
D-galactose	–	–	–	–	–
Lactose	–	–	–	–	–
Raffinose	–	–	–	–	–
Melibiose	–	–	–	–	–

Table 2 : Effect of change in pH on haemagglutinating reactivity of the anti-LH lectin (n=25)

Erythr-	*Range of pH level tested*																	
ocytes	*1.00*	*1.25*	*1.30*	*1.75*	*2.00*	*2.25*	*2.75*	*3.25*	*3.75*	*4.25*	*4.75*	*5.00*	*5.20*	*5.50*	*5.75*	*6.25*	*6.75*	*6.95*	*7.00*
A/LH⁺	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
B/LH⁺	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
O/LH⁺	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–

Table 3 : Haemagglutinatinating activity of the anti-LH lectin at different concentrations (n=25)

Erythrocytes	*Lectin prepared in different concentrations*										
	1:5	*1:6*	*1:7*	*1:8*	*1:9*	*1:10*	*1:11*	*1:12*	*1:14*	*1:16*	*1:18*
A\LH⁺	1:512	1:512	1:512	1:128	1:32	1:32	1:32	1:16	1:16	1:8	1:8
LH-status	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	–ve	–ve	–ve
A\LH⁻	1.128	1:128	1:128	1:32	1: 16	1:16	1:8	1:8	1:4	1:2	1:2
B\LH⁺	1:512	1:512	1:512	1:128	1:32	1:32	1:32	1:32	1:16	1:8	1:8
LH-status	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve
B\LH⁻	1:128	1:128	1:128	1:32	1:16	1;16	1:16	1:8	1:8	1:4	1:4
O\LH⁺	1:512	1:512	1:512	1:256	1:64	1:64	1:64	1:32	1:32	1:16	1:16
LH-status	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve

lectin would react, we prepared the dilutions of anti-LH lectin in ratios of 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:11, 1:12, 1:14, 1:16 and 1:18, respectively. Lectins prepared in the ratios of 1:5, 1:6 and 1:7 exhibited titres upto 1:512 with A/LH$^+$, B/LH$^+$ and O/LH$^+$ cells and upto 1:128 with A/LH$^-$ and B/LH$^-$ cells. Lectin at concentrations 1:16 and 1:18 showed the lowest titres, 1:8 with A/LH$^+$ and B/LH$^+$, 1:16 with O/LH$^+$, 1:2 with A/LH$^-$ and 1:4 with B/LH$^-$ cells. When tested with the lectin diluted to 1:18, A and B cells lost their LH$^+$ status but O cells retained it. In fact O cells were found invariably LH$^+$ in a number of earlier studies (Shrivastava et al., 1979; Sehajpal and Shrivastava, 1981; Kaur, 1983; Reddy et al., 1981 and Koley, 1992). The differentiation between LH$^+$ and LH$^-$ cells was most clear when the lectin was diluted at the ratio of 1:9.

Temperature exhibited a limited range of variability in the agglutination pattern of lectins against human erythrocytes (Indravathamma and Seshadri, 1984; Bose and Bhalla, 1989). The anti-LH lectin treated with temperature like 4°C to 56°C showed best haemagglutination activity. At 80°C the anti-LH lectin did react weakly, and at 100°C it completly lost the haemagglutination activity.

Besides seeds, we prepared lectins from *E. lithosperma* leaves, barks and pods. Lectins prepared from *E. lithosperma* leaves and pods reacted rather weakly with red blood cells and failed to differentiate LH$^+$ cells from LH$^-$ ones. Lectin prepared from *E. lithosperma* leaves reacted with A/LH$^+$ and A/LH$^-$ cells at the titre of 1:4. Lectin prepared from *E. lithosperma* pods reacted with above five types of red cells at the titre of 1:1 only. Lectin prepared from *E. lithosperma* barks failed to show any agglutinating activity with human red blood cells.

The results of sugar inhibition tests indicate that the lectin has strong affinity to bind those sugars which contain molecules of galactose as one of their components (*e.g.* D-galactose, lactose, raffinose and melibiose). Whereas in glucose containing sugars (*e.g.* L-arabinose, D-glucose, N-acetyl-D-glucosamine, maltose, salicin and sucrose) were not inhibited by the anti-LH lectin.

Change in pH did affect the haemaggl-utinating activity of the anti-LH lectin. The results indicate that the lectin becomes totally inactive to react with red cell surface antigens when the pH is lowered to 1.25 and again at pH 7.0. At pH 1.25, perhaps there is a change in the membrane potential of erythrocytes which may be responsible for negative reaction. At high pH 7.0, almost all ions (Na^+ and Cl^-) present in normal saline (major ingradient of lectin preparing medium) are dissociated which may interfere with the agglutination raction of the anti-LH lectin on erythrocyte membrane surface receptors. Dissociation of ions at high pH can affect greatly the haemagglutination activity (Paulova et al., 1971) and our results tend support to this hypothesis.

The differentiation between LH$^+$ and LH$^-$ cells is best found when the lectin saline ratio is 1:9. Thus, it seems that an optimum concentration of lectin is necessary for the best differentiation between LH$^+$ and LH$^-$ types of reaction. Below and above these concentration, this property of the anti- LH lectin is severely affected.

Though various biological properties of the anti-LH lectin have been reported, many more immunochemical properties are yet to be explored for the better understanding of the LH-system and its applications.

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