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Effects of Physico-chemical Agents on the Biological Activities of the Mulberry Seed Lectins

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Abstract: Three lectins were extracted and purified from mulberry seeds i.e., MSL-1, MSL-2 and MSL-3.These lectins exhibited strong cytotoxic effect in brine shrimp lethality bioassay. The biological activities of the lectins MSL-1, MSL-2 and MSL-3 were studied after various physical and chemical treatments. The biological activities of the lectins were affected greatly with the changes of pH and temperature and the lectins showed maximum hemagglutinating activities around pH 7.2-7.6 and temperature 20-35°C. The biological activities of the lectins were abolished with the higher concentration of acetic acid and denaturants, guanidine-HCl and urea. The activities of all the three lectins were affected more pronouncedly by guanidine-HCl than urea. In the presence of metal chelator, EDTA and-SH group binding agent, $HgCl_2$, the heamagglutinating activities of the mulberry seed lectins were destroyed completely while in the presence of Ca^{2+} salts that of the lectins were increased remarkably.

Key words: Heamagglutination activity, mulberry seed lectin, physical and chemical agents

Introduction

Lectins are glycoproteins of mainly plants and animal origin that bind specific saccharides and agglutinate cell of various types (Lis and Sharon, 1986). The binding of lectins to surface carbohydrate initiates several interesting activities on cells. These include blood group specific hemagglutination activity on lymphocytes, tumor-cell specific agglutination activity and insulin like activity on adiposities (Lis and Sharon, 1973; Goldstein and Hayes, 1987). They also serve as valuable tools in the diverse areas of bio-medical research. So, lectin is currently attracting much interest more than 70 lectins have been isolated from leguminous plants, mostly from seeds. It was reported that lentil seeds contained at least two lectins, which agglutinated albino rat red blood cells (Samad and Absar, 2001).

The three dimensional structure of a protein is governed by its primary structure and its environment. The organized native structure (conformation) of a protein is known to be affected from the effect of external environmental changes such as temperature, acidity, urea or detergent solutions and a number of other chemicals.

In structural studies of proteins it is often necessary to established conditions for reversible denaturation. The choice of denaturation condition depends on the stability of the protein of interest. Among the techniques used for reversible denaturation are lowering of the pH (Itano and Singer, 1958), freezing and thawing in concentration of salt (Market, 1963) and adding denaturants such as urea and Guanidine-HCl (Chilson *et al*., 1964, 1965; Meighen and Schachman, 1970a, b).

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In the present study, the lectins have been subjected to various physical and chemical treatments and their effects on the hemagglutination activities were observed. The study is expected to provide important information regarding some of the physico-chemical properties such as pH stability, thermal stability and the stability of the lectins towards denaturating agent.

Materials and Methods

Mulberry seeds were collected from experimental plot of Bangladesh Sericulture Research and Training Institute, Rajshahi, Bangladesh and were dried in the sunlight and preserved in the desiccators for experimental purposes. Sephadex G-75, DEAE-cellulose, CM-cellulose and Sepharose 4B were purchased from Sigma Chemicals Co. USA.Acetic acid and urea were the products of British Drug House (BDH). Poole, England. Guanidine-HCl was the product of Bio-rad Laboratories, Richmond, California, USA. All the other reagents used were of analytical grade. Unless otherwise specified, all the operation was performed at 4°C.

Purification of Lectins

Mulberry seed lectins were purified in biologically active form by gel filtration of 100% $(NH_4)_2$ SO_4 saturated crude extract on Sephadex G-75follwe by ion exchange chromatography on DEAE Cellulose and CM cellulose chromatography (Yeasmin *et al*., 2001).

Hemagglutinating Activity

The hemagglutination tests were performed using albino rat red blood cells as described by Lin *et al*. (1981). Lectin solution 90.2 mL in 5 mM phosphate buffer saline, pH 7.2 was mixed with 0.2 mL of 4% albino rat red blood cells and incubated at 37°C for an hour the extent of hemagglutnation were observed under microscope.

Physical Treatments of the Lectins

Effect of pH

Lectin solutions (0.25-0.35%) in respective buffer of pH ranges from 2.0-10.5 were incubated for 10 h at 28°C. The hemagglutinating activity was determined after dialysis lectin solution against 5 mM phosphate buffer, pH 7.2 for 18 h at 4°C.

Effect of Temperature

The lectin solutions (0.2-0.3%) in 5 mM phosphate buffer, pH 7.2 were heated at temperature ranges of 20-80 °C in a temperature controlled water bath. After cooling the heated lectin solutions in an ice bath, the hemagglutinating activity was determined following the method as described earlier.

Chemical Treatments of the Lectins

Treatment with Acetic Acid

Lectin solutions (200 μL) in 5 mM phosphate buffer, pH 7.2 were mixed with acetic acid at different concentrations. After an incubation period of 1 h at 4°C, the lectin solutions were dialyzed against 5 mM phosphate buffer, pH 7.2 and then hemagglutinating activity was determined.

Treatment with Urea

Solid urea at different concentrations of 2, 4, 6 and 8 M was added to lectins (in 10 mM Tris-HCl buffer, pH 7.6). The solutions were incubated at 15°C for 12 h and then dialyzed against 5 mM phosphate buffer, pH 7.2 for overnight at 4°C. The hemagglutinating activity of the dialyzed solution was determined.

Treatment with Guanidine-HCI

To the lectin solutions (in 10 mM Tris-HCl buffer, pH 7.6), were added solid guanidine-HCl to the concentrations of 0.25, 0.5, 1, 2, 4 and 6 M. After incubation at 20°C for 12 h, the solutions were dialyzed against 5 mM phosphate buffer, pH 7.2 for 12 h at 4°C and the hemagglutinating activity was determined.

Treatment with Various Metal Ions and Salts

The metal salts of different concentrations (in 5 mM phosphate buffer, pH 7.2) were added to the lectin solutions (200 μL) and incubated for 30 min at room temperature. The hemmaglutinating activity was assayed after incubating at 37°C for 1 h. In this experimental procedure deionized water was used.

Results

Effect of pH on Hemagglutinating Activity

pH stability of mulberry seed lectins were summarized in Table 1. and the results demonstrated that the biological activities of MSL-1, MSL-2 and MSL-3 were markedly influenced by the pH changes. The hemagglutinating activities of the lectins were higher in the netural pH regions than that in the acidic pH and basic pH regions.The lectin MSL-1, MSL-2 and MSL-3 gave optimum hemagglutinating activity at pH 7.2, 7.2-7.6 and 7.2, respectively. Beyond these pH values the activities of the lectins decreased at the acidic as well as basic pH regions and the lectins lost their activities almost completely around pH 2.0 and 10.5.

Effect of Temperature on Hemagglutinating Activity

The hemagglutinating activities of MSL-1, MSL-2 and MSL-3 were also affected remarkably by temperature changes. As given in Table 2 the lectins gave maximum hemagglutinating activities around

Table 1: pH stability of mulberry seed lectins

	Relative hemagglutinating activity (%)		
pH (Buffer composition)	MSL-1	MSL-2	MSL-3
2.0 (KCl-HCl)	5	5	0
3.0 (ACONa-HCl)	10	15	5
4.0 (ACONa-CH_3COOH)	15	20	15
5.0 (ACONa-CH_3COOH)	20	35	25
6.0 (NaH_2PO_4-Na_2HPO_4)	40	55	50
6.5 (NaH_2PO_4-Na_2HPO_4)	60	75	70
7.2 (NaH_2PO_4-Na_2HPO_4)	100	100	100
7.6 (NaH_2PO_4-Na_2HPO_4)	90	100	80
8.0 (NaH_2PO_4-Na_2HPO_4)	60	80	60
9.5 ($Na_2B_4O_7$-HCl)	50	40	25
10.5 ($Na_2B_4O_7$-Na_2CO_3)	5	5	0

Table 2: Temperature stability of mulberry seed lectins

	Relative hemagglutinating activity (%)		
Temperature (°C)	MSL-1	MSL-2	MSL-3
20	100	100	100
25	100	100	100
30	100	100	100
35	100	100	90
40	90	90	85
50	60	70	65
60	40	30	35
70	20	15	15
80	0	0	0

the temperature 20-35°C. With further rise of temperature, the activities of the lectins decreased gradually and all the three lectins lost their activities completely around 80° C.

Effect of Acetic Acid on the Hemagglutinating Activity

The lectins, MSL-1and MSL-2 retained their activities almost completely while MSL-3 lost about 50% of its activity even after treatment with 5% acetic acid (Table 3). The hemagglutinating activities of the lectins decreased rapidly with further increase of acetic acid concentration and more than 75% activities of MSL-1 and MSL-2 were lost at 20% acetic acid concentration while MSL-3 lost its activity completely at 10% acetic acid concentration.

Effect of Urea on the Hemagglutinating Activity

The hemagglutinating activities of MSL-1, MSL-2 and MSL-3 were decreased sequentially with increased in urea concentration and the activities were abolished almost completely after treatment with 8 M urea (Table 4). The results also indicated that the MSL-3 is more sensitive to urea than that of MSL-1 and MSL-2.

Effect of Guanidine-HCl on the Hemagglutinating Activity

The hemagglutinating activities of MSL-1, MSL-2 and MSL-3 were affected markedly after treatment with guanidine hydrochloride. The results presented in Table 5. indicated that MSL-1, MSL-2 and MSL-3 retained only 10, 25 and 5% activities, respectively after treatment of the lectins with 2 M guanidine hydrochloride and the activities of MSL-1, MSL-2 and MSL-3 were abolished completely after treatment with further higher concentration of guanidine-HCl.

Table: 3 Effect of acetic acid on the hemagglutinating activity of mulberry seed lectins

	Relative hemagglutinating activity(%)		
Concentration of acetic acid (%)	MSL-1	MSL-2	MSL-3
0	100	100	100
2.5	100	100	70
5.0	95	90	50
10.0	70	65	0
20.0	25	20	0
30.0	0	0	0

Table 4: Effect of urea on the hemagglutinating activities of mulberry seed lectin

	Relative hemagglutinating activity(%)		
Concentration of urea (M)	MSL-1	MSL-2	MSL-3
0	100	100	100
2	80	80	60
4	70	60	40
6	40	40	25
8	0	10	0

Table 5: Effect of Guanidine-HCl on the hemagglutinating activities of mulberry seed lectins

	Relative hemagglutinating activity(%)		
Concentration of guanidine-HCl (M)	MSL-1	MSL-2	MSL-3
0	100	100	100
0.25	85	95	80
0.50	50	70	50
1.0	20	40	10
2.0	10	25	5
4.0	0	10	0
6.0	0	0	0

Table 6: Effect of metallic salts on the hemagglutinating activities of mulberry seed lectins

		Relative hemagglutinating activity (%)		
Salt added	Concentration (mM)	MSL-1	MSL-2	MSL-3
Control	-	100	100	100
EDTA	100	-	-	0
	50	10	-	0
	25	40	5	0
$CaCl_2$	100	120	130	120
	50	110	110	110
$MnCl_2$	100	100	100	20
	50	100	100	50
KCl	100	100	100	60
	50	100	100	100
$MgSO_4$	100	100	100	50
	50	100	100	70
Na_2SO_4	100	100	100	80
	50	100	100	100
$NaNO_3$	100	100	100	80
	50	100	100	100
$HgCl_2$	10	0	0	0

Effect of Various Metallic Salts on the Hemagglutinating Activity

As presented in Table 6. the hemagglutinating activities of all the three lectins, MSL-1, MSL-2 and MSL-3, were abolished completely after treatment with 100 mM EDTA solution. Significantly, the activities of all the lectins were also abolished completely in presence of only 10 mM of $HgCl_2$. While, the activities of the lectins were enhanced significantly in the presence of Ca^{++}salts.

Discussion

The present study has been done to determine the stability of MSL-1, MSL-2 and MSL-3 by using physical and chemical means and to establish the condition for chemical modifications of mulberry seed lectins. The present data concluded that the hemagglutinating activities of mulberry seed lectins were affected with the changes of pH and temperature. The results showed that MSL-1, MSL-2 and other metallic salts such as Mn^{++}, K^+, Mg^{2+} and Na^+ produced no effect on the activities of MSL-1 and MSL-2 but the activity of MSL-3 was decreased remarkably in the presence of $Mn^{2+}$and Mg^{2+} salts.

MSL-3 were more stable in slightly basic pH (i.e., pH 7.2-7.6) than the acidic pH region. The activities of all the three lectins were found to be active upto 35°C and the activities then decreased rapidly with further rise of temperature suggesting the denaturation or disorganization of the structure of lectins at higher temperature.

The biological activities of MSL-1, MSL-2 and MSL-3 were found to be inactivated almost completely after treatment with 30% acetic acid, which might also be due to denaturation or destruction of the native structure of the lectin.

The activities of mulberry seed lectins were affected sequentially with the increase in concentration of denaturant such as urea and Guanidine-HCl. The results clearly demonstrated that the mulberry seed lectins are more sensitive to guanidine-HCl than urea. The lectins, MSL-1, MSL-2 and MSL-3 were inactivated almost completely after treatment with 4 M guanidine-HCl and 8 M urea. It was also found that the hemagglutinating activities of the lectins were enhanced significantly in the presence of metallic salts Ca^{2+} while in the presence of EDTA, a metal chelator, the activities of the lectins were abolished completely, suggesting that Ca^{2+} is essential for hemagglutination of

mulberry seed lectins which is released completely from the lectins after treatment with EDTA. The inhibitory effect of EDTA on the hemagglutinating activities of TM (Tora-mame) lectin have also been reported (Itoh *et al*., 1980).

Some lectins have been reported to be metalloprotein (Goldstein and Hayes, 1978) and a part of metal is essential for the activities of lectins (Takahashi *et al*., 1971; Alford, 1970; Paulova *et al*., 1971a, b; Tunis, 1965), polysaccharide precipitation (Paulova *et al*., 1971a, b) and lymphocyte transformation (Takahashi *et al*., 1971; Alford, 1970).

In mulberry seed lectins calcium may be present in low concentration and a part of the metal might have been removed from the lectin molecules during the purification steps. This possibility may be supported from the observation that the hemagglutinating activity was increased significantly by the addition of calcium to the purified lectins. Further, the hemagglutinationg activities of all the lectins were abolished completely after treatment with Hg^{2+} salt suggesting the participation of -SH groups in the activities of lectins.

It can be concluded from these above mentioned experimental results that MSL-1 is slightly more stable than MSL-2 and MSL- 3.

References

Alford, R.H., 1970. Metal cation requirements for phytohemagglutinin-induced transformation of human peripheral blood lymphocytes. J. Immunol., 104: 698-703.

Chilson, O.P., L.A. Costello and N.O. Kaplan, 1964. Hybridization of Lactic- Dehydrogenase *in vitro* by concentrated sodium chloride and by reversible inactivation in urea. J. Mol. Biol., 10: 349-352.

Chilson, O.P., G.B. Kitto and N.O. Kaplan, 1965. Mechanism of hybridization of Lactic Dehydrogenase, *in vitro*. Biochemistry, 4: 271.

Goldstein, I.J. and C.E. Hayes, 1978. The lectins: Carbohydrate-binding proteins of plants and animals. Adv. Carbohydr. Chem. Biochem., 35: 127-340.

Itano, H.A. and S.J. Singer, 1958. Dissociation and recombination of human adult hemoglobins A. Proc. Nat. Acad. Sci., 10: 349.

Itoh, M., K. Kondo, H. Komada, K. Izutsu, Y. Shimabayashi and T. Takahashi, 1980. Purification and characterization of a lectin from *Phaseolus vulgaris* seed. Agric. Biol. Chem., 44: 125-133.

Lin, J.Y., T.C. Lee, S.T. Hu and T.C. Tung, 1981. Isolation of four isotoxic proteins and one agglutinin from Jequiriti bean (*Abrus precatorious*). Toxicin, 19: 41-51.

Lis, H. and N. Sharon, 1973. The biochemistry of plant lectins (phytohemagglutinins). Annu. Rev. Biochem., 42: 541-574.

Lis, H. and N. Sharon, 1986. Lectins as molecules and as tools. Annu. Rev. Biochem., 55: 35-67.

Lis, H. and N. Sharon, 1989. Lectins. Chapman and Hall, London, pp: 127.

Market, C.L., 1963. Production of replicable persistent changes in zygote chromosomes of Rano pipiens by injected proteins from adult liver nuclei. Science, 140: 1329.

Meighan, E.A. and H.K. Schachman, 1970a. Hybridization of native and chemically modified enzymes.I. Development of general method and its application to study of the subunit of the structure of aldolase. Biochemistry, 9: 1163.

Meighan, E.A. and H.K. Schachman, 1970b. Hybridization of native and chemically modified enzymes. II. Native and succinylated glyceraldehyde 3 phosphate dehydrogenase. Biochemistry, 9: 1177.

Paulova, M.G., M. Entlcher, J. Ticha, V. Kosti and J. Kocourek, 1971a. Phytohemagglutinins - VII. Effect of Mn^{2+} and Ca^{++} on hemagglutination and polysaccharide precipitation by phytohemagglutinin of *Pisum sativum*. Biochem. Biophys. Acta., 23: 513.

Paulova, M.G., M. Entlcher, J. Ticha, V. Kosti and J. Kocourek, 1971b. Studies on Phytohemagglutinins-IX. Metal content and activity of the hemagglutininfrom the lentil (*Lens esculenta moench*). Biochimica et Biophys. Acta., 252: 388-395.

Samad, M.A. and N. Absar, 2001. Purification and characterization of two lectins from lentil (*Lens esculenta monch*) seed. J. Biol. Sci., 9: 91-96.

Takahashi, T., Y. Shimabayashi, K. Iwomoto and I.E. Leiner, 1971. The role of metal ions in the hemag-glutinating activity of the wax bean hemagglutinin. Agric. Biol. Chem., 35: 1274-1279.

Tunis, M., 1965. The inhibitory action of EDTA on erythrocyte agglutination by lectins. J. Immunol., 95: 876-879.

Yeasmin, T., A. Kashem Tang, A. Razzaque and N. Absar, 2001. Purification and Characterization of three Galactose specific lectins from Mulberry seeds (*Morus* sp.). Eur. J. Biochem., 268: 6005-6010.