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RESEARCH ARTICLE

Effect of Temperature, pH and Denaturing Agents on Biological Activity of MCJ Lectin

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Abstract: *Momardica Charantia* L. var. Jhalri (MCJ) lectin has been isolated from wild bitter gourd and partially characterized in the previous study. The present work reveals the effect of temperature, pH and denaturing agents on haemagglutination activity of MCJ lectin. MCJ lectin sustained its haemagglutination activity up to 45 ˚C. Above 45 ˚C, the activity continuously decreased and at 65 ˚C it was completely disappeared. The lectin showed its haemagglutination activity in the pH range 3-11. The activity was maximum (100%) at the optima of pH range 5-8. During treatment of MCJ lectin with denaturing agents *viz.* Urea, Thiourea and Guanidine-HCl, it lost its haemagglutination activity at high concentration of agents.

Keywords: Lectin, Haemagglutination, Temperature, Denaturing agents

Introduction

An understanding about plant lectins is that they are the proteins (or Glycoproteins) which possess at least one noncatalysing domain that specifically and reversibly binds to mono and oligosaccharides. These proteins other than enzyme and antibody have non-immune origin[1-3]. Lectins have ubiquitous expressions in almost all living species involving plant kingdom, animals and mammalians[4]. In plants their presence has been detected in bark, leaves, roots, fruits and seeds. Lectins have also been isolated from bacteria, fungi, snails and eels[5-8]. Because of their ability to bind sugars, they can precipitate various glycocojugates, can identify cell surface sugars and separate glycoproteins[9]. The same virtue attribute them with a biological activity of agglutinating human and animal erythrocytes. Lectins specially from plants, therefore, have become important tools in glycobiology for advanced studies. These proteins, now a days, have provoked incipient effects such as antiviral activity, antibacterial activity, antifungal activity, anti-insect activity and plant defence activity that has intrigued the researchers to settle methodologies for social welfare[1,9].

Addition of various chemicals including denaturing agents, variation of temperature and nature of medium have been found to affect tertiary structure and henceforth haemagglutination activity of lectins[10-17]. MCJ (*Momardica Charantia* L. var. Jhalri) lectin had been isolated from wild bitter gourd and belongs to cucurbitaceae family. In the previous studies this wild

fruit lectin has been partially characterized[18,19]. Here in the same experimental series MCJ lectin has been investigated and examined for alteration in its haemagglutination activity due to change in temperature, nature of medium and presence of different denaturing agents.

Experimental

Phosphate buffered saline (PBS) was purchased from Bangalore Genei Pvt.Ltd.(15 mM phosphate buffer, 138 mM NaCl, 2.7 mM KCl and pH was 7.4). The human RBC's of blood group 'O' were collected in Alsever's solution and centrifuged at 800 g for 10 min. After decanting the serum, the RBC's were washed and diluted with PBS to get a 2% (v/v) suspension of RBC's. pH providing buffers 0.1 N HCl (pH 1.0), 50 mM glycine-HCl (pH 2-3), 50 mM, sodiumacetate-acetic acid (pH 4-5), 50 mM maleic acid-NaOH (pH 6), 50 mM tris-HCl (pH 7-8) and 50 mM glycine-NaOH (pH 9-11) were prepared according to the data described in the standard manual[20]. Buffer A is 20 mM tris-HCl buffer (pH 8.0) containing 0.3 M NaCl and 1 mM of phenylmethylsulfonyl chloride.

Effect of temperature on haemagglutination activity of MCJ lectin

1 mL MCJ lectin solution (C=1.87 mg/mL) was taken in a washed clean glass test tube and was heated in a water bath from 30 ˚C (room temperature) to 80 ˚C. At intervals of 5 ˚C, 25 µL aliquot was withdrawn and checked for haemagglutination activity[19]. The inactivation kinetics of MCJ protein was performed using 1 mL MCJ lectin solution, by heating at 45 ˚C in an oven constantly. Agglutination activity was checked after every 10 min. using 50 µL aliquots over a period of nearly 2 h. An aliquot taken before heating served to estimate as control, which was considered to have 100% activity. The effect of different temperatures on the haemagglutination activity of MCJ lectin has been shown in Figure 1.

Effect of pH on haemagglutination activity of MCJ lectin

The lectin in volume of 10 µL (C=1.87 mg/mL) was incubated with 90 µL of various buffers in a microtitre plate and 50 µL aliquot was diluted serially with 0.3 M NaCl. Agglutination assay was performed by adding 50 µL of human erythrocytes (2%) suspension to each well after incubation for 1 h at room temperature. The agglutination value obtained by assaying the MCJ lectin using buffer a (pH 8.0) was taken as control and was considered 100%. The graph was also plotted between the haemagglutination activity (%) and pH values as shown in Figure 2.

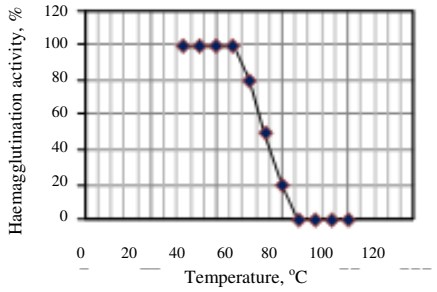

Figure 1. Thermal stability curve for haemagglutination activity of MCJ lectin

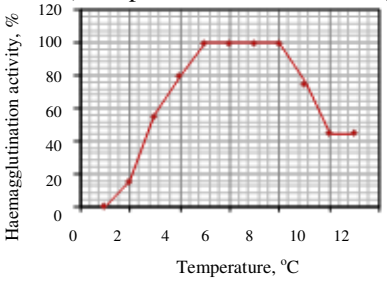

Figure 2. pH stability curve for haemagglutination activity of MCJ lectin

Effect of denaturing agents on haemagglutination activity of MCJ lectin

1 mL of MCJ lectin (C=1.87 mg/mL) solution was taken in a glass test tube and heated till boiling. The protein was precipitated. After cooling to room temperature the solution was subjected to haemagglutination activity.

50 µL of each protein-denaturing agents (Thiourea, Urea and Guanidine-HCl) in different concentration (0-9 M) were taken in a microtitre plate. To these agents 50 µL of lectin solution were added and incubated for 1 h and haemagglutination activity was performed by adding 50 µL of erythrocyte suspension [2%(v/v)] to each well. The native haemagglutination of lectin in the absence of protein denaturing agent was taken as control. The results obtained are shown in the Figure 3.

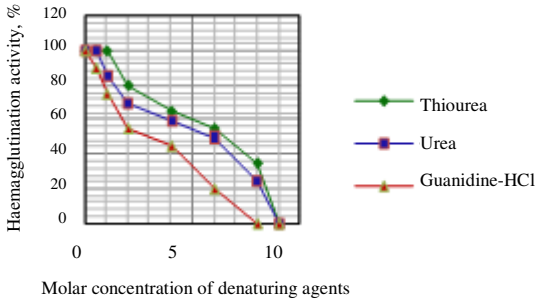

Figure 3. Effect of denaturing agents on haemagglutination activity of MCJ lectin

Results and Discussion

Effect of temperature on haemagglutination activity of MCJ lectin

The effect of temperature on haemagglutination activity was observed. MCJ lectin retained its native haemagglutination activity from room temperature, 30 ˚C to the increased temperature 45 ˚C. A further increase in temperature from 45 ˚C reduced the haemagglutination activity of MCJ lectin continuously. At 55 ˚C, 50% haemagglutination activity was lost whereas at 65 ˚C the activity of protein was completely destroyed. At temperature about 65 ˚C, protein did not show any haemagglutination activity and at 100 ˚C the MCJ lectin got precipitated strongly. The loss of biological activity of MCJ lectin with increased temperature is due to destabilisation of sporadic weak interactions of tertiary structure responsible for native conformation of lectin.

At 55 ˚C the lectin was successful to maintain its 50% haemagglutination activity for 12 min. While at 45 ˚C the lectin could retain its 100% activity strongly for 50 min. The lectin activity at this temperature (45 ˚C) could not be inactivated completely even after heating for 95 min. The MCJ lectin, once lost its agglutinationactivity at 65 ˚C could not regain it when cooled to room temperature. This shows that the inactivation kinetics of haemagglutination activity of lectin at this temperature (65 ˚C) to be an irreversible process. Earlier, other Momardica Charantia species[21] has been reported to retain its haemagglutination activity at maximum temperature of 55 ˚C while MCJ lectin from this wild fruit exhibits the same at comparatively lesser temperature of 45 ˚C. It may be attributed to the climatic conditions and geographical origin of plant that organises comparatively thermally less stable tertiary structure of MCJ lectin.

Effect of pH on haemagglutination activity of MCJ lectin

A haemagglutination assay of MCJ lectin with different pH shows that the lectin is active from pH 3-11. The haemagglutination activity of lectin increases with the increase in the pH and the activity becomes maximum during the pH range 5 to 8 *i.e*. MCJ lectin shows a broad pH optima of pH 5-8. With further increase in pH, the haemagglutination activity decreases and retained only less than 50% agglutination activity at pH 10 to 11. Thus more acidic and more alkaline medium are less favourable conditions for haemagglutination activity of MCJ lectin.

Effect of denaturing agents on haemagglutination activity of MCJ lectin

All protein denaturing agents (Urea, Thiourea and Guanidine-HCl) used here are chaotropic agents and could prevent the haemagglutination activity of MCJ lectin. The high concentration of denaturing agents are supposed to allow water molecules to disrupt the hydrophobic interactions in the interior of lectin that support its native conformations. Under similar conditions of molar concentration distorted sigmoid type curves were obtained during denaturation of MCJ lectin. Complete loss of haemagglutination activity of MCJ lectin by guanidine-HCl (8M) than urea (9M) and thiourea (9M) indicates guanidine-HCl to be more potent denaturing agent for MCJ lectin.

Conclusion

Lectin from wild fruit of *Momardica charantia* L. var. Jhalri in the present study is found to be heat labile and has good thermal resistance power. Furthermore, thermal stability of MCJ lectin up to temperature <65 ˚C makes its extraction easy from seeds of fruit at room temperature without undergoing denaturation. In narrow range of slightly acidic and alkaline medium lectin can preserve its maximum biological activity. Denaturation of MCJ lectin is also much susceptible towards high concentration of Urea, Thiourea and Guanidine-HCl.

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