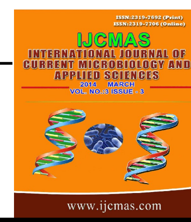

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Original Research Article

Comparative Serological properties of partially purified Haemagglutinins from an Anthropophilic dermatophyte (*Trichophyton rubrum)* and a Zoophilic dermatophyte (*Trichophyton mentagrophytes*)

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A B S T R A C T

Keywords

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The activities of hemagglutinins against human A, B, O erythrocytes, rabbit and sheep erythrocytes could be removed by absorption for 12 hours at 5^0C to the test erythrocytes as mentioned above. The results clearly suggested the presence of a single hemagglutinin in intra and extracellular forms of *Trichophyton rubrum* and *Trichophyton mentagrophytes.* Cross absorption and hemagglutination inhibition studies revealed that hemagglutinins of both species possess a single agglutinin of similar nature having binding affinity for sialoglycoconjugates only.

Introduction

Agglutinins are ubiquitous ranging from microorganisms to higher animals. Agglutinines can agglutinates cells and precipitate glycoconjugates due to their specific binding to certain carbohydrates present on cell surface. In regard to fungal agglutinins most have been isolated and characterized from the group basidiomycetes (Kawagishi et al., 1991). Very little work has been done yet on agglutinins from pathogenic fungi. Rafai and Shalaby (1961) examined several fungi and representing dermatophytes for Their hemagglutination activities. Some studies regarding preliminary serological properties have been made on some dermatophytes like *Trichophyton rubrum, Trichophyton violaceum,* etc. Refai, *et al.* (1977). They also studied haemagglutinin from *Microsporum gupseum (*Refai., 1985). Some haemagglutinin had been isolated from *Chrysosporium keratinophilum* and *Anixiopsis stercoraria* (Chabasse, *et al.,* 1986). The present study deals with isolation and partial purification along with the comparison of serological properties of two haemagglutinins one from anthrophilic dermatophyte (*Trichophyton rubrum)* and another from zoophilic dermatophyte *Trichophyton mentagrophytes*.

Materials and Methods

The strains of *Trichophyton rubrum* (MTCC-296) and *Trichophyton mentagrophytes* were obtained from the Institute of Microbial Technology, Chandigarh, India and from the Department of Mycology, School of Tropical Medicine, Calcutta, respectively. The organisms were maintained on Sabouraud's dextrose agar slopes (Sabouraud, 1911). The culture was maintained at $30^0 \pm 0.5^0$C for three weeks. *Trichophyton rubrum* shows heavy growth with creamy white spores accumulation on the surface and dark red pigmentation. No significant pigmentation appeared in case of *Trichophyton mentagrophytes* below the growth layer. The cultures were preserved at 4^0C. They were transferred to the fresh media at two months intervals.

To obtain uniform inoculum, growth on Sabouraud's agar slope (15 days old)was harvested with sterile distilled water, washed twice and finally suspended in 5ml of sterile water. 0.2ml of the suspension was used as inoculum. Dry mycelia, containing intracellular agglutinin were homogenized with Sea sand (E.Mark) in Morter and Pestle with 10mM PBS, pH=7.0, followed by sonication in Raytheon 10m.c. 250 watt sonic oscillation for 5 minutes. The extract was obtained by removing the debris by centrifugation at 10,000 r.p.m. in a refrigerated centrifuge and finally concentrated by lyophilization. The extracellular and intracellular agglutinins from the culture filtrate and mycelial extract were precipitated with solid ammonium sulphate upto 80% saturation and centrifuged. The precipitate after dissolving in small amount of 50mM tris-buffer, pH=8.0 was exhaustively dialyzed against the same buffer. The volume of the dialyzed materials was reduced to 10ml by lyophilization and stored at -20^0C until used (Mitra et al., 1994).

Samples of human blood were obtained from Ashok Laboratory, Kolkata and were collected in Citrate-dextrose solution. Blood from rat, mouse, rabbit and guineapig was obtained by vein and cardiac puncture. Blood from duck, chicken and pigeon was collected after sacrificing the birds and from goat, sheep, pig was collected from slaughter houses. Blood samples from human and animals were centrifuged at 2000 r.p.m for 5 minutes and the serum and buffy coat were removed by aspiration. The packed erythrocytes were washed twice with saline (0.9%, w/v) and finally 2% (V/V) suspension in saline was prepared. 2.5ml of 0.1%(w/v) Pronase solution were added to 0.2 ml of packed erythrocytes. The suspension was incubated at 37^0C for 30 min. The enzyme treated erythrocytes were subsequently washed thrice with saline and finally a 2% suspension (v/v) was prepared in 0.9% saline solution.

To 0.2 ml of packed erythrocytes were added to 2 ml of saline and 100µl of neuraminidase from *C. perfrinigens* and the mixture was incubated at 37^0C for 30 minutes. The neuraminidase treated erythrocytes were then washed thrice with saline and resuspended at a concentration of 2% (v/v) in 0.9% saline soloution. All glycoproteins tested (100mg each) were desialylated by treatment with 0.05 mole H_2SO_4 at 80^0C for 1 hour to give asialo derivative of each glycoprotein. The reaction was stopped by neutralization with 1M NaOH. The acid treated glycoproteins were sufficiently dialyzed against distilled water and then lyophilized. The extent of desialylation was measured by the method of Aminoff (1961).

Hemagglutination and hemagglutination-inhibition assays were performed in 96 well polystyrene 'V' bottomed plate according to Chaterjee *et al.* (1979). Two fold serially diluted hemagglutinin (25 µl) in 10mM Tris-buffered saline (TBS, 10mM Tris-HCl, 150 mM NaCl, pH=7.5) was added an equal volume of 2% suspension of washed erythrocytes, untreated or enzyme treated and the mixture was incubated at 27^0C for 1 hour after gentle shaking. Agglutination was observed visually and reciprocal of the highest dilution of the lectin solution showing visible agglutination was recorded as titer. The experiment was performed in duplicate and the controls were wet up using TBS.

The hemagglutination-inhibition assay was carried out as follows. All inhibitors to be tested were dissolved in 0.9% saline at concentrations upto 200mM for mono- and oligosaccharides and 1% (w/v) for glycoproteins. To two fold serially diluted test substances (25µl) in 0.9% saline was added on equal volume of diluted lectin (titer 2) solution. After incubation of 12 hours at 27^0C, 25 µl of 2% suspension of untreated erythrocytes were added and the mixture was allowed to stand for 1 hour more at the above temperature. The degree of hemagglutination was examined and maximum dilution of inhibitor solution showing inhibition was recorded.

Cross-absorption test was carried out using *Trichophyton rubrum* and *Trichophyton mentagrophytes* hemagglutinins as follows. The hemagglutinins were mixed with one-third of its volume in washed, packed and untreated human A, B, O erythrocytes, rabbit and sheep erythrocytes separately and incubated for 12 hour at 5^0C with occasional shaking. After centrifugation at 2000xg the supernatant was tested against some erythrocytes used for absorption and against the other erythrocytes tested.

Results and Discussion

Hemagglutinins from *Trichophyton rubrum* and *Trichophyton mentagrophytes* agglutinated human A,B and O erythrocytes almost equally as well as other animal erythrocytes as shown in Table-1.

Among humans, blood group O and rabbit erythrocytes showed titer a little more. Chicken, sheep, goat and rat erythrocytes were agglutinated considerably well whereas horse erythrocytes were weakly agglutinated. No agglutination was recorded with duck erythrocytes. These results suggested that a common receptor for the agglutinins is present on all types of erythrocyte surface. Unlike chicken, duck erythrocytes contained no receptor for these two types of hemagglutinins derived from the two dermatophyte species.

The results of hemagglutination inhibition assays (table-2) shows that both intra and extracellular hemagglutinins did not recognize any simple sugar or its derivative as inhibitor, whereas several glycoproteins were found to be inhibitors of both hemagglutinins. Among the glycoproteins tested, fetuin was found to be the most potent inhibitor. Besides, procine stomach mucin, procine thyroglobulin, bird nest's glycoprotein also inhibited the hemagglutination, where as free sialic acid, bovine submaxillary mucin, all asialoglycoproteins except fetuin, failed to inhibit the hemagglutination. The hemagglutination inhibition experiment clearly indicated that both intra and extracellular

Table.1 Hemagglutination profiles of intra and extracellular hemagglutinins of *Trichophyton rubrum* and *Trichophyton mentagrophytes*

Erythrocytes	Titer (at 150 µg/ml.)			
	Trichophyton rubrum		***Trichophyton mentagrophytes*.**	
	Intracellular	**Extracellular**	**Intracellular**	**Extracellular**
Human A	8	8	8	8
Human B	8	8	16	16
Human 0	16	16	16	16
Chicken	8	8	8	8
Duck	0	0	0	0
Sheep	8	8	8	8
Goat	8	8	4	4
Rat	8	8	4	5
Rabbit	16	16	16	16
Horse	4	4	4	4

Table.2 Hemagglutination- inhibition of intra and extracellular hemagglutitins[a] of *Trichophyton rubrum* and Hemagglutinins *Trichophyton mentagrophytes* by selected glycoproteins.

Glycoproteins	Minimum Inhibitory Concentration or MIC (mg/ml) required for complete inhibition of two hemagglutinating doses of agglutinins.			
	Trichophyton rubrum		***Trichophyton mentagrophytes*.**	
	Intracellular	**Extracellular**	**Intracellular**	**Extracellular**
Fetuin	0.625	0.625	0.625	0.625
Asialofetuin	1.25	1.25	1.25	1.25
Procine thyroglobulin	1.25	1.25	2.5	2.5
Procine Stomach mucin	1.25	1.25	1.25	1.25
Bird's nest glycoprotein	2.5	2.5	2.5	2.5

a. All hemagglutinis used with two hemagglutination doses.

hemagglutinins of *Trichophyton rubrum* and *Trichophyton mentagrophytes* seemed to have recognize complex carbohydrates present in sialoglycoproteins, whereas simple sugars or their derivatives, free sialic acid failed to inhibit the interaction. Thus both hemagglutinins of these two fungal species showed similar recognition phenomenon towards complex carbohydrates like other dermatophytes agglutinins (Chabasse and Robert, 1986; Bouchara et al., 1987) and other fungal species (Tsuda, 1979; Eifler and Ziska. 1980; Lin, and Chou. 1984; Kawagishi et al., 1988).

The activities of hemagglutinins against human A, B, O erythrocytes, rabbit and sheep erythrocytes could be removed by absorption for 12 hours at 5^0C to the test erythrocytes as mentioned above. The results clearly suggested the presence of a single hemagglutinin in intra and extracellular forms of *Trichophyton rubrum* and *Trichophyton mentagrophytes.* Cross absorption and hemagglutination inhibition studies revealed that hemagglutinins of both species possess a single agglutinin of similar nature having binding affinity for sialoglycoconjugates only.

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