Isolation and partial characterization of haemagglutinins from plasmodia of *Physarum polycephalum*

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The soluble haemagglutinins produced by plasmodia of *Physarum polycephalum* were purified by chromatographic methods and resolved into haemagglutinins I and II. On SDS-PAGE, purified haemagglutinins I and II each gave a single band with an apparent molecular mass of 6 and 11 kDa, respectively. The results of gel-filtration chromatography suggested that both haemagglutinins were dimers of the respective subunits under non-denaturing conditions. Rabbit erythrocytes were preferentially agglutinated by both haemagglutinins. The human type A, B and O erythrocytes were agglutinated by haemagglutinin II to an equal degree but were not agglutinated by haemagglutinin I. Simple sugars failed to inhibit the activities of both haemagglutinins. The activities, however, were effectively inhibited by the addition of thyroglobulin. Other glycoproteins such as fetuin, orsomucoid and transferrin inhibited the activity of haemagglutinin I but not that of haemagglutinin II. These haemagglutinins were detected in a slime fraction obtained from the culture media of starved plasmodia, suggesting that they are released to the outside of the plasmalemma to become associated with the slime layer on the plasmodial surface.

Introduction

Lectins that specifically bind sugars and thus agglutinate erythrocytes have been found in many species of organisms ranging from bacteria (Sharon, 1987) to higher plants and animals (Drickamer, 1988). These lectins are thought to be involved in some physiological events such as infection, adhesion or migration of cells (Lis & Sharon, 1986). The functions of some integral membrane lectins have been revealed. The lectin of mammalian liver membranes that binds galactose is believed to function in recovering asialoglycoproteins from serum (Ashwell & Harford, 1982). The mannose-6-phosphate-specific lectin on the lysosomal membrane mediates the sorting of hydrolytic enzymes to the lysosome (von Figura & Hasilik, 1986). On the other hand, the physiological roles of soluble lectins are still unclear (Barondes, 1984) though soluble β -galactoside-binding lectins have been found in a wide range of vertebrate tissues. In the cellular slime mould, *Dictyostelium discoideum*, a developmentally regulated lectin termed discoidin has been studied in detail (Barondes & Springer, 1987). In a previous paper (Morita & Nishi, 1991) we have shown that the slime layer on the

plasmodial surface of *Physarum polycephalum* also contained haemagglutinating activity toward rabbit erythrocytes. To elucidate the biological role of the haemagglutinins of the true slime mould, we attempted to purify these proteins. In this paper, we report the isolation and partial characterization of these haemagglutinins from *P. polycephalum*.

Methods

Cultivation of plasmodia. Axenic cultures of *Physarum polycephalum* were grown as suspensions of microplasmodia in semi-defined medium containing glucose, yeast extract and tryptone (Daniel & Baldwin, 1964). The cultures were incubated at 26 °C with shaking. Plasmodial growth was estimated by measuring the protein content of the culture, using the Lowry method.

Haemagglutinating assay. Haemagglutinating activity was measured with trypsin-treated, formalinized rabbit erythrocytes. Rabbit erythrocytes were treated with trypsin and then fixed with formalin according to the methods of Nowak *et al.* (1976) and Butler (1963), respectively. Human erythrocytes type A, B and O were donated by Toyama Medical and Pharmaceutical University Hospital, Toyama, Japan. Erythrocytes of other animals were purchased from Nippon Biosupply Center, Japan. Haemagglutinating activity was determined by a serial twofold dilution method with microtitre V-plates as described previously (Morita & Nishi, 1991).

Inhibition assays were done as follows. Monosaccharides, polysaccharides and glycoproteins to be tested were dissolved in 0.15 M-NaCl at appropriate concentrations. The solutions (25 μ l) were mixed with 25 μ l of samples containing four minimum haemagglutinating doses.

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Abbreviations: ABS, acetate-buffered saline; CBB, Coomassie brilliant blue.

After incubation for 30 min at room temperature, 25 µl trypsin-treated, formalinized rabbit erythrocyte suspension was added to test the haemagglutinating activity. Thyroglobulin (porcine), fetuin (fetal calf serum), orsomucoid (human), ovomucoid (chicken egg white), ovalbumin (egg), mucin (bovine submaxillary glands) and transferrin (human) were purchased from Sigma, and other sugars were purchased from Nakarai tesque.

Preparation of thyroglobulin-immobilized Toyopearl HW-55. Thyroglobulin (100 mg, type III, Sigma) was dissolved in 10 ml 0.1 M-NaHCO₃, pH 8.0, containing 0.5 M-NaCl. The thyroglobulin solution was added to 2 g AF-Tresyl-Toyopearl HW-55 (TOSOH) and incubated at 25 °C for 4 h with gentle shaking. After the incubation, excess thyroglobulin was removed by washing with 0.5 M-NaCl. The reaction was blocked by the addition of 0.1 M-Tris/HCl, pH 8.0, containing 0.5 M-NaCl. After incubation at 25 °C for 1 h, thyroglobulin-immobilized Toyopearl was packed in a column (0.5 × 5 cm) and washed with excess ABS.

Purification of haemagglutinin. All procedures were carried out at 4 °C unless otherwise stated. The microplasmodia in 200 ml liquid medium at late growth phase were removed by centrifugation and washed with phosphate-citrate buffer (14.7 mM-potassium phosphate, 16.7 mM-citric acid, pH 4.6). The plasmodia were then resuspended in 100 ml acetate-buffered saline (ABS; 75 mM-sodium acetate buffer containing 75 mM-NaCl, pH 5.0) and disrupted with a Teflon-glass homogenizer. The homogenate was centrifuged at 100 000 g for 1 h and solid ammonium sulphate was added to the supernatant to give 40% saturation. The mixture was then centrifuged at 15 000 g for 45 min and the clear supernatant was dialysed against ABS for 4 h.

The extract was chromatographed on an affinity column of thyroglobulin-Toyopearl equilibrated with ABS (bed volume 10 ml). Unbound materials were eluted with the same buffer and with 1 M-NaCl in the buffer until the A_{280} was reduced to less than 0.01. Adsorbed materials were then eluted with 0.05 M-glycine/HCl, pH 2.0, containing 0.5 M-NaCl. A portion of each fraction was removed for haemagglutination assay. Under these conditions, virtually all haemagglutinating activity was retained on the column and eluted in a sharp peak upon decreasing the pH. To avoid denaturation, the pH of the active fractions was immediately adjusted to about pH 5.0–6.0 with 1 M-NaOH and dialysed against 20 mM sodium acetate buffer, pH 5.0, for 4 h.

The haemagglutinins were further purified by ion-exchange chromatography. The dialysate of the active fraction was then applied onto a column (1 × 8 cm) of SP-Toyopearl (TOSOH) equilibrated with the same buffer (20 mM-sodium acetate, pH 5.0). After the unadsorbed protein was eluted with the same buffer, the protein was deadsorbed by a linear gradient of 0–0.5 M-NaCl in the same buffer. The active fractions were concentrated by ultrafiltration using a YM-5 membrane (Amicon) and dialysed against 10 mM-sodium phosphate buffer, pH 6.0, for 4 h. The dialysate was then applied to a column (1 × 8 cm) of hydroxylapatite (BioRad). After the column was washed with the same buffer, the adsorbed proteins were eluted with a linear gradient of 5–100 mM-sodium phosphate buffer.

In some experiments, the slime fraction was used as the starting material. The slime fraction was prepared from the culture media of starved plasmodia by ethanol precipitation as described previously (Morita & Nishi, 1991). The pellet was dissolved in 20 mM-Tris/HCl containing 0.5 M-NaCl, pH 7.3, and applied to a column (3.5 × 50 cm) of Toyopearl HW-75. Under these conditions, slime substances were eluted at the void volume whereas haemagglutinating activities were retained in the column. The active fractions were collected and subjected to purification as above.

PAGE. PAGE under non-denaturing conditions was carried out in a 15% (w/v) gel at pH 9.5 by the method of Davis (1964), or at pH 4.3 by

the method of Reisfeld *et al.* (1962). About 5 µg protein was applied to each lane. Electrophoresis was carried out at constant voltage of 150 V at 4 °C and the gel was stained with Coomassie brilliant blue (CBB) as described previously (Morita & Nishi, 1991).

SDS-PAGE was carried out by the methods of Schagger & von Jagow (1987). Proteins were dissolved in the sample buffer [4% (w/v) SDS, 12% (w/v) glycerol, 50 mM-Tris/HCl, 2% (v/v) β-mercaptoethanol, pH 6.8] and incubated at 40 °C for 30 min. The concentrations of polyacrylamide in the stacking gel and separating gel were 4 and 16.5%, respectively. Electrophoresis was carried out at 30 V for 1 h and when the tracking dye entered the separating gel, the voltage was increased to 95 V. After about 16 h, the gels were stained with CBB. The molecular mass standards used were phosphorylase *b* (M_r 94 000), albumin (67 000), ovalbumin (43 000), carbonic anhydrase (30 000), trypsin inhibitor (20 100) and α-lactalbumin (14 000) (all Sigma). Myoglobin fragments (M_r 2512, 6214, 8159, 14 404, 16 949; Pharmacia) were also co-electrophoresed.

Gel-filtration. The column (1.5 × 100 cm) of Toyopearl HW-55 (superfine, TOSOH) was equilibrated with ABS prior to application of the sample. Chromatography was performed at 4 °C and fractions of 1.65 ml were collected. The column was calibrated with the following markers: bovine serum albumin (M_r 66 000), carbonic anhydrase (29 000), cytochrome *c* (12 400), aprotinin (6500) and cyanocobalamin (3500) (all Sigma) and blue dextran was used for determination of the void volume of the column.

Results

Purification of haemagglutinin

Fig. 1 shows the changes in haemagglutinating activities in extracellular and cytosolic fractions during plasmodial growth. The activity in the extracellular fraction was only detected after the plasmodia entered into the stationary phase. In the exponential phase, in contrast, the activities were mainly found in the intracellular soluble fraction. It seems that haemagglutinins in the intracellular fraction were released outside the plasmodium and associated with the slime layer when the plasmodia reached maximal growth. To characterize the haemagglutinins, we first attempted to purify the haemagglutinin obtained from cytosolic fractions, because the slime substances in the extracellular fraction sometimes interfered with column chromatography. The cytosolic fraction of growing plasmodia contains only a small amount of slime substances.

Plasmodia were disrupted by homogenization and the soluble extract was subjected to ammonium sulphate precipitation to remove slime molecules. At 40% saturation, no loss of activity was observed. Since previous experiments with crude extracts or slime fractions (Morita & Nishi, 1991) indicated that the haemagglutinating activity was effectively inhibited in the presence of thyroglobulin, we attempted to purify this haemagglutinin by affinity chromatography with thyroglobulin immobilized on Toyopearl HW-55. Fig. 2a shows a typical elution profile of the crude extract from

Fig. 1. Haemagglutinating activity in the intra- and extra-cellular fractions of *P. polycephalum*. Microplasmodia were grown in a semi-defined liquid medium. At 24 h intervals, a portion of the culture was centrifuged at 750 *g* for 2 min and the supernatant was saved as an extracellular fraction. The pellet was washed and disrupted in 3 vols ABS by sonication. The sample was then centrifuged at 15000 *g* for 15 min and the supernatant was removed. The pellet was washed once and the washing solution was combined with the supernatant and saved as the intracellular fraction. Haemagglutinating activities in the extra- (Δ) and intra-cellular (○) fractions were assayed with trypsin-treated, formalinized rabbit erythrocytes as described in Methods. The activity was expressed as the reciprocal of the titre of each fraction. The plasmodial growth (●), as expressed by protein content in the culture, is also shown.

the thyroglobulin-Toyopearl affinity column. When the sample was loaded on the affinity column, all the activity was bound to the column and eluted with 0.05 M-glycine/HCl containing 0.5 M-NaCl, pH 2.0. Since no simple sugar was found to inhibit the haemagglutinating activity, we adopted the acidic buffer to elute haemagglutinin from the affinity column. The eluate was immediately neutralized with 1 M-NaOH because the haemagglutinating activity was unstable in the low pH range as described below. The haemagglutinins were further purified by ion-exchange chromatography with SP-Toyopearl (Fig. 2b).

At this stage of the purification, the specific activity was about 50-fold higher than that of crude extracts.

Fig. 2. Purification of haemagglutinins by column chromatography. Plasmodia in the growth phase were washed with phosphate-citrate buffer, then disrupted in ABS with a Teflon/glass homogenizer. The homogenate was centrifuged (100000 *g*, 1 h) and the supernatant was fractionated with ammonium sulphate precipitation as described in Methods. The soluble extract was dialysed against ABS and loaded on an affinity column of thyroglobulin-Toyopearl (bed volume 10 ml) equilibrated with ABS (a). The column was eluted with ABS followed by ABS containing 1 M-NaCl (arrow A). Finally, the active fraction was eluted with 0.05 M-glycine/HCl, pH 2.0, containing 0.5 M-NaCl (arrow B). Fractions of 2 ml were collected. A portion of each sample was removed to measure A_{280} (●) and haemagglutinating activity toward trypsin-treated, formalinized rabbit erythrocytes (○). The

active fractions were dialysed against 20 mM-sodium acetate buffer, pH 5.0, and loaded on a column of SP-Toyopearl (b). The adsorbed materials were eluted with a linear gradient of 0–0.5 M-NaCl in the same buffer. Fractions of 4 ml were collected. The active fractions were collected and dialysed against 10 mM-sodium phosphate buffer, pH 6.0. The dialysate was loaded on a column (1 × 8 cm) of hydroxylapatite equilibrated with the same buffer (c). The column was eluted with a linear gradient of 5–100 mM-sodium phosphate buffer, pH 6.0. Fractions of 2 ml were collected.

Fig. 3. SDS-PAGE of purified haemagglutinins I and II. Purified haemagglutinins I (1) and II (2) were subjected to SDS-PAGE after treatment with β -mercaptoethanol. The active fraction eluted from the SP-Toyopearl column (3) (see Fig. 4) was also co-electrophoresed. Molecular mass markers (R) used were phosphorylase b (M_r 97400), bovine serum albumin (66200), ovalbumin (45000), carbonic anhydrase (31000), soybean trypsin inhibitor (21500) and lysozyme (14400). Myoglobin fragments (r) were also run on the same gel. After electrophoresis, the gel was stained with CBB.

Table 1. Purification scheme of haemagglutinins from plasmodia

Purification step	Protein (mg)	Total HA* (titre ⁻¹)	HA/protein (titre ⁻¹ mg ⁻¹)	Recovery (%)	Purification
Crude extract†	263.8	157440	597	100	1
(NH ₄) ₂ SO ₄ -precipitation	240.0	256000	1067	163	1.8
Thyroglobulin-Toyopearl	1.17	19200	15410	12.2	27.5
SP-Toyopearl	0.625	18336	29338	11.6	49.1
Hydroxylapatite	—	—	—	7.2	—
Peak I	0.183	5376	29377	—	49.2
Peak II	0.110	6016	54691	—	91.6

* Haemagglutinating activity (HA) was determined using serial twofold dilutions. The titre⁻¹ expressed is the reciprocal of the highest dilution needed for haemagglutination with trypsin-treated, formalized rabbit erythrocytes.

† Two-day old microplasmodia (30 ml packed volume) were collected just prior to the stationary phase in a liquid culture and homogenized in 100 ml ABS.

However, SDS-PAGE of this active material revealed that the fraction contained two distinct components with molecular masses of about 6 and 11 kDa (Fig. 3, lane 3). These two molecules were effectively separated by hydroxylapatite column chromatography (Fig. 2c) and termed haemagglutinin I and II, respectively. A summary of the purification is shown in Table 1. Recovery of haemagglutinating activity was greater than 100% at the ammonium sulphate precipitation step. This step might have removed some interfering substances. From 30 ml (packed volume) of the plasmodia, about 0.3 mg of the haemagglutinins were obtained.

Biochemical properties of haemagglutinins

The purified haemagglutinins were analysed by PAGE under non-denaturing conditions (Fig. 4). Haemagglutinin I or II were found to be homogeneous either at pH 4.3 or 9.5. The haemagglutinins were also subjected to SDS-PAGE after the treatment with β -mercaptoethanol. The electrophoretic profile was not changed by the treatment with β -mercaptoethanol. Both haemagglutinins I and II migrated as single polypeptide bands with apparent molecular masses of 6 and 11 kDa, respectively (Fig. 3, lanes 1 and 2). On the other hand,

Fig. 4. PAGE of haemagglutinins under non-denaturing conditions. Haemagglutinins I and II eluted from the hydroxylapatite column were subjected to PAGE at pH 4.3 and 9.5. After electrophoresis, the gels were stained with CBB.

when the purified haemagglutinins were subjected to gel-filtration with columns of Toyopearl HW-55 (superfine) under non-denaturing conditions, haemagglutinins I and II were eluted at positions corresponding to molecular masses of about 12.5 and 21 kDa, respectively (data not shown). It appears, therefore, that these haemagglutinins, I and II, obtained by gel-filtration were dimers of identical subunits with molecular masses of 6 or 11 kDa, respectively. The haemagglutinin purified from the slime fraction also showed a similar profile upon SDS-PAGE (data not shown), suggesting that the slime haemagglutinins are identical to those in the intracellular fraction.

The purified haemagglutinins showed no detectable loss of activity when heated at 60 °C for 10 min in PBS though the activity was gradually decreased at higher temperatures. The activity was stable in a pH range between 5.0 and 11.0 at 4 °C, but it rapidly decreased at lower pH values. The activity of these haemagglutinins showed a pH optimum at 5–6. At pH > 8, however, the activity was severely depressed.

The haemagglutinating activity remained unchanged after trypsin treatment (5 mg ml⁻¹, for 30 min at 37 °C in PBS). Treatment with EDTA (100 mM) or β -mercaptoethanol (4 mM) did not affect the haemagglutinating activity. These results indicate that these haemagglutinins require neither divalent cations nor free thiol residues for their activities.

Table 2. Activity of purified haemagglutinins with different erythrocytes

Purified haemagglutinins I and II (150 μ g protein ml⁻¹) were used for the assay. The titre is expressed as the maximum dilution of the sample for detecting positive haemagglutinating activity. The assay was done using formalinized erythrocytes from different animals.

Erythrocyte	Haemagglutinating activity (titre ⁻¹)	
	Haemagglutinin I	Haemagglutinin II
Human A	ND	8
Human B	ND	8
Human O	ND	8
Rabbit	64	64
Sheep	16	2

ND, Not detected.

Table 3. Inhibition of purified haemagglutinin I and II with various sugars and glycoproteins

Trypsin-treated, formalinized rabbit erythrocytes were used in this assay.

Sugars and glycoproteins	Minimum amounts (mg ml ⁻¹) inhibiting four haemagglutinating doses	
	Haemagglutinin I	Haemagglutinin II
Simple sugars*	Not inhibitory	
Ovalbumin	> 5	> 5
Ovomucoid	> 5	> 5
Orsomucoid	0.55	> 5
Horseradish peroxidase	> 5	> 5
Mucin	> 5	> 5
Fetuin	2.97	> 5
Transferrin	5	> 5
Thyroglobulin	0.22	0.31

* Simple sugars tested which were not inhibitory up to 0.2 M concentration included D-glucose, D-galactose, D-mannose, D-fucose, L-rhamnose, D-xylose, N-acetylglucosamine, N-acetylgalactosamine, D-glucuronic acid, D-galacturonic acid, α -methylglucoside, α -methylmannoside, β -methylglucoside, cellobiose, mellobiose, raffinose, maltose and lactose.

Agglutinating property and carbohydrate-binding specificity

Both purified haemagglutinins preferentially agglutinated formalinized rabbit erythrocytes rather than other animal erythrocytes (Table 2). The agglutinating activities toward human and sheep erythrocytes were also detected when the purified haemagglutinins were used in this assay. The sheep erythrocytes were effectively agglutinated by haemagglutinin I rather than II whereas the human type O, A and B were agglutinated equally by haemagglutinin II but not by haemagglutinin I. These results suggest that the two haemagglutinins are distinct

molecules which recognize different domains of saccharide structures on the erythrocyte membrane surface.

Carbohydrate-binding specificities of the haemagglutinins were determined by inhibition assays with a series of simple sugars and some glycoproteins (Table 3). None of the simple sugars tested had any detectable effect on haemagglutination up to a concentration of 0.2 M. The activities of both haemagglutinins I and II, however, were effectively inhibited in the presence of thyroglobulin. The activity of haemagglutinin I, but not II, was inhibited by other glycoproteins such as fetuin, orsomucoid or transferrin. A clear difference between haemagglutinin I and II was observed when orsomucoid was used as an inhibitor. The activity of haemagglutinin I was effectively inhibited by the glycoprotein at a concentration of about 0.55 mg ml⁻¹ though the activity of haemagglutinin II was not inhibited even at 5 mg ml⁻¹. In contrast, ovalbumin, horseradish peroxidase, ovomucoid and mucin were not inhibitory to either haemagglutinin. When trypsin-digested thyroglobulin was used, the inhibitory effect was greatly reduced (about 25%) relative to native thyroglobulin.

Discussion

We have previously reported that the slime fraction from the culture of starved plasmodia contained agglutinating activity toward rabbit erythrocytes (Morita & Nishi, 1991). In the present study, we found that these activities were also detected in the soluble extracts of growing plasmodia. It is believed that the intracellular soluble haemagglutinins are released to the outside of the plasmalemma and then they associated with the slime layer on the plasmodial surface. When plasmodia reached maximal growth, these haemagglutinins may be released to the medium together with slime substances.

The data presented here indicated that plasmodia produced two distinct haemagglutinins, termed haemagglutinins I and II, each of which is a dimer of identical subunits having molecular masses of 6 and 11 kDa, respectively. These haemagglutinating activities were inhibited by glycoproteins such as thyroglobulin but not by simple sugars. However, tryptic digestion of thyroglobulin reduced its inhibitory effect. These results suggest that plasmodial haemagglutinins recognize complex saccharide moieties. Lectins which are not inhibited by these simple sugars have been found in some species (Giga *et al.*, 1985; Sueyoshi, 1985). The two haemagglutinins seem to recognize different domains of saccharide structure because each haemagglutinin showed different affinity to orsomucoid and to sheep and human erythrocytes. It is possible that they have different roles in some physiological event such as growth and differentiation.

In *Dictyostelium discoideum*, when vegetatively growing amoebae are starved, they become adhesive and began to aggregate into multicellular organisms. These adhesive cells, but not vegetative cells, have been reported to synthesize two lectins termed discoidin I and II (Rosen *et al.*, 1973; Ma & Firtel, 1987). In contrast, *P. polycephalum* myxamoebae fuse in pairs and form a multi-nucleate plasmodia without a cell-aggregation step. Aldrich & Reiskind (1987) suggested that the surface of myxamoebae contained soluble endogenous lectins. It is not known, however, whether the putative lectins on the surface of myxamoebae play a role in fusion. Though we did not examine the haemagglutinating activity at the myxamoebae stage, the activities in plasmodia were detected not only in the vegetative growing stage but also in the stationary phase. It was reported that both discoidin I (Barondes *et al.*, 1985) and muscle lectin (Cooper & Barondes, 1990) are synthesized as soluble cytoplasmic proteins which are then externalized during differentiation. At that stage, the lectins become prominently localized in the extracellular matrix such as slime coat and laminin, where they are presumed to function. These situations are comparable to the plasmodial haemagglutinins of the true slime mould which were found in the intracellular fraction of actively growing plasmodia and were detected in the slime layer of starved plasmodia. It is, therefore, possible that these molecules are functioning in the extracellular space by interacting with glycoconjugates on the plasmodial surface.

The complementary glycoconjugates that bind to discoidin (Ray & Lerner, 1982; Cooper *et al.*, 1983, 1986) and pallidin (Drake & Rosen, 1982) have been investigated and it has been found that the cellular slime mould lectins show an affinity to glycoconjugates containing *N*-acetylgalactosamine or galactose (Barondes *et al.*, 1987). Recently, it was reported that the carbohydrate binding site of the discoidin I participates in its externalization but not in cell to cell adhesion (Barondes & Springer, 1987). Discoidin I was reported to have a role for cell-substratum adhesion and ordered cell migration similar to that of fibronectin in higher organisms (Springer *et al.*, 1984; Gabius *et al.*, 1985). Unlike the cellular slime mould (Rosen *et al.*, 1975), the plasmodial haemagglutinins showed no affinity to monosaccharides and, therefore, the function of plasmodial haemagglutinins seems to be different from that of the lectin in the cellular slime mould. Thyroglobulin (Fukuda & Egami, 1971), orsomucoid (Schwarzman *et al.*, 1978), fetuin (Spiro & Bhoyroo, 1974) and transferrin (Jamieson *et al.*, 1971), which showed an inhibitory effect on the haemagglutinin, are reported to have complex type N-linked oligosaccharides in their structure. In contrast, mucin from bovine submaxillary glands (Ozeki & Yoshizawa, 1971), which is a glycoprotein containing

heterogeneous O-linked oligosaccharide, was not inhibitory. Thus, it is likely that plasmodial haemagglutinins recognize specific sugar sequences in complex-type N-linked oligosaccharides. In previous studies (Kuroda *et al.*, 1989; Morita *et al.*, 1986; Morita & Nishi, 1989), we showed that membrane proteins are generally glycosylated with N-linked oligosaccharides ranging from high-mannose type to complex-type, which are candidates for interacting with these haemagglutinins. Plasmodia are naked masses of protoplasm, which migrate on the solid substratum to search for nutrients in natural environments. The outer surface of migrating plasmodia is surrounded by slime layers. When plasmodia come into contact with each other, they fuse rapidly. It is tempting to speculate that the plasmodial haemagglutinin may be involved in the migration process or in fusion by interacting with specific carbohydrate receptors present on the cell surface. Characterization of the receptors of these haemagglutinins on plasmodial surfaces is being investigated.

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