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Carbohydrate-binding specificities and physico-chemical properties of lectins in various tissue of phlebotominae sandflies

Lucie Palánová and **Petr Volf**

Department of Parasitology, Charles University, Viničná 7, 128 44 Prague 2, Czech Republic

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Abstract. Physico-chemical properties and carbohydrate-binding specificity of hemagglutination activity (HA) were compared in tissue lysates and haemolymph of unfed and bloodfed females of five sandfly species. Sandfly gut lectins were found to be heat-labile, sensitive to dithiotreitol treatment, freezing/thawing procedures and were affected by divalent cations. The pH optimum of HA ranged between 7.0–7.5. Specificity of gut HA of all species studied was directed towards aminosugars and some glycoconjugates, mainly lipopolysaccharide from *Escherichia coli* K-235, heparin and fetuin. Gut HA of *Phlebotomus papatasi* (Scopoli, 1786) was strongly inhibited by lipophosphoglycan (LPG) from *Leishmania major* promastigotes. In females, that took blood, the HA was higher but the carbohydrate-binding specificity remained the same; this suggests that the same lectin molecule was present, at different levels, both in unfed and fed flies. High HA was found in ovaries of fed females of *Lutzomyia longipalpis* (Lutz et Nieva, 1912), *P. papatasi* and *P. duboscqi* Neveu-Lemaire, 1906. In *P. papatasi* and *P. duboscqi* the HA was present also in the haemolymph and head lysates of both fed and unfed females. Carbohydrate-binding specificity of HA present in these tissues was similar with the gut lectin.

Lectins (hemagglutinins) are ubiquitous sugar-binding proteins that occur in plants, animals and microorganisms. Lectins found in various tissue of blood-sucking insects have been found to play a special role in vector-parasite interactions (for review see Ingram and Molyneux 1991). In phlebotominae sandflies the lectin activities have been described in the gut and head lysates of various *Phlebotomus* and *Lutzomyia* species (Wallbanks et al. 1986, Volf et al. 1994).

Our preliminary experiments on *P. duboscqi* Neveu-Lemaire, 1906 showed that the hemagglutination activity (HA) is present also in other tissues and led us to hypothesise that the lectin is present in sandfly haemolymph. In this paper we have therefore studied HA of various tissue lysates and haemolymph of 5 sandfly species and we focussed our attention on binding specificity and selected physico-chemical properties of the hemagglutinins. As the activity is strongly sex-dependent (Volf and Killick-Kendrick 1996) only female flies were used for experiments. In female sandflies fed on blood and protein solutions the midgut HA increased (Volf and Killick-Kendrick 1996, Volf and Palánová 1996). To distinguish if these activity changes were caused by increased secretion of the same lectin or by additional effect of the second hemagglutinin, we have compared sugar-binding specificities of hemagglutinins in unfed and fed females.

MATERIALS AND METHODS

Tissue lysates and haemolymph samples. Sandfly colonies were maintained at the Department of Parasitology, Charles University on 50% sucrose at 26°C, 14/10 LD photoperiod and relative humidity greater than 65%. The flies used to initiate the colonies were provided by Prof. R. Ward (*L. longipalpis*) and by Prof. R. Killick-Kendrick, Imperial College, Ascot (other species). Groups of 20 females of *Lutzomyia longipalpis* (Lutz and Nieva, 1912), *Phlebotomus duboscqi*, *P. papatasi* (Scopoli, 1786), *P. perniciosus* Newstead, 1911 and *P. halepensis* Theodor, 1958 were dissected in hemagglutination buffer (20 mM Tris, 150 mM NaCl, pH 7.5). The heads, mid and hindguts with Malpighian glands (= "guts") were separated from other tissue (= "body"). Lysates were prepared as described by Volf et al. (1994) in 100 µl hemagglutination buffer.

Groups of females (= "fed flies") dissected 1, 2, 3 and 6 days after feeding on hamster (for *P. halepensis*) or mice (for other species) were used for comparison with controls which did not take blood (= "unfed flies"). Ovaries and haemolymph were obtained from females of *L. longipalpis*, *P. duboscqi* and *P. papatasi*. Haemolymph was obtained from groups of 20 females immobilized at 4°C. Females were used unfed or 2 days after blood-feeding. Flies were bled by cutting legs in 5 µl of cold hemagglutination buffer, diluted haemolymph exudates were pooled, centrifuged at 6,000 g for 3 minutes to remove haemocytes and cell debris and stored in –20°C.

Ovaries were dissected from groups of 20 females 2 days after blood feeding and made into lysates in 100 µl of hemagglutination buffer. Protein concentration in haemolymph samples was determined according to Bradford (1976) using bovine serum albumin in hemagglutination buffer as a standard.

Preparation of red blood cells (RBC). Previous observations on *L. longipalpis* revealed that gut HA gave high titres with rabbit RBC (RaRBC) only (Volf 1993). For the present study, nine RBC types, different from those used in the above mentioned paper, were used, and RaRBC served as a positive

Address for correspondence: P. Volf, Department of Parasitology, Charles University, Viničná 7, 128 44 Prague 2, Czech Republic; Phone: (420) 02219553196; Fax: (420) 299713; E-mail: volf@beba.cesnet.cz

Table 1. Carbohydrates and glycoconjugates tested as potentital inhibiors of gut HA of unfed females of *Lutzomyia longipalpis* and *Phlebotomus duboscqi*.

Monosacharides:
D-Glucose, D-Galactose, D-Mannose, D-Xylose, D-Arabinose
D-Ribose, D-Fructose, L-Fucose, D-Tagatose, L-Rhamnose
Deoxy sugars:
2-deoxy-D-Glucose, 2-deoxy-D-Galactose
Amino sugars:
D-Lyxosylamine, D-Glucosamine,
D-Mannosamine,D-Galactosamine,
N-Acetyl-D-Glucosamine, N-Acetyl-D-Mannosamine,
N-Acatyl-D-Galacosamine, N-Acetyl-Neuraminic acid
Sugar acids:
D-Glucuronic acid, D-Galacturonic acid
Methyl glycosides:
Methyl-β-D-Xylopyranoside, Methyl-α-D-Glucopyranoside,
Methy-α-D-Mannopyranoside, Methyl-β-D-Galactopyranoside
Sugar alcohols:
Mannite, L-Arabinite, D-Arabinite, D-Sorbite, Dulcite, Ribitol
Disacharides:
Maltose, Trehalose, α-Lactose, β-Lactose, Sucrose, Turanose,
Melibiose, Lactulose, Cellobiose, Stachyose,
6-O-β-Galactopyranosyl-D-galactose
4-O-α-D- Galactopyranosyl-D-galactopyranose,
4-O-β-Galactopyranosyl-D-mannopyranose
Trisacharides:
Raffinose, Melezitose
Polysacharides:
Laminarin
Glycoproteins:
Fetuin, Bovine submaxillary mucin, Ovalbumin

control. Trypsin and glutaraldehyde treatment: RBC were incubated with 0.01% (w/v) bovine trypsin in PBS (Léčiva, Prague) for 1 hr at room temperature and repeatedly washed in PBS. Then, RBC were fixed with 1% (v/v) glutaraldehyde (Serva) in PBS for 1 hr at room temperature, washed in PBS, incubated with blocking solution of 0.1 M glycin and repeatedly washed with hemagglutination buffer. Neuraminidase treatment: RBC were incubated with 1% (v/v) neuraminidase from Vibrio cholerae (SEVAC, Prague), gently shaken for 2 hrs at 37°C and then washed with hemagglutination buffer.

Hemagglutination tests. Fifty µl double dilutions of haemolymph or tissue lysates were prepared in hemagglutination buffer in U-well microtitre plates to give final dilution ranges of 1 : 4 to 1 : 2048. The same volume of 2% suspension of RBC in saline (150 mM NaCl) was added and agglutination was scored after 2 hrs at room temperature. The end point titre was defined as the last dilution causing agglutination visible by unaided eye. This dilution was deemed to contain approximately one hemagglutination unit. Rabbit RBC were used as positive control in tests with other types of RBCs. All tests were repeated twice.

Effect of temperature. In order to determine the thermal stability of HA, aliquots of gut lysates of four species were incubated at 4, 22, 37, 45, 50, 55, 60, 65, 70 and 80°C for 30 min. prior to hemagglutination assay. Samples were also repeatedly frozen (–20°C) and thawed (25°C) or stored for one, two or three months at –20°C.

Effect of pH. In order to determine the optimum pH for agglutination activity PBS (pH 5.5–8.0) and Tris-HCl buffer (20 mM Tris, 150 mM NaCl, pH 7.5–9.0) were used for preparation of sample dilution series in hemagglutination tests. Gut lysates of unfed *L. longipalpis* females were used.

Dithiothreitol (DTT) reduction and exposure to urea. DTT splits disulfide linkages and urea breaks hydrogen bonds in protein molecules. Gut lysates of unfed *L. longipalpis* females were incubated overnight at 25°C with an equal volume of either 10 mM DTT dissolved in Tris-HCl buffer (10 mM Tris-HCl, 150 mM NaCl, pH 8.0) or 8 M urea in PBS (pH 7.4).

Effect of protease inhibitors. To ascertain if addition of protease inhibitors to samples affects hemagglutination titres, gut lysates of four sandfly species were incubated overnight at 4°C with equal volume of 2 or 5 mM EDTA in hemagglutination buffer or 30 min. at 25°C with 2mM phenylmethylsulfonyl fluoride (PMSF) or 5 µg/mg of leupeptine in hemagglutination buffer. Gut lysates of *L. longipalpis, P. duboscqi, P. papatasi* and *P. perniciosus* females dissected unfed and of *L. longipalpis* and *P. duboscqi* females dissected two days after blood feeding were used in experiments with PMSF and leupeptin. In experiments with EDTA gut lysates of unfed *L. longipalpis* and *P. papatasi* and blood-fed *L. longipalpis* females were used. Controls were incubated with buffer only and the HA in all samples was examined as described above.

Hemagglutination inhibition test. Doubling dilutions of potential inhibitors (each of 50 µl) were prepared in hemagglutination buffer. Fifty µl of lysates adjusted to contain approximately 1.5 hemagglutination units and 50 µl of 2% suspension of RaRBC were added and incubated for 2 hrs at room temperature. The lowest concentration of the inhibitor blocking hemagglutination was expressed as a 50% inhibitory concentration. Tests were repeated twice. Fifty four carbohydrates and glycoconjugates (Sigma) were used as potential inhibitors of HA in gut lysates of unfed females of *L. longipalpis* and *P. duboscqi* (Table 1). Six carbohydrates and two glycoconjugates (D-glucose, D-galactose, D-glucosamine, D-galactosamine, D-mannosamine, D-lyxosylamine, fetuin and heparin) were chosen for the comparison of sugar specificity of hemagglutinins present in tissue lysates and haemolymph of four sandfly species (Table 5). Lipohosphoglycan (LPG) of *Leishmania major*, clone FV1 (MHOM/IL/80 Friedlin) was tested as inhibitor of sandfly midgut lectin activity. LPG of stationary phase promastigotes (about 4–5 × 109 cells) purified on a column of octyl-Sepharose and LPG extracted from delipidated log-phase (procyclic) promastigotes (about 1.5 × 109 cells) according to McConville et al. (1987) were kindly provided by Dr. D. Rangarajan. Amount of procyclic LPG in sample did not exceed 5 mg (Dr. Rangarajan, personal communication). Samples were diluted in 100 µl of hemag- glutination buffer and used as described above. Due to limited amount of material available, we studied LPG effect using one sandfly species only; *P. papatasi*, one of the natural vector of *Leishmania major*, was chosen for this purpose.

RESULTS

Agglutination of different erythrocytes Ten types of erythrocytes were used in hemagglutination tests with tissue lysates (head, gut and body) of unfed *Lutzomyia longipalpis* females and seven types of erythrocytes were were used in tests with gut lysates of unfed females of *Phlebotomus papatasi.* Gut lysates of both species exhibited high activity (titre 128 for *L. longipalpis* and 64–128 for *P. papatasi*) against untreated and neuraminidase-treated rabbit erythrocytes, very low levels (titre 8 for both species) were detected against trypsinized and fixed human A RBC. Other types of erythrocytes (trypsinized and fixed rabbit, mouse and human B,O RBC for both species and untreated dog, chicken and human AB RBC for *L. longipalpis*) were not agglutinated. HA of head and body lysates of *L. longipalpis* was detected using RaRBC only (titre 4 for head and 4–8 for body).

Hemagglutination activity (HA) in unfed flies. In unfed females (day 0) of all five species studied the highest HA was detected in the gut lysates (titres 64–256) (see Table 2). Significant differences were found between HA in the head and body lysates of various species. Only very low activity levels (titre 8 or lower) were detected in the head and body lysates of *L. longipalpis*, *Phlebotomus perniciosus* and *P. halepensis*. Moderate titres (16–64) were found in head and body lysates of *P. papatasi* and *P. duboscqi* (Table 2).

Haemolymph of *L. longipalpis* females did not agglutinate RaRBC while haemolymph of *P. papatasi* and *P. duboscqi* exhibited HA titres 16–32 (Table 3). Protein concentration in haemolymph samples ranged between 200 and 300 μg/ml.

HA in fed flies. In fed flies the HA of the gut lysates was elevated, reaching peak titres 2 days after blood meal; activity levels were from 4–8 fold (for *P. papatasi* and *P. perniciosus*) to 16 fold (for *P. duboscqi*) higher than that in unfed flies. After day 3, the HA dropped and six days after blood-feeding it was similar to prefeeding level (Table 2). Post-engorgement increase of HA was found also in body lysates. High HA titres (64–128) were detected even in species where hemagglutinin was almost undetectable before blood meal intake (*L. longipalpis*, *P. perniciosus* and *P. halepensis*). The activity increase in body lysates was slower than that observed in the gut lysates; the highest titres were detected 3–6 days after blood-feeding (Table 2). When ovaries were separated from the rest of the body of *L. longipalpis*, *P. papatasi* and *P. duboscqi* females, the high HA was found in ovaries and only low HA titres were detected in the rest of the body (Table 3). In head lysates, no significant changes of HA were found; difference in HA between head lysates of unfed and fed flies did not exceed one titre (Table 2). Slight post-engorgement increase of HA was detected in haemolymph of females of *P. duboscqi* and *P. papatasi*. On the other hand, no HA was found in haemolymph of fed females of *L. longipalpis* (Table 3).

Table 2. Hemagglutination activities (hemagglutination titres = $\log_2$ n) of tissue extracts of five sandfly spacies against native rabbit red blood cells.

Species			Head	Gut	Body
L. longipalpis	unfed	0	2	7	2
	fed	1	2	8	5
	fed	2	3	10	6–7
	fed	3	3	6	7
	fed	6	2	7	7
	unfed	6	2	7	3
P. duboscqi	unfed	0	4–5	7	6
	fed	1	4–5	9	6–7
	fed	2	5	11	6–7
	fed	3	5	9	7–8
	fed	6	5	7	7–8
	unfed	6	4–5	7	6–7
P. papatasi	unfed	0	4	6–7	4
	fed	1	4	7–8	4–5
	fed	2	4	9	6–7
	fed	3	4	8	6–7
	fed	6	4	6–7	6
	unfed	6	4	6–7	5
P. perniciosus	unfed	0	<2	6–7	3
	fed	1	<2	7–8	3
	fed	2	2	9	3
	fed	3	2	8	6
	fed	6	3	7	6
	unfed	6	3	7	3
P. halepensis	unfed	0	2	8	2
	fed	1	2	9	3–4
	fed	2	2	10	5
	fed	3	2	10	5
	fed	6	2	8	6
	unfed	6	2	8	4–5

*Lysates were prepared from 5–7 days old females which did not take a blood (unfed 0), from females maintained another 6 days without blood-feeding (unfed 6) and from females fed on the host at the age of 5–7 days and dissected 1,2,3 and 6 days later (fed 1, 2, 3 and 6).

Effect of temperature. The process of repeated freezing and thawing of gut samples reduced 2–4 fold the HA titres against RaRBC. On the other hand, reduction of HA in gut lysates frozen for up to 3 months did not exceed one titre. Incubation of gut lysates in temperature up to 45°C did not effect HA (Table 4). When samples were heated from 50 to 65°C a progressive reduction in titres was observed. Heating at 70 and 80°C completely negated HA titres in all samples studied (Table 4).

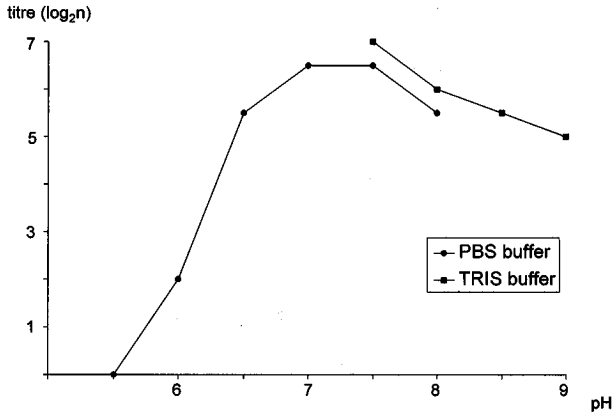

Fig. 1. Hemagglutination activity of *Lutzomyia longipalpis* gut lysates at different pH conditions.

Effect of pH. Midgut HA of *L. longipalpis* showed high activity in the pH range 6.5–9.0. The highest titres occurred at pH 7.0–7.5. Strong decrease of HA was observed at pH below 6.5 and no activity was found at pH 5.5 (Fig. 1).

Exposure to DTT and urea. Four-fold decrease of *L. longipalpis* gut HA titres was observed following DTT treatment. Urea either had no effect on HA or reduction of HA did not exceed one titre (data not shown).

Exposure to PMSF, leupeptin and EDTA. Leupeptin-treated samples gave the same titres as non-treated controls for all gut lysates used. Similarly, treatment by PMSF did not lower HA titres or resulted in 2-fold decrease of HA. EDTA in concentration 2 mM decreased HA titres 8–16 fold, 5 mM EDTA completely negated HA titres in gut lysates of nonfed and fed females of *L. longipalpis* and *P. duboscqi.*

Carbohydrate inhibition. Sugar-binding specificity of hemagglutinins was studied by hemagglutination inhibition assay. From 54 carbohydrates and glycoconjugates tested, eight inhibited gut HA of *L. longipalpis* and *P. duboscqi* females (Table 5). The most effective monosaccharide inhibitor was galactosamine, the most effective glycoconjugate inhibitor was lipopolysaccharide (LPS) of *Escherichia coli* K-235 which inhibited in concentration 19.5 μg/ml. Strong inhibitory effect was achieved by fetuin and heparin, weak inhibition was observed by glucosamine, mannosamine and lyxosylamine and bovine submaxillary mucin (Table 5). Only very small quantitative differences were found in sugar-binding specificity of HA from various tissue lysates and the haemolymph (BSM) (Table 5). Six carbohydrates and glycoconjugates inhibited, in same or similar concentrations, the HA of tissue lysates and haemolymph; in all samples the most effective inhibitory monosaccharide was galactosamine. Similarly, no significant differences were found between specificities of HA in gut lysates prepared from unfed and fed flies (Table 5). Gut HA of

Table 3. Comparison of hemagglutination activitie (hemagglutination titres = log_2 n) of haemolymph ovaries and the rest of the body (without ovaries) of unfed and fed females of three sandfly species.

Sandfly species		haemolymph	ovaries	rest of the body
L. longipalpis	unfed*	<2		
	fed 2	<2	7-8	4
P. duboscqi	unfed	5		
	fed 2	6	8	6
P. papatasi	unfed	4–5		
	fed 2	5	8	5–6

*Lysates prepared from 5–7 days old females which did not take blood (unfed) and from females dissected two days after bloodfeeding (fed 2).

Table 4. Effect of heat treatments on sandfly midgut hemagglutination activities (hemagglutination titres = log_2n).

sandfly species		Temperature									
		4	22	37	45	50	55	60	65	70	80
L. longipalpis	unfed*	7	7	7	7	6	5	<3	<3	<3	<3
	fed 2		10			8	6		4	<3	
P. duboscqi	unfed	7	7	7		7	5	4	<3	<3	<3
	fed 2	11	11	11		10	8	5	4	<3	<3
P. papatasi	unfed		6			6	6		<3	<3	
	fed 2		9			9	7	4	<3	<3	
P. perniciosus	unfed		6			6	5		<3	<3	
	fed 2	9	9	9		9	8	5	4	<3	<3

*Abbreviations same as in Table 3.

P. papatasi was strongly inhibited by LPG from *Leishmania major.* Semipurified LPG from procyclic promastigotes inhibited in dilution 1 : 1280 (corre- sponds to concentration less than 40 μg/ml of LPG), purified LPG from metacyclic promastigotes in dilution 1 : 160.

DISCUSSION

Our experiments showed that *Lutzomyia longipalpis* and *Phlebotomus papatasi* agglutinins reacted mainly with rabbit erythrocytes and neuraminidase treatment had no effect on agglutination. They did not react neither with most of erythrocytes used in this study nor with untreated mouse and human O RBC and gave only low titres with human A RBC (Volf 1993, Volf and Palánová, unpublished). These results differ from those of other authors probably due to different hemagglutination technique. Wallbanks et al. (1986) and Ingram and Molyneux (1991) who worked on HLA plates by microhemagglutination technique demonstrated high lectin activities against human and canine RBC in three *Phlebotomus* species including *P. papatasi.* Our laboratory used this technique for parasite-agglutination tests (Svobodová et al. 1996) but for hemagglutination tests we prefere to work with larger volumes in U-shape microtitre plates. This technique was more

Table 5. Carbohydrate inhibition of the hemagglutination activities of tissue extracts and haemolymph of *Lutzomyia longipalpis* (LO), *Phlebotomus duboscqi* (DU), *P. papatasi* (PA) and *P. perniciousus* (PE).

Samples		carbohydrates (mM)				glycoconjugates (μg/ml)			
		N-gal	N-Glc	N-Man	N-Lyx	heparin (IU)	fetuin	BSM	LPS
Head	DU	31	125	125	nd	376	312	nd	nd
	PA	31	125	250	nd	376	625	nd	nd
Gut unfed*	LO	63	250	250	250	188	312	625	19.5
	DU	31	125	125	250	188	156	625	19.5
	PA	31	125	250	nd	376	3,312	nd	nd
	PE	31	125	125	nd	376	625	nd	nd
Gut fed 2	LO	63	250	250	250	188	312	nd	nd
	DU	31	125	125	250	188	156	nd	nd
Body	DU	63	250	500	nd	376	625	nd	nd
	PA	63	250	250	nd	376	312	nd	nd
Haemolymph	DU	63	125	125	250	188	156	nd	nd
	PA	31	125	63	nd	188	625	nd	nd
Ovaries	LO	63	125	125	125	nd	156	nd	nd
	DU	31	125	63	125	nd	156	nd	nd
	PA	31	125	63	125	nd	78	nd	nd

material-consuming but gave us standard results clearly visible by unaided eye.

Midgut HA had a broad pH optimum with high activities at neutral and slightly alkaline pH. The peak of activity occurred at pH 7.0–7.5 that correspond with pH conditions naturally occurring in sandfly midgut just after blood-feeding (Y. Tang and P. Bates, personal communication). The effect of DTT reduction indicates that sandfly lectins possess a limited amount of methionine and/or cysteine residues and that disulfide linkages may be responsible, in part, for structural configuration of the lectin molecule. Experiments with EDTA addition suggested that cations are needed for agglutination activity of the molecule. The influence of cations, sensitivity to freezing/thawing and heat-lability of the agglutinin correspond to properties of most other lectins known from bloodsucking Diptera (for review see Ingram and Molyneux 1991) and together with sensitivity to DTT are also an indication of the protein nature of the agglutinin molecule.

Addition of protease inhibitors did not significantly change agglutination levels. In sandflies, lectins are secreted into midgut lumen (Volf and Killick-Kendrick 1996) and, similarly with other blood-sucking arthropods with extracellular digestion, come into contact with the midgut proteinases. Therefore, the resistance of lectin molecule to protease might be a prerequisite for maintaining its biological activity. On the other hand, in ticks, where digestion and proteases are intracellular, midgut lectins seems to be sensitive to protease effect (Uhlíř et al. 1996).

Some lectins found in gut tissue and haemolymph of hematophagous insects are inducible (for review see Ingram and Molyneux 1991). Secretion of gut lectin activities in tsetse flies and sandflies was stimulated by blood-meal (Maudlin and Welburn 1987, Volf and Killick-Kendrick 1996) or ingestion of protein solutions (Volf and Palánová 1996). In some *Glossina* species, there is evidence that two gut lectins with different sugar specificity participate in increased postengorgement HA levels (Welburn et al. 1994, Grubhoffer et al. 1994). In sandflies, differences observed in lectin specificities between unfed and fed females were not significant and we suppose that the same lectin molecule is present, in different levels, in unfed as well fed flies.

In *P. papatasi* and *P. duboscqi* the HA was present in various tissues including the haemolymph. This corresponded with previous observations on HA in head lysates of these two species (Volf et al.1994) and with results obtained by isolation and structural characterization of the lectin; a polypeptide reacting with anti-lectin antibodies was found in the haemolymph of *P. duboscqi* but not in the haemolymph of *L. longipalpis* (Volf and Palánová, unpublished). Wallbanks et al. (1986), who reported the first finding of lectin in *P. papatasi* head lysates, expected that this activity is associated with foregut of the insect. From our experiments, it seems more likely that HA in head lysates resides in the haemolymph. In our experiments, moreover, HA was detected in ovaries of fed females of *L. longipalpis, P. papatasi* and *P. duboscqi*. Carbohydrate-binding specificity of HA present in ovaries, haemolymph, head

and body lysates did not differ from that of the previously described gut lectin (Volf et al. 1994). Possible explanation could be that in females of all sandfly species the lectin is present in high concentrations in ovaries and gut. However, in some species (*P. papatasi*, *P. duboscqi*) it circulates in haemolymph in higher concentrations that in the other ones (e.g. *L. longipalpis*).

The site of *de novo* biosynthesis of sandfly lectins is unclear at the present time. Insect agglutinins are synthetized mainly by haemocytes and the fat body, but they were also reported to be produced by sexual organs (Stiles et al. 1988). For detailed study of the site of biosynthesis of sandfly lectins hybridization *in situ* will be required, however, ovaries or gut tissue seem to be the most probable candidates.

Lectins of *P. papatasi* and other species studied, agglutinate *Leishmania* promastigotes by the carbohydrates present on the promastigote surface (Wallbanks et al. 1986, Svobodová et al. 1996). The major glycoconjugate present on the surface membrane of promastigotes is lipophosphoglycan (LPG). This molecule includes stage-specific terminal sugars which are, according to Pimenta et al. (1992) and Sacks et al. (1994), involved in midgut adhesion of procyclic promastigotes. To ascertain whether sandfly lectin could serve as a LPG receptor, we have used LPG of *Leishmania major* in carbohydrate inhibition tests with *P. papatasi* gut lysate. Both crude procyclic and purified metacyclic LPG inhibit HA and especially the procyclic LPG had a very strong inhibitory effect. However, for detailed study of the role of sandfly lectins in LPG-mediated attachment, purified and precisely quantified glycoconjugates from both promastigote forms will be needed.

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