Lectin-Binding Patterns in the Testes of the Java Fruit Bat (*Pteropus vampyrus*) and the Japanese Lesser Horseshoe Bat (*Rhinolophus cornutus*)

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**Abstract.** Lectin binding patterns in the testes of the Java fruit bat, *Pteropus vampyrus*, and the Japanese lesser horseshoe bat, *Rhinolophus cornutus*, were investigated by light microscopy. Binding patterns were similar in both species, except for some slight differences. Con A and WGA gave a diffuse reaction all over the seminiferous epithelium in both species. In addition to this binding pattern, PHA-E exhibited a granular reaction within the cytoplasm of pachytene spermatocytes. PNA and RCA-I gave an intense reaction in the acrosomal region from Golgi to acrosome-phase spermatids in both species, but, in addition to this binding pattern, RCA-I reacted in the cytoplasm of spermatocytes and spermatids in the Java fruit bats. PSA revealed a granular reaction in the cytoplasm of spermatids in the Japanese lesser horseshoe bats. These binding patterns were similar to those of mammals studied before, except that a few specific bindings were detected in the bats.

**Key words:** Lectin, Japanese lesser horseshoe bat, Java fruit bat, Spermatid, Testis.


Since lectins have a specific binding affinity for sugar residues, they have been used as histochemical reagents to investigate the distribution of glycoconjugates in various tissues. Until now, several studies have been done on lectin-binding patterns in the testes of mammalian species, such as the man [1–4], rodents [5–9], insectivore [10], common tree shrew [11], bull [12] and goat [13], but, there are no reports on the Order Chiroptera. In this study, two kinds of bats were selected: one (Java fruit bat) belongs to the Suborder Megachiroptera, and the other (Japanese lesser horseshoe bat) belongs to the Suborder Microchiroptera. The lectin-binding patterns in the testes of these two species were investigated by light microscopy. To detect the specificity in both species, the lectin binding patterns were compared with each other and with those of other mammalian species.

**Materials and Methods**

**Animals**

Two adult Java fruit bats obtained in East Java,
Indonesia and three adult Japanese lesser horseshoe bats captured in Aomori prefecture, Japan were used in the present study. All of these bats showed signs of active spermatogenesis according to the histological analysis described below. Body weight and forearm length information are shown in Table 1.

**Light microscopy**

Under pentobarbital anesthesia, the animals were perfused with 0.9% physiological saline followed by Bouin’s fixative through the left ventricle. The testes were excised, sliced into slabs and immersed in the same fixative overnight. They were dehydrated in a graded series of ethanol, infiltrated in xylene and embedded in paraffin wax. Sections (4 µm) were deparaffinized, stained with periodic acid-Schiff (PAS)-hematoxylin and examined by light microscopy to confirm active spermatogenesis in these animals.

**Lectin histochemistry**

The following lectins were used in the present study: *Ulex europaeus* agglutinin I (UEA-I), soybean (*Glycine max*) agglutinin (SBA), peanut (*Arachis hypogaea*) agglutinin (PNA), *Ricinus communis* agglutinin I (RCA-I), *Dolichos biflorus* agglutinin (DBA), *Phaseolus vulgaris* agglutinin (PHA-E), *Bandeiraea simplicifolia* I (BSL-I), *Concanavalin* (*Canavalia ensiformis*) agglutinin (Con A), *Pisum sativum* agglutinin (PSA) and wheat germ (*Triticum vulgaris*) agglutinin (WGA).

The sections (4 µm) obtained from the paraffin block described above were deparaffinized, rehydrated and treated with 0.01 M phosphate-buffered saline (PBS) and 1% bovine serum albumin (BSA) -PBS. The sections were incubated with biotinylated lectins (Vector stain, CA, USA, 25 µg/ml) in 1% BSA-PBS for 60 min. After washing with PBS, they were treated with 1% BSA-PBS and incubated with avidin-biotin peroxidase complex (ABC, Vector stain, CA, USA) for 30 min. They were then washed again with PBS, immersed in diaminobenzidine (DAB, 0.2 mg/ml)-H₂O₂ (0.005%) for 5 min and rinsed in distilled water. They were stained with hematoxylin and observed by light microscopy.

Negative controls without lectins were processed in parallel.

**Results**

**Java fruit bat**

The spermatids of the Java fruit bats could be easily subdivided into 4 phases (Golgi, cap, acrosome and maturation-phases). Six lectins (PNA, PHA-E, Con A, WGA, RCA-I and PSA) exhibited a positive reaction in spermatogenic cells, whereas four lectins (UEA-I, SBA, DBA and BSL-I) revealed a negative reaction in the testes. PNA and RCA-I showed an intense reaction in the acrosomal region of spermatids. In short, PNA was intensely positive in the acrosomal region from Golgi to acrosome-phase spermatids (Fig. 1a). This reaction disappeared in maturation-phase spermatids. RCA-I gave an intense reaction in the acrosomal region from Golgi to acrosome-phase spermatids and also reacted in the cytoplasm of spermatocytes and spermatids (Fig. 1b). In addition to a diffuse reaction in the seminiferous epithelium, PHA-E exhibited a granular reaction within the cytoplasm of pachytene spermatocytes (Fig. 1c). Although Con A and WGA revealed a diffuse reaction all over the seminiferous epithelium, WGA showed an intense reaction in the cytoplasm of acrosome-phase spermatids and Sertoli cells (Fig. 1d). PSA showed a weak reaction in the interstitial region, Sertoli cells and elongate spermatids.

**Japanese lesser horseshoe bat**

The spermatids of the Japanese lesser horseshoe bats could also be subdivided into 4 phases (Golgi, cap, acrosome and maturation-phases). In most cases, the lectin binding patterns in the testes of the Japanese lesser horseshoe bats were similar to those of the Java fruit bats (Fig. 2a, b), but they were different in the RCA-I and PSA reactions. In the Japanese horseshoe bats, RCA-I was strongly positive in the acrosomal region from Golgi to acrosome-phase spermatids as well as in the Java fruit bat and the Japanese lesser horseshoe bat

<table>
<thead>
<tr>
<th>Species</th>
<th>weight (g)</th>
<th>forearm length (mm)</th>
</tr>
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<tbody>
<tr>
<td>Java fruit bat ①</td>
<td>613</td>
<td>175</td>
</tr>
<tr>
<td>Java fruit bat ②</td>
<td>521</td>
<td>175</td>
</tr>
<tr>
<td>Japanese lesser horseshoe bat ①</td>
<td>7.2</td>
<td>41.0</td>
</tr>
<tr>
<td>Japanese lesser horseshoe bat ②</td>
<td>6.8</td>
<td>41.4</td>
</tr>
<tr>
<td>Japanese lesser horseshoe bat ③</td>
<td>6.9</td>
<td>41.4</td>
</tr>
</tbody>
</table>
Fig. 1. Lectin binding sites in the testis of the Japanese lesser horseshoe bat. (a) PNA is strongly positive in the acrosomal region. (b) PHA-E exhibits a granular reaction within the cytoplasm of pachytene spermatocytes in addition to a diffuse reaction in the seminiferous epithelium. (c) RCA-I reacts strongly in the acrosomal region and reacts in the cytoplasm of spermatocytes and spermatids. (d) WGA reveals a diffuse reaction all over the seminiferous epithelium and reacts strongly in the cytoplasm of acrosome-phase spermatids and Sertoli cells. × 320.
Fig. 2. Lectin binding sites in the testis of the Java fruit bat. Binding patterns of PNA(a) and PHA-E(b) are similar to those of the Japanese lesser horseshoe bat, but the reaction of RCA-I and PSA is different. (c) RCA-I is strongly positive in the acrosomal region and reacts in the cytoplasm of spermatids. (d) PSA has an intense and granular reaction in the cytoplasm of spermatids. × 320.
fruit bats, but a granular reaction was observed in pachytene spermatocytes at stages VIII to IX only in the Japanese lesser horseshoe bats (Fig. 2c). PSA gave an intense and granular reaction in the cytoplasm from Golgi to acrosome-phase spermatids in the Japanese lesser horseshoe bats in contrast to a weak reaction in spermatids of the Java fruit bats (Fig. 2d).

Cytochemical controls
In both species, negative controls showed no reaction.

Discussion
The binding patterns of 10 lectins in the testes showed a similarity between Java fruit bats and Japanese lesser horseshoe bats. Although UEA-I, SBA, DBA and BSL-I gave no reaction in any regions of the testes in either species, PNA, RCA-I, PHA-E, Con A, PSA and WGA showed a positive reaction in the testes of both species. PHA-E, Con A and WGA exhibited a diffuse reaction in the testes. The reaction of PNA and RCA-I was strongly detected in the acrosomal region of spermatids. PSA, representing $\alpha$-Mannose and complex glycan with a fucosylated core, weakly bound to the interstitial cells, Sertoli cells and elongate spermatids, but the binding patterns of RCA-I and PSA were somewhat different. RCA-I showed a wide affinity for spermatocytes in the Java fruit bats, but not in the Japanese lesser horseshoe bats. PSA exhibited a granular reaction in the cytoplasm from Golgi to acrosome-phase spermatids in the Japanese lesser horseshoe bats, but not in the Java fruit bats. This granular reaction of PSA has not been observed in any other lectins. These findings, therefore, suggest that PSA may detect some specific glycoconjugates in a certain organelle of the Japanese lesser horseshoe bats. The UEA-I reaction, representing the appearance of $\alpha$-Fucose residues, have been reportedly detected in the acrosomal region of the rat [5, 6], mouse [7, 8], nutria [8] and bull [12], but no reaction was or has previously been observed in the testes of the Java fruit bat, the Japanese lesser horseshoe bat, the guinea pig [9] or the musk shrew [10]. It has been demonstrated that SBA reacts strongly in the acrosomal region of many mammalian species, such as man [1–4], rat [5, 6], mouse [7, 8], nutria [8], guinea pig [9], common tree shrew [11], bull [12] and shiba goat [13], but this reaction could not be detected in either the Java fruit bat or the Japanese lesser horseshoe bat. The DBA binding pattern has varied among the various species. In shiba goats [13], DBA is negative, as it is in bats. In musk shrews [10], DBA has reacted in the cytoplasm of primary spermatocytes, whereas in rats [5, 6] it has reacted in the acrosome of maturation-phase spermatids. Therefore, such differences in lectin bindings in the acrosomal region may reflect different acrosomal components of sugar residues in each mammalian species. In contrast to the variation in lectin bindings in acrosome, PNA had a conserved binding pattern in the acrosomal region of all mammalian species examined so far [1–13]. In concurrence, the present study also showed an intense reaction in the acrosomal region of bats. Since PNA specifically recognizes $\beta$-Galactose residues, the acrosomal region in mammals should be $\beta$-Galactose-rich.

Although most lectin binding patterns in bats were similar to those in mammals studied before, there were some lectins such as SBA and PSA with specific binding patterns. More information is necessary to confirm whether these findings are specific to the Order Chiroptera.

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References


