We identified a novel horseshoe crab hemocyte-derived lectin, which we named tachylectin-4. It has more potent hemagglutinating activity against human A-type erythrocytes than a previously identified hemocyte lectin with an affinity to N-acetylgalactosamine, tachylectin-2. The purified tachylectin-4 is an oligomeric glycoprotein of 470 kDa, composed of subunits of 30 and 31.5 kDa. Ca\(^{2+}\) at 10 mM enhanced the hemagglutinating activity 4-fold, and the activity was inhibited by EDTA and o-phenanthroline. L-Fucose and N-acetylmuraminic acid at 100 mM completely inhibited the activity of tachylectin-4. The activity was also inhibited more strongly by bacterial S-type lipopolysaccharides (LPS) but not by R-type LPS lacking O-antigen. The most effective S-type LPS was from *Escherichia coli* O111:B4, and the minimum concentration required for inhibiting agglutination against human A-type erythrocytes (0.1 \(\mu\)g/ml) was 160-fold lower than those of S-type LPS from *Salmonella minnesota*. Therefore, colitose (3-deoxy-L-fucose), a unique sugar present in the O-antigen of *E. coli* O111:B4 with structural similarity to L-fucose, is the most probable candidate for a specific ligand of tachylectin-4.

A cDNA coding for tachylectin-4 was isolated from a hemocyte cDNA library. The open reading frame of the 1344-base pair cDNA coded for the mature protein with an estimated molecular mass of 307 kDa. The purified tachylectin-4 is an oligomeric glycoprotein of 470 kDa, composed of subunits of 30 and 31.5 kDa. Ca\(^{2+}\) at 10 mM enhanced the hemagglutinating activity 4-fold, and the activity was inhibited by EDTA and o-phenanthroline. L-Fucose and N-acetylmuraminic acid at 100 mM completely inhibited the activity of tachylectin-4. The activity was also inhibited more strongly by bacterial S-type lipopolysaccharides (LPS) but not by R-type LPS lacking O-antigen. The most effective S-type LPS was from *Escherichia coli* O111:B4, and the minimum concentration required for inhibiting agglutination against human A-type erythrocytes (0.1 \(\mu\)g/ml) was 160-fold lower than those of S-type LPS from *Salmonella minnesota*. Therefore, colitose (3-deoxy-L-fucose), a unique sugar present in the O-antigen of *E. coli* O111:B4 with structural similarity to L-fucose, is the most probable candidate for a specific ligand of tachylectin-4.

A cDNA coding for tachylectin-4 was isolated from a hemocyte cDNA library. The open reading frame of the 1344-base pair cDNA coded for the mature protein with 232 amino acids. There is no significant sequence similarity to any other known LPS-binding lectins, whereas tachylectin-4 is homologous to the NH\(_2\)-terminal domain of unknown functions of *Xenopus laevis* pentraxin 1.

Arthropods have developed a unique immune system without the acquired immunoglobulin-dependent immunity found in vertebrates. Therefore, innate immunity, the pre-existing and immediate ability to prevent and limit invading microbes and pathogens, is likely to be a major host defense system in arthropods. The hemolymph of horseshoe crabs contains three abundant proteins, hemocyanin, C-reactive protein, and \(\alpha\)-macroglobulin, and one type of granular cell, accounting for 99% of the total hemocytes (1, 2). The granular cells are extremely sensitive to bacterial endotoxin, *i.e.* lipopolysaccharides (LPS), and the cells release granular components in response to LPS stimulation (3–6). This response is thought to be important for host defense in engulfing and killing invading microbes, in addition to preventing the leakage of hemolymph. The hemocytes contain large and small granules that selectively store proteins and defense molecules, including serine proteasezymogens, a clottable protein that participates in the coagulation cascade, protease inhibitors, antibacterial peptides, lectins, and others (6).

Many kinds of lectins play crucial roles in innate immunity and host defense not only in vertebrates but also in invertebrates, with involvement in processes such as non-self recognition, inflammation, opsonization, cell-cell or cell-extracellular matrix interactions, fertilization, development, and regeneration (7–11). To better understand the biological role of lectins in host defense of the horseshoe crab, we have now identified various lectins found in hemolymph of this animal (6). Recently, we have now identified new horseshoe crab hemocyte-derived lectins, named tachylectin-1 (L6) (12) and tachylectin-2 (L10) (13). Tachylectin-1 displays LPS-binding potential with antibacterial activity toward Gram-negative bacteria, but it has no hemagglutinin activity for human, sheep, and rabbit erythrocytes. On the other hand, tachylectin-2 has hemagglutinating activity against human A-type erythrocytes with specificity for N-acetylgalactosamine. Furthermore, tachylectin-2 has agglutination activity against *Staphylococcus saprophyticus* KD. Both tachylectin-1 and tachylectin-2 are composed of unique tandem sequence repeats with no significant sequence similarity with other known proteins, including various animal and plant lectins. While continuing these studies on horseshoe crab lectins, we have now identified a new hemocyte lectin, which we named tachylectin-4; it has more potent hemagglutinating activity than tachylectin-2, shows a unique binding specificity to O-antigen of LPS, and has sequence similarity to the NH\(_2\)-terminal domain with unknown functions of *Xenopus* pentraxin 1.

**EXPERIMENTAL PROCEDURES**

**Materials**—Hemocytes lysate from the Japanese horseshoe crab (*Tachypleus tridentatus*) was prepared as described (14). LPS isolated from *Escherichia coli* O111:B4, *Salmonella minnesota* (smooth), and *S. minnesota* R595 (Re) were from List Biological Laboratories, Inc., Campbell, CA and those isolated from *E. coli* J5 (Rc), *E. coli* F583 (Rd), *S. minnesota* R7 (Rd.), *Salmonella typhimurium* (smooth), *S. typhimurium* TV119 (Ra), and *S. typhimurium* SL684 (Rc) were from Sigma. ConA-Sepharose and molecular weight standards were from Pharmacia Biotech Inc. L-Fucose-immobilized agarose was from EY Laboratories, Inc., San Mateo, CA. Lysyl endopeptidase was from Wako Pure Chemical Industries, Ltd., Tokyo, Japan. Restriction endonucleases and DNA-modifying enzymes were from Nippon Gene Co. (Toyama, Japan), Toyobo Co., Ltd. (Osaka, Japan), and Takara Shuzo Co. (Kyoto, Japan). \(\alpha\)-[\(^{32}\)P]dCTP was from Amersham Japan, Tokyo. Sugars and glycoproteins were from Sigma, Nacalai Tesque, Co., Ltd. (Kyoto, Japan), and Seikagaku Kogyo, Co., Ltd. (Tokyo, Japan). A \(\lambda\)ZipLox cDNA library was constructed from poly(A\(^{+}\)) RNA extracted from hemocytes, using a poly(A\(^{+}\)) selection procedure and \(\lambda\)ZipLox packaging system. The nucleotide sequence(s) reported in this paper has been submitted to the GenBank\(^{TM}\)EBI Data Bank with accession number(s) AB005542.

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The abbreviations used are: LPS, lipopolysaccharide(s); HPLC, high performance liquid chromatography; PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction; ConA, concanavalin A.

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Hemagglutinating and LPS Binding Activities—Hemagglutinating (13) and LPS binding (12) activities were determined, as described.

Glycosidase F Treatment—The samples were reduced and S-alkylated with iodoacetamide in 50 mM Tris-HCl, pH 7.5, containing 8 M urea (15). The S-alkylated protein (5 μg) was incubated with 0.1 unit of N-glycosidase F (Boehringer Mannheim) in 50 mM Tris-HCl, pH 7.5, containing 2 M urea at 37 °C for 18 h and subjected to SDS-PAGE under reducing conditions.

Gel Filtration—Purified tachylectin-4 was applied to a Superdex 200 HR10/30 column, equilibrated with 20 mM Tris-HCl, pH 7.5, containing 0.15 M NaCl at a flow rate of 0.5 ml/min. Reference proteins for molecular weight determination were thyroglobulin (Mr = 669,000), apoferritin (Mr = 443,000), alcohol dehydrogenase (Mr = 150,000), bovine serum albumin (Mr = 67,000), and carbonic anhydrase (Mr = 30,000).

SDS-PAGE—SDS-PAGE was performed in 15% slab gels, according to Laemmli (16). The gels were stained with Coomassie Brilliant Blue R-250. The reference proteins were phosphorylase b (Mr = 94,000), bovine serum albumin (Mr = 67,000), ovalbumin (Mr = 43,000), carbonic anhydrase (Mr = 30,000), soybean trypsin inhibitor (Mr = 20,000), and α-lactoalbumin (Mr = 14,400).

Isolation of Tachylectin-4-derived Peptides—Tachylectin-4 (100 μg) was reduced and then S-alkylated with iodoacetamide (15). The S-alkylated protein was digested with lyssyl endopeptidase (enzyme/substrate = 1/100, w/w) in 0.1 M NH₄HCO₃ containing 2 M urea at 37 °C for 24 h. The peptides were separated by reverse-phase HPLC using a Chemcosorb 5-ODS-H column (2.1 × 150 mm, Chemco Scientific Co., Ltd., Osaka). Peptides were eluted from the column with a linear gradient of 0–80% acetonitrile in 0.06% trifluoroacetic acid at a flow rate of 0.2 ml/min. Absorbance was monitored at 210 nm. Amino acid sequence analyses were performed using an Applied BioSystems 473A or 477A sequencer with the chemicals and programs supplied by the manufacturer (Perkin-Elmer Japan Co., Ltd., Tokyo, Japan). Amino acid sequence analyses were performed using an Applied Biosystems 473A or 477A sequencer with the chemicals and programs supplied by the manufacturer (Perkin-Elmer Japan Co., Ltd., Tokyo, Japan) (17).

Amino Acid Analysis—Samples were hydrolyzed in 6 M HCl containing 1% phenol at 110 °C for 20 h in evacuated tubes. The hydrolysates were analyzed using a Pico-Tag system (Waters Japan Co., Ltd., Tokyo, Japan) (17).

Tachylectin-4-specific DNA Probes and Screening of cDNA Library—The degenerate nucleotide sequences of the primers used for PCR were based on the peptides derived from lyssyl endopeptidase digestion (-Asn-Ala-Tyr-Val-Glu-Thr- and -Ile-Thr-Asp-Asp-Tyr-Val-) of tachylectin-4. Sense and antisense nucleotides were synthesized with an EcoRI site at the 5’ end. Reactions for PCR contained the cDNA template (corresponding to 0.1 μg of poly(A)+ RNA) and 100 pmol each of the primer were carried out in a Perkin-Elmer Cetus thermal cycler. The PCR products were treated with EcoRI and purified with agarose gel electrophoresis. Fractions of interest were then ligated into plasmid BlueScript II SK (Stratagene, La Jolla, CA) for sequence analysis, as described (18). One clone (0.4 kilobase), containing the sequence of tachylectin-4, was used as a probe to screen the λZipLox cDNA library. The PCR fragment, labeled with [α-32P]dCTP using a Ready-To-Go™ DNA-labeling kit (Pharmacia Biotech Inc.) served as a probe. After secondary screening, the plasmids containing the cDNA insert were prepared from the positive plaques, following the manual supplied by the manufacturer.

Homology Search—Amino acid sequence was compared with all entries in the SWISS-PROT protein data base by the FASTA homology search system of the European Bioinformatics Institute.

RESULTS

Purification of Tachylectin-4—The lysate prepared from 100 g (wet weight) of hemocytes was first fractionated on a dextran sulfate-Sepharose CL-6B column (4.5 × 20 cm) with increasing concentrations of NaCl from the range of 0.15–2.0 M (14), and a typical elution pattern and hemagglutinating activity against human A-type erythrocytes is shown in Fig. 1A. The activity in the flow-through fraction is derived from tachylec- tin-2 (13). The 0.15 M NaCl fractions containing a new lectin, named tachylectin-4, indicated by a bar were pooled and applied to a ConA-Sepharose column (3 × 15 cm), equilibrated with 20 mM Tris-HCl, pH 7.5, containing 0.15 M NaCl. After washing with equilibration buffer, proteins were eluted with the same buffer containing 1 M α-methyl-D-glucoside (Fig. 1B). The lectin fractions indicated by a bar were pooled and dialyzed against 20 mM Tris-HCl, pH 7.5, containing 0.15 M NaCl. The dialyzed sample was then applied to an 1-fucose-immobilized
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**TABLE I**

Purification of tachylectin-4 from horseshoe crab hemocyte lysate

| Purification step                  | Total protein$^a$ (mg) | Total activity (titer) | Specific activity (titer/mg) | Purification $^b$-fold | Yield (%) |
|-----------------------------------|------------------------|------------------------|-----------------------------|------------------------|-----------|
| Hemocyte lysate                   | 21,152                 | 604,160                | 29                          | 1                      | 100       |
| Dextran sulfate-Sepharose 6B      | 1,208                  | 243,200                | 201                         | 1                      | 63        |
| ConA-Sepharose                    | 160                    | 153,600                | 962                         | 4.8                    | 611       |
| Fucose-agarose                    | 1.1                    | 135,171                | 122,328                     | 1.0                    | 56        |

$^a$ For the determination of protein concentrations, A$_{280}$ = 10 for 1% protein solution, was assumed.

**TABLE II**

Hemagglutinating activity of tachylectin-4

| Erythrocytes Minimum agglutination concentration $^a$ | µg/ml |
|-------------------------------------------------------|-------|
| Human                                                 |       |
| A-type                                                | 0.014 |
| B-type                                                | 1.7   |
| O-type                                                | 0.86  |
| Horse                                                 | Not agglutinated |
| Rabbit                                                | Not agglutinated |
| Sheep                                                 | Not agglutinated |

$^a$ Not agglutinated at 12.5 µg/ml.

![Fig. 2. SDS-PAGE of S-alkylated tachylectin-4 after treatment with N-glycosidase F.](image)

The purified tachylectin-4 gave a doublet on SDS-PAGE under reducing ($M_r = 30,000$ and $31,500$) and non-reducing ($M_r = 23,500$ and $26,000$) conditions (Fig. 1D). On the other hand, an apparent molecular weight of tachylectin-4 in solution was determined by gel filtration to be 470,000, indicating an oligomeric protein. The two subunits of 31.5 and 30 kDa could be separated by reverse-phase HPLC on a YMC C4 column (4.6 × 150 mm, Yamamura Chemical Laboratories Co., Ltd., Kyoto, Japan) (data not shown), and their NH$_2$-terminal sequences proved to be identical up to 18 residues as follows: Trp-Arg-Leu-Tyr-Leu-Pro-Val-Ile-Val-Lys-Tyr-Gly-X-Met-Lys-Leu-Asp-. Furthermore, amino acid compositions of 20 h hydrolysates and peptide mapping patterns of the two subunits were almost indistinguishable (data not shown). The purified tachylectin-4 was treated with N-glycosidase F and subjected to SDS-PAGE (Fig. 2). The N-glycosidase F treatment resulted in disappearance of the upper band, and the lower band was only observed in the gel with the same mobility as the untreated lower band (lanes 2 and 3), indicating that the different molecular weights between the subunits is caused by partial modification of the subunit with N-linked sugars.

The extinction coefficient of tachylectin-4 at 280 nm for 1.0% solution in Tris-HCl buffer (pH 7.5) was calculated from data derived from amino acid analysis. The value of 14.0 was used for subsequent determinations of tachylectin-4 concentrations.

**Hemagglutinating Activity of Tachylectin-4**—Tachylectin-4 agglutinated all types of human erythrocytes and A-type erythrocytes were most sensitive (Table II). In the presence of 10 mM CaCl$_2$, tachylectin-4 agglutinated A-type erythrocytes at the minimum concentration of 0.014 µg/ml, and 60- and 120-fold higher concentrations were required for O-type and B-type erythrocytes, respectively. No hemagglutination was observed for erythrocytes derived from horse, rabbit, and sheep (Table II). The hemagglutinating activity for A-type erythrocytes was enhanced 4-fold by 10 mM Ca$^{2+}$, but other divalent cations including Zn$^{2+}$, Co$^{2+}$, Cu$^{2+}$, and Mn$^{2+}$ at 0.1 mM and 10 mM had no effects. The hemagglutinating activity was completely inhibited by 10 mM EDTA and 0.2 mM o-phenanthroline, suggesting the presence of metal ion(s) other than Ca$^{2+}$ in tachylectin-4.

**Effects of Carbohydrates on Hemagglutination**—Effects of various carbohydrates on the hemagglutinating activity of tachylectin-4 are shown in Table III. For mono- and disaccharides, L-fucose and N-acetylgalactosamine at the minimum concentration of 100 µg/ml inhibited the hemagglutination. On the other hand, a bacterial cell wall component, LPS, was a more potent inhibitor. An LPS derived from *E. coli* O111:B4 was the most potent inhibitor at the minimum concentration of 0.1 µg/ml. Interestingly, LPS isolated from rough mutants of *E. coli* O111:B4 (smooth) such as *E. coli* J5 (Rc) and *E. coli* F583 (Rd) showed no inhibitory effect, suggesting that the O-antigen of the S-type *E. coli* is a determinant for carbohydrate recognition by tachylectin-4. This was the case for other Gram-negative bacteria. LPS derived from S-types of both *S. minnesota* and *S. typhimurium* inhibited the hemagglutinating activity at the minimum concentrations of 15.6 and 7.8 µg/ml, respectively, but LPS isolated from several rough mutants had no effect. To confirm the binding activity to O-antigen, hemagglutinating activity was measured using sheep erythrocytes coated with LPS derived from a S-strain (*E. coli* O111:B4) and a rough mutant (*S. minnesota* R595 (Re)). Tachylectin-4 signif-
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Inhibition of agglutinating activity of tachylectin-4

| Component                        | Minimum inhibitory concentration<sup>a</sup> |
|----------------------------------|---------------------------------------------|
| J-Δ-Fucose                       | 100 μg/ml                                   |
| J-Δ-Glucose                      | NI<sup>b</sup>                              |
| J-Δ-Galactose                    | NI                                          |
| J-Δ-Mannose                      | NI                                          |
| J-Δ-Rhamnose                     | NI                                          |
| J-Δ-Xylose                       | NI                                          |
| J-Δ-Glucosamine                  | NI                                          |
| J-Δ-Galactosamine                | NI                                          |
| N-Acetylneuraminic acid          | 100 mM                                      |
| N-Acetyl-D-glucosamine           | NI                                          |
| N-Acetyl-D-galactosamine         | NI                                          |
| N-Acetyl-D-mannosamine           | NI                                          |
| Lactose                          | NI                                          |
| N-Acetyllalloctosamine           | NI                                          |
| Methyl β-D-mannoside             | NI                                          |
| Methyl β-D-glucoside             | NI                                          |
| Hyaluronic acid                  | NI                                          |
| Laminarin                       | NI                                          |
| LPS: E. coli O111:B4 (smooth)    | 0.1 μg/ml                                   |
| LPS: E. coli O111:B4 (Rd)        | NI                                          |
| LPS: S. minnesota (smooth)       | 15.6 μg/ml                                  |
| LPS: S. minnesota R595 (Re)      | NI                                          |
| LPS: S. minnesota R7 (Rd)        | NI                                          |
| LPS: S. pykminumirum (smooth)    | 7.8 μg/ml                                   |
| LPS: S. pykminumirum TV119 (Ra)  | NI                                          |
| LPS: S. pykminumirum SL684 (Re)  | NI                                          |
| Lipoteichoic acid: Staphylococcus aureus | 31.3 μg/ml                              |
| Lipoteichoic acid: Streptococcus faecalis | 31.3 μg/ml                              |
| Lipoteichoic acid: Streptococcus mutans | 62.5 μg/ml                              |
| Lipoteichoic acid: Streptococcus sanguis | 62.5 μg/ml                              |
| Lipoteichoic acid: Streptococcus pyogenes | 125 μg/ml                              |
| Lipoteichoic acid: B. subtilis   | 500 μg/ml                                   |

<sup>a</sup> Minimum concentrations required for inhibition of two hemagglutinating titers of tachylectin-4 against human A-type erythrocytes.

<sup>b</sup> NI, not inhibited at 100 μg/ml or 1000 μg/ml.

DISCUSSION

We previously identified two kinds of lectins with no significant sequence similarity with other known proteins. These were named tachylectin-1 with LPS binding activity (12) and tachylectin-2 with hemagglutinating activity against human A-type erythrocytes (13). In the present study, we identified a horseshoe crab hemocyte lectin, tachylectin-4, with hemagglutinating activity against human erythrocytes and with binding specificity to O-antigen of bacterial LPS. Tachylectin-4 has different characteristics from both tachylectin-1 and tachylectin-2. 1) The minimum agglutination concentration of tachylectin-4 required for human A-type erythrocytes (0.014 μg/ml) is about 100-fold lower than that of tachylectin-2 (1.6 μg/ml) (13). 2) The addition of Ca<sup>2+</sup> at 10 mM enhances the hemagglutinating activity of tachylectin-4 by 4-fold, whereas Ca<sup>2+</sup> is not required for the activity of tachylectin-2 and EDTA has no effect on the activity of tachylectin-2 (13). However, the hemagglutinating activity of tachylectin-4 was completely inhibited by EDTA or α-phenanthroline, indicating that tachylectin-4 contains metal ion(s) with an important role for the sugar binding. 3) The activity of tachylectin-2 is inhibited by 0.1 mM N-acetylglucosamine (13), which had no effect on the activity of tachylectin-4 at 100 mM. On the other hand, D-Δ-fucose (6-deoxy-D-galactose) specifically inhibits the activity of tachylectin-4, with no inhibition for tachylectin-2 (1.6 μg/ml) (13). 4) Tachylectin-2 (27 kDa) exists as a monomer in solution (13), whereas tachylectin-4 is present as a high molecular mass oligomer of 470 kDa. Based on an assumption of 30 kDa for one subunit, tachylectin-4 is composed of 15–16 subunits, under physiological conditions. 5) Tachylectin-1 agglutinates sheep erythrocytes coated with LPS derived from both a wild-type (smooth) and a Re mutant of S. minnesota (12). In contrast, the hemagglutinating activity of tachylectin-4 was inhibited by S-type LPS, not by R-type LPS lacking O-antigen.

The LPS from E. coli O111:B4 was most effective, and the minimum concentration required to inhibit hemagglutination for human A-type erythrocytes was 160-fold and 80-fold lower than those of LPS from S. minnesota (smooth) and S. typhimurium (smooth), respectively. The O-antigen of E. coli O111:B4 is built up by a unique repeating unit of a main chain containing 3,6-dideoxy-L-galactose, 3-β-D-glucose, and 1→N-acetylgalactosamine, and a monosaccharide side chain of colitose (3,6-dideoxy-L-galactose or 3-deoxy-L-fucose) (21). In these monosaccharides, colitose is the most probable candidate for a specific ligand of tachylectin-4, since it is a unique monosaccharide present in the O-antigen of E. coli O111:B4, its structure is similar to that of L-fucose, and three other monosaccharides had no effect on the hemagglutinating activity of tachylectin-4.
On the other hand, the O-antigen of *S. typhimurium* is composed of a main chain containing D-mannose, L-rhamnose, and D-galactose, and two monosaccharide side chains of D-glucose and abequose (3,6-dideoxy-D-galactose or 3-deoxy-D-fucose) (21). Abequose is also a candidate for another ligand, since the hexoses except abequose have no effect on the activity of tachylectin-4 (Table III). Abequose is the D-isomer of colitose, which may cause a reduced affinity to tachylectin-4.

A sequence homology search showed no significant sequence similarity between tachylectin-4 and other known LPS-binding lectins, such as *Limulus* endotoxin-binding protein-protease inhibitor (22), mammalian plasma LPS-binding protein (23), and *Periplaneta* lectins from the American cockroach (11, 24, 25), whereas the search indicates the sequence similarity of tachylectin-4 to *X. laevis* pentraxin 1 (20) (Fig. 4). Pentraxins are decameric or dodecameric proteins composed of identical protomers, arranged in two pentameric or hexameric rings interacting face-to-face (26, 27). They constitute a family of carbohydrate binding proteins with divalent cation dependence, and some pentraxins, such as human C-reactive protein, belong to acute phase proteins, rapidly increasing their concentrations in response to stress, injury or infection (7, 28–30). However, *Xenopus* pentraxin 1 gene, identified from a *Xenopus* cDNA library using pentraxin-specific oligonucleotide probes, unexpectedly encodes a novel fusion protein with an NH₂-terminal domain of the mature protein is shown by an arrowhead. A dotted line and an asterisk represent a poly(A) additional signal and the termination codon, respectively.

Fig. 3. Nucleotide and deduced amino acid sequences of tachylectin-4. A, a restriction map and sequencing strategy. B, the nucleotide and deduced amino acid sequences. Nucleotides and amino acid residues are numbered on the right. A single underline represents sequences determined by amino acid sequence analysis of isolated peptides. Asn-108 circled is an attachment site for N-linked sugar chain. The NH₂-terminal domain of the mature protein is shown by an arrowhead. A dotted line and an asterisk represent a poly(A) additional signal and the termination codon, respectively.
suggest that they serve synergistically to accomplish an effective host defense system against invading microbes and foreign substances.

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FIG. 4. Alignment of the amino acid sequence of tachylectin-4 with that of Xenopus pentraxin 1. A, manual alignment was performed with appropriate gaps. Residues identical to tachylectin-4 are boxed. B, schematic presentation of Xenopus pentraxin 1 and tachylectin-4. Homologous regions are represented by black bars.