Isolation and Characterization of Rhamnose-binding Lectins from Eggs of Steelhead Trout (Oncorhynchus mykiss) Homologous to Low Density Lipoprotein Receptor Superfamily

(Received for publication, February 23, 1998, and in revised form, May 1, 1998)

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Two L-rhamnose-binding lectins named STL1 and STL2 were isolated from eggs of steelhead trout (Oncorhynchus mykiss) by affinity chromatography and ion exchange chromatography. The apparent molecular masses of purified STL1 and STL2 were estimated to be 84 and 68 kDa, respectively, by gel filtration chromatography. Sodium dodecyl sulfate polyacrylamide gel electrophoresis and matrix-assisted laser desorption ionization time of flight mass spectrometry of these lectins revealed that STL1 was composed of noncovalently linked trimer of 31.4-kDa subunits, and STL2 was noncovalently linked trimer of 21.5-kDa subunits. The minimum concentrations of STL1, a major component, and STL2, a minor component, needed to agglutinate rabbit erythrocytes were 9 and 0.2 μg/ml, respectively. The most effective saccharide in the hemagglutination inhibition assay for both STL1 and STL2 was L-rhamnose. Saccharides possessing the same configuration of hydroxyl groups at C2 and C4 as that in L-rhamnose, such as L-arabinose and D-galactose, also inhibited. The amino acid sequence of STL2 was determined by analysis of peptides generated by digestion of the S-carboxymethylated protein with Achromobacter protease I or Staphylococcus aureus V8 protease. The STL2 subunit of 195 amino acid residues proved to have a unique polypeptide architecture; that is, it was composed of two tandemly repeated homologous domains (STL2-N and STL2-C) with 52% internal homology. These two domains showed a sequence homology to the subunit (105 amino acid residues) of D-galactoside-specific sea urchin (Anthocidaris crassispina) egg lectin (37% for STL2-N and 46% for STL2-C, respectively). The N terminus of the STL1 subunit was blocked with an acetyl group. However, a partial amino acid sequence of the subunit showed a sequence similarity to STL2. Moreover, STL2 also showed a sequence homology to the ligand binding domain of the vitellogenin receptor. We have also employed surface plasmon resonance biosensor methodology to investigate the interactions between STL2 and major egg yolk proteins from steelhead trout, lipovitellin, and β'-component, which are known as vitellogenin digests. Interestingly, STL2 showed distinct interactions with both egg yolk proteins. The estimated values for the affinity constant (Kd) of STL2 to lipovitellin and β' component were 3.44 × 10^6 and 4.99 × 10^6, respectively. These results suggest that the fish egg lectins belong to a new family of animal lectin structurally related to the low density lipoprotein receptor superfamily.

Lectins are a group of sugar-binding proteins that recognize specific carbohydrate structures and agglutinate a variety of animal cells by binding to cell-surface glycoproteins and glycolipids. The present state of knowledge permits us to organize the known animal lectins into several categories depending on sequence similarity and common characteristics such as sugar binding specificity, conserved carbohydrate recognition domains, and ion requirements, i.e., C-type, I-type, galectins, pentraxins, and P-type lectin (1–4). Although a number of egg lectins have been isolated from various fish families such as Salmonidae (5–9), Clupeidae (10), and Cyprinidae (11), they have not been classified to any animal lectin family. The fish egg lectins are characterized by their binding affinity to L-rhamnose, with a few exceptions (8, 11). In other species, L-rhamnose-binding lectins have been found only in American cockroach (Periplaneta americana) (12) and Streptomyces 27S5 (13).

The physiological significance of the existence and the structure of the L-rhamnose-binding proteins in fish eggs have not been clarified yet. However, they may be involved in a variety of biological functions, including regulation of carbohydrate metabolism (14), prevention of polyspermy (15, 16), cross-linking of carbohydrate-rich proteins of the fertilization envelope (17), bactericidal effects (6, 18), mitogenesis (19), lectin-mediated cellular cytotoxicity (20), and opsonization of pathogens (7).

In the present paper, we report the isolation and characterization of L-rhamnose-binding lectins from the egg of steelhead trout (Oncorhynchus mykiss) and the complete amino acid sequence of the lectin, STL2. Surprisingly, its tandemly repeated domains are homologous to the ligand-binding domain of the vitellogenin receptor, which belong to the low density lipoprotein (LDL) receptor superfamily. Furthermore, the interaction

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* This work was supported in part by Grants-in-aid for Scientific Research 08306011 from the Ministry of Education, Science, Sports, and Culture of Japan. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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§ The abbreviations used are: LDL, low density lipoprotein; CAM, S-carboxamidomethylated protein; Lv, lipovitellin; MALDI-TOF, matrix-assisted laser desorption ionization time of flight; PAGE, polyacrylamide gel electrophoresis; VLDL, very low density lipoprotein; VTG, vitellogenin; HPLC, high performance liquid chromatography; STLs, steelhead trout lectins; SUEL, sea urchin egg lectin.

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of STL2 with two major egg yolk proteins, lipovitellin (Lv) and β9 component, which derived from vitellogenin, was demonstrated by a biosensor based on surface plasmon resonance (21). Based on their structural properties and kinetic measurements, we discuss the biological functions of the L-rhamnose-specific lectins from fish eggs.

EXPERIMENTAL PROCEDURES

Materials—The eggs were obtained from mature steelhead trout (5–6 years old, 2–3 kg weight) cultured in Tomakomai Experimental Forest Station, Hokkaido University, Hokkaido, Japan. Achromobacter protease I, Staphylococcus aureus V8 protease, and α-L-rhamnose monohydrate were purchased from Wako Pure Chemical Ind. (Osaka, Japan). Epoxy-activated Sepharose 6B, Superdex 200, and HiTrap Q column were purchased from Amersham Pharmacia Biotech. An L-rhamnose-Sepharose 6B gel was prepared according to the directions by Amersham. All other reagents were the purest grade commercially available.

Isolation of Steelhead Trout Egg Lectins—Eggs (5 kg) was homogenized and defatted with acetone and filtered through filter paper, and the filtrate was dried at room temperature to yield 1.7 kg of the acetone powder. The acetone powder (50 g) was homogenized with 300 ml of ice-cold 0.15 M NaCl containing 0.15 M NaCl and eluted with a linear gradient of 0 to 1 M NaCl in the same buffer. AUFS, absorbance units at full scale.

FIG. 1. Purification of steelhead trout egg lectins by affinity chromatography on a L-rhamnose-Sepharose 6B column. The fish egg lectins were adsorbed on 50 ml of L-rhamnose-Sepharose 6B gel for 10 h at 4 °C batchwise, and then the gel was placed in a glass column (3 × 10 cm). STLs were eluted with 0.2 M L-rhamnose in 50 mM sodium acetate buffer (pH 5.5) containing 0.15 M NaCl. Lectin fractions indicated by a bar were combined and further purified.

FIG. 2. Purification of STLs by anion exchange chromatography on a HiTrap Q column. STLs obtained from affinity chromatography were further purified by anion exchange chromatography on a HiTrap Q column (5 ml) equilibrated with 20 mM Tris-HCl buffer (pH 9.0) and eluted with a linear gradient of 0 to 1 M NaCl in the same buffer. AUFS, absorbance units at full scale.

FIG. 3. SDS-PAGE of STL1 and STL2 before and after reduction with 2-mercaptoethanol. Lanes 1 and 3 are STL1 and lanes 2 and 4 are STL2, respectively. Lanes 3 and 4 are reduced with 2-mercaptoethanol. Bovine albumin (66,000), ovalbumin (45,000), carbonic anhydrase (31,000), and soybean trypsin inhibitor (21,500) were used as standard markers.

FIG. 4. Thermostability of STL1 and STL2. Hemagglutinating activities were measured after preincubation of STLs in 20 mM Tris-HCl (pH 7.0) containing 0.15 M NaCl for 0 to 120 min at different temperatures. ■, 40 °C; □, 50 °C; ●, 60 °C; ○, 70 °C.

of STL2 with two major egg yolk proteins, lipovitellin (Lv) and β' component, which derived from vitellogenin, was demonstrated by a biosensor based on surface plasmon resonance (21). Based on their structural properties and kinetic measurements, we discuss the biological functions of the L-rhamnose-specific lectins from fish eggs.

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Isolation of Steelhead Trout Egg Lectins—Eggs (5 kg) was homoge-
sorbed substances were removed by washing the gel with 50 mM sodium acetate buffer (pH 5.5) containing 0.15 M NaCl (ABS buffer). The gel was packed in a column (3.0 × 10 cm) and washed with ABS, and then the adsorbed substance was eluted with 0.2 M l-rhamnose in ABS. The fractions with significant absorption at 280 nm were collected and dialyzed against 20 mM Tris-HCl buffer (pH 7.5). The lectin fraction was further purified by anion exchange chromatography on a HiTrap Q column (5 ml, Amersham) pre-equilibrated with 20 mM Tris-HCl buffer (pH 8.0). The lectin solution was tested for hemagglutination activity in the absence or presence of Ca$^{2+}$ or Mg$^{2+}$ ions in 0.15 M NaCl.

Molecular Weight Determination of Proteins and Proteolytic Products—The molecular weights of STLs and their subunits were determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), gel filtration and matrix-assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry (Kompakt MALDI I, Shimadzu, Kyoto, Japan). SDS-PAGE was performed according to the method of Laemmli (49) using 15% separating gel in the presence or absence of 2-mercaptoethanol, and protein bands were stained by Coomassie Brilliant Blue R-250. Gel filtration was performed on a Superdex 200 column (1 × 30 cm) eluted with 20 mM Tris-HCl buffer (pH 7.5) containing 0.15 M NaCl and 10 mM l-rhamnose.

For MALDI-TOF mass spectrometry, proteins were embedded in a sinapinic acid (3, 5-dimethoxy-4-hydroxycinnamic acid) matrix, which absorbs UV-light, for analysis. Desorption and ionization of the mixed sample and matrix were induced by a nitrogen laser at 337 nm with a pulse width of 3 ns. 100 single-shot spectra were averaged to improve the signal-to-noise ratio. A second measurement was performed with insulin (M, 5,734.5) (bovine pancreas, Sigma) as an internal standard. Thus, it was possible to determine the molecular weight of the samples with an accuracy of 0.1%.

Preparation of S-Carboxamidomethylated (CAM)-STL2—STL2 was reduced with 10 mM dithiothreitol in 0.25 M Tris-HCl buffer (pH 8.6) containing 10 mM EDTA and 6 M guanidine hydrochloride at 37 °C for 2 h and reacted with 20 mM iodoacetamide for 30 min at room temperature with shielding from light to convert freshly generated cysteine residues into S-carboxamidomethyl cysteine. Excess reagent was removed by gel filtration on a HiTrap desalting column (5 ml, Amersham) pre-equilibrated with 0.1 M NH$_4$HCO$_3$ (pH 8.0).

Enzymatic Digestion and Separation of Peptides—CAM-STL2 (50 nmol) was dissolved in 1 ml of 50 mM Tris-HCl (pH 9.0) containing 1 M urea and digested with Achromobacter protease 1 (S/E = 100:1) at 37 °C for 16 h. CAM-STL2 (50 nmol) was also digested with S. aureus V8 protease (S/E = 50:1) in 1 ml of 50 mM sodium phosphate buffer (pH 7.8) in the presence of 1 M urea at 37 °C for 12 h. Each digest was separated by reversed-phase HPLC on a TSKgel ODS 120T column (5 μm, inner diameter 4.6 mm × 250 mm) ( Tosoh, Tokyo, Japan) using a gradient of acetonitrile in 0.1% trifluoroacetic acid.

\[
\begin{array}{|c|c|c|}
\hline
\text{Saccharide}^a & \text{STL1} & \text{STL2} \\
\hline
\text{l-Rhamnose} & 0.3 & 0.3 \\
\text{Mellibiose} & 12.5 & 3.1 \\
\text{l-Arabino} & 25 & 100 \\
\text{D-Galactose} & 50 & 50 \\
\text{Lactose} & 100 & 100 \\
\text{Others}^b & >200 & >200 \\
\hline
\end{array}
\]

\[\text{Saccharide}^a \text{ Minimum concentration of saccharides required for complete inhibition.}]

\[\text{Saccharide}^b \text{ p-Glucose, } \text{D-mannose, } \text{D-xylose, } \text{D-fucose, } \text{D-acetylglucosamine, } \text{D-acetylgalactosamine, mucine type 1, asialomucine type 1, and fe-}

\text{tain.}]

\text{mulated by the minimum concentration of sugar needed to cause negative hemagglutination.}

The thermostability of the lectins was examined by the hemagglutination assay described above after incubating for various periods at 40, 50, 60, or 70 °C. To test the dependence of divalent cations on hemagglutination, the lectins were treated in 0.1 M EDTA for 15 min at room temperature and dialyzed against 0.15 M NaCl at 4 °C overnight. The lectin solution was tested for hemagglutination activity in the absence or presence of Ca$^{2+}$ or Mg$^{2+}$ ions in 0.15 M NaCl.

Rhamnose-binding Lectins from Steelhead Trout Eggs

FIG. 5. Determination of the molecular masses of STLs by matrix-assisted laser desorption/ionization time of flight mass spectrometry. Int., intensity.
Amino Acid Sequence Analysis—Amino acid compositions were determined by the precolumn labeling method (23, 24) using 4-dimethylaminoazobenzene-4-sulfonyl chloride (Pierce). The amino acid sequences of purified proteins and proteolytic peptides were determined by a gas-phase protein sequencer (Shimadzu PSQ-1). Molecular mass determination of the peptides was performed by using MALDI-TOF mass spectrometry as described above. Homologous sequences were searched by FASTA program (25) accessed by Genome Net WWW2.1. Hydropathy profile analysis and the secondary structure prediction of STL2 were performed on the basis of the primary structure according to the methods of Kyte and Doolittle (26) and Chou and Fasman (27), respectively.

Surface Plasmon Resonance Analysis of the Interaction between STL2 and Egg York Proteins—BIAcoreTM (Amersham), which is based on

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FIG. 6. Separation of peptides generated by digestion of the CAM-STL2 with Achromobacter protease I (A) and S. aureus V8 protease (B). Peptides were separated by reversed-phase HPLC on a TSKgel ODS 120T column (5 μm, inner diameter 4.6 mm × 250 mm) using a gradient of acetonitrile in 0.1% trifluoroacetic acid. The flow rate was 1 ml/min. AUFS, absorbance units at full scale.

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were diluted to a concentration of 10 and 50 g/ml. A single-site binding model was adopted for the procedure of Hara and Hirai (28). Purified Lv and S. aureus L-rhamnose were analyzed by nonlinear least squares curve fitting using the BIAevaluation software. Purified Lv showed the purification results of STLs. The yields of STL1 and STL2 from 50 g of saline extract of eggs were 170 and 120 μg/ml, respectively. The low recovery of lectins from affinity chromatography was mainly due to the precipitation during dialysis. Isolation and Molecular Characterization of STLs—Amino Acid Sequence Analysis—The N-terminal amino acid sequence of CAM-STL2 identified the first 38 residues including three CAM-cysteine. The enzymatic digest of CAM-STL2 (50 nmol) with Achromobacter protease I and S. aureus V8 protease was separated by reversed-phase HPLC as shown in Fig. 6. Each generated peptide, designated L1–L8 for Achromobacter protease I and V1–V9 for S. aureus V8 protease, was isolated and subjected to amino acid analysis and MALDI-TOF mass spectrometry (Table III). The N-terminal sequence of L8 was identical to that of native protein (Fig. 6). The successive amino acid sequences of L1–L6, V1–V7, and V9 were determined up to their C terminus by a gas phase protein sequencer. Gel filtration on Superdex 200 showed that the approximate molecular masses of STL1 and STL2 were 84 and 68 kDa, respectively (data not shown). Each STL gave single bands at 28 kDa (STL1) and 22 kDa (STL2) on SDS-PAGE in the absence of 2-mercaptoethanol and at 35 kDa (STL1) and 28 kDa (STL2) in the presence of 2-mercaptoethanol (Fig. 3), suggesting that STL1 and STL2 possess internal disulfide bonds. Furthermore, the MALDI-TOF mass spectrometry of STL1 gave a predominant peak at 31.4 kDa and minor peaks at 63 and 94 kDa, and STL2 gave a predominant peak at 21.5 kDa and minor peaks at 43 and 64.5 kDa (Fig. 4). These results indicate that STL1 is a noncovalently linked trimer consisting of the 31.4-kDa subunits and that STL2 is a noncovalently linked trimer consisting of 21.5-kDa subunits. The isoelectric points of STL1 and STL2 were measured to be 6.75 and 4.70, respectively, by isoelectric focusing gel electrophoresis (data not shown).

RESULTS

Isolation and Molecular Characterization of STLs—A fraction containing lectin was obtained from the acetone powder of the steelhead trout eggs by means of affinity chromatography on L-rhamnose-Sepharose 6B (Fig. 1). The fraction obtained from affinity chromatography gave two peaks on a HiTrap Q anion exchange chromatography (Fig. 2). Since both peaks contained protein showing strong agglutination activity on rabbit erythrocytes and gave a single protein band on SDS-PAGE (Fig. 3), proteins isolated from the first and second peaks were designated as STL1 and STL2, respectively. Table I summarizes the purification results of STLs. The yields of STL1 and STL2 from 50 g of saline extract of eggs were 170 and 120 μg/ml, respectively. The low recovery of lectins from affinity chromatography was mainly due to the precipitation during dialysis before ion exchange chromatography. Purified STL2 showed more potent hemagglutinating activity (0.2 μg/ml) than did STL1 (9 μg/ml).

Sugar Specificity—In the hemagglutination inhibition assay using rabbit erythrocytes, L-rhamnose was the most potent monosaccharide inhibitor for both STL1 and STL2 (Table II). Melibiase, L-arabinose, and D-galactose, which possess the same hydroxyl group orientation at C2 and C4 of the pyranose ring structure of L-rhamnose, also showed inhibitory effects. On the other hand, other monosaccharides tested (Table II) showed no inhibitory activity even at concentrations of 0.2 M. Glycoproteins such as mucine type 1, asialomucine type 1, and fetuin also did not show any inhibition at concentrations up to 0.1%.

Amino Acid Sequence Analysis—The N-terminal amino acid sequence of CAM-STL2 identified the first 38 residues including three CAM-cysteine. The enzymatic digest of CAM-STL2 (50 nmol) with Achromobacter protease I and S. aureus V8 protease was separated by reversed-phase HPLC as shown in Fig. 6. Each generated peptide, designated L1–L8 for Achromobacter protease I and V1–V9 for S. aureus V8 protease, was isolated and subjected to amino acid analysis and MALDI-TOF mass spectrometry (Table III). The N-terminal sequence of L8 was identical to that of native protein (Fig. 6). The successive amino acid sequences of L1–L6, V1–V7, and V9 were determined up to their C terminus by a gas phase protein sequencer. Gel filtration on Superdex 200 showed that the approximate molecular masses of STL1 and STL2 were 84 and 68 kDa, respectively (data not shown). Each STL gave single bands at 28 kDa (STL1) and 22 kDa (STL2) on SDS-PAGE in the absence of 2-mercaptoethanol and at 35 kDa (STL1) and 28 kDa (STL2) in the presence of 2-mercaptoethanol (Fig. 3), suggesting that STL1 and STL2 possess internal disulfide bonds. Furthermore, the MALDI-TOF mass spectrometry of STL1 gave a predominant peak at 31.4 kDa and minor peaks at 63 and 94 kDa, and STL2 gave a predominant peak at 21.5 kDa and minor peaks at 43 and 64.5 kDa (Fig. 4). These results indicate that STL1 is a noncovalently linked trimer consisting of the 31.4-kDa subunits and that STL2 is a noncovalently linked trimer consisting of 21.5-kDa subunits. The isoelectric points of STL1 and STL2 were measured to be 6.75 and 4.70, respectively, by isoelectric focusing gel electrophoresis (data not shown).

STL1 completely lost its hemagglutinating activity by heating at 50 °C for 90 min, whereas STL2 retained half of the activity under the same conditions (Fig. 5). However, STL2 was inactivated by heating at 70 °C for 10 min. STLs maintained their hemagglutination activities between pH 4 and 7 (data not shown). No appreciable change was seen in the hemagglutinating activities of STL1 and STL2 after treatment with 0.1 M EDTA, whereas no enhanced activity was observed by the addition of Ca²⁺ or Mg²⁺ ion (data not shown).

FIG. 7. Detailed summary of sequence determination of STL2. The proven sequences of specific peptides are given in one-letter code (Table III) below the summary sequence. L and V demonstrate the peptides generated by cleavage of the CAM protein with Achromobacter protease I and S. aureus V8 protease, respectively. Peptides sequences in uppercase letters were proven by Edman degradation, and those in lowercase letters indicate tentative identification or that deduced from amino acid compositions and MALDI-TOF mass spectrometry.

FIG. 8. Hydrophathy profiles of STL2 and SUEL. Analysis was performed by the method of Kyte and Doolittle (29).
L7 and L2 and between L2 and L1. Thus, the complete amino acid sequence of CAM-STL2 was determined as shown at the top of Fig. 7. STL2 was composed of 195 amino acid residues with a molecular mass calculated to be 21,349 Da, which is in good agreement with the value (21,500 Da) obtained from MALDI-TOF mass spectrometry. STL2 contained 16 half-Cys and 22 Thr residues. On the other hand, no His, Met, or Trp residues were present. Sugar analysis of CAM-STL2 showed no evidence for the existence of sugar chains in the molecule as the representative sensorgrams (7.8–62.5 μg/ml) are shown in Fig. 10. The binding parameters were estimated by nonlinear least squares curve fitting on the bases of the subunit monomer (21.5 kDa). The association rate constant (kₐss) of STL2 with Lv and β component were 1.10 × 10⁴ M⁻¹ s⁻¹ and 8.04 × 10³ M⁻¹ s⁻¹, respectively. The affinity constants, Kᵣ, for the STL2-Lv and STL2-β component interactions were calculated to be 4.99 × 10⁶ M⁻¹ and 3.44 × 10⁵ M⁻¹, respectively. These interactions were completely inhibited by 0.2 M L-rhamnose (Fig. 11). When 0.2 mM glucose was co-injected with STL2, however, no significant decrease in surface plasmon resonance was observed (data not shown). These results suggested that the interactions between STL2 and egg yolk proteins, Lv and β component, were L-rhamnose-specific.

**DISCUSSION**

Two L-rhamnose-binding lectins, STL1 and STL2, were isolated from eggs of steelhead trout. The complete amino acid sequence of STL2 has been determined (Fig. 7). STL2 was found to consist of 195 amino acid residues consisting of two tandem repeat domains with 52% amino acid homology (Fig. 9). Fifty-two percent of amino acids were identical between the N-terminal domain (STL2-N) and the C-terminal domain (STL2-C). All half-Cys residues were conserved between two tandem repeats. The hydrophobicity profiles were also similar to each other (Fig. 8).

To explore the sequence homology between STL1 and STL2, STL1 was subjected to a gas-phase protein sequencer after S-carboxamidomethylation. Initial attempts at amino acid sequencing revealed that the N terminus of STL1 is blocked. Therefore, CAM-STL1 was unblocked by treating with trifluoroacetic acid according to the method of Gheorghe et al. (29) to remove a possible N-acetyl group, repurified, and sequenced successfully through the first 34 residues. The N-acetyl group of STL1 was confirmed by MALDI-TOF mass spectrometry of the N-terminal peptide of CAM-STL1, prepared by digesting with Achromobacter protease I (data not shown).

**Interaction of STL2 with Immobilized Egg Yolk Proteins**—Specific dose-dependent sensorgrams of STL2 were observed in both cases of the Lv- and β component-immobilized surfaces. The representative sensorgrams (7.8–62.5 μg/ml) are shown in Fig. 10. The binding parameters were estimated by nonlinear least squares curve fitting on the bases of the subunit monomer (21.5 kDa). The association rate constant (kₐss) of STL2 with Lv and β component were 1.10 × 10⁴ M⁻¹ s⁻¹ and 8.04 × 10³ M⁻¹ s⁻¹, respectively. The affinity constants, Kᵣ, for the STL2-Lv and STL2-β component interactions were calculated to be 4.99 × 10⁶ M⁻¹ and 3.44 × 10⁵ M⁻¹, respectively. These interactions were completely inhibited by 0.2 M L-rhamnose (Fig. 11). When 0.2 mM glucose was co-injected with STL2, however, no significant decrease in surface plasmon resonance was observed (data not shown). These results suggested that the interactions between STL2 and egg yolk proteins, Lv and β component, were L-rhamnose-specific.
terminal sequences of fish egg lectins including STL1 with the amino acid sequences of the two tandem domains of STL2 and SUEL. The N-terminal sequence homologies among the fish egg lectins were not so high, with only 21 to 46% identities. However, the segment Tyr-Gly-Arg is conserved throughout the lectins. Fish egg lectins have very characteristic properties compared with other animal lectins, because most of them have specific binding affinity to L-rhamnose. Some exceptions include β-glucosyl/-fucose-binding lectins from Perca fluviatilis and Dicentrarchus labrax and lectin from Petromyzon marinus, which is specific to sialoglycoconjugate (11). Furthermore, it has been reported that fish eggs or serum contains multiple L-rhamnose-binding isolectins that widely vary in molecular weight and other properties (38, 39). In the present study, it was found that steelhead trout eggs contain two distinct isolectins with subunits of different molecular sizes, different hemagglutinating activities, and different binding specificities for some saccharides (Table II). STL2 showed greater affinity for melibiose than did STL1, whereas STL1 showed greater affinity for L-arabinose than did STL2. In addition, STL1 was blocked by an N-acetyl residue. These observations suggest that fish eggs possess multiple isolectins with diverse molecular properties.

Further studies are needed to determine the physiological significance of multiple lectins as well as their physiological function. The hemagglutination activity of STLs was most effectively inhibited by L-rhamnose and weakly inhibited by D-galactose and L-arabinose, which have the same hydroxyl group orientation at C2 and C4 as L-rhamnose. On the other hand, SUEL is exclusively specific to D-galactose. Therefore, valuable information concerning the relationship between the structure and sugar binding specificity must be obtained by investigating the carbohydrate recognition domains of STLs and SUEL. Since L-rhamnose is not a naturally occurring sugar in vertebrates, the physiological significance of the L-rhamnose-binding lectin is puzzling. However, the ligands for L-rhamnose-binding lectins in animals are not necessary to have a L-rhamnose moiety in the molecules if their binding activities to unknown glycoconjugates are taken into account. One of the physiological roles of fish egg lectins is as a defense mechanism against pathogenic bacteria. The fish egg lectins from Oncorhynchus tschawytscha and Rutilus rutilus showed antibacterial activity (6, 8), those from Oncorhynchus rhodurus

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**Fig. 11.** Sensorgrams showing the inhibitory effect of L-rhamnose on the interactions of STL2 with immobilized Lv (A) and β component (B). STL2 was injected onto the sensor chip at the concentration of 62.5 μg/ml in the presence or absence of 0.2 M L-rhamnose.

**Fig. 12.** Comparison of amino acid sequences of STL2 and mammalian VLDL receptors. Partial amino acid sequences of mammalian VLDL and VTG/LDL receptors corresponding to ligand binding domain (Glu-103→Pro-274, repeat 3→6) are aligned. Boxes show identical residues, and the stippled box shows the conserved half-cystine residues.

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showed an opsonic effect (7), and those from Oncorhynchus keta showed bacterial agglutination activity (5). We have not obtained any evidence that STLs play a role in defense mechanisms, as STLs did not show an antibacterial activity against Escherichia coli or Bacillus subtilis (data not shown).

A new family of animal lectins homologous to STL2 are present not only in fish but also in other animals such as sea urchin. SUEL has been suggested to be involved in the fertilization and development of the embryo (41). SUEL was first accumulated in the cortical cytoplasm of embryos and then incorporated into the extracellular hyaline layer at least until the mid gastrula stage. For fish egg lectins, their involvement in the fertilization and development of the embryo has not been demonstrated yet. An immunofluorescence study showed that some fish egg lectins were associated exclusively with the content and surrounding membrane of cortical vesicles situated within the cytoplasm of maturing oocytes (16). We observed that the hemagglutinating activity of chum salmon egg homogenerate varied with the stages of development (5). The activity was reduced markedly after the eyed stage and disappeared just before hatching.

It should be noted that STL2 showed sequence similarity to the ligand binding domains of the mammalian LDL receptor and very low density lipoprotein (VLDL) receptor (Fig. 12). The LDL receptor superfamily has a ligand binding domain composed of cysteine-rich repeats, epidermal growth factor precursor-type repeats, an O-linked sugar domain, a transmembrane domain, and a cytoplasmic domain (42). Although the sequence homology is less than 30%, it is apparent that the sequence of STL2 can be matched to the region between repeat 3 and repeat 6 in the ligand binding domain of the LDL receptor (Fig. 12). Recently, it was discovered that the receptor for yolk lipoprotein, VLDL and vitellogenin (VTG) in chicks belongs to the LDL receptor superfamily (43, 44). VTG is a lipophosphoglycoprotein that is produced under female hormonal control in liver and is transported in the circulation to the female gonads as a precursor of egg proteins. Recently, it was reported that the VTG in fish is immediately processed into the Lv, β' component, and phosvitin by cathepsin D like protease in eggs (45). The present study showed that STL2 interacted to the major egg yolk proteins, Lv and β' component, with Ks of 10^4 M levels. Although the VTG receptor has been isolated from the egg of rainbow trout (46, 47), we do not know the relationship between the VTG receptor and STL2 because of the lack of sequence information of the fish VTG receptor. However, it is not probable that STL2 is derived from the VTG receptor, since STL does not have the consensus sequences for the modular repeats characteristic of LDL receptor superfamily members as revealed with the mosquito VTG receptor (48). In mammals, VTG receptors localized in coated pits on the surface of growth component oocytes are able to accumulate the VTG and other ligands in the yolk with high concentrations (42). Nosek et al. (16) confirmed that fish egg lectins are associated with the cortical vesicles of maturing oocytes in immunofluorescence studies. The most attractive hypothesis regarding the biological functions of fish egg lectins is that they could decode the carbohydrate moieties of glycoproteins and shuttle them into the proper compartment (14). Our observations that STL2 interacts with egg yolk proteins supports the hypothesis.

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