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Purification and Partial Characterization of 76 kDa Transglutaminase in the Egg Envelope (Chorion) of Rainbow Trout, *Oncorhynchus mykiss*[1]

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Transglutaminase (TGase), responsible for crosslinking between proteins, is known to be localized exclusively in the egg envelope (chorion) of rainbow trout, *Oncorhynchus mykiss*, and probably participates in the post-fertilization chorion hardening. We purified the TGase from unfertilized egg chorions by sequential chromatography using SP-Sepharose, Q-Sepharose, and TSK-gel G3000SWXL columns. The purified enzyme was a monomeric protein having the molecular mass of 76 kDa. It promoted incorporation of monodansylcadaverine into chorion protein and catalyzed the polymerization of chorion subunit proteins. The effect of various reagents suggested that the chorion TGase is a Ca^{2+}-dependent SH-enzyme similar to the well-characterized TGases of various animals. The highest activity was observed at pH 6.0. The amines examined in the present study inhibited the TGase activity of the purified enzyme. However, they did not necessarily cause effective inhibition of its activity. These properties of the chorion TGase were essentially consistent with our previous observations on polymerization of chorion proteins, resulting in chorion hardening. We compared the amino acid composition of the purified TGase with those of the previously characterized TGases of fishes, such as chum salmon and red sea bream. The results suggest that the chorion 76 kDa TGase is not homologous with those liver TGases in terms of amino acid composition.

Key words: chorion hardening, egg envelope, purification, rainbow trout, transglutaminase.

In various animals, the vitelline envelope or unfertilized egg envelope is known to change into fertilized egg envelope with the participation of enzymes such as ovoperoxidase (*9, 20, 42*), transglutaminase (*2, 4, 9*), and a protease (*59*) in sea urchin eggs, a protease, ovochymase, in *Xenopus* eggs (*11, 12, 31, 32*), or a protease (*38, 60*) and ovoperoxidase (*14, 49*) in mouse eggs. In addition, cortical granular lectin (*41, 61, 62*) participates in the change of *Xenopus* egg envelope.

In some fishes, the change involves 2 processes: polymerization of subunit proteins of egg envelope (chorion) by transglutaminase (TGase) (*17, 18, 24, 26, 36, 37, 44*) and limited hydrolysis of a chorion subunit protein by metalloprotease (*16*). The polymerization by TGase is suggested to result in hardening of the chorion by formation of ε-(γ-glutamyl)lysine crosslinks between the chorion subunit proteins. Recent studies showed that the TGase is localized exclusively in unfertilized egg chorion of medaka, *Oryzias latipes* (*25, 37*) and rainbow trout, *Oncorhynchus mykiss* (*17, 24*). In the previous study (*17*), we extracted TGase activity from the chorions and examined some of its properties. However, the TGase of fish egg chorion, including sea urchin egg TGase, has never been isolated as a homogeneous form.

Transglutaminase (protein-glutamine γ-glutamyltransferase; EC 2.3.2.13) is well known to participate in blood clotting (*5, 51*), hair formation (*35*), keratin formation (*57*), or copulatory plug formation (*50*). Although its physiological functions are not precisely clarified, mainly because the natural substrates have not been clearly identified, the occurrence of other types of TGase is known in liver (*10*), in erythrocytes (*52*), and in various types of cells (*27*). Recently, it has been suggested that TGase plays a role in apoptotic death of certain types of cells (*8*). As for invertebrates, there are some reports that *Limulus* hemocyte TGase participates in blood coagulation (*58*), and that annulin of grasshopper embryo is homologous to TGase and participates in some morphogenetic activities of the cells (*53*). In the case of fishes, such as red sea bream (*63*) and chum salmon (*47*), the amino acid sequences of liver cell type TGases were deduced from the analysis of their cDNAs. However, the physiological function of those TGases is not yet clear. The chorion TGase purified in the present study plays a significant role in fertilization/activation-associated chorion hardening. This is the first purification of a fish chorion TGase.

MATERIALS AND METHODS

Unfertilized eggs of the rainbow trout, *O. mykiss*, were obtained from the Irikawa Trout Hatchery (Tokyo), the

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Oizumi Experimental Station of Tokyo University of Fisheries (Yamanashi) and the Fuji Trout Farm (Shizuoka). After artificial spawning, the eggs, kept in coelomic fluid, were transported to our laboratory at low temperature (0-4°C). When the unfertilized eggs were stored in an ice-cold water bath, they showed neither any sign of activation such as perivitelline space formation, nor any sign of egg degeneration such as oil droplet assembly, for several days.

Isolation of Egg Envelope (Chorion)—Unfertilized eggs were put into 143 mM NaCl-10 mM Tris·HCl (pH 7.2) and cut up with small scissors. The egg contents were removed by pipetting, then the chorions were washed with the same solution and stored in a freezer until use.

Determination of TGase Activity—TGase activity was determined essentially according to the method of Lorand and Gotoh (*34*) or Ha and Iuchi (*17*). The standard reaction mixture (1 ml) consisted of 0.5 mM monodansyl-cadaverine (MDC), 0.2% dimethylcasein, 5 mM 2-mercaptoethanol (2-ME), 5 mM $CaCl_2$, 50 mM Tris·HCl (pH 7.2) and various amounts of enzyme preparation. After incubation at 10°C, the reaction was stopped by adding ice-cold trichloroacetic acid (final concentration, 5%). The precipitate was washed 3 times with 3-5 ml of ethanol-ether (1 : 1), dried with the aid of a vacuum pump and dissolved in 3 ml of 8 M urea-0.5% sodium dodecylsulfate (SDS)-50 mM Tris·HCl (pH 8.0). After the dried precipitate was completely dissolved, the intensity of fluorescence of the solution was measured on a fluorescence spectrophotometer (F-2000, Hitachi, Tokyo) with excitation at 355 nm and emission at 525 nm. Fluorescence of 1 nmol MDC/ml was used as a standard. For human blood coagulation factor XIII (fibrinoligase), the unit of TGase activity has been defined as AIU (amine incorporation unit per min at 37°C) (*34*). However, in the present study, 1 unit of chorion TGase activity was tentatively defined as 1 nmol of MDC incorporated into dimethylcasein per hour at 10°C (MIU: MDC incorporation unit).

Determination of Protein Content—Protein content was measured according to the method of Smith *et al.* (*55*) using bovine serum albumin as a standard (BCA Protein Assay Reagent, Pierce, Rockford). Throughout the purification, the protein amount was monitored by measuring the absorbance at 280 nm.

Purification Procedures—We extracted and purified the TGase from the isolated chorions. All the procedures were performed at low temperature (about 4°C). The standard procedure was as follows:

Step1: About 11,000 isolated chorions (about 48 g in wet weight) were homogenized with about 95 ml of 143 mM NaCl-10 mM Tris·HCl (pH 7.2), and the homogenate was centrifuged at 28,000×*g* for 30 min. The extract thus prepared was used as the starting material for purification.

Step 2: The crude extract from the chorions was dialyzed against 10 mM Tris·HCl (pH 7.2)-5 mM $CaCl_2$-5 mM 2-ME (Buffer A) overnight at 4°C. A small amount of precipitate was frequently formed during the dialysis. After centrifugation at 28,000×*g* for 10 min, the supernatant was applied to an SP-Sepharose column (9×1.7 cm; column volume, 15 ml; Pharmacia, Uppsala) equilibrated with Buffer A and the run-off fraction was collected.

Step 3: The run-off fraction was applied to a Q-Sepharose column (4.5×2 cm; column volume, 15 ml; Pharmacia) equilibrated with Buffer A. The column was washed with about 60 ml of Buffer A, and elution was performed with a linear gradient of 0-500 mM NaCl buffered with Buffer A. Fractions of 2.6 ml were collected. The chromatographic pattern is shown in Fig. 1. TGase activity-containing fractions eluted at the NaCl concentration of about 260-320 mM were pooled and subjected to the next step.

Step 4: The TGase fractions thus prepared were dialyzed against 60%-saturated ammonium sulfate buffered with Buffer B (143 mM NaCl-10 mM Tris HCl-5 mM $CaCl_2$-5 mM 2-ME, pH 7.2) overnight at 4°C. Fifty to 60% of the TGase activity was found in the precipitate fraction. After centrifugation at 28,000×*g* for 30 min, the precipitate was dissolved in about 0.75 ml of Buffer B, applied to a TSK-gel G3000SWXL column (0.78×30 cm, Tosoh, Tokyo) equilibrated with Buffer B and fractionated by HPLC. Gel filtration chromatography presented two major protein peaks, P1 and P2, as shown in Fig. 2a. The P2 fraction had the TGase activity. The fractions were pooled and rechromatographed by the same method. A final fractionation profile is shown in Fig. 2b. To stabilize the TGase activity, 5 mM $CaCl_2$ and 5 mM 2-ME were added to all TGase samples (*17, 54*).

Incorporation of MDC into Chorion and Polymerization of Chorion Subunits Catalyzed by Purified TGase—Chorions isolated from unfertilized eggs were incubated in 143 mM NaCl-100 mM ethylenediaminetetraacetic acid (EDTA, pH 7.2) at 60°C for 20 min in order to inactivate the endogenous TGase activity (*17*). The chorions thus prepared were washed several times with the same solution and finally with 10 mM Tris·HCl (pH 7.2), and homogenized in the 10 mM Tris·HCl buffer (pH 7.2). The chorion homogenate and the P2 fraction rechromatographed on the gel filtration column were mixed with 0.5 mM MDC-5 mM $CaCl_2$-5 mM 2-ME-50 mM Tris·HCl (pH 7.2) and incubated at 10°C. The amount of MDC incorporated into chorion proteins was determined by the same method as described in "*Determination of TGase Activity.*"

Polymerization of chorion subunits was examined essentially according to the method of Ha and Iuchi (*17*). We

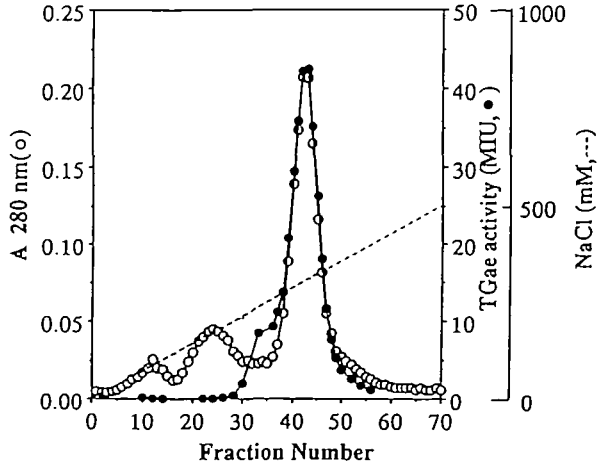

Fig. 1. **Q-Sepharose column chromatographic pattern at purification step 3.** ○, ●, and ···· show protein content measured in terms of absorbance at 280 nm, TGase activity expressed in MIU (MDC incorporation unit), and the concentration gradient of NaCl in the eluent, respectively. Abscissa, fraction number.

used unfertilized egg chorions whose TGase activity had been inactivated by incubation at 60°C for 20 min. The chorion homogenate and the P2 fraction were mixed with 5 mM $CaCl_2$-5 mM 2-ME-10 mM Tris·HCl (pH 7.2) and incubated at 10°C. After the reaction was stopped by adding EDTA (final concentration, 200 mM), the chorion homogenate was boiled in 12 M urea-100 mM phosphate (pH 6.8). The chorion sample thus prepared was subjected to SDS-polyacrylamide gel electrophoresis.

SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)—SDS-PAGE was carried out essentially according to the method of Laemmli (*29*) using 7.5% gel. Protein samples for SDS-PAGE were boiled in 1% SDS-3.1% 2-ME-100 mM phosphate buffer (pH 6.8) for 5 min. To estimate the molecular mass, the SDS molecular weight marker kit (MW-SDS-200, Sigma, St. Louis) was used as standard proteins: myosin (205 kDa), β-galactosidase (116 kDa), phosphorylase *b* (97.4 kDa), bovine serum albumin (66 kDa), egg albumin (45 kDa), and carbonic anhydrase (29 kDa). Gels were stained with Coomassie Brilliant Blue R250 (Sigma).

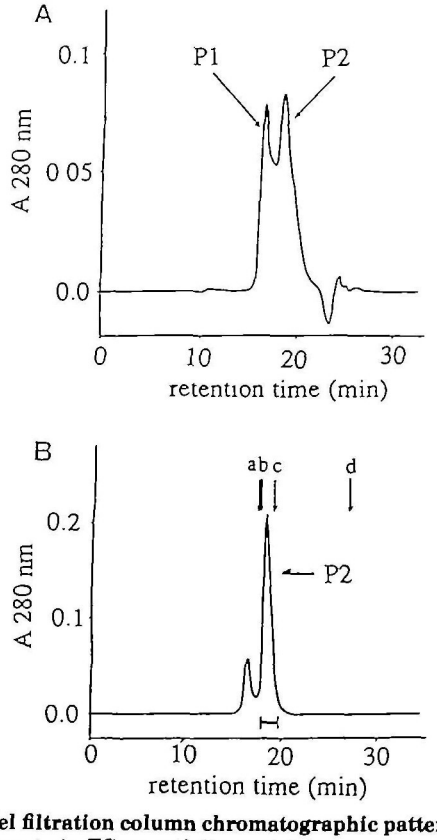

Fig. 2. **Gel filtration column chromatographic pattern at purification step 4.** A: TGase activity-containing fractions obtained at step 3 (Fig. 1) were combined, applied to TSK-gel G3000SWXL column (0.78×30 cm, Tosoh, Tokyo), and fractionated by HPLC as described in the text. The protein amount was monitored by measuring the absorbance at 280 nm. Two major protein peaks were obtained and tentatively named P1 and P2 in the present study. Abscissa, retention time (min). B: The fractions P2 in A were pooled and rechromatographed by the same method as in A. Arrows a, b, c, and d show retention times for standard proteins, alcohol dehydrogenase (150 kDa), bovine serum albumin (66 kDa), egg albumin (45 kDa), and chymotrypsinogen (25 kDa), respectively. Ordinate, absorbance at 280 nm; abscissa, retention time (min). Fractions indicated by a bar under peak P2 were collected and used as the purified chorion TGase.

Estimation of Apparent Molecular Mass of Native TGase by Gel Filtration—To estimate the apparent molecular mass of the P2 protein, we used a TGK-gel G3000-SWXL HPLC column (0.78×30 cm) equilibrated with Buffer B. Standard proteins were alcohol dehydrogenase (150 kDa), bovine serum albumin (66 kDa), egg albumin (45 kDa), and chymotrypsinogen A (25 kDa) (Sigma, St. Louis).

Amino Acid Analysis—The purified P2 protein was hydrolyzed in 6 N HCl containing 2% thioglycolic acid for 22 h at 110°C. Amino acid analysis was performed on a Hitachi amino acid analyzer L-8500 at Toray Research Center (Tokyo).

Examination of Enzymatic Properties—The pH dependency of the purified TGase was examined using various buffers. Fifty millimolar Tris·HCl buffer (pH 7.2) in the standard reaction mixture was replaced by 50 mM acetate buffer (pH 4.0-6.0), 50 mM imidazole buffer (pH 6.0-7.5), 50 mM Tris·HCl buffer (pH 7.2-9.0), or 50 mM glycine buffer (pH 9.0-11.05).

To examine the effects of various reagents on the TGase activity, we used hydroxylamine, cadaverine, spermidine, spermine, lysine, poly-lysine (5 mer), GTP (Sigma, St. Louis), or ATP (Wako, Tokyo), adjusted to pH 7.2. The activity was determined by the same method as described in "*Determination of TGase Activity.*"

RESULTS

Purification—As previously reported (*17, 26*), the activity of transglutaminase (TGase), which is responsible for ε-(γ-glutamyl)-lysine crosslink formation between proteins, was localized exclusively in the unfertilized egg envelope (chorion) of the rainbow trout, *O. mykiss*. Therefore, we purified the enzyme from crude extract of isolated chorions as described in "MATERIALS AND METHODS." Seventy to 80% of the TGase activity could be extracted from the chorions. As indicated in Fig. 2a, the gel filtration profile at purification step 4 exhibited two protein peaks, P1 and P2. Rechromatography of P2 using TSK-gel G3000SWXL column provided a peak with a symmetrical profile. The final P2 fraction contained the TGase activity. As estimated by gel filtration, the molecular mass was 52 kDa. As shown in Fig. 3, SDS-PAGE analysis showed that P2 consisted of

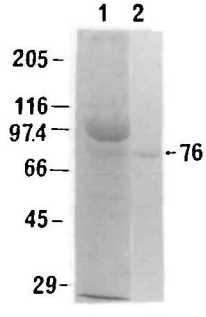

Fig. 3. **Sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE).** Lanes 1 and 2 show SDS-PAGE patterns of the crude extract of chorion and the purified P2 protein, respectively. Numbers on both sides indicate molecular masses (kDa).

only a 76 kDa protein. Therefore, the results suggested that a chorion TGase had been purified to homogeneity and it was a 76 kDa monomeric protein.

Table I summarizes a typical purification of the chorion TGase. The extent of purification was not high (a 5-6-fold purification), suggesting that the starting crude extract of chorions does not contain large amounts of other proteins. In fact, the 76 kDa TGase band was observable in the SDS-PAGE pattern of the crude extract (Fig. 3).

TABLE I. **Summary of purification of chorion TGase.**

	Activity (MIU)	Protein (mg)	Specific activity (MIU/mg protein)	(Fold)	Yield (%)
Crude extract	4,910	8.67	567	1	100
SP-Sepharose run-off fraction	3,630	8.74	414	0.73	74
Q-Sepharose TGase fraction	2,280	2.0	1,140	2.0	46
Gel filtration P2 fraction	1,038	0.53	1,983	3.5	21
Rechromatographed P2 fraction	423	0.138	3,070	5.4	8.6

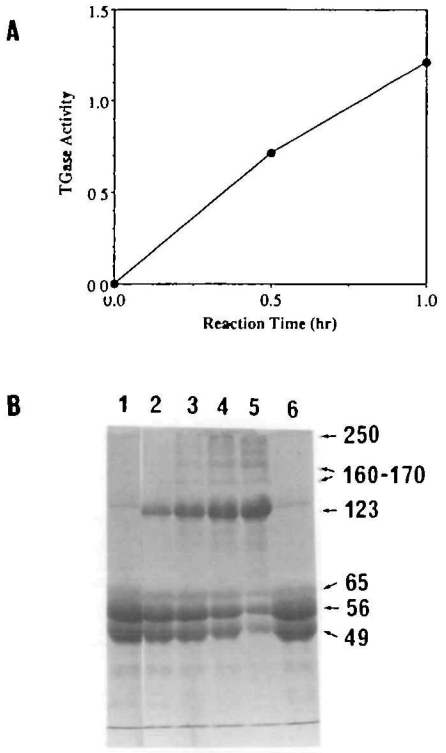

Fig. 4. **Incorporation of monodansylcadaverine (MDC) into chorion and polymerization of chorion proteins catalyzed by purified P2 protein.** A: Incorporation of MDC (nmol) into chorion catalyzed by the purified P2 protein (●) was measured as a function of time at 10°C. Amounts of P2 of 4.67 units (MIU) and chorion of 2.74 mg were used in the present study. Ordinate, activity expressed in nmol of MDC incorporated into the chorion homogenate; abscissa, incubation time (h). B: Time course of polymerization of chorion subunit proteins catalyzed by the purified P2 protein was examined by SDS-PAGE as described in the text. Lanes 1, 2, 3, 4, and 5 show SDS-PAGE patterns of chorion proteins 0, 1, 3, 6, and 24 h after incubation, respectively. Lane 6 shows the pattern 24 h after incubation in the absence of the P2 enzyme. Numbers on the right side indicate molecular masses (kDa). Amounts of P2 protein of 2.22 units (MIU) and chorion of 1.92 mg were used in the present study.

Incorporation of MDC into Chorion and Polymerization of Chorion Subunits Catalyzed by the Purified Enzyme— Throughout the course of purification, we used an unnatural substrate, MDC or dimethylcasein, to assay the TGase activity. Therefore, we examined whether or not the P2 fraction actually catalyzed both incorporation of MDC into the natural substrate, chorion, and polymerization of chorion subunits. Figure 4a shows that P2 protein catalyzed the time-dependent incorporation of MDC into chorions. In addition, polymerization of chorion subunit proteins was catalyzed by the P2 fraction, as shown in Fig. 4b. During the incubation, major 49, 56, and 65 kDa subunits of unfertilized egg chorions gradually disappeared, and 123, 160-170, and larger-than-250-kDa proteins increased. The polymer-

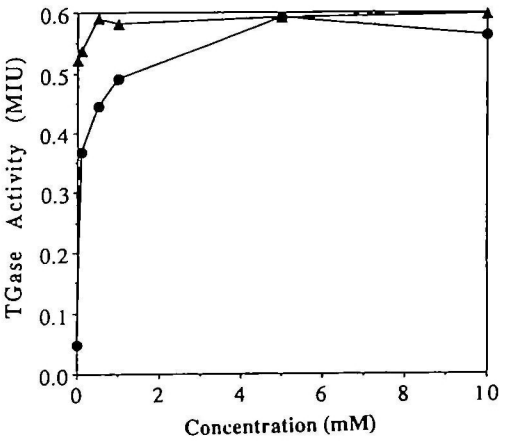

Fig. 5. **Effect of $CaCl_2$ or 2-mercaptoethanol on the TGase activity of the purified P2 protein.** ● and ▲ show the TGase activities of the purified P2 protein determined as a function of concentration of $CaCl_2$ and 2-mercaptoethanol, respectively. The activity was expressed in MIU (MDC incorporation unit). Abscissa, concentration of $CaCl_2$ or 2-mercaptoethanol (mM).

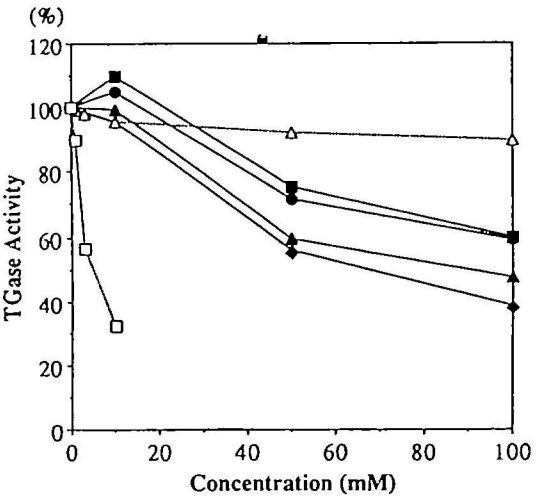

Fig. 6. **Effects of various reagents on the TGase activity of P2 protein.** ●, ▲, ■, ◆, △, and □ show the TGase activities affected in the presence of cadaverine, spermidine, spermine, hydroxylamine, lysine, and poly-lysine (5-mer), respectively. The TGase activity not treated with the reagents was represented as 100%. Abscissa, concentration of the reagents (mM).

ization profiles observed in the present study were essentially the same as in our previous reports (*17, 24*).

Enzymological Properties of Chorion TGase—In the present study, we used the final P2 fraction as purified chorion TGase and examined some of its properties. The TGase activity of the P2 fraction was completely inhibited by 0.1 mM EDTA or 0.01 mM iodoacetamide. Figure 5 shows the concentration-activity relationship of the P2 fraction for $CaCl_2$ or 2-ME. The results suggest that the chorion TGase is a Ca^{2+}-dependent SH-enzyme similar to the well-characterized TGases such as blood coagulation factor XIII (*5, 51*), guinea pig liver TGase (*10*), *Limulus* haemocyte TGase (*58*), and others (*52*).

Using 0.5 mM MDC and chorion homogenate as substrates, we examined the effects of various amines, lysine or poly-lysine on the TGase activity of the P2 fraction. Amines are well known as competitive inhibitors for various TGases. As shown in Fig. 6, the amines examined in the present study inhibited the activity. However, they were not necessarily effective inhibitors for the chorion TGase. Lysine did not inhibit the TGase activity, while poly-lysine (5 mer) was one of the most powerful inhibitors of the TGase.

It is well known that the activity of cellular TGase in guinea pig liver (*1*) and human erythrocytes (*3*), or that of blood coagulating factor XIII (*6*) is significantly inhibited by ATP or GTP, while that of secretory TGases in guinea pig prostate gland (*50*) and in *Limulus* hemocyte is not (*58*). The activity of the P2 fraction was not affected by either ATP or GTP.

As shown in Fig. 7, the highest peak of the activity was observed at pH 6.0 with a minor peak at pH 10.0. Such a pH dependency profile supports our previous finding that the activity of chorion protein polymerization occurring during hardening of the chorion isolated from unfertilized eggs is highest under an acidic condition (*24*).

Amino Acid Composition—Figure 8 shows a comparison of amino acid compositions between the rainbow trout chorion TGase and the TGases in various animals, including some fishes. As for fish enzymes, the amino acid sequences of the liver TGases of red sea bream (*63*) and chum salmon (*47*) have recently been deduced from cDNAs. As shown in Fig. 8, their amino acid compositions closely resembled those of some mammalian TGases, such as liver TGase of guinea pig (*23*) and tissue-type TGase of bovine aorta (*40*).

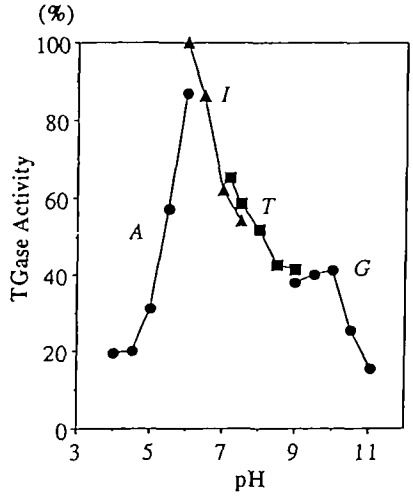

Fig. 7. **pH-dependency of the TGase activity of P2 protein.** A, I, T, and G indicate acetate, imidazole, Tris·HCl, and glycine buffer, respectively. The highest value of the activity was taken as 100%.

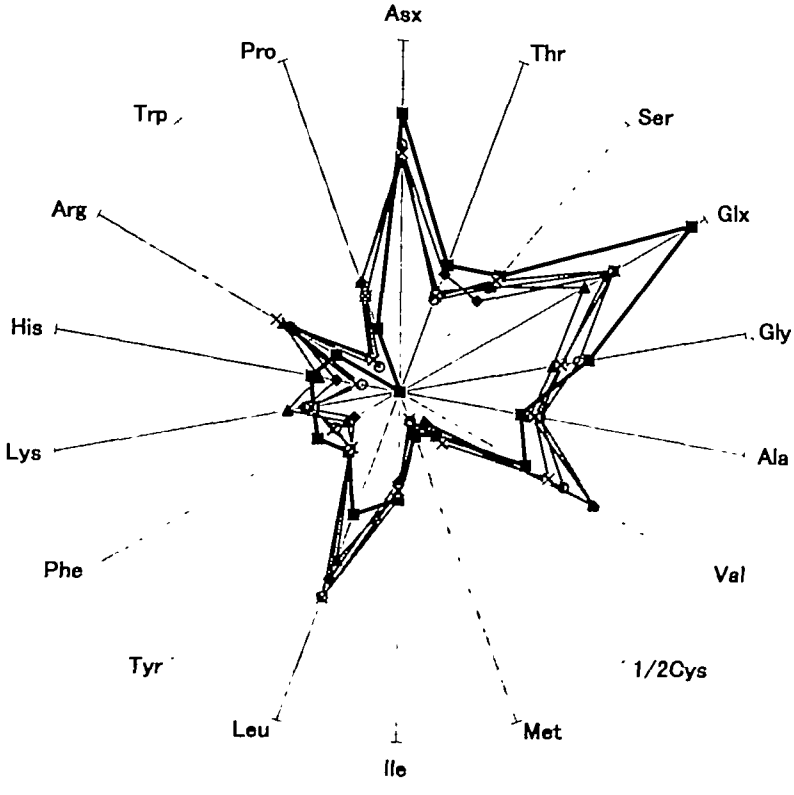

Fig. 8. **Comparison of amino acid compositions of various TGases.** All the amino acid compositions were represented as star diagrams (max. 16%). ■, ◆, ▲, ○, and × indicate the rainbow trout chorion TGase purified in the present study, liver TGase of chum salmon (*47*), liver TGase of red sea bream (*62*), liver TGase of guinea pig (*23*), and bovine aorta TGase (*40*), respectively.

However, the rainbow trout chorion TGase seemed to be significantly different from these liver and cellular type TGases in the contents of Glx (glutamine/glutamic acid), Val, and Leu residues. In addition, there were minor differences in the contents of Phe, His, Arg, or Trp residues. These results suggest that the chorion TGase may not be a member of the liver and cellular type TGases.

DISCUSSION

Our previous studies have suggested that a factor participating in the fertilization/activation-associated chorion hardening is localized in the egg envelope (chorion) of unfertilized egg of the rainbow trout, *O. mykiss*, and that it is a transglutaminase (TGase) responsible for ε-(γ-glutamyl)-lysine crosslink formation between proteins (*16, 24, 26*). However, the enzyme has never been isolated in a homogeneous form. In the present study, we purified the chorion TGase and characterized some of its properties.

The purified enzyme was a monomeric protein having a molecular mass of 76 kDa. We carried out agar gel electrophoresis at pH 8.6, incubated the gel with 0.2% dimethylcasein and 0.5 mM MDC at 4°C overnight and visualized the fluorescent band of MDC by UV irradiation. Only one band could be observed. Thus, the preliminary study shows that the purified 76 kDa protein has TGase activity.

The amino acid composition of the chorion 76 kDa TGase was characterized by high contents of Glx, Phe, and His residues, and low contents of Val, Leu, Lys, Arg, and Trp residues. We performed a computer-aided search of the amino acid sequences of liver TGases of red sea bream (*63*), chum salmon (*47*), and guinea pig (*23*), cellular type TGase of bovine aorta (*40*), human blood coagulation factor XIIIa (*15, 56*), keratinocyte TGases of human (*45*), rat (*45*), and rabbit (*48*), prostate TGases of human (*7*) and rat (*21*), and erythrocyte TGases of human (*28*), mouse (*46*), and chicken (*33*). However, we could not find any TGase that was highly homologous to the chorion TGase of rainbow trout in terms of amino acid composition. Although more studies are required to draw a definitive conclusion, the results suggest that chorion TGase of rainbow trout may be a unique and new type of TGase. At least, it does not seem to be a member of the group of previously characterized fish liver TGases in terms of amino acid composition.

Amines such as monodansylcadaverine (MDC) and cadaverine are well known as competitive inhibitors of various TGases (*13*). Further, MDC severely inhibited both the polymerization of chorion subunit (*17*) and the post-fertilization chorion hardening at the concentration of 2 mM (*26*). In contrast, relatively high concentrations of cadaverine (20–100 mM) were required for inhibiting both polymerization of chorion subunits and chorion hardening (*24*). When TGase activity was assayed using MDC as substrate, cadaverine was not necessarily one of the best inhibitors of the chorion TGase. The difference between the inhibitory effect of lysine and that of poly-lysine may be important to an understanding of the mode of action of the TGase in chorion hardening. Although the TGase activity was assayed using an unnatural substrate, its maximum activity at pH 6 was consistent with the pH dependency of chorion protein polymerization during hardening of the chorion isolated from unfertilized eggs (*24*).

Chorion proteins are known to be produced as precursor forms (choriogenins) in the liver of spawning female of medaka (*Oryzias latipes*) (*19, 39*), rainbow trout (*22, 43*), or other fishes (*30*). They are transported into the ovary, and accumulated as an intercellular matrix between the oocytes and the follicle cells. The process of intrahepatic production is responsive to estradiol-17β. In the course of formation of the chorion structure, the TGase is considered to be simultaneously incorporated or integrated into the structure. However, we have no information as to the origin of the chorion TGase or its integration into the chorion. As the amino acid composition of the chorion TGase did not seem to be similar to that of liver TGase of the fish, red sea bream (*63*) or chum salmon (*47*), there is a possibility either that its origin is not the liver, or, if it is, that the chorion TGase is a molecule expressed specifically in vitellogenic female liver.

We tentatively extracted TGase from chorions of activated eggs by the same method as described in the present study. The specific activity of the TGase was higher than that of the enzyme from unfertilized egg chorion. Thus, the preliminary study suggests the possibility of fertilization-associated activation of chorion TGase. In the near future, we will examine the post-fertilization activation, the amino acid sequence and the origin of chorion TGase.

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