Transglutaminase Activity Correlates to the Chorion Hardening of Fish Eggs

Akinori Fukuda,[†] Nobuyuki Kanzawa,[†] Toru Tamiya,[†] Katsuya Seguro,[‡] Tomoko Ohtsuka,[‡] and Takahide Tsuchiya*[,†]

Department of Chemistry, Faculty of Science and Technology, Sophia University, Tokyo 102, Japan, and Ajinomoto Company, Inc., Food Research and Development Laboratories, Kanagawa 210, Japan

The texture of fish eggs changes into a unique chewy texture, for example, caviar, during preservation in salt solution. In this work, the changes in fish eggs after preservation in salt solution were investigated. After salt preservation, fish eggs stiffened, and an increase of ϵ-(γ-glutamyl)lysine (GL) cross-linked products in the chorion fraction was observed. Transglutaminase (TGase) also activated after salt preservation. Therefore, it can be hypothesized that the change of breaking strength after salt preservation was due to the increment of the GL cross-linked products, which was produced by the activation of TGase. Additionally, two kinds of TGase isoforms localized in the chorion fraction of fish egg.

Keywords: *Transglutaminase; chorion; hardening; fish egg; salt preservation*

INTRODUCTION

The texture of fish eggs changes into a unique chewy texture, for example, caviar (salted sturgeon eggs), during preservation in salt solution. This phenomenon may correlate to a change in the membrane structure of the fish egg, which is considered to depend on intracellular chemical reactions. Although these chemical reactions have not yet been established, many kinds of fish eggs are processed by the salt treatment to develop some unique chewy textured foods.

In the eggs of rainbow trout (*Oncorhynchus mykiss*), formation of covalent cross-links between constituent proteins was recognized in the chorion during the hardening (Iuchi et al., 1991). The ϵ-(γ-glutamyl)lysine (GL) cross-links were also discovered in the egg envelope of medaka (*Oryzias latipes*) after exposure to fresh water (Iuchi et al., 1995). Transglutaminase (glutaminyl-peptide:amine γ-glutamyltransferase, TGase, EC 2.3.2.13) catalyzes the formation of the GL cross-links in proteins and is distributed in many tissues or organisms (Kumazawa et al., 1996; Yasueda et al., 1994; Icekson and Apelbaum, 1987; Ando et al., 1989).

In this study, we describe the change of the breaking strength, GL contents, and TGase activity of fish eggs before and after salt treatment to demonstrate the relationship between chorion hardening and TGase activity. Additionally, we report the localization of TGase in salmon egg detected by polyclonal antibodies against guinea pig liver TGase.

MATERIALS AND METHODS

Materials. Unfertilized eggs of landlock salmon (*Oncorhynchus masou*), Japanese char (*Salvelinus leucomaenis*), and rainbow trout (*O. mykiss*) were obtained by artificial spawning. The eggs obtained were stored in isotonic saline at 4 °C. Polyclonal antibodies against guinea pig liver TGase (type II) were purchased from Upstate Biotechnology Inc. (Lake Placid, NY). Guinea pig liver TGase was purchased form Sigma Chemical Co. (St. Louis, MO).

Analytical Procedures. *Salt Preservation.* Eggs were washed with isotonic saline and preserved in 7% NaCl solution by gently stirring for 3 days at 4 °C.

Breaking Strength. The breaking strength of an egg was measured by the puncture test using a rheometer (NRM-2010J-CW, Fudoh Kogyo Co., Ltd., Tokyo) with a plane plunger (10 mm in diameter).

TGase Activity and GL Content. Fish eggs were crushed and suspended in isotonic saline and centrifuged at 1000*g* for 10 min. To isolate the chorion fraction, the precipitate was resuspended in isotonic saline, and this procedure was repeated until the yolk proteins were completely removed. Measurements of GL contents were performed according to the previously reported methods (Tarca and Fesus, 1990; Griffin and Wilson, 1984; Kumazawa et al., 1993). TGase activity was determined according to reported methods (Takagi et al., 1986; Lorand et al., 1971; Lorand and Gotoh, 1970).

Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis (SDS−PAGE) and Western Blotting. Proteins were extracted from whole eggs, chorion, and yolk of the salmon eggs with isotonic saline containing 5% Triton X-100 by the Teflon homogenizer. Samples (40 μg each) were analyzed by 7.5% SDS−PAGE (Laemmli, 1970).

The protein bands were transferred onto Immobilon nylon membranes (Millipore, Bedford, MA). Immunodetection was performed by enhanced chemiluminescence (ECL, Amersham) using anti-TGase antibodies (1:500 dilution) according to the manufacturer's instruction. Protein concentration was determined according to the protein−dye binding method of Bradford (1976), using bovine serum albumin as a standard.

RESULTS AND DISCUSSION

The breaking strength, GL contents, and TGase activity of the eggs from landlock salmon, Japanese char, and rainbow trout before and after salt preservation were examined and are shown in Table 1. All of the fish eggs stiffened after salt preservation. An ϵ-(γ-glutamyl)lysine cross-linked product was detected in

* Author to whom correspondence should be addressed (telephone +81-3-3238-3365; fax +81-3-3238-3361; e-mail t-tsuchi@hoffman.cc.sophia.ac.jp).

[†] Sophia University.

[‡] Ajinomoto Co., Inc.

S0021-8561(97)01058-3 CCC: $15.00
Published on Web 05/08/1998

Table 1. Breaking Strength, GL Contents, and TGase Activity of Fish Egg[a]

	breaking strength[b] (g·cm)		GL contents[c] (μmol/100 g of dry egg)		TGase activity[d] (units/mg)	
	not preserved	salt preserved	not preserved	salt preserved	not preserved	salt preserved
landlock salmon	13.6 ± 3.6	22.7 ± 4.4	14.4 ± 2.1	21.4 ± 2.3	2.21 ± 0.08	3.50 ± 0.24
Japanese char	23.1 ± 3.3	39.6 ± 4.1	11.9 ± 0.9	16.1 ± 3.3	2.75 ± 0.05	4.09 ± 0.13
rainbow trout	12.3 ± 3.9	32.5 ± 10.1	20.1 ± 2.1	38.1 ± 3.4	4.28 ± 0.33	8.87 ± 0.23

[a] All values are given as mean ± SD. [b] $n = 20$. Breaking force (g) and depth of indentation (cm) were recorded. [c] $n = 3$. GL contents were determined using synthetic ε-(γ-gltamyl)lysine as standard. [d] $n = 3$. TGase activity within the chorion fraction was detected by the amount of monodansylcadaverine (MDC) incorporated into *N,N′*-dimethylcasein (DMC) by incubation for 30 min at 37 °C. One unit of the enzyme was defined as the amount that incorporated 1 nmol of MDC in DMC per minute.

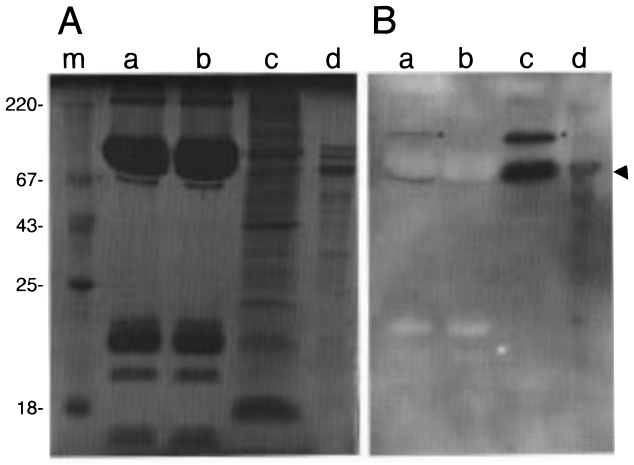

Figure 1. Detection of TGase in salmon egg by Western blotting. Proteins were prepared as described under Materials and Methods: (A) Coomassie Brilliant Blue stain; (B) immunodetection with anti-TGase antibodies; m, molecular size marker; a, whole egg extracts; b, yolk extracts; c, chorion extracts; d, guinea pig liver TGase. Arrowhead indicates TGase. Asterisked peptide was unknown peptide, which may be an autocatalytic product or reacted nonspecifically with the antibodies. Molecular masses in kilodaltons are indicated on the left of the gel.

both salt-preserved and nonpreserved fish eggs. In all chorion fractions of the fish eggs, GL contents increased after salt preservation. TGase activities within the chorion fraction of all the fish eggs were also activated after salt preservation, but in the yolk fraction it was not detectable before or after salt preservation (data not shown). We therefore conclude that the change of breaking strength after salt preservation was due to the increment of the GL contents, which was produced by the activation of TGase.

Each 40 μg of whole, chorion, and yolk proteins of salmon eggs was subjected to SDS−PAGE. As shown in Figure 1, the antibodies reacted with peptides in the whole egg's fraction and with doublet peptides of the chorion fraction, whose apparent molecular masses were 70 kDa. The mass is similar to that of rat liver TGase. The doublet peptides may be caused by the existence of two kinds of TGase isoforms in fish eggs. The doublet peptides in three preparations did not change the molecular ratio (data not shown). The asterisked peptide (∼140 kDa) in the whole and chorion fractions also reacted with the antibodies, which may be an autocatalytic product or reacted nonspecifically with the antibodies. The amount of the peptide changed in every preparation (data not shown). No peptides were detected in the yolk fraction. These results showed that the TGase was localized specifically in the chorion of fish egg.

In this paper we study the changes after preservation of fish eggs in salt solution to research the relationship between the chewy texture of fish eggs after salt preservation and TGase activity or breaking strength of fish eggs. Additional research is needed to elucidate the mechanisms that explain the relationship between chorion hardening and TGase activity of fish eggs.

LITERATURE CITED

Ando, H.; Adachi, K.; Umeda, K.; Matsuura, A.; Nonaka, M.; Uchio, R.; Tanaka, H.; Motoki, M. Purification and characterics of a novel transglutaminase derived from microorganisms. *Agric. Biol. Chem.* **1989**, *53*, 2613−2454.

Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248−254.

Griffin, M.; Wilson, J. Detection of ε-(γ-glutamyl) lysine. *Mol. Cell. Biochem.* **1984**, *58*, 37−49.

Icekson, I.; Apelbaum, A. Evidence for transglutaminase activity in plant tissue. *Plant Physiol.* **1987**, *84*, 972−974.

Iuchi, I.; Masuda, K.; Yamagami, K. Change in component proteins of the egg envelope (chorion) of Rainbow Trout during hardening. *Dev. Growth Diff.* **1991**, *33*, 85−92.

Iuchi, I.; Ha, C. R.; Masuda, K. Chorion hardening in medaka (*Oryzias latipes*) egg. *Fish Biol. J. MEDAKA* **1995**, *7*, 15−20.

Kumazawa, Y.; Seguro, K.; Takamura, M.; Motoki, M. Formation of ε-(γ-glutamyl)lysine cross-link in cured horse mackerel meat induced by drying. *J. Food Sci.* **1993**, *58*, 1062−1064.

Kumazawa, Y.; Nakanishi, K.; Yasuda, H.; Motoki, M. Purification and characterization of transglutaminase from Walleye Pollack liver. *Fish. Sci.* **1996**, *62*, 959−964.

Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **1970**, *227*, 680−685.

Lorand, L.; Gotoh, T. The fibrin stabilizing factor system. *Methods. Enzymol.* **1970**, *19*, 770−782.

Lorand, L.; Lockridge, O. M.; Campbell, L. K.; Myhrman, R.; Bruner-Lorand, J. A continuous fluorescent method suited for automating measurements of factor XIII in plasma. *Anal. Biochem.* **1971**, *44*, 221−231.

Takagi, J.; Saito, Y.; Kikuchi, T.; Inada, Y. Modification of transglutaminase assay: use of ammonium sulfate to stop the reaction. *Anal. Biochem.* **1986**, *153*, 295−298.

Tarca, E.; Fesus, L. Determination of ε-(γ-glutamyl)lysine cross-link in proteins using phenylisothiocyanate derivatization and high-pressure liquid chromatographic separation. *Anal. Biochem.* **1990**, *186*, 135−140.

Yasueda, H.; Kumazawa, Y.; Motoki, M. Purification and characterization of a tissue-type transglutaminase from red sea bream (*Pagrus major*). *Biosci., Biotechnol., Biochem.* **1994**, *58*, 2041−2045.

Received for review December 15, 1997. Revised manuscript received March 30, 1998. Accepted April 1, 1998.

JF9710589