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Studies on Rainbow Trout Egg (*Salmo gairdnerii irideus*)

II. Carbohydrate in the Egg Protein

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The presence of carbohydrate in teleost fish egg in the process of vitellogenesis has been determined mainly by histochemical technique. It has for long been believed that several forms of carbohydrates are present in the eggs. Glycogen and glucose have been found in the cellular structures of larvae and embryos at the various development stages of teleost fish egg[1,2]. However there is so far little evidence of the presence of the carbohydrate in the yolk reserve.

ITO *et al.*[3] reported the presence of the anthrone reaction positive carbohydrate in lipoprotein hydrolysate of rainbow trout egg. The authors[4] also found the carbohydrate in lipoprotein by the qualitative test. NEUBERGER[5] found the bound-carbohydrate in ovalbumin, the carbohydrate-containing proteins have been studied by many workers, and the carbohydrate has been isolated from several proteins of a hen's egg yolk and white[6,7,8,9]. Therefore, the same ideas might be applied to the teleost fish egg.

The chemical composition and physiological importance of bound-carbohydrate in protein have been studied by many investigators. Glycoprotein which is suggested to be distributed widely in nature has attracted special interest recently, since it becomes clear that the carbohydrate attached to protein was important not only on carbohydrate metabolism, but also with respect to its physiological activity.

The present paper deals with the carbohydrate in the protein of the rainbow trout immature egg.

EXPERIMENTAL AND RESULTS

Materials: The egg proteins from the rainbow trout, component I and component II, were prepared by the method described previously[4]. The component I was defatted by the method of HILLYARD *et al.*[10], washed several times with a small amount of distilled water to exclude the salt and contaminants, and dried over P_2O_5 under reduced pressure. The component II was desalted by dialyzing with water, lyophylized, and dried.

Hydrolysis of the component I and the component II: The component I was hydrolyzed with 0.2 N hydrochloric acid in an ampoule at 110°C. The quantity of carbohydrate released from the protein was determined at intervals by the anthrone method[11]. The Figure 1 indicates that the amount of carbohydrate reaches to a

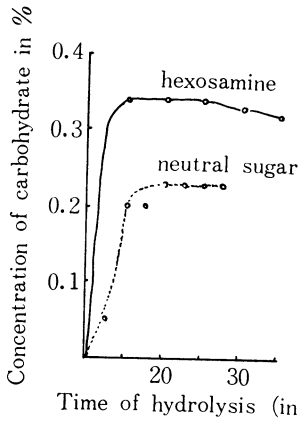

Fig. 1. Release of carbohydrates from protein.

constant level after 20 hours. Therefore 25 hours was calculated as the time of hydrolysis of protein in the following experiments.

Paper chromatography of neutral carbohydrate: The fish egg proteins were hydrolyzed, filtered and the hydrolysates were applied to the columns of Amberite IR−120 (H^+ form) and IR−410 (COO^- form). Then the eluates were concentrated under the reduced pressure at 40°C. A small portion of the eluates were spotted

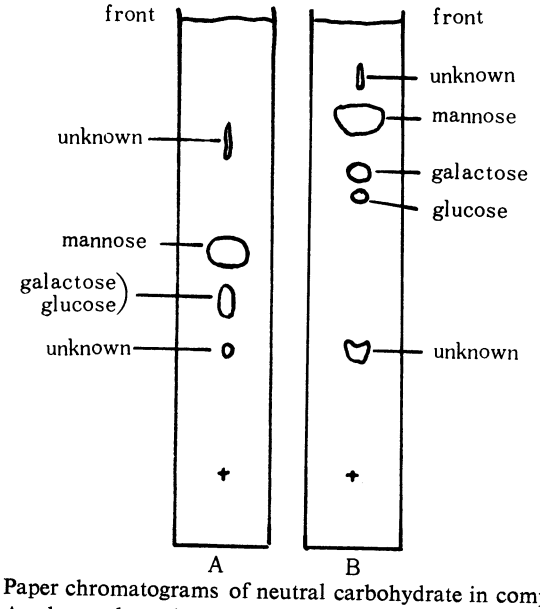

Fig. 2. Paper chromatograms of neutral carbohydrate in component I.
A: butanol, acetic acid, water (4 : 1 : 5)
B: pyridine, butanol, water (2 : 3 : 1.5)

on Toyo filter paper No 51 (20cm × 10cm). Ascending paper chromatography was carried out by the method of multiple developments[12] (twice) with *n*-butanol,

acetic acid, water (4: 1: 1.5 v/v), and pyridine, *n*-butanol, water (2: 3: 1.5 v/v) as solvent system at 30°C. Carbohydrate spots were detected by spraying the solution of aniline hydrogen phthalate or ammonium silver nitrate. Four to five spots were detected in the component I, and identified as D-mannose, D-galactose, and D-glucose. Mannose was predominant in them. Among two unknown spots, the one which was trace in amount and high in Rf was posed to be rhamnose, but the other one which was low in Rf was incided with neither monosaccharide nor disaccharide. Aldopentose was insufficiently recognized in the component I, while the orcinol-ferric chloride reaction was quite positive as to the component II.

Quantitative analysis of hexose and pentose: During the estimation of neutral sugar in the egg protein, it became necessary to determine pentose and hexose when present together. The amount of neutral carbohydrate, hexose and pentose, in the hydrolysates were determined by the following procedures. The absorption spectra of authentic carbohydrate solution treated by the anthrone method[11] shows the maximum at 625 mμ in hexose, and 430 mμ in pentose as seen in Figure 3. On the

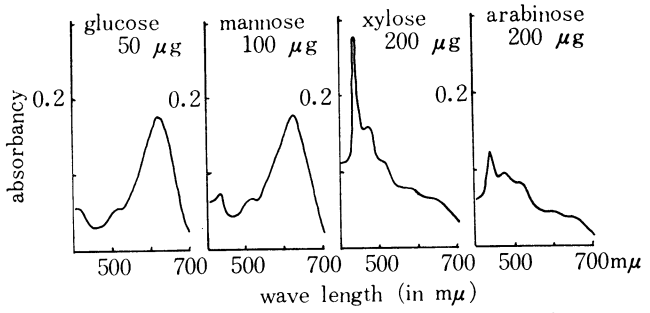

Fig. 3. The absorption spectra of authentic sugars with anthrone reagent.

other hand, the hexose treated with the orcinol-ferric chloride method[13] has the absorption maximum at 660 mμ, and the pentose at 445 mμ respectively (Fig. 4).

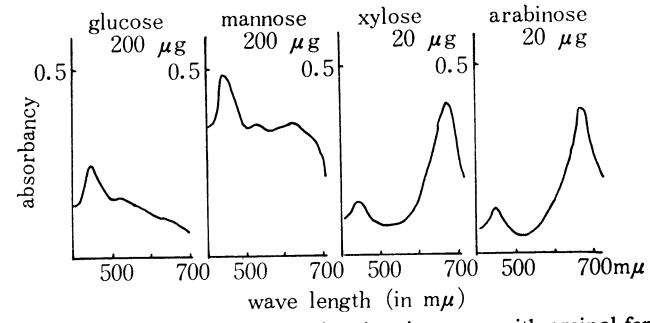

Fig. 4. The absorption spectra of authentic sugars with orcinol-ferric chloride reagent.

The relative color intensity of each monosaccharide developed by the anthrone and the orcinol-ferric chloride reagents are shown in Table 1. The values of galactose,

fructose, and rhamnose (not shown in Figure 2 and 3) are also shown.

Table 1. Sensitivities of various carbohydrates for anthrone and orcinol-ferric chloride reagents.

	anthrone*	orcinol-$FeCl_3$*
glucose	100%	2%
galactose	53%	8%
mannose	47%	4%
fructose	53%	2%
xylose	6%	100%
arabinose	2%	98%
rhamnose	67%	7%

* calculated from absorbancy for 40μg of sugar

Applying the different sensitivities of hexose and pentose for two procedures, the amount of each carbohydrate can be obtained by solving the following equations:

$$A \cdot x + B \cdot y = Ea$$

$$C \cdot x + D \cdot y = Eo$$

in which constant A and B are the extinction coefficient of hexose and pentose measured by the anthrone method, C and D the value by the orcinol-ferric chloride method respectively. x and y are the concentration of hexose and pentose in μg per ml respectively. Ea is the absorbancy of the solution developed by the anthrone method, and Eo by the orcinol-ferric chloride method. Recovery tests were made on the mixture of glucose and xylose. Table 2 shows that the recovery is satis-

Table 2. Recovery tests for determination of xylose and glucose.

glucose μg	xylose μg	absorbancy		glucose		xylose	
		anthrone 625mμ	orcinol 660mμ	found μg	recovered %	found μg	recovered %
35.7	0.0	0.145	0.015	35.7	100.0	0.0	100.0
35.7	9.3	0.148	0.218	35.7	100.0	9.4	100.1
26.7	18.6	0.113	0.412	26.6	100.4	18.6	100.0
17.8	27.9	0.082	0.606	18.4	103.4	27.8	99.6
8.9	37.2	0.049	0.814	8.7	98.0	37.5	100.8
0.0	37.2	0.007	0.810	0.0	100.0	37.2	100.0

factory at a level of 98.0 to 103.4%. Therefore the carbohydrate bound with protein was analyzed by using this method before applying to the ion-exchange resins. The results obtained are summarized in Table 3. It indicates that 0.23 per cent of hexose and 0.02 per cent of pentose in the component I, and 0.24 per cent of hexose and 0.33 per cent of pentose in the component II respectively.

Table 3. Carbohydrate composition of egg yolk proteins of rainbow trout.

	component I	component II	vitellin*
hexose	0.23%	0.24%	1.3 %
pentose	0.02%	0.33%	—
hexosamine	0.34%		0.67%
sialic acid	0.14%		0.38%

* cited from ABRAHAM *et al.*[8]

Estimation of hexosamine and sialic acid: Amino sugar of the hydrolysate was determined by the modification of ELSON-MORGAN procedure[14]. The content of it was 0.34 per cent in terms of glucosamine in the component I, but not determined in the component II.

Sialic acid was estimated by the method of SVENNERFOLM[15]. In this case, however, the fish egg protein was hydrolyzed by 0.1N sulfuric acid, filtered, passed through the ion-exchange resin, Dowex 50×8 (H^+form), and washed with M acetate buffer solution (pH 4.6). The eluate thus obtained was applied for the analysis of sialic acid. The amount of sialic acid was calculated by using the molar absorbancy of N-acetylneuraminic acid cited from the value of SVENNERFOLM[15]. Table 3 indicates that the content of sialic acid is 0.14 per cent in the component I.

DISCUSSION

The quantitative estimation of pentose and hexose in natural products has been made by many workers. The colorimetric method of FERNELL and KING[13] is suitable for the simultaneous determination of hexose and pentose, although there is a small error due to the orcinol-sulfuric acid procedure. Therefore a modified procedure was presented for the determination of hexose and pentose. It was founded on the combination of the anthrone procedure with the orcinol-ferric chloride procedure. Under the conditions employed the anthrone reagent reacts specifically with hexose producing the color reactant, but does not react sensibly with pentose. On the other hand the orcinol-ferric chloride reagent is sensitive for pentose but insensitive for hexose. However methylpentose, rhamnose, had somewhat different sensitivity from aldopentose as seen in Table 2. With this method the methylpentose, when contaminated in test solution, gave a color that was impossible to distinguish from that of aldohexose or ketohexose.

The recovery test of this procedure was made and the accuracy was high at 98.0–103.4%. The ion-exchange treatment of the hydrolysates was necessary for the elimination of interfering substances.

It is shown that the component I isolated from egg yolk contains hexosamine, sialic acid, and hexoses of which mannose predominates over galactose, and glucose. It is well known that protein-bound mannose widely distributes in nature, namely, ovalbumin[9,16,17], lipovitellin[7,8], fetuin[18,19], and fungal amylase[20,21] *etc.* Moreover galactose and glucose have been found in protein. According to ABRAHAM *et*

al.[8], presence of pentose was not demonstrated in vitellin. The carbohydrate content in the component I coincided with the value of mature egg lipoprotein of rainbow trout[3]. The amount of pentose was detected at a level of 0.02 per cent. However this might be due to the experimental error.

The component II, which was suggested to be a livetin-like protein, contains hexose and pentose more than the component I. Therefore the component II might be called a glycoprotein. JARED and WALLACE[22] obtained three proteins from rainbow trout egg by TEAE-cellulose, and proposed that the protein, which corresponds to the component II presented here, was serum protein. WILLIAMS[23], and MOK and COMMON[24] established that the hen's livetins were serum proteins by immunoelectrophoretic identification. It is well known that the livetins also contain a small amount of carbohydrate[6,25]. It is necessary to carry out further experiments for the identification of the component II with serum protein.

Both hexosamine and sialic acid have been found in some proteins as important constituents. ABRAHAM *et al*[8].) found D-glucosamine in vitellin. An appreciable amount of amino sugar was also detected in the component I. Thus it is suggested that the component I of the trout egg is similar to the lipovitellin of hen's egg. The sialic acid is generally bound to either protein or polysaccharide. However free acid has not been detected in nature except a few cases[26]. WARREN[27] found that a large amount of free sialic acid was contained in trout egg. There occured a small amount of sialic acid at a level of 0.14 per cent in the component I.

SUMMARY

(1) The component I which is a lipoprotein isolated from immature egg of rainbow trout contained hexose, hexosamine, and sialic acid as the carbohydrate constituent, Mannose predominated over galactose and glucose. The carbohydrate content of the component I was lower than that of lipovitellin of hen's egg.

(2) The component II contained both hexose and pentose. It is suggested that the substance is a kind of glycoprotein similar to livetin of hen's egg yolk.

(3) The method for simultaneous determination of hexose and pentose was discussed.

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虹鱒卵の生化学的研究
II 卵蛋白質中の炭水化物について

中川平介

虹鱒未成熟卵から分離したリポ蛋白質，コンポネントI，にヘキソース，ヘキソサミン，シアル酸の存在することを認めた．ヘキソース中マンノースが最も多く，他にガラクトース，グルコースが存在する．このリポ蛋白質の炭水化物含量は鶏卵リポビテリンより低い値を示した．

コンポネントIIはペントース，ヘキソースを含み，鶏卵のリベチン類似の一種の糖蛋白質であろうと考える．

同一試料中に存在するヘキソース，ペントースの定量方法について検討した．