ISOLATION OF A PHOSPHOPROTEIN OF HIGH PHOSPHORUS CONTENT FROM THE EGGS OF BROWN BROOK TROUT.*

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Received October 22, 1964

Phosphoprotein fractions containing approximately 10% phosphorus were first prepared by Levene and Alsberg from defatted yolks of hen and fish eggs and were designated respectively as vitellinic and as ichthulinic acids. Owing to the drastic extraction procedure employed by these authors, the occurrence of these fractions as genuine yolk constituents remained undecided. In 1949, Mecham and Olcott obtained from hen's egg yolk a phosphoprotein of a phosphorus content of 10% under conditions excluding its origin by secondary degradation. It was designated as phosvitin and accounts for approximately 50% of the total phosphoprotein phosphorus of yolk. It contains 14 different amino acid residues (Lewis et al. (1950), Connelly et al. (1961)). Ito, Fujii and Yoshioka reported in 1963 the isolation of a new phosvitin from the eggs of rainbow trout (Salmo irideus). It contained 4% of phosphorus and yielded 6 different amino acids as hydrolysis products. Barman, Nguyen-Kim Bai and Nguyen-Van Thoai (1964) obtained from the eggs of herring (Clupeus harengus) a phosphoprotein containing 12% of phos-

* This study was supported by grants from the National Science Foundation, the Godfrey H. Hyams Trust, Boston, and the Charlton Fund, Boston.

phorus and composed of only 5 different amino acid groups. We wish to report the isolation from eggs of the brown brook trout (Salmo trutta) of a phosvitin[1] which differs from those mentioned above with regard to phosphorus content and amino acid composition.[2] A novel procedure for the purification of phosvitins has been developed in the course of the study.

Trout eggs were obtained from the Plymouth Rock Trout Hatchery, Plymouth, Massachusetts. We wish to thank Mr. W.E. Whiting, Plymouth, for his generous cooperation. An acetone powder was prepared from the clear yolk liquid obtained by sedimentation of the homogenized eggs at 100,000 x G for one hour. Thorough washing of the acetone powder with diethyl ether is important for the removal of the phospholipids. A 30% aqueous suspension of the powder was brought to pH 8 by addition of 0.1 N sodium hydroxide. After mechanical shaking for 30 minutes, an equal volume of a saturated solution of ammonium sulfate was added to the mixture, and the suspension was filtered on a fluted Whatman No. 1 filter. The salts were removed by dialysis, the dialyzed liquid was clarified by centrifugation, and the supernatant was mixed with one third volume of 15% trichloroacetic acid for the precipitation of contaminating proteins. The filtrate was adjusted to pH 7.5 by the addition of a solution of sodium hydroxide, the solution was concentrated in a flash evaporator and thoroughly dialyzed against water at 5°. Lyophilization

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[1] The word phosvitin is used in this paper as a generic term for water-soluble phosphoproteins occurring mainly in egg yolks. The phosphoprotein described by Ito et al. (1963) will be designated in the following as trout phosvitin a.

[2] A preliminary report of a part of this investigation has been published (G. Bartsch, J. Knolle, G. Schmidt and S. J. Thannhauser, Abstr., Meeting of the American Chemical Society, Washington, D. C., 1962, page 3C).

of the material prepared from 100 gm of eggs yielded 80 mg of a white powder containing 7% phosphorus all of which was alkali-labile. Up to 10 ml of a 3% solution of the phosphoprotein in water were subjected to continuous flow electrophoresis in 0.03 M sodium barbital-HCl buffer of pH 8.5 in the Elphor VaP apparatus of Grassmann and Hannig (Dr. Bender and Dr. Hobein, Inc., Munich, Germany). For scanning, comparable amounts of the 48 fractions were applied to Whatman No.1 paper and sprayed with ninhydrin reagent. Trout phosvitin b was the only major peak and was distributed over 4 to 6 fractions near the anode. The protein was recovered from the combined dialyzed peak fractions by lyophilization. It was precipitated from a 5% solution with three volumes of 95% ethanol.

Properties. Figures 1 and 2 show that trout phosvitin b migrates as one peak during electrophoresis in a Tiselius apparatus and during sedimentation in the ultracentrifuge.

The amino acid composition of trout phosvitin b was determined according to the procedure of Moore and Stein (as modified in 1963). In Table I, the composition of trout phosvitin b is compared with that of trout phosvitin a on the basis of the data reported by Ito et al. (1963).

Alanine and glycine respectively were found to be the only N-terminal groups in trout phosvitin b and in hen phosvitin.

In contrast to hen phosvitin (Sundararajan et al., (1960)), trout phosvitin b is not precipitated from its aqueous solutions by HCl at pH 1.8 or by trichloroacetic acid. This property which facilitates its purification must be considered in the analysis of trout tissues.

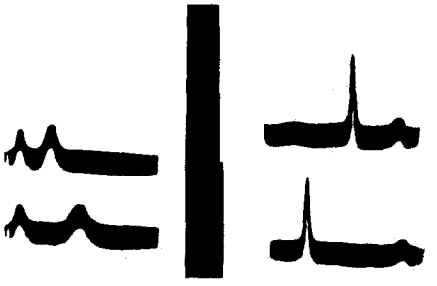

Fig. 1. Tiselius Electrophoresis of trout phosvitin b. Left: Ascending boundaries after 5' and 10'. Right: Descending boundaries after 11' and 24'. Perkin-Elmer Apparatus Model 38 A. 2 ml cell, 0.03 M sodium barbital-HCl buffer pH 8.6, 200 Volts, 10 mA, 9.6×10^{-4} $ohm^{-1}cm^{-1}$.

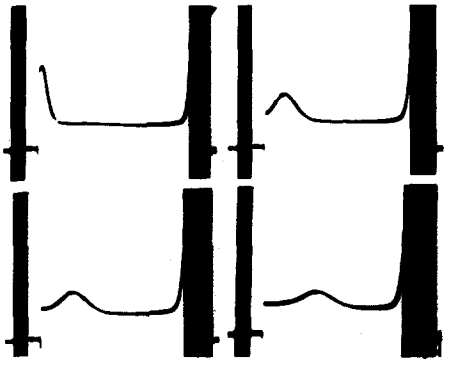

Fig. 2. Sedimentation of trout phosvitin b. Bar angle 70°. 16', 80', 128' and 208' at 59,780 rpm in 0.1 M sodium chloride solution containing 0.03 M sodium barbital-HCl buffer of pH 7.5. Temperature 17°.

The differences between the respective amino acid composition of trout phosvitin a (from Trutta irideus) and that of trout phosvitin b (from Salmo trutta) are remarkable. The available observations do not permit as yet an evaluation of their possible biological significance. The possibility cannot be ruled out that these differences might be related to the different procedures used for the isolation of both proteins.

Table I.

Composition of trout phosvitins a and b.

constituent group(s)	trout phosvitin a (Ito et al.)	trout phosvitin b
N/P (gram atoms)	8.2	3.3
gm P/100 gm protein	4.0	8.5
gm N/100 gm "	14.8	12.6
Sedim. Constant	1.9 S	1.5 S
Number of residues per 50 serine groups[3]	trout phosvitin a	trout phosvitin b
serine	+	50
threonine	0	2
alkali-labile P	+	50
alkali-resistent P	0	0
lysine	0	5
arginine	0	14
histidine	0	trace
aspartic	+	10
glutamic	+	4
amide	not det.	15
glycine	+	3
alanine	+	2
valine	0	trace
leucine	0	0
isoleucine	0	2
proline	0	5
tyrosine	0	2
phenyl alanine	0	1
tryptophane	not det.	+
S-containing am. acids	0	0

[3] The assumed figure of 50 serine residues per molecule of trout phosvitin b corresponds to a molecular weight of 18,500 -- a value which is compatible with its sedimentation constant.

Summary. A new phosvitin (trout phosvitin b) has been isolated from the eggs of Salmo trutta. It accounts for at least 20% of the total phosphoprotein phosphorus of these eggs. It differs from the trout phosvitin a described by Ito et al. by its higher phosphorus content and by the presence of amino acid groups that are lacking in the latter. The composition of trout phosvitin b resembles that of hen's phosvitin rather than that of trout phosvitin a.

The initial steps of the novel procedure for the isolation of phosvitins used in this study are more generally applicable than the techniques employed hitherto for this purpose.

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