

STUDY ON THE CRYSTALLINE STYLE OF THE FRESHWATER MUSSEL, *PARREYSIA CORRUGATA*

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Abstract

The crystalline style of *Parreysia corrugata* is a flexible, gelatinous glyco-protein, protrudes from the style sac and impinges on the gastric shield. The style is insoluble in glycerine, alcohol, acetic acid and other dilute acids but it dissolves in distilled water at a pH 7.0. It contains twelve free amino acids and fourteen protein-bound amino acids. The enzyme amylase was found to be present.

Introduction

Most all lamellibranchs except septibranchs, possess a crystalline style. The physiology of the crystalline style in freshwater bivalves has been studied, as well as the chemical composition.

In the present study, the chemical composition, the amino acids and the enzymes of the crystalline style of *Parreysia corrugata* have been worked out.

Material and methods

Specimens of *Parreysia* used in the study were collected from the Kham river near Aurangabad. As soon as the specimens were brought to the laboratory the crystalline style was removed and an extract was prepared in distilled water. Different chemical tests were performed.

For separating the style with a sufficient quantity of 80% ethanol, to precipitate the protein, the free amino acids were separated. The supernatant was treated with three

times the quantity of chloroform after centrifugation and the upper layer alone was used. The protein-bound amino acids were obtained by hydrolysis of the precipitated protein in 6N HCl for 18 hours under reflux. After hydrolysis, the contents were evaporated in a steam bath to dryness for removing the acid. To the residue a few drops of isopropyl alcohol were added because it serves as preservative. The spotting was then done with this extract. The amino acids were studied by circular, ascending and two dimensional paper partition chromatography. Whatman No. 1 filter paper was used for spotting and 0.5% ninhydrin prepared in acetone was used as a colour developing agent. Simultaneously, the control chromatograms of known quantities of amino acids were run. To study the nature of protein, paper electrophoresis using Whatman No. 1 was also employed on the aqueous solution of the style.

For the enzyme study the specimens were carefully dissected to take out the crystalline style. Since the style readily dissolves in distilled water, its aqueous solution was prepared for carrying out the experiments. The style extract was incubated for 24 hours with different substrates like starch, lactose, maltose, saw dust, gelatin pieces and bromophenol blue milk.

Results

The crystalline style

The crystalline style of *Parreysia corrugata* is a flexible gelatinous rod which protrudes from the style sac and impinges on the gastric shield. It is a glycoprotein and is insoluble in glycerine, alcohol, acetic acid and other dilute acids. It dissolves in distilled water and has a pH 7.0. The

Table 1. Chemical composition of the crystalline style of *Parreysia corrugata*.

Test	Observation	Inference
Iodine	Appearance of Red colour	Presence of carbohydrates
Fehling's	No reduction	Absence of soluble carbohydrates
Extract : 5% CuSO ₄	No precipitation	Absence of soluble carbohydrates
Biuret	Appearance of pinkish violet colour	Presence of protein
Ninhydrin	Development of blue colour	Presence of protein
Arginine	Appearance of intense carmine colour	Presence of arginine
Xanthoprotein	Deepening of yellow colour to orange	Presence of tyrosine and tryptophan
Heating	No coagulation	No mucin
Extract : TCA	No precipitation	No globulin
Extract : Strong acetic acid	No precipitation	No mucin, albumen and globulin
Extract : Saturated (NH ₄) ₂ SO ₄	No turbidity	No globulin and albumen
Extract : absolute alcohol	Precipitation	Glucoprotein

colour of the style varies from pale yellow to light flesh, depending upon the nature of food of the animal. The style is about 30-35 mm. long and 0.5-0.7 mm. in diameter. It dissolves completely within a short time, on removal of the mussel from the water.

Chemical composition of the style

The results of the tests carried out with aqueous extract of the crystalline style of *Parreysia* are presented in Table 1. The style is not a globulin as it dissolves in distilled water. It shows Lieberman's reaction, in which the solution in 6N HCl becomes at first colourless, then turns violet and finally becomes dark blue. When the style is treated with saturated ammonium sulphate solution, its insoluble nature indicates that it contains no mucins, albumins and globulins. The aqueous solution of the style is precipitated by absolute alcohol so it is a glycoprotein. The crystalline style protein in *Mactra* was a sort of mucin which had its components uronic acid, glucosamine, galactose, mannose, xylose, fucose and an unknown sugar (Hashimoto *et al.*, 1954).

Amino acids of the style of *Parreysia*

Free and protein-bound amino acids in the crystalline

Table 2. Amino acids of the crystalline style of *Parreysia corrugata*.

+ = Present
- = Absent

Name of amino acid	Free amino acid	Protein bound amino acid
Leucine	+	+
Phenyl alanine	+	+
Valine	+	+
Methionine	-	+
Proline	+	+
Tyrosine	+	+
Alanine	+	+
Threonine	-	+
Glycine	+	+
Serine	+	+
Glutamic acid	+	+
Aspartic acid	-	+
Histidine	+	-
Arginine	+	+
Asparagine	-	-
Lysine	-	-
Ornithine	-	-
Cystine	+	+

Table 3. Action of crystalline style enzymes on various carbohydrates, protein and fat in *Parreysia corrugata*.

Experiment	Intensity of enzyme action		Remark
	Experiment	Control	
2% starch + 2% extract + Benedict's qualitative reagent	Strong reduction	No reduction	Amylase present
2% lactose + 2% extract + Barfoed's reagent	No reduction	No reduction	Lactase absent
2% maltose + 2% extract + Barfoed's reagent	No reduction	No reduction	Maltase absent
Saw dust + 2% extract + Benedict's qualitative reagent	No reduction	No reduction	Cellulase absent
Few gelatin pieces + 5 ml. of extract	No liquefaction of gelatin pieces	No reaction	Protease absent
Bromophenol blue milk + 5 ml. of extract	No development of yellow colour	No reaction	Lipase absent

represented in Table 2. The crystalline style of *Parreysia* contains twelve free amino acids. They are leucine, alanine, valine, proline, tyrosine, alanine, glycine, glutamic acid, histidine, arginine and cystine. Fourteen proteinbound amino acids on the protein of the crystalline style are present. They are, phenylalanine, valine, methionine, proline, alanine, threonine, glycine, serine, glutamic acid, aspartic acid, arginine and cystine. In *Mactra sulcataria* amino acids are present (Hashimoto *et al.*, 1954). Alanine, arginine and proline, which are found in *Parreysia corrugata*, are lacking in *Mactra*. The interesting fact is that the sulphur containing amino acids, methionine and cystine are invariably present in all bivalves. Other amino acids may vary in different forms and may not be identical.

Enzymes of the style

Experiments carried out for the detection of enzymes showed that the crystalline style of *Parreysia* contained amylase. Other enzymes like lactase, maltase, cellulase and lipase were not present. The results are given in Table 3.

Chemistry of the crystalline style of the bivalves have been made by a number of workers like Mitra (1901) in *Anodonta*, Yonge (1926) in *Mya* and *Ostrea*, Nair (1955) in *Bankia* and Venugopalan (1955) in *Sanguinolaria*. The chemical nature of the crystalline style was worked out by various investigators such as Barraix (1889), List (1902), Nelson (1918), Berkeley (1935), Laevine (1946) and Venugopalan (1955). They showed that the style chiefly contained globulin with an amylase and a weak maltase (Yonge, 1926). The style of *Parreysia* contains glycoprotein (Table 1) and it also possesses an enzyme amylase, other carbohydrases being absent (Table 3).

Hashimoto *et al.* (1954) and Hashimoto & Sato (1955), who carried out investigation on the crystalline style of *Mactra sulcataria*, showed that the style contained mucins which had several component sugars. The amino acids of the style of *Mactra* were also determined by these workers. The style of *Mactra* contained only 10 amino acids. In *Parreysia* four more amino acids namely leucine, valine, arginine and proline were present (Table 2). Free and protein-bound amino acids of the crystalline style of *Sanguinolaria* were studied by Venugopalan (1953). Hashimoto *et al.* (1954) and Venugopalan (1955) found that the style was rich in mucopolysaccharides. The style of *P. corrugata* was found to possess glycoprotein.

The crystalline style is invariably present in almost all bivalves except septibranchs. Studies on the phys-

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