A Blue-Green Pigment Isolated from Blood Plasma of the Arctic Sculpin (*Myoxocephalus scorpioides*)

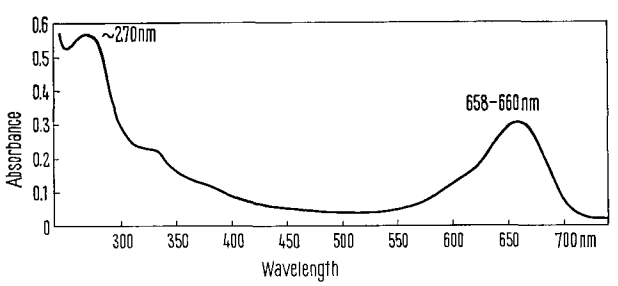
Blue-green pigments in the blood serum and plasma of several fish were first reported by NOLF[1] in 1907. Since then, several investigators have reported similar findings in the serum[2,3], skin[4], and bones[5] of a number of species of fish. The physico-chemical properties of a blue-green protein isolated from the serum of an eel, *Anguilla japonica*, have recently been reported[6,7].

While collecting blood plasma samples from several species of Arctic fish during the 1968 Bering Sea Expedition of the R/V *Alpha Helix*, I noticed that the plasma of *Myoxocephalus scorpioides*, a sculpin found in the Bering Sea and Arctic Ocean from Alaska to Labrador, was blue-green. This report describes the purification and partial characterization of this blue-green plasma pigment.

Materials and methods. The sculpin were caught by hook and line at Nunivak Island (Alaska) during the months of March and June. Blood samples were obtained either by cardiac puncture, or from the severed caudal vein. The blue-green plasma was obtained by centrifugation of the heparinized blood. The plasma[8] was fractionated with ammonium sulfate, and the blue-green pigment precipitated between 70 and 80% saturation. The precipitate was collected, dissolved in water and dialyzed; the blue-green pigment was not dialyzable. Although the pigment could be further purified by polyacrylamide disc gel electrophoresis, it was found that it could be more highly purified by the method of isoelectric focusing[9]. Using a pH gradient[10] of 3 to 6, a sharp blue-green band formed near the anode after approximately 48 h. This band was collected from the isoelectric focusing column, and the isoelectric point of the pigment determined by measuring the pH of the solution. The value determined for the isoelectric point was 3.1. The blue-green solution was next dialyzed and lyophilized. A very small amount (< 0.5 mg) of blue-green material was isolated from about 6 ml of plasma. The purified pigment was found to be homogeneous when it was subjected to polyacrylamide disc gel electrophoresis[11] at pH 8.6 and stained with amido black 10B.

The absorption spectrum of the lyophilized pigment dissolved in a small volume of water is shown in the Figure. The absorption spectrum of the crude plasma was found to have absorption maxima at ~412 nm and 658–660 nm. The purified pigment has only the one maximum at 658–660 nm in the visible region. The 412 nm maximum in the spectrum of the crude plasma is due to the Soret band of oxyhemoglobin[12] and is the result of a small amount of hemolysis in the plasma samples.

Pieces of skin from the sculpin, after being freed from adhering flesh, were homogenized in a Waring Blendor. The purification procedure described above was carried out on the homogenized solution. A blue-green pigment which had an isoelectric point and absorption spectrum identical to that purified from the plasma was isolated.



Absorption spectrum of purified blue-green pigment from blood plasma of the Arctic sculpin, *Myoxocephalus scorpioides*.

Results and discussion. The UV-absorption spectrum and the physical properties of the blue-green pigment are characteristic of a chromoprotein. The approximate amino acid composition and molecular weight of the chromoprotein were determined. A small amount of purified blood plasma chromoprotein was hydrolyzed in a sealed ampule with 6*N* HCl under vacuum for 48 h at 100 °C. The hydrolysate was analyzed on a modified Beckman-Spinco amino acid analyzer[13]. Approximately 50% of the recoverable amino acids[14] after the acid hydrolysis were aspartic acid (~15%), glutamic acid (~12%), serine (~11%), and glycine (~8%). Analysis[15] of a solution which had been hydrolyzed in 6*N* HCl for 2 h at 100 °C indicated the presence of carbohydrate moieties associated with the chromoprotein. The molecular weight estimate was determined from sedimentation equilibrium experiments performed in a Beckman Model E analytical ultracentrifuge. A weight-average-molecular weight of ~46,000 was calculated based on an assumed partial specific volume of 0.725.

The prosthetic group of the chromoprotein isolated from eel blood serum was found to be biliverdin[7]. The eel serum chromoprotein has absorption maxima[6] at 702–704 nm and 383–384 nm which differs considerably from the absorption spectrum of the purified Arctic sculpin plasma chromoprotein. This difference suggests that the prosthetic group of the sculpin chromoprotein is not biliverdin. Since only a very small amount of material was isolated, no attempts were made to split the prosthetic group from the purified sculpin chromoprotein. In experiments with the crude plasma, however, it was found that the prosthetic group could be extracted into chloroform following treatment of the plasma with 10 vol. of HCl-methanol (1:9) and removal of the denatured protein by centrifugation. The color and failure of the prosthetic

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group to extract into hexane when this solvent was used instead of chloroform indicates it is not a carotenoid. The extraction into chloroform after protein denaturation with acidic methanol is similar to that of chromoproteins having bilin prosthetic groups[4,7,16]. However, attempts to isolate the prosthetic group from the chloroform solution were not successful. The solution gradually changed from blue-green to yellow and finally the color disappeared completely.

Two temperate-water fishes, *Clinocottus analis* (wooly sculpin) collected in tidal pools along the Southern California coast and *Scorpaenichthys marmoratus* (cabezon), which was collected[17] off the coast of Southern California on the ocean bottom at a depth of about 20 m, were also found to have blue or blue-green blood plasma. Preliminary experiments indicate that a blue-green protein, with properties similar to those of the chromoprotein found in the blood plasma and skin of the Arctic sculpin, could be isolated from the plasma of both species[18]. The blue-green protein therefore appears to occur in the blood plasma of 3 different species of fish. The 3 species belong to the family *Cottidae* and further studies of the blood plasma and skin of fishes in this family may reveal other examples of the occurence of the chromoprotein. The biosynthetic pathway and the physiological function, if any, of the blue-green protein are not known[19].

Zusammenfassung. Aus dem Blutplasma und der Haut des arktischen Spinnenfisches *Myoxocephalus scorpioides* wurde ein blaugrünes Chromoprotein isoliert.

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[18] Besides the blue-green pigment, there also appears to be a reddish pigment present in the plasma of *Scorpaenichthys marmoratus*. Except for some ammonium sulfate fractionations, no attempt was made to purify this pigment.

[19] This work was supported in part by Grant No. 07011-03 from the United States Public Health Service. I wish to thank Dr. P. SCHOLANDER who extended the invitation to participate in Phase I of the R/V *Alpha Helix* Bering Sea Expedition (supported by NSF Grant No. GB7173); Dr. S. SCHWARTZ for his many helpful suggestions concerning the purification and characterization of the pigment; Mr. W. MOLLER who operated the ultracentrifuge; Dr. D. L. FOX for helpful discussions; and Dr. S. L. MILLER in whose laboratory this work was performed.

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Hydrolysis of Amino Acid β-Naphthylamides by Aminopeptidases in the Parotid Gland

In a previous paper[1], we reported the enzymic hydrolysis of amino-acid β-napthylamides by aminopeptidases in human parotid saliva. The hydrolysis of glycyl-L-prolyl β-naphthylamide in parotid saliva was relatively higher than those of other amino-acid β-napthylamides, indicating the presence of a newly described kidney enzyme by HOPSU-HAVU and GLENNER[2] in parotid salivary fluid.

We have recently found that an aminopeptidase which hydrolyzes glycyl-L-prolyl β-naphthylamide was predominantly present in bovine parotid gland[3]. This communication describes the comparison of the substrate specificities with aminopeptidases in bovine parotid gland and the intracellular distribution of the enzymes.

Bovine parotid gland was obtained fresh, packed in ice, from the slaughterhouse. The tissue was homogenized by the use of an Ultra-Turrax homogenizer with 9 vol. of 0.25 *M* sucrose. After removing cell debris and nucleus by low-speed centrifugation, mitochondrial and microsomal fractions and soluble supernatant were separated by differential centrifugation. The substrate amino-acid β-naphthylamides, which were synthetized as described by GLENNER et al.[4], were kindly supplied from Dr. G. G. GLENNER. Glycyl-L-prolyl β-naphthylamide hydrobromide was kindly synthetized by Drs. S. SAKAKIBARA and K. TAKADA by the method of GLENNER et al.[2]. The incubation mixture contained 90 μmoles *Tris*-maleate buffer, pH 7.0, 0.45 μmole amino-acid β-naphthylamide and water to 0.90 ml. The activity for the hydrolysis of α-L-glutamyl β-naphthylamide and α-L-aspartyl β-naphthylamide was measured in the presence of 1 m*M* of Ca^{2+}[5]. Incubation was carried out at 37 °C for 60 min. Increase of fluorescence intensity of 410 nm of β-naphthylamine released by enzymic hydrolysis of amino-acid β-naphthylamide was measured with the excitation light at 335 nm using an Aminco-Bowman spectrophotofluorometer[6].

Results are shown in the Table. Aminopeptidases hydrolyzing amino-acid β-naphthylamides were distributed mainly in the microsomal fraction, as well as in the soluble fraction. The enzyme activities in mitochrondrial and nuclear fractions were low and probably due to contamination of the microsomal fraction. Among 21 amino-acid β-naphthylamides, naphthylamides of glycyl-proline, alanine, leucine, methionine, arginine, norleucine, and norvaline were good substrates for aminopeptidases in the parotid gland. These amino-acid β-naphthylamides were also good substrates for salivary aminopeptidases[1].

The enzyme which hydrolyzes glycyl-prolyl β-naphthylamide was the most active aminopeptidase in the parotid gland, as well as in parotid saliva[1]. This enzyme was mainly localized in the microsomal fraction as shown in the Table. Analysis of the reaction product in the hydrolysis of glycyl-prolyl β-naphthylamide by paper chromatography demonstrated that N-terminal glycyl-proline was liberated from the substrate. This result indicated that the aminopeptidase in the salivary gland is similar to the enzyme in the kidney newly described by

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