THE ROLE OF PITUITARY IN REGULATING ANTIFREEZE PROTEIN SYNTHESIS IN THE WINTER FLOUNDER

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1. Introduction

The antifreeze proteins are a unique class of serum proteins found in some polar and cold water fish. These proteins prevent the fish from freezing and are essential for their survival [1,2]. At present, there are two different types of antifreeze proteins known; the glycoproteins isolated from some Antarctic [3] and Arctic fishes [4] and the polypeptide antifreeze from the winter flounder [5,6]. The glycoproteins consist of repeating tripeptide units of alanyl–alanyl–threonine with a disaccharide linked to the threonyl residue. Flounder antifreeze protein, on the other hand, contains 8 amino acids and no carbohydrate moiety. The only similarity between these two types of antifreezes are the abundance of alanine in their amino acid composition.

The occurrence of the antifreeze protein (AFP) in the winter flounder inhabiting Newfoundland seawater is seasonal [7]. It appears in the plasma during November and disappears during May of each year. Both temperature and photoperiod are involved in regulating this annual cycle [8]. We have recently shown that the pituitary is necessary for the seasonal disappearance of the antifreeze from the plasma [9]. However the detailed mechanisms whereby the pituitary regulates plasma antifreeze has yet to be investigated. Here we describe our findings on the effect of hypophysectomy on AFP synthesis using both in vivo and in vitro procedures. Our results show that while control animals lose the ability to synthesis AFP during the summer months, the hypophysectomized animals continue to synthesize the protein.

2. Materials and methods

Winter flounder, *Pseudopleuronectes americanus*, were collected from Chapel's Cove, Newfoundland in February 1978, and maintained in 240 l aquaria supplied with flowing seawater (ambient temperature and photoperiod). The fish were hypophysectomized in April by the methods in [10]. The biosynthesis of AFP was studied in July (water temp. 8–10°C) when all traces of plasma antifreeze had disappeared from normal control fish. The in vivo biosynthesis in normal and hypophysectomized flounder was investigated by intravenously injecting 50 μCi [^{14}C]alanine (spec. act. 120 mCi/mmol, New England Nuclear, Lachine, Quebec). The animals were bled 48 h later. The serum was precipitated with equal vol. 20% trichloroacetic acid. Acid-soluble materials were first desalted on a G-75 column in 0.05 M NaCl and the non-retarded materials were later rechromatographed on a G-75 column (16 × 86 cm) in 0.05 N NH_4HCO_3 [5,11]. Aliquots were taken for radioactive counting in 10 ml Aqusol 2 (New England Nuclear).

For the in vitro incorporation experiments, livers from the control and hypophysectomized fish were cut into 0.5 mm thick slices using a mechanical tissue chopper (Mickle Lab. Engineer. Co. Gomshall, England). The slices (1 g each) were placed in 25 ml Erlenmeyer flask containing 2 ml Krebs-Ringer bicarbonate buffer and preincubated for 10 min at 15°C. The tissues were then transferred to another flask containing 2 ml buffer, 10 μCi [^{14}C]alanine and 100 μl Trasylol (FBA Pharmaceut. Co., Montreal). The flasks were gassed with O_2:CO_2 (95:5, v/v)

stoppered and incubated at 15°C for 4 h. After homogenization, the homogenates were treated with an equal vol. 20% trichloroacetic acid. Acid-soluble materials were then desalted, the protein fractions were later chromatographed on the Sephadex G-75 column as described earlier.

The freezing point of the serum was determined using a freezing point osmometer (Advanced Instr., Needham Heights, MA) which senses the heat of fusion on freezing [5]. One mOsm/kg corresponds to 1.856 °C.

3. Results and discussion

The appearance of AFP in flounder serum is seasonal. The osmolality of the fish serum increases from 330 mOsm in October to > 600 mOsm in January and February when the average water temperature is at its lowest of −1°C to −1.3°C. The osmolality starts to decline when the seawater warms up and reaches a minimum value of 320–330 mOsm again in June and July [11]. The increase in osmolality in the winter is mainly due to the presence of AFP [7].

The osmolality of the control and hypophysectomized animals used in this experiment is shown in table 1. The animals were hypophysectomized in April (~600–650 mOsm) and the experiments were carried out in July. The control animals had a mean value of 332 mOsm which is normal for July. The hypophysectomized animals, however, retained high levels of AFP in their plasma. These results are essentially the same as those in [9]. It appears that pituitary gland is necessary for the disappearance of the AFP from the plasma. The maintenance of high levels of AFP in the hypophysectomized animals at summer water temperatures could be due to the failure of the fish to degrade the protein or to the continued synthesis of the protein by the liver. Preliminary

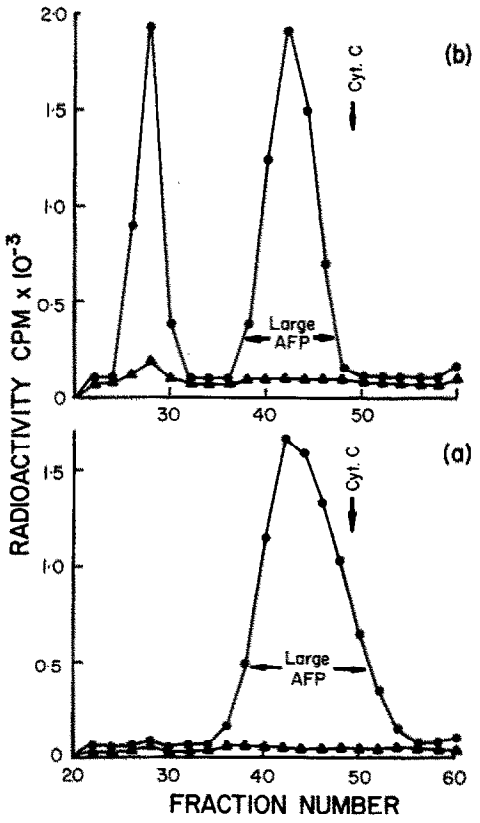

Fig.1. Biosynthesis of the antifreeze protein in control and hypophysectomized winter flounder. (a) In vivo studies. The control (▲——▲) and hypophysectomized flounder (●——●) were each injected with 50 μCi [^{14}C]alanine. The animals were bled and sacrificed 48 h later. After desalting, the acid-soluble materials from the plasma samples were chromatographed on a Sephadex G-75 column (1.6 × 86 cm) in 0.05 M NH_4HCO_3. The fraction size was 2.3 ml. Fractions corresponding to the 15 000 dalton component were indicated as 'large AFP'. (b) In vitro studies. Liver slices from control (▲——▲) and hypophysectomized flounder (●——●) in fig.1(a) were incubated 15°C for 4 h in Krebs-bicarbonate buffer. The tissues were processed with trichoroacetic acid. After desalting, acid-soluble materials were chromatographed on the G-75 columns as in fig.1A.

Table 1
Freezing point depression of control and hypophysectomized winter flounder at warm temperature (8–10°C)

	No animals used	Serum osmolality mOsm	Freezing point depression
Control animals	3	332	−0.62°C
Hypophysectomized animals	3	590	−1.09°C

experiments in our laboratories have indicated that the clearance of radioactive antifreeze from the plasma of hypophysectomized animals is similar to that of the controls (G.L.F., C.L.H., unpublished).

Our earlier in vivo [11] and in vitro [12] experiments have demonstrated that flounder AFP (10 000 daltons) is synthesized via a larger precursor protein of 15 000 daltons. The present experiments indicate that the control animals lost the ability to synthesize the AFP, as judged by the absence of the radioactive 15 000 dalton precursor both in vivo and in vitro (fig.1). This is in agreement with a current seasonal study of antifreeze biosynthesis [12]. Hypophysectomized animals, on the other hand, continued to synthesize the precursor component in both cases. Since the control animals did not have the ability to synthesize the AFP even under optimal incubation conditions [12], this suggests the absence of a readily translatable antifreeze mRNA. The transcription of the mRNA in the control animals would presumably be suppressed. Therefore, it is conceivable that the removal of the pituitary removed or inactivated a repressor for the AFP gene, thus allowing the transcription and translation of the mRNA. These studies suggest that the pituitary gland inhibits the synthesis of flounder AFP during the summer months and that this inhibition is removed annually with the approach of winter seawater temperatures.

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