The Journal of Experimental Biology 208, 2609-2613
Published by The Company of Biologists 2005
doi:10.1242/jeb.01666

Antifreeze activity in the gastrointestinal fluids of *Arctogadus glacialis* (Peters 1874) is dependent on food type

Kim Præbel[1,*] and Hans Ramløv[2]

[1]*University of Tromsø, Norwegian College of Fishery Science, N-9037 Tromsø, Norway* and [2]*Roskilde University, Department of Life Sciences and Chemistry, PO Box 260, DK-4000 Roskilde, Denmark*

*Author for correspondence (e-mail: kim.praebel@nfh.uit.no)

Accepted 26 April 2005

Summary

The influence of two food types, *Boreogadus saida* (Bs) and crustaceans (Cr), on the osmolality, ion concentrations, antifreeze activity and antifreeze glycoprotein (AFGP) distribution in the gastrointestinal fluids of the Arctic gadoid *Arctogadus glacialis* was determined. The gastrointestinal fluids were hyperosmotic to serum but no significant differences in osmolality were found between the two food types. The food type significantly affected the antifreeze activity of the mid-gut fluids. The hysteresis freezing points, –3.27±0.30°C and –2.44±0.11°C for *B. saida* and crustaceans, respectively, were significantly lower than that of serum (–1.99±0.07°C). Furthermore, an exceptionally large thermal hysteresis ranging from 1.47±0.19°C to 2.04±0.30°C was observed in the intestinal fluids of fish feeding on *B. saida*. Native gel electrophoresis revealed that the gastrointestinal fluids contained AFGPs in all the different size groups. However, differences in band intensities for the two food types suggest that the ingested food has an influence on the concentration of the different AFGP-sizes in these fluids. A decrease in band intensities combined with a drop in thermal hysteresis from mid-gut to hind-gut fluid suggests that absorption of AFGP or possibly degradation occur during digestion.

Key words: *Arctogadus glacialis*, *Boreogadus saida*, antifreeze glycoproteins, freezing avoidance, osmolality, ion concentrations, gastrointestinal fluids.

Introduction

The gastrointestinal tract and the gills are probably the two main sites for entry of ice crystals in fish exposed to ice-laden waters, because the integument has been shown to be a relatively good barrier to ice propagation at temperatures below the freezing point of the blood (Turner et al., 1985; Valerio et al., 1992). Although the osmolality of the gastrointestinal fluids in polar fishes is elevated compared with that of temperate fishes (Hickman, 1968; O'Grady et al., 1982b), the melting point is still above that of seawater (–1.9°C). It has been shown that the Antarctic notothenioids avoid ice propagation from ingested ice crystals by accumulating antifreeze glycoproteins (AFGP) in their intestinal fluid and, in part, the source is the bile (O'Grady et al., 1983). Presence of antifreeze proteins in nearly all the body fluids and tissues (Ahlgren et al., 1988; DeVries, 1971; DeVries et al., 1974; DeVries and Wohlschlag, 1969; Dobbs et al., 1974; O'Grady et al., 1982b; O'Grady et al., 1983) demonstrate their importance in the freezing avoidance of the polar fishes. Antifreeze proteins lower the non-equilibrium freezing point of an aqueous solution without significantly affecting the melting point, thus producing a difference between the freezing and melting points. This difference is called the thermal hysteresis or the antifreeze activity, and is thought to be the result of an adsorbtion inhibition mechanism (Raymond and DeVries, 1977). The AFGPs appear in at least eight sizes with molecular masses ranging between 2.6–33.7 kDa, but recent high resolution gradient gels show at least 15 molecular isoforms (Chen et al., 1997). The AFGP1–5 (33.7–10.5 kDa) have the greatest antifreeze activity (Kao et al., 1986; Osuga et al., 1978; Schrag et al., 1982), whereas the smaller AFGP6–8 (7.9–2.6 kDa) only exhibit up to a third of the antifreeze activity of AFGP1–5 (Kao et al., 1986; Osuga et al., 1978; Schrag et al., 1982). Furthermore, it has been shown that the antifreeze activity strongly depends on the AFGP concentration (O'Grady et al., 1982a).

Although AFGP comprises 3–4% (w/v) of the blood in polar fishes (Ahlgren et al., 1988), and is, thereby, energetically costly to maintain, no mechanism has been identified that prevents rectal loss of AFGPs in the Antarctic notothenioids (O'Grady et al., 1983). However, studies of temperate fishes have shown that the fish rectum has the capacity to absorb small amounts of intact proteins and peptides with different molecular size and shape (<40 kDa) (McLean and Ash, 1987; McLean et al., 1999).

The aim of this study was to determine the effect of the ingested type of food on the osmolality, ion concentrations, antifreeze activity and AFGP distribution of the fluids in the gastrointestinal tract of the Arctic gadoid *Arctogadus glacialis*.

Additionally, the hypothesis of AFGP absorption in the intestine of *A. glacialis* is discussed.

Materials and methods

Animal and sampling procedure

Specimens of *Arctogadus glacialis* (Peters 1874) were caught from a hole in the sea ice in April by hook and line at Ujaraqsuit (70°38′55N, 51°48′28W), Uummannaq district, Greenland. The fish were caught in 14–40 m of water at water temperature of –1.8°C.

The specimens were anaesthetized by 30 mg l^{-1} MS-222 (Sigma Chemical Co., St Louis, MO, USA) and blood was collected from the caudal vein by syringe using a 19-gauge needle. Samples were allowed to clot at approximately 20°C, and thereafter stored overnight at 4°C before centrifugation. After centrifugation at 4000 ***g*** for 10 min the serum was removed by pipette and stored at –25°C for later analysis.

The intestinal tract of the dead fish was dissected out, and the oesophagus and rectal end of the tract clamped. The tract was then separated into a stomach, mid-gut and hind-gut portion by clamping between the segments.

Stomach fluid was collected by syringe using a 16-gauge needle puncturing through the stomach wall. The mid-gut and hind-gut fluids were collected by draining the content into 1.9 ml Eppendorf tubes. The fluids were then centrifuged at 4000 ***g*** for 10 min and the supernatant was removed by pipette and stored at –25°C.

Observed food content in the stomach was correlated with the colour of the supernatant. Green samples indicated *Boreogadus saida* (Polar cod) was ingested whereas red samples indicated that crustaceans (Amphipods and Copepods) had been the prey.

Ion concentrations, osmolality and melting-point and freezing-point determinations

Cation concentrations in the body fluids were determined as triplicates with a FLM3 Flame Photometer (Radiometer, Copenhagen, Denmark). Chloride concentrations were measured as triplicates with a CMT 10, Chloride Titrator (Radiometer, Copenhagen, Denmark). Osmolality in the various body fluids was measured as duplicates with a Wescor 5100C vapor pressure osmometer (Wescor Inc., Logan, UT, USA).

The freezing point of the body fluids was measured using a Clifton Nanolitre Osmometer (Clifton Technical Physics, Hartford, NY, USA), mounted on a Zeiss STEMI SV11 APO microscope (Carl Zeiss AG, Oberkochen, Germany. The samples were loaded with a capillary micropipette into the center of oil-filled wells and sample size was approximately a third of the well size. The samples were then quickly cooled to –40°C and the temperature was slowly raised until the last ice crystal disappeared, which was taken as the observed melting point. After refreezing the samples, the temperature was slowly raised again until approximately 0.09°C lower than the melting point. The temperature was then lowered 0.19°C and allowed to stabilize for 1 min. Further temperature decrease was then performed at a rate of 0.19°C min^{-1} until explosive growth of ice spicules occurred. The temperature at which the spicular growth occurred is the hysteresis freezing point, and the temperature difference between the melting point and the hysteresis freezing point is defined as the antifreeze activity.

The measured melting and hysteresis freezing points were given in mOsm, and by multiplying the osmolality values by 0.001858°C $mosmol^{-1}$ kg^{-1} (Levine, 1995), the corresponding temperature (°C) was found. The maximal thermal hysteresis was then determined as described by Sørensen and Ramløv (2001). Statistical significance was tested at the $P \leq 0.05$ significance level by using a two-tailed Student's *t*-test. Data are given as means ± S.E.M. (N=3–6).

Electrophoresis

The antifreeze glycoproteins were isolated from the blood and gastrointestinal fluids by adding cold trichloroacetic acid (TCA; Merck, Germany) up to a final concentration of 5% (v/v) [antifreeze glycoproteins are soluble in TCA (DeVries and Wohlschlag, 1969; Van Voorhies et al., 1978)]. After centrifugation at 9500 ***g*** for 5 min, the supernatant was transferred to a Spectrapor-3 dialysis tubing (molecular mass cut off 3.5 kDa; Spectrum Laboratories Inc., Rancho Dominguez, CA, USA), dialyzed against distilled water at 4°C for 36 h and then lyophilized. The AFGP were then resuspended in distilled water and fluorescently labelled with fluorescamine (Sigma Chemical Co.) (Chen et al., 1997) and run on 10–20% non-denaturing acrylamide gradient gel using borate in the buffer system (O'Grady et al., 1982b), for 1 h at 200 V and 4 h at 30 mA in a cold room. Purified AFGP from the Antarctic notothenioid *Dissostichus mawsoni* (kindly provided by A. L. DeVries) was used as standard. All samples were loaded on the gel in the same total concentration (250 μg). The gel was viewed with transmitted UV-light and images captured with an Eagle-eye imaging system (Stratagene, La Jolla, CA, USA).

Results

Ion concentrations, osmolality and melting point and freezing point determinations

There was no significant difference in osmolality and antifreeze activity in the stomach fluids for the two food types. Surprisingly, the hysteresis freezing points [–1.82±0.05°C (Bs) and –1.76±0.08°C (Cr)], were near or above that of seawater (–1.8 °C) (Fig. 1 and Table 1). The concentrations of Na^+, K^+ and Cl^- in the stomach fluids from fish that had eaten crustaceans (667±26 mmol l^{-1}) were significantly higher than from those fish that had eaten *Boreogadus saida* (526±14 mmol l^{-1}).

The food type had an influence only on the antifreeze activity of the mid-gut fluids. *Arctogadus glacialis* that had eaten *B. saida* showed significantly higher hysteresis (antifreeze activity) (2.04±0.31°C) than specimens feeding on crustaceans (1.17±0.08°C) (Fig. 1 and Table 1). The hysteresis freezing points [–3.27±0.30°C (Bs) and –2.44±0.11°C (Cr)]

Table 1. *Comparison of osmolality, melting and freezing points, thermal hysteresis and ion concentrations in serum and gastrointestinal fluids from* Arctogadus glacialis *that had ingested* Boreogadus saida *(Bs) and crustaceans (Cr)*

		N	OSM (mosmol kg^{-1})	Calculated MP (°C)	TH (°C)	HFP (°C)	Na^+ (mmol l^{-1})	K^+ (mmol l^{-1})	Cl^- (mmol l^{-1})
Serum		10	511±7	–0.95	1.04±0.05	–1.99	234±5	4±0.7	173±2
Stomach fluid	Bs	5	720±36	–1.34	0.48±0.09	–1.82	160±17	23±2.1	343±6
	Cr	3	775±64	–1.44	0.32±0.01	–1.76	275±49	17±2.7	376±30
Mid-gut fluid	Bs	6	661±36	–1.23	2.04±0.30	–3.27	171±11	14±2.0	121±8
	Cr	5	681±35	–1.27	1.17±0.08	–2.44	191±8	13±2.5	161±18
Hind-gut fluid	Bs	6	685±24	–1.27	1.47±0.19	–2.74	197±6	16±1.5	137±13
	Cr	5	695±44	–1.29	1.24±0.20	–2.53	200±10	9±0.9	169±8

Values are means ± S.E.M. Statistical significance was tested at the $P \leq 0.05$ significance level by using a two-tailed Student's *t*-test. The melting point (MP) was calculated on the basis of the osmolality value (OSM): MP = osmolality × (0.001858°C mosmol^{-1} kg^{-1}). Thermal hysteresis (TH) was calculated as the difference between the observed freezing and melting point. The hysteresis freezing point (HFP) was calculated by adding the calculated melting point to the observed thermal hysteresis.

for both food types were significantly lower than that of serum (–1.99±0.07°C).

The osmolality of the mid-gut fluids was not significantly different from the stomach fluids for the two types of food, but the ion concentrations in the mid-gut fluids were significantly lower than in the stomach fluid, showing the absorption of ions in the mid-gut.

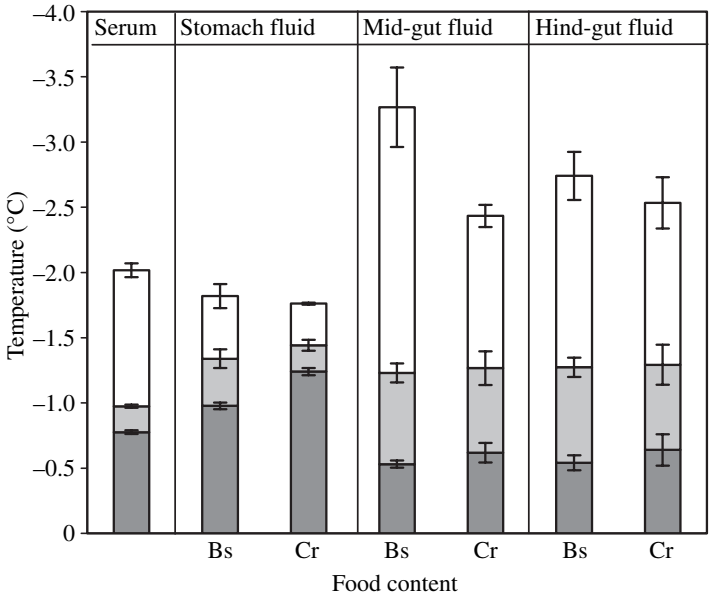

Fig. 1. The hysteresis freezing points of the gastrointestinal fluids and serum from *Arctogadus glacialis* that had ingested *Boreogadus saida* (Bs) and crustaceans (Cr). The dark gray boxes represent the melting point depression due to measured ions, light gray boxes represent the difference between the measured melting point and the melting point depression due to measured ions. The open boxes represent the thermal hysteresis due to AFGP. Data are given as mean ± S.E.M. Statistical significance was tested at the $P \leq 0.05$ significance level by using a two-tailed Student's *t*-test. The food type only had significant influence on the antifreeze activity of the mid-gut fluids.

Neither ion concentrations, osmolality nor antifreeze activity of the hind-gut fluids showed significant differences for the two food types. Nevertheless the hysteresis freezing points were significant lower (Bs: –2.74±0.19°C, Cr: –2.53±0.20°C) than the hysteresis freezing point of the serum (–1.99±0.07°C) (Fig. 1 and Table 1).

Gel electrophoresis

Native gel electrophoresis revealed that the stomach fluids and the intestinal fluids contained AFGP in all the different size groups (Fig. 2). A distinctive smear is seen below each known AFGP-size showing that the AFGPs are degraded into many sub-sizes (>40) during the digestion process.

When comparing the bands from *A. glacialis* serum (lane 5), and the stomach and intestinal fluids (lanes 1–3, 7–9), it is clear that AFGP synthesized by *A. glacialis* is present throughout the whole digestive system (Fig. 2, arrow a). By comparing the bands from *B. saida* serum (lane 6) and those of the stomach and intestinal fluids, it is obvious that *B. saida* AFGP is present in both food groups (Fig. 2, arrow b).

Comparison of band intensities of AFGP7 and 8 in the stomach fluids (lanes 1 and 7) for the two types of food shows that the food type has an influence on the concentration of the different AFGP sizes in these fluids. The stomach fluid of *A. glacialis* that had ingested *B. saida* contains higher concentrations of AFGP7 and 8 (Fig. 2, arrow c and d) than when *A. glacialis* had ingested crustaceans (Fig. 2, arrow e and f).

Furthermore, the gel electrophoresis, where all lanes were loaded in equal total concentration of AFGP, indicated that absorption or possible degradation of AFGP occur during the food passage through the digestive system. This is seen by the decrease in intensity of the AFGP7 and 8 bands from stomach to hind-gut fluid (Fig. 2, lanes 1–3 and 7–9), indicating the disappearance

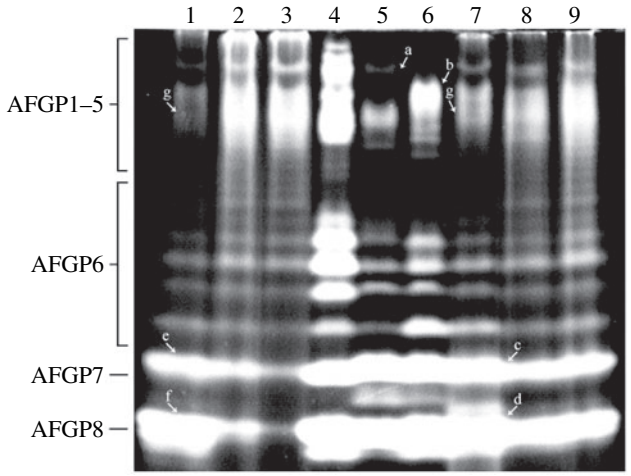

Fig. 2. Native gradient polyacrylamide gel showing the AFGP composition in the gastrointestinal fluids and the serum of *Arctogadus glacialis* and in the serum of *Boreogadus saida.* Lane 1, stomach fluid, food: crustaceans; lane 2, mid-gut fluid, food: crustaceans; lane 3, hind-gut fluid, food: crustaceans; lane 4, purified AFGP from *Dissostichus mawsoni*, used as standard; lane 5, *A. glacialis* serum; lane 6, *B. saida* serum; lane 7, stomach fluid, food: *B. saida*; lane 8, mid-gut fluid, food: *B. saida*; lane 9, hind-gut fluid, food: *B. saida*. All lanes were loaded with equal amount of AFGP (250 g). The main AFGP sizes for the *D. mawsoni* standard are indicated. Arrow a indicates an AFGP synthesized by *A. glacialis* and that is present in all the gastrointestinal fluids; arrow b shows an AFGP in *B. saida* serum that can be found in all the gastrointestinal fluids; arrow c indicates that AFGP7 has a higher intensity than the AFGP7 in lane 1 (arrow e); arrow d indicates that AFGP8 has higher intensity than AFGP8 in lane 1 (arrow f); arrow g shows the increase in concentration of the high molecular mass AFGPs in the gastrointestinal tract, which originate in the disappearance of the low molecular mass AFGPs.

of AFGP7 and 8 from the fluids. The observation is supported by the increase of the intensities of the high molecular AFGPs (Fig. 2, lanes 1–3 and 7–9, arrow g).

Discussion

Previous (unpublished) work on freshly caught specimens of *Arctogadus glacialis* has shown that the digestive system is fortified with antifreeze glycoproteins that lower the freezing point of the digestive fluid to below that of seawater (K. Præbel, C.-H. C. Cheng, A. L. DeVries and H. Ramløv, unpublished). Thus, growth of ingested ice crystals is hindered and the fish is, thereby, protected against ice propagation from the intestinal lumen. Earlier work on intestinal antifreeze glycoproteins in polar fishes have only been carried out on starved specimens (Ahlgren et al., 1988; O'Grady et al., 1982b, 1983) and in detail only in the Antarctic notothenioids (O'Grady et al., 1983).

During the dissection and sample preparation of the specimens of *A. glacialis* it was noticed that about 50% of the specimens had ingested *Boreogadus saida*, indicating that *A. glacialis* does not feed exclusively on crustaceans as reported elsewhere (Süfke et al., 1998). This observation is supported by diet studies of *A. glacialis* and large specimens of *B. saida* captured in Dove Bay, Northeast-Greenland (K. Præbel, unpublished), which revealed that both species are piscivorous and cannibalistic predators.

Our results show that there is a correlation between ingested food and the thermal hysteresis in the intestinal fluids of *A. glacialis*. Only non-significant osmolality differences of the gastrointestinal fluids were observed when the two food types were compared. Corresponding comparison of ion concentrations revealed that only stomach fluid differed significantly. This is probably due to the high ionic content in the crustaceans as they are iso-osmotic to seawater, and to the large amount of protons needed to buffer the carbonate originating from the cuticle of those animals.

One interesting question that arises from the results concerns the extremely high antifreeze activity (2.04±0.31°C, HFP: –3.27±0.30°C) found in the mid-gut fluids where the ingested food was *B. saida*. The physiological significance in terms of freezing avoidance is doubtful, because the average freezing point of Arctic seawater is –1.8°C (Garrison, 1998). The polyacrylamide gel electrophoresis showed that the AFGPs are degraded into many sub-sizes during the digestion process. As pointed out in other studies, the size composition of AFGP have crucial influence on the antifreeze activity (Ahlgren and DeVries, 1984; Kao et al., 1986; Osuga et al., 1978; Schrag et al., 1982). Thus, a possible explanation for the high antifreeze activity can be the combination of many sub-sizes of AFGP and the fact that the concentration of AFGP increases in the intestine due to water uptake by the gut wall (O'Grady et al., 1982b).

An increase in antifreeze activity was observed from the stomach to the mid-gut with a decrease in the hind-gut. This observation is consistent with the digestion, absorption and evacuation pattern. The antifreeze activity is low in the stomach due to little release of AFGP from the partly digested food. The increasing antifreeze activity in the mid-gut must be a consequence of increased release of AFGP from the digestion of the AFGP-laden polar cod, many sub-sizes of AFGP and increasing AFGP-concentration due to water uptake. Thus, the lower antifreeze activity found in the hind-gut might be caused by further degradation of the large sizes of AFGP and to the presence of less active smaller sizes. Nevertheless, the hysteresis freezing point of the hind-gut fluids is still well below of that of seawater.

From the gel electrophoretic study it is clear that AFGP from digested *B. saida* were present in all the samples, also in the samples where the food was believed to be crustaceans. Sæther et al. (1999) showed that the total gastrointestinal time for evacuation of an inert marker in *B. saida* was approximately 400 h at 0.5°C. A similar evacuation time could be expected for *A. glacialis*, since they live in colder environmental temperatures and the digestion time decreases with temperature. Therefore, the AFGP from *B. saida* found in all the samples confirms that *A. glacialis* feed on other prey than crustaceans.

In view of the fact that fish are able to absorb intact proteins up to at least 40 kDa (Berge et al., 2003; McLean and Ash, 1987; McLean et al., 1999), it is an open question whether or not absorption of antifreeze glycoproteins from the intestinal fluids occurs in *A. glacialis*. Experiments conducted by O'Grady et al. (1983) indicated that antifreeze glycoproteins are not absorbed as intact molecules in the intestinal system of the Antarctic notothenioids. Nevertheless, several observations shown in the present study indicate that absorption of AFGP might occur in *A. glacialis*. First, the gel electrophoretic study shows decreasing intensity of the low molecular mass AFGP from stomach to hind-gut, suggesting that absorption might be taking place. Secondly, our results show that the antifreeze activity is lower in the hind-gut fluids compared with the mid-gut fluid. Third, the high AFGP concentration combined with a long evacuation rate will increase the possible AFGP absorption (Hirst, 1993). Thus, further experiments on the relation between AFGP concentration, absorption and food type are needed to answer the question of whether AFGPs are absorbed or not.

In conclusion, the findings illustrate that the antifreeze activity in the intestinal tract of *A. glacialis* is dependent on food type. Furthermore, the results indicate that absorption of AFGP might occur in the digestive system of *A. glacialis* unlike that reported in the Antarctic notothenioids.

The authors would like to thank Karl Markussen, Uummannaq, Greenland for his help in localization of *Arctogadus glacialis*. Anne Kettunen and Jørgen S. Christiansen, University of Tromsø, Norway, is thanked for valuable comments to the manuscript and Chi-Hing Christina Cheng and Arthur DeVries, University of Illinois, for their enthusiastic discussions concerning freezing avoidance in fish and for providing standard mixtures of AFGP for the gel-electrophoretic study. This work was supported by The Danish Natural Science Foundation (Grant 21-01-0518) to H.R.

References

Ahlgren, J. A. and DeVries, A. L. (1984). Comparison of antifreeze glycopeptides from several Antarctic fishes. *Polar Biol.* **3**, 93-97.

Ahlgren, J. A., Cheng, C.-H. C., Schrag, J. D. and DeVries, A. L. (1988). Freezing avoidance and the distribution of antifreeze glycopeptides in the body fluids and tissues of Antarctic fish. *J. Exp. Biol.* **137**, 549-563.

Berge, G. E., Goodman, M., Espe, M. and Lied, E. (2003). Intestinal absorption of amino acids in fish: kinetics and interaction of the in vitro uptake of – methionine in Atlantic salmon (*Salmo salar* L.). *Aquaculture* **229**, 265-273.

Chen, L., DeVries, A. L. and Cheng, C.-H. C. (1997). Convergent evolution of antifreeze glycoproteins in Antarctic notothenioid fish and Arctic cod. *Proc. Natl. Acad. Sci. USA* **94**, 3817-3822.

DeVries, A. L. (1971). Glycoproteins as biological antifreeze agents in Antarctic fishes. *Science* **172**, 1152-1155.

DeVries, A. L. and Wohlschlag, D. E. (1969). Freezing resistance in some Antarctic fishes. *Science* **163**, 1073-1075.

DeVries, A. L., DeVries, Y. L., Dobbs, G. H. and Raymond, J. A. (1974). Studies of the Antarctic cod, *Dissostichus mawsoni*. *Antarctic J.* **9**, 107-108.

Dobbs, G. H., Lin, Y. and DeVries, A. L. (1974). Aglomerularism in Antarctic fish. *Science* **185**, 793-794.

Garrison, T. (1998). *Oceanography*. London: Wadsworth Publishing Company.

Hickman, C. P. (1968). Ingestion, intestinal adsorption, and elimination of seawater and salts in the southern flounder, *Paralichthys lethostigma*. *Can. J. Zool.* **46**, 457-466.

Hirst, B. H. (1993). Dietary regulation of intestinal nutrient carriers. *Proc. Nutr. Soc.* **52**, 315-324.

Kao, H. M., Fletcher, L. G., Wang, C. N. and Hew, L. C. (1986). The relationship between molecular weight and antifreeze polypeptide activity in marine fish. *Can. J. Zool.* **64**, 578-582.

Levine, I. N. (1995). *Physical Chemistry*. New York: McGraw-Hill.

McLean, E. and Ash, R. (1987). Intact protein (antigen) absorption in fishes: mechanism and physiological significance. *J. Fish Biol.* **31**, 219-223.

McLean, E., Rønsholdt, B., Sten, C. and Najamuddin (1999). Gastrointestinal delivery of peptide and protein drugs to aquacultured teleosts. *Aquaculture* **177**, 231-247.

O'Grady, M. S., Schrag, D. J., Raymond, A. J. and DeVries, L. A. (1982a). Comparison of antifreeze glycopeptides from Arctic and Antarctic fishes. *J. Exp. Zool.* **224**, 177-185.

O'Grady, S. M., Ellory, J. C. and DeVries, A. L. (1982b). Protein and glycoprotein antifreezes in the intestinal fluid of Polar fishes. *J. Exp. Biol.* **98**, 429-438.

O'Grady, S. M., Ellory, J. C. and DeVries, A. L. (1983). The role of low molecular weight antifreeze glycopeptides in the bile and intestinal fluid of Antarctic fish. *J. Exp. Biol.* **104**, 149-162.

Osuga, D. T., Ward, F. C., Yeh, Y. and Feeney, R. E. (1978). Cooperative functioning between antifreeze glycoproteins. *J. Biol. Chem.* **253**, 6669-6672.

Raymond, J. A. and DeVries, A. L. (1977). Adsorption inhibition as a mechanism of freezing resistance in polar fishes. *Proc. Natl. Acad. Sci. USA* **74**, 2589-2593.

Schrag, J. D., O'Grady, S. M. and DeVries, A. L. (1982). Relationship of amino acid composition and molecular weight of antifreeze glycopeptides to non-colligative freezing point depression. *Biochim. Biophys. Acta* **717**, 322-326.

Süfke, L., Piepenburg, D. and von Dorrien, C. F. (1998). Body size, sex ratio and diet composition of *Arctogadus glacialis* (Peters, 1874) (Pisces: Gadidae) in the Northeast Water Polynya (Greenland). *Polar Biol.* **20**, 357-365.

Sæther, B.-S., Christiansen, J. S. and Jobling, M. (1999). Gastrointestinal evacuation of particulate matter in polar cod *Boreogadus saida*. *Mar. Ecol. Prog. Ser.* **188**, 201-205.

Sørensen, T. F. and Ramløv, H. (2001). Variations in antifreeze activity and serum inorganic ions in the eelpout *Zoarces viviparus*: antifreeze activity in the embryonic state. *Comp. Biochem. Physiol. A.* **130**, 123-132.

Turner, J. D., Schrag, J. D. and DeVries, A. L. (1985). Ocular freezing avoidance in Antarctic fish. *J. Exp. Biol.* **118**, 121-131.

Valerio, P. F., Kao, M. H. and Fletcher, G. L. (1992). Fish skin – an effective barrier to ice crystal propagation. *J. Exp. Biol.* **164**, 135-151.

Van Voorhies, W. V., Raymond, J. A. and DeVries, A. L. (1978). Glycoproteins as biological antifreeze agents in the cod, *Gadus ogac* (Richardson). *Physiol. Zool.* **51**, 347-353.