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Iron transport across the skin and gut epithelia of Pacific hagfish: Kinetic characterisation and effect of hypoxia

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ABSTRACT

In most animals, the acquisition of the essential trace metal iron (Fe) is achieved by the gut, but in hagfishes, the skin is a nutrient absorbing epithelium, and thus may also play a role in Fe uptake. In the current study, the absorption of Fe, as Fe(II), across the intestinal and cutaneous epithelia of Pacific hagfish (*Eptatretus cirrhatus*) was investigated. Both epithelia absorbed Fe, with saturation at lower tested concentrations, superseded by a diffusive component at higher Fe exposure concentrations. Affinity constants (K_m) of 9.4 and 137 μM, and maximal Fe transport rates (J_{max}) of 0.81 and 0.57 nmol cm^{-2} h^{-1} were determined for the skin and the gut, respectively. This characterises the skin as a relatively high-affinity Fe transport epithelium. The majority of the absorbed Fe in the skin remained in the tissue, whereas in the gut, most absorbed Fe was found in the serosal fluid, suggesting distinct mechanisms of Fe handling between the two epithelia. To determine if reduced dissolved oxygen altered Fe transport, hagfish were subjected to hypoxia for 24 h, before Fe transport was again assessed. Hypoxia had no effect on Fe transport across gut or skin, likely owing to the relative lack of change in haematological variables, and thus an unaltered Fe demand under such conditions. These data are the first to kinetically characterise the absorption of a nutritive trace metal across the epithelia of hagfish and add to the growing understanding of the role of the skin in nutritive transport in this group.

1. Introduction

The absorption of nutrients is an essential process in all animals. Among vertebrates, this is a role that is restricted to the gastrointestinal epithelium, with a single exception. Hagfishes are primitive vertebrates that have the unique ability to use their cutaneous epithelium for organic nutrient uptake (Glover et al., 2011a, 2016; Stephens, 1968). In fact, the skin of hagfish performs a number of transport functions including the elimination of nitrogenous waste (Braun and Perry, 2010; Clifford et al., 2014), the uptake of the inorganic nutrient phosphorus (Schultz et al., 2014), and the potentially harmful absorption of the toxic metal nickel (Glover et al., 2015).

While there is growing evidence of the importance of the hagfish skin as a transport epithelium (Glover et al., 2013), there is remarkably little understanding of transport processes in the hagfish gastrointestinal tract (Glover and Bucking, 2015). To date, physiological evidence has shown that phosphate (Schultz et al., 2014), glucose (Bucking et al., 2011), and the amino acids L-alanine and glycine (Glover et al., 2011b) are absorbed by the gut of hagfish. However, there is nothing known regarding the absorption of nutritive trace metals across the hagfish digestive tract, or indeed, any hagfish epithelium.

Iron (Fe) is an important nutritive trace metal. It has critical functions as a co-factor in a range of enzymes and is particularly important in respiration where it acts as the oxygen-binding entity in haemoglobin. In general, Fe absorption in teleost fishes is similar to that in mammals (Bury et al., 2003). Molecular and physiological evidence suggests that apical Fe transport is achieved in the ferrous state (Fe(II)), via a proton-dependent transporter (divalent metal transporter-1; DMT1, also known as Nramp2 (SLC11A2); Cooper et al., 2006b, 2007; Dorschner and Phillips, 1999; Kwong and Niyogi, 2008; Kwong et al., 2010, 2013), which also facilitates the uptake of a number of other divalent metals (Cooper et al., 2007; Kwong and Niyogi, 2009). Basolateral transport is achieved by IREG (iron-regulated transporter, also known as ferroportin (SLC40A1); Cooper et al., 2006b; Donovan et al., 2000). The principal route of Fe uptake is likely to be the diet, due to the higher concentration and bioavailability of Fe via this route (Bury et al., 2003).

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However, although bioavailable Fe levels are very low in natural waters (Stumm and Morgan, 1996), the gills of teleost fish also play an important role in Fe acquisition (Bury and Grosell, 2003a, 2003b), especially in developing fish without viable digestive tracts (Andersen, 1997). The mechanisms of Fe absorption in hagfish, and the relative importance of the gut and alternative transport epithelia in this process, are unstudied.

A remarkable characteristic of hagfish is their extreme hypoxia tolerance (Forster, 1998). While buried in sediments, or burrowing into a decaying seafloor carcass, hagfish may experience significantly reduced environmental oxygen concentrations (Martini, 1998). In mammals, hypoxia is linked to changes in Fe metabolism (Chepelev and Willmore, 2011; Haase, 2010). For example, DMT1 contains hypoxia regulatory elements in its promotor region (Shah et al., 2009), and following hypoxia DMT1 expression increases (Li et al., 2008). Hypoxia-induced changes in Fe metabolism are mechanisms that aid in improving oxygen transport, which relies on the Fe-based respiratory pigment haemoglobin (Windsor and Rodway, 2007). Previous work has shown that hagfish epithelial transport processes are responsive to hypoxia (Bucking et al., 2011). Following exposure to 24 h of hypoxia, Pacific hagfish exhibited increases in glycine transport across the gill and the gut epithelia. This effect was accompanied by an increase in brain glycine, which is thought to induce metabolic depression and/or act as a cytoprotective mechanism (Bucking et al., 2011).

The current study sought to characterise the epithelial transport of Fe in the Pacific hagfish (*Eptatretus stoutii*). Specifically, the concentration-dependent kinetics of Fe uptake were examined in the skin and intestine utilising *in vitro* techniques. In addition, by analogy with mammals, it was hypothesised that characteristics of Fe transport in hagfish would change with hypoxia, a mechanism that might act to increase oxygen-carrying capacity of the blood and contribute to their remarkable tolerance of this phenomenon. This study highlighted key differences in the magnitude of uptake and handling of Fe between transport epithelia, but showed that transport was independent of environmental oxygen concentrations.

2. Materials and methods

2.1. Animals

Hagfish (*Eptatretus stoutii*) were collected via baited traps from Barkley Sound (Fisheries and Oceans Canada permits XR107 2012 and XR107 2013), off the west coast of Vancouver Island, near Bamfield. Following capture, fish were held in 500-L tarpaulin-covered tanks, receiving flow-through natural seawater at 12 °C, at Bamfield Marine Sciences Centre (BMSC). Hagfish were held for at least a week, unfed, prior to any experimentation. All procedures were approved by the BMSC Animal Care and Use Committee (RS-12-8; RS-13-11).

2.2. Transport assays

Hagfish (N = 6; ~70 g) were euthanised via anaesthetic overdose (2 g L^{-1} 3-aminobenzoic acid ethylester; MS222). The gut (posterior to hepatic portal vessel) and dorsal medial skin were then removed, and each was divided into six sections for Fe transport assays. For each animal, a complete kinetic curve was run with one concentration per tissue section, with sections randomised between test concentrations. For the gut, the nominal Fe concentrations tested were 2, 5, 20, 50, 200, and 500 μM, while for the skin, the nominal concentrations were 1, 2, 5, 10, 20, and 100 μM, all made up in filtered seawater (in mM: Na, 492; K, 9; Ca, 12; Mg, 50; Cl, 539; pH 8.0). These concentrations were achieved by dilution of a stock solution consisting of $FeCl_3$ and ascorbic acid in a 1:20 ratio, that was made fresh daily, and which was spiked with ~5–10 $\mu C_i\ mL^{-1}$ of ^{59}Fe (as $FeCl_3$; Perkin Elmer). The ascorbate ensured that Fe was presented to the fish as Fe(II) (Bury et al., 2001).

Gut transport was assessed using an intestinal gut sac technique (Glover et al., 2011b). Briefly, intestinal sections of ~3–5 cm were sutured closed at one end with surgical silk, and in the other was placed a short section (~3 cm) of cannula tubing (PE50; Intramedic, Clay Adams), which was flared at the inserted end. This was secured in place with surgical silk, and the cannula was used to introduce a small volume (0.5–3 mL) of the test Fe concentration in filtered sea water. The cannula was then heat-sealed and the sac was suspended in 50-mL of aerated hagfish Ringer (in mM: NaCl, 474; KCl, 8; $CaCl_2 \cdot 2H_2O$, 5; $MgSO_4 \cdot 7H_2O$, 3; $MgCl_2 \cdot 6H_2O$, 9; $NaH_2PO_4 \cdot H_2O$, 2.06; $NaHCO_3$, 41; and glucose, 5; pH 7.6). After a 2-h assay, a sample of the hagfish Ringer (serosal fluid) was taken for assessment of transported radiolabel. The gut sac was split open, rinsed in a high Fe solution (10 mM as Fe(II)) to remove any adsorbed ^{59}Fe, scraped free of any adhering mucus and epithelial tissue, and surface area was measured (see Glover et al., 2003). Tissue was then placed in a scintillation vial to which was added 2 mL of 2 N HNO_3 and the samples were digested for 48 h at 65 °C. Samples of Fe stock solutions (for determination of specific activity), the serosal fluid, and the digested tissue had scintillation fluor added (Optiphase for water samples, UltimaGold for tissue digests; Perkin Elmer), before they were counted in a liquid scintillation counter (LS6500; Beckman Coulter). Quench correction was applied based on the external standards ratio method.

Skin transport was assessed via a modified Ussing technique that has been described in detail previously (Glover et al., 2011a). Briefly, skin was stretched across the top of a 15 mL vial, and secured by screwing the lid, with an aperture cut into it, back in place. Ten millilitres of hagfish Ringer was added inside the vial, and the apparatus was secured upside-down, such that the external skin surface was immersed in an aerated bathing medium containing the appropriate radiolabelled Fe concentration in filtered seawater (prepared as described above). Transport was assessed for 2 h after which 5 mL of hagfish Ringer was removed for scintillation counting, and the skin was digested in 2 mL of 2 N HNO_3 for 48 h at 65 °C. Bathing medium, serosal fluid, and tissue digest samples were then assessed for ^{59}Fe activity as described above.

Uptake of Fe in both tissues was calculated using the following equation:

$$\text{Uptake}\left(\text{nmol cm}^{-2}\text{h}^{-1}\right) = (\text{cpm}/\text{SAct})/\text{SA}/\text{t}$$

where cpm is accumulation of ^{59}Fe radioactivity in the tissue and serosa, SAct is the specific activity (cpm $nmol^{-1}$), SA is surface area (cm^{-2}), and t is time (2 h).

2.3. Hypoxia exposure

To examine the effects of hypoxia on Fe transport, individual hagfish were put in sealed 600-mL plastic containers containing seawater, and placed in a water bath to maintain the temperature at 12 °C. Hagfish designated as normoxic (N = 7) had aeration provided through a hole in the lid, whereas chambers containing hypoxic hagfish (N = 7) were not aerated, and thus became hypoxic as the animal gradually consumed the oxygen in the chamber. After 24 h, hypoxic hagfish had all depleted oxygen partial pressure (PO_2) to levels less than 10 mmHg. Both groups were then removed, euthanised by anaesthetic overdose (2 g L^{-1} MS222), and a 1–5 mL blood sample was withdrawn from the subcutaneous caudal sinus. A heparinised capillary tube was used to draw a subsample of whole blood that was then spun at 5000 × *g* in a haemofuge, for determination of haematocrit. A 100 μL subsample was removed, added to Drabkin's reagent, and analysed for haemoglobin content (mg mL^{-1}) via measuring absorbance at 540 nm after a 24 h incubation in the dark at 4 °C. A 20 μL subsample was removed for an erythrocyte count via a haemocytometer. The remaining blood was immediately analysed for PO_2 (mmHg) via an oxygen electrode (Cameron Instruments) connected to a AM Systems Polarographic Amplifier