

Short sequence report

Unique multimeric immunoglobulin crosslinking in four species from the family Gadidae

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The basic architectural unit of immunoglobulin (Ig) is a disulphide-associated heterodimer of two heavy chains and two light chains (H_2L_2), hereto referred to as a monomer. All species that possess Ig (all vertebrates except the agnathan cyclostomes) have multimeric serum Ig. Multimeric serum Ig in mammals is the IgM pentamer ($H_{10}L_{10}$). The elasmobranchs also have a pentameric ($H_{10}L_{10}$) multimeric form [1]. The teleosts have a tetrameric (H_8L_8) serum Ig with a native molecular mass estimated to be between 700 and 1000 kDa depending on the species. This has been determined by both size-exclusion chromatography and non-reducing, low percentage acrylamide slab gel electrophoresis for a number of phylogenetically diverse teleosts: bluefin tuna, *Thunnus maccoyii* [2]; common carp, *Cyprinus carpio* [3]; sheephead, *Archosargus probatocephalus* [4]; Atlantic salmon, *Salmo salar* [5,6]; rainbow trout, *Oncorhynchus mykiss* [7]; Atlantic cod, *Gadus morhua* [8]; sea-bass, *Dicentrarchus labrax* [9], sea-bream, *Sparus aurata* [9]; toadfish, *Spheroides glaber* [10]; Atlantic menhaden, *Brevoortia tyrannus* [11]; channel catfish, *Ictalurus punctatus* [12]; striped bass (*Morone saxatilis*), barramundi (*Lates calcarifer*), Mozambique tilapia (*Oreochromis mossambicus*), Nile tilapia (*Oreochromis niloticus*) [6]. Teleost Ig had been considered to exist as a single isotype, given that a single constant region gene, C_μ , codes for the heavy chain. More recently a second C gene, C_δ , that appears to be analogous to mammalian IgD has been described in Atlantic salmon [13], Atlantic cod [14] and the channel catfish [15]. There are also recent reports of two distinct teleost isotypes, C_ζ , in the zebra-fish, *Brachydanio rerio* [16] and C_τ in the rainbow trout and possibly other species, including the Atlantic salmon [17].

Mammalian IgM is always fully disulphide cross-linked and cannot be secreted if it is not [18]. Multiple isoforms of the teleost H_8L_8 tetramer exist, however, in the serum of the common carp [3], rainbow trout [7], sheephead [4], channel catfish [12] and the striped bass, barramundi, Mozambique tilapia and Nile tilapia [6]. In rainbow trout this

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diversity is generated by random disulphide cross-linking between Ig H₂L₂ monomers [19]. The composition of the H₈L₈ tetramer ranges from four covalently bound (disulphide-crosslinked) monomers (H₈L₈) to four non-covalently associated H₂L₂ monomers with intermediate forms (trimers, dimers and monomers). As it has also been demonstrated in rainbow trout that each unique Ig was composed of all the isoforms (tetramer, trimer + monomer and so on), each isoform must have been produced by the same B-cell, each isoform must have used the heavy chain therefore these differences are not isotypic per se [7]. The differential role, if any, which these isoforms play in the adaptive immune response of fish, is unclear.

Members of the Gadidae including the Atlantic cod, haddock (*Melanogrammus aeglefinus*) and pollock (*Pollachius pollachius*) do not produce a diversified immunoglobulin repertoire in response to antigen [20,21]. They belong to a more derived superorder (the Paracanthopterygii) than the salmonids (Protacanthopterygii) [22] and other fish for which the production of specific immunoglobulin upon vaccination has been demonstrated extensively. Furthermore both the haddock and the Atlantic cod have high levels of circulating Ig, a phenomena shared with supposedly “primitive” teleosts such as gars [21,23].

As part of our studies into the immune responses of gadids we compared the quaternary structure of immunoglobulin from the Atlantic cod, haddock, pollock and cusk (*Brosme brosme*). In this paper we report reduced isoform diversity in all of these Gadiformes when compared to Atlantic salmon.

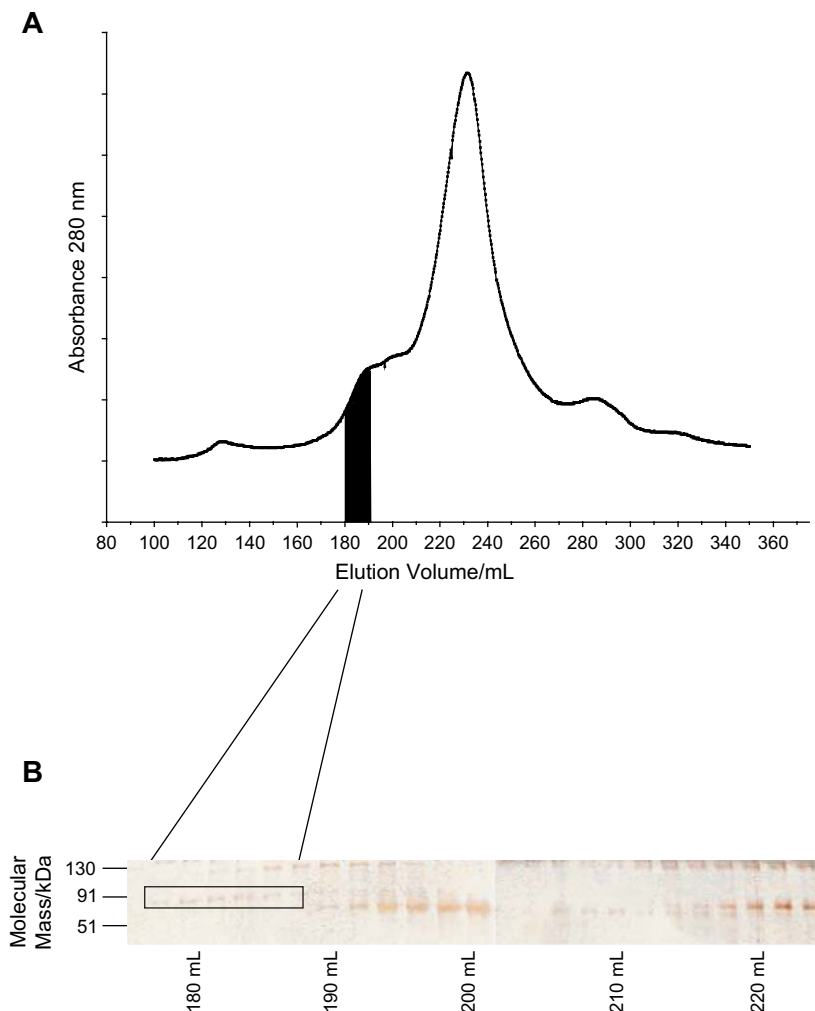


Fig. 1. Purification and analysis of Atlantic cod (*Gadus morhua*) plasma (A) Sephacryl S400 26/60 size exclusion chromatograph showing eluant absorbance at 280 nm (A280). Ig containing fractions are shaded. (B) Silver stained reducing, denaturing SDS-PAGE of selected fractions. Immunoglobulin heavy chain containing fractions are boxed.

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