Isolation of two C-reactive protein homologues from cod (*Gadus morhua* L.) serum

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**Abstract**  
Pentraxins are important molecules in innate defence and play a role in the acute phase response of both mammals and fish. Isolation of cod pentraxins by affinity chromatography using phosphorylcholine agarose revealed two pentraxin-like proteins, referred to as PI and PII proteins. These varied in their overall charge, pentamic and subunit molecular size, glycosylation and N-terminal amino acid sequences. The PI protein was homologous with the CRP-like pentraxin previously described in cod whereas the PII protein was a new CRP homologue, which was characterized by substantial individual heterogeneity with regard to subunit size and relative density. The results indicate considerable genetic variations in the cod pentraxins.

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**Introduction**

The pentraxins, serum amyloid P (SAP) and C-reactive protein (CRP), are pattern recognition proteins found in both invertebrates and vertebrates. Pentraxins belong to the innate immune system and are key factors in acute phase response to infection, injury or trauma. They can activate the classical complement pathway as well as take part in other immunological and biological processes [1]. SAP and CRP are the prototypical acute phase proteins in mammals, their serum levels increasing drastically during an acute phase response [2]. Decreasing, as well as increasing serum levels have been reported during acute phase response of different fish species [3–8].

Pentraxins are pentameric, planar molecules. The five subunits are commonly non-covalently linked, often glycosylated and with a recognition- and effector-face; dimeric forms (decamers) and larger aggregates have also been described [9–11]. They show characteristic calcium dependent ligand specificity, CRP binds primarily to phosphorylcholine, a pneumococcal C-polysaccharide, and SAP shows specificity for phosphoethanolamine, agarose, zymosan and other glycanas as well as DNA and chromatin [9]. The ligand specificity is generally accepted as being the defining characteristic of the pentraxin type. The two pentraxins, SAP and CRP, are both present in mammalian serum and...
human SAP and CRP show about 70% amino acid sequence similarity when conservative substitutions are taken into account [9]. SAP- and/or CRP-like proteins have been described in the serum of several fish species, for example, plaice (Pleuronectes platessa L.) [3], rainbow trout (Oncorhynchus mykiss) [12,13], halibut (Hippoglossus hippoglossus) [14] and channel catfish (Ictalurus punctatus) [15]. Pentraxin homologue of cod has been described by Lund and Olafsen [14]. According to their data a CRP-like pentraxin was present in cod serum. This was essentially based on the binding specificity for phosphorylcholine (PC).

During recent studies at our laboratory on the role of pentraxin in the acute phase response of cod, additional data was obtained and a previously noted heterogeneity of the cod pentraxins [16] was confirmed, which is the subject of this paper.

Materials and methods

Fish and serum

All fish came from the Marine Institute’s Experimental Station, Stadur, Grindavik, Iceland, originating from wild gamete fertilized and hatched at the station and maintained at 7 °C under optimal cultural conditions [17]. For the isolation of pentraxin from serum, cod weighing 600–700 g was used. Blood, 2–10 ml, was collected from the caudal vessel of anaesthetized (40 μg tricaine methanesulphonate l−1) fish and allowed to coagulate overnight at 4 °C. Serum was then collected, pooled from several fish, and stored at −20 °C until use.

To examine the individual heterogeneity of pentraxins, serum from 12 fish, weighing about 100 g and originating from the same hatch, were used.

To study the possible change with time serum collected at intervals over a period of 18 months from one individual cod as well as serum collected from six individuals at the beginning and the end of the 18 month period was used. These samples came from a previous experiment on cod [18].

Affinity chromatography

The isolation of pentraxins by affinity chromatography, using phosphorylcholine (PC)-agarose and ethylenediaminetetraacetic acid (EDTA) elution was based on previously published protocols [6,12–14,19,20].

PC-agarose from Pierce Chemicals (USA) was used. Ten milliliters of the PC-agarose was equilibrated with several changes of excess 50 mM Tris–HCl buffer, pH 8.0, containing 150 mM NaCl (TBS) and 2 mM CaCl2, (TBS-Ca). The gel was then mixed with 13 ml of TBS-Ca buffer and 13 ml of pooled cod serum and gently rotated overnight at 4 °C. The supernatant was discarded and the gel washed once with the TBS-Ca buffer. The gel was packed onto a chromatographic column ( XK16, Amersham Pharmacia Biotech, Sweden), connected to the AKTA FPLC system from Amersham Pharmacia Biotech and washed in TBS-Ca buffer until the absorbance of the eluate was back to zero background. Bound protein (crude pentraxins) was then eluted with 10 mM EDTA in TBS buffer.

Ion exchange chromatography

MonoQ anion ion exchange column (HR5/5, Amersham Pharmacia Biotech) was used. The column was equilibrated in 20 mM Tris–HCl, pH 7.5 start buffer. The crude pentraxin isolated above was equilibrated in the start buffer using a PD buffer exchange column (Amersham Pharmacia Biotech) and then loaded onto the MonoQ column and eluted with start buffer followed by a salt gradient of 0–1 M NaCl in the start buffer.

Size-exclusion chromatography

Superose 12 (HR10/30, Amersham Pharmacia Biotech) was used and the elution buffer was 100 mM Tris–HCl, 150 mM NaCl, pH 8.0. A size calibration graph was plotted using a gel filtration calibration kit from GE Healthcare UK Limited, UK, and the molecular weight of the pentraxins extrapolated from this.

SDS-PAGE analysis

Standard SDS-PAGE analysis of reduced and non-reduced samples was carried out using the Mini-PROTEAN II system from Bio-Rad Laboratories (USA) according to the manufacturer’s instructions. The separation gel was 12% or 14% and the stacking gel 4.5% acrylamide. Reduced samples were mixed with an equal volume of 0.125 M Tris, pH 6.8 sample buffer containing 0.5% 2-mercaptoethanol and 2% SDS and heated for 2–5 min at 100 °C. Non-reduced samples were mixed with the same buffer, omitting the mercaptoethanol and the heating. Non-reduced samples were also analysed in homogeneous 4% SDS-PAGE acrylamide gel strengthened with 0.6% agarose according to a method described by Aavallion and Mor [21]. Native-PAGE analysis in a 12% gel, omitting SDS from all solutions, was also carried out on non-reduced samples. Proteins were stained with Silver Stain Plus (Bio-Rad) or Phast Gel Blue R (Sigma–Aldrich, USA). Prestained molecular weight markers from Fermentas Life Sciences, Germany, or Invitrogen, Germany, were used for estimating molecular weight.

Western blotting

Following standard SDS-PAGE separation, proteins were transferred to a nitrocellulose (NC) membrane (Immobilon-P transfer membrane, Millipore, UK), using a semi-dry MilliBlot Graphite Electroblotter (Millipore) according to the manufacturer’s instructions. Transfer was for 1 h at room temperature. Residual sites of the NC membrane were blocked with 0.1% semi-skimmed milk powder in 0.1 M Tris buffered saline, pH 7.8, containing 0.1% Tween20, for 1 h at room temperature or overnight at 4 °C. Incubations with the primary and the secondary peroxidase or alkaline phosphatase conjugated antibodies, diluted in the blocking solution, were for 1 h at room temperature with extensive washing between each step. When using peroxidase conjugated secondary antibody, the blots were developed using the ECL system from Amersham Pharmacia Biotech. When using alkaline phosphatase conjugated secondary antibody the blots were developed in a substrate buffer of 100 μg ml−1.
NBT (nitro blue tetrazolium), 60 μg ml⁻¹ BCIP (5-bromo-4-chloro-3-indonylphosphate) and 4 mM MgCl₂ in 0.1 M ethanamine–HCl buffer, pH 9.6.

Electron microscopy

A drop of the protein solution, 900 μg ml⁻¹, was placed onto a 200 mesh Formvar-coated copper grid and left for 2 min. Excess fluid was removed by touching the edge with filter paper. A drop of 1% phosphotungstic acid, pH 6.5, was added and left for 2 min before excess fluid was removed as before. This was allowed to dry and then viewed using an FEI Tecnai Spirit G2 Bio Twin transmission electron microscope (TEM) (FEI UK Ltd.).

Ligand specificity

Approximately 10 mg of agarose (BDH Chemicals, Ltd., UK), zymosan (insoluble preparation of yeast, Saccharomyces cerevisiae, prepared at our laboratory according to Llanos et al. [22]) or PC-agarose were washed extensively in TBS-Ca buffer and then incubated with about 20 mg protein in 100 ml TBS-Ca buffer or buffer only for 1 h at room temperature with gentle shaking. One microlitre of the supernatant was dotted on a pair of nitrocellulose membranes, dried and then blocked in 0.1% semi-skimmed milk powder as described in “Western Blotting”. One membrane was incubated with anti-pentraxin antibody and the other with anti-PII antibody (see “Antibodies”), this was followed by incubation with alkaline phosphatase conjugated antibody and the colour developed using the NBT/BCIP substrate buffer as described in “Western Blotting”.

Amino acid sequence analysis

A total of six subunits, two from the PI protein and four from the PII protein, were subjected to sequence analysis. For N-terminal amino acid sequencing, using Edman’s degradation method, samples were excised from PVDF membrane following SDS-PAGE and Western blotting and protein staining using red ponceau S or amido black. This analysis was kindly carried out by J. d’Alayer at the Institut Pasteur, Paris, France. For mass spectrometry based protein identification (proteomics), using a Waters QTOF2 mass spectrometer, samples were excised from acrylamide gel following SDS-PAGE and Phast Gel Blue staining. This analysis was kindly carried out by K. Bailey, School of Biomedical Sciences, Queens Medical Centre, Nottingham, UK.

Deglycosylation

A kit from New England BioLabs (USA) containing the enzyme PNGase, which removes N-linked oligosaccharides from proteins under reducing or non-reducing conditions, was used following the manufacturer’s instructions. The protein sample was treated with the enzyme both before and after reduction in reducing buffer containing mercaptoethanol (from the kit).

Antibodies

Three polyclonal antibodies were produced in mice according to Overkamp et al. [23]: (a) anti-pentraxin antibody produced against crude pentraxin preparation isolated by affinity chromatography, (b) anti-PI produced against peak I from the MonoQ separation and (c) anti-PII produced against peak II from the MonoQ separation.

Results

Affinity isolation and ion exchange chromatography

Thirteen milliters of cod serum, pooled from several individuals, following affinity chromatography, yielded about 1 mg of crude pentraxin or approximately 80 μg ml⁻¹ serum. Separation on an anion exchange column gave two peaks, I and II (Fig. 1). Peak I was eluted in a single 0.5 ml fraction with 0.48 M NaCl, peak II had a broader elution profile and was eluted in three 0.5 ml fractions with 0.54–0.64 M NaCl. The proportional protein yield of peaks I and II was about 1:1. These will be referred to as PI and PII proteins below.

Fractions were analysed in SDS-PAGE followed by silver staining. The result is shown as an insert in Fig. 1. The PI protein was composed of two subunits whereas the PII protein was composed of several (here 4) subunits, the number of these varied from one serum pool to another (not shown).

Size estimation of the PI and PII proteins

Various methods were used to estimate the molecular size of the PI and PII proteins. Some degree of discrepancy was observed between these methods reflecting the variable behaviour of proteins, reduced or non-reduced, in different media [24].

In aqueous phase (size-exclusion chromatography, results not shown) both proteins were eluted as a single, symmetrical peak. The PI protein was eluted with 11.54 ml and the PII with 12.46 ml. The corresponding molecular size

Figure 1  Ion exchange chromatography (MonoQ anion exchanger) of crude cod pentaxins. Two distinct types of C-reactive proteins were eluted. The PI protein (peak I) was eluted with 0.48 M NaCl and the PII protein (peak II) with 0.54–0.64 M NaCl. The insert shows the SDS-PAGE/silver staining analysis of the fractions from peak I (PI protein, 1 fraction) and peak II (PII protein, three fractions) under reducing conditions.
extrapolated from the standard graph was 158 and 90 kDa, respectively.

Using standard SDS-PAGE the two subunits of the PI protein were 28 and 31 kDa under reducing conditions (Fig. 2b) and under non-reducing conditions 49 and 54 kDa subunits were seen as well as two larger proteins, approximately 200 and 240 kDa (Fig. 2a). This indicated the presence of some inter-subunit disulfide bonds. When examined in 4% homogeneous SDS-PAGE gel 110 and 125 kDa proteins were seen and in native-PAGE and 118 and 158 kDa proteins were observed (results not shown).

Using standard SDS-PAGE, the PII protein separated into several protein subunits, 22–29 kDa under reducing conditions (Fig. 2b) and 20–26 kDa under non-reducing conditions (Fig. 2a), indicating the absence of inter-subunit disulfide bonds. The slightly larger size observed under reducing conditions probably reflects the effects of reduced intra-subunit disulphide bonds changing the protein shape and movement through the gel. No proteins larger than 70 kDa were seen in 4% homogeneous SDS-PAGE whereas in native-PAGE a major 82 kDa protein and a minor 158 kDa protein were seen.

All the PI and PII subunits seen in Fig. 2 were also detected in Western blotting using anti-pentraxin antibody.

Electron microscopy

Results from the EM analysis of the PII protein showed a pentameric-like form, which was 8.18 nm and 7.96 nm across (Fig. 3). Analysis of the PI protein was unsuccessful.

Antibody production

The mouse antibody produced against crude pentraxin, isolated by affinity chromatography (anti-pentraxin antibody), reacted in Western blotting with both the PI and PII proteins (Fig. 4). The mouse antibodies produced against the PI protein or against the PII protein were monospecific and did not cross react. Ample quantity was obtained of the anti-pentraxin and anti-PII antibody but only a limited amount of the anti-PI antibody, which also showed relatively weak activity.

Ligand specificity

The Phosphorylcholine-agarose removed both the PI and PII proteins from the solution, agarose alone reduced the protein density slightly, in particular of the PI protein, and zymosan had no effect on either the PI or the PII proteins.
protein (Fig. 5). The monospecificity of the anti-PII antibody was again demonstrated (Fig. 5b).

Glycosylation of cod pentraxins

The PNGase removed about 2 kDa from the larger subunit of the PI protein following reduction making it the same size as the smaller subunit but the enzyme did not remove carbohydrate from the non-reduced PI protein (Fig. 6). The PNGase removed carbohydrate from the largest subunit of the PII protein both before and after reduction. Although in the case of the PII protein it was not possible to ascertain the amount of carbohydrate removed it must be at least 2 kDa.

Sequence analysis of the PI and PII proteins

The following protein subunits (see insert in Table 1) were subjected to N-terminal analysis: PI subunit 2 and PII subunit 4 and the following to mass spectrometric analysis: PI subunit 1 and PII subunits 1–3. Both types of analysis demonstrated a significant similarity to pentraxins from other species (Fig. 7, Table 1).

The N-terminal sequence of the PI subunit 2 was identical to the N-terminal sequence of cod CRP homologue, published by Lund and Olafsen [14] but also showed relatively high identity to SAP from, for example, wolfish (Anarhichas lupus) and halibut. Lower identity was generally shown to CRP of other species. (Fig. 7). Mass spectrometric analysis of the PI subunit 1 gave four sequences, three of which showed significant identity to pentraxins (SAP) from other species. One of these (QDLSGK) matched a proportion of the N-terminal sequence of the PI subunit 2 (Table 1).

The N-terminal sequence of the PII subunit 4 showed significant similarity to a CRP-like protein isolated from rainbow trout, published by Murata et al. [13] and to CRP described in several other species (Fig. 7). Mass spectrometric analysis of the PII subunits 1 gave six, four and three sequences, respectively (Table 1). With the exception of two sequences from the PII subunit 1, all the sequences showed significant identity to CRP from other species. One sequence, VFPETANSFVELTPAK, from each of the three PII subunits (PII-1, -2 and -3) matched a proportion of the N-terminal sequence of the PII subunit 4 (Table 1).

Two sequences were shared by the PI subunit 1 and the PII subunits 1. One of these, ALFSLATR, also showed significant identity to SAP from salmonids whereas the other, FQAVTATTGR, showed no significant match to pentraxins (Table 1).

Heterogeneity of cod pentraxin

Twelve sera from individual cod of a similar size and of the same origin were tested in Western blotting using the monospecific anti-PII antibody (Fig. 8a). With regard to the number of subunits, their size and relative density most of the subunit patterns were different from one individual to another.

Sera from six individuals from another group of cod retained the same subunit pattern at the beginning and end of a period of 18 months (Fig. 8b) and when one of these (number 1) was tested at intervals during this period the same subunit pattern was seen throughout although the density varied (Fig. 8c).

When these sera were tested with the weak anti-PI antibody there were indications that such heterogeneity was not associated with the PI protein (results not shown).

Discussion

In the present study, two types of calcium dependent phosphorylcholin (PC) binding proteins, referred to as PI and PII proteins, were isolated from cod serum. The total quantity of pentraxin in serum, 80 μg ml⁻¹, was comparable to that of other fish species [6,8].

The PC agarose used for the affinity isolation would be expected to retain both PC (CRP) and agarose (SAP) binding proteins. Further examination of the ligand specificity
Table 1  The amino acid sequence analysis of subunits from protein PI and PII as shown in Fig. 7 and examples of percentage identity (Blast analysis, GenBank accession number shown when relevant)

<table>
<thead>
<tr>
<th>Subunits from proteins PI and PII</th>
<th>Type of analysis</th>
<th>Results</th>
<th>Blast analysis: examples of % identity (Swiss-Prot and NCBI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PI subunit 1</strong></td>
<td><strong>MS</strong></td>
<td>TPGNVLNWR</td>
<td>78% identity to SAP from Atlantic salmon [5] and 67% identity to SAP from rainbow trout [5]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ALFSLATR*</td>
<td>75% identity to SAP from Atlantic salmon and rainbow trout [5]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FQAVTATTGR**</td>
<td>No significant match to pentraxin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>QDLSGK /C14</td>
<td>100% identity to CRP from cod and SAP from Atlantic salmon, wolffish [14] and rainbow trout [12]</td>
</tr>
<tr>
<td><strong>PI subunit 2</strong></td>
<td><strong>N-terminal</strong></td>
<td>IPQDLSDKMLTFPKE</td>
<td>100% identity to CRP from cod [14]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>73% identity to SAP from wolffish [14] and 57% identity to SAP from human [27]</td>
</tr>
<tr>
<td><strong>PII subunit 1</strong></td>
<td><strong>MS</strong></td>
<td>LGQDPDNFLGFQAK***</td>
<td>67% identity to pentraxin from carp [26] and zebrafish (CAM46930)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VFPEETANSFVELTPAK**</td>
<td>71% identity to CRP from rainbow trout [13] and 65% identity to CRP from plaice [25]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FQAVTASATR**</td>
<td>No significant match to pentraxin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AVADFHMASLPLNK</td>
<td>No significant match to pentraxin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PLTLGGQEDSYGGDFLK</td>
<td>53% identity to SAP from Atlantic salmon and rainbow trout [5]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ALFSLATR*</td>
<td>75% identity to SAP from Atlantic salmon and rainbow trout [5]</td>
</tr>
<tr>
<td><strong>PII subunit 2</strong></td>
<td><strong>MS</strong></td>
<td>LGQDPDNFLGFQAK***</td>
<td>67% identity to pentraxin from carp [26] and zebrafish (CAM46930)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VFPEETANSFVELTPAK**</td>
<td>71% identity to CRP from rainbow trout [13] and 65% identity to CRP from plaice [25]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EVLFFAYR</td>
<td>75% identity to pentraxin from zebrafish (CAM46925)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DELNVWR*</td>
<td>100% identity to pentraxin from carp [26] and zebrafish (CAM46924)</td>
</tr>
<tr>
<td><strong>PII subunit 3</strong></td>
<td><strong>MS</strong></td>
<td>LGQDPDNFLGFQAK***</td>
<td>67% identity to pentraxin from carp [26] and zebrafish (CAM46930)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VFPEETANSFVELTPAK**</td>
<td>71% identity to CRP from rainbow trout [13] and 65% identity to CRP from plaice [25]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DELNVWR*</td>
<td>100% identity to pentraxin from carp [26] and zebrafish (CAM46924)</td>
</tr>
<tr>
<td><strong>PII subunit 4</strong></td>
<td><strong>N-terminal</strong></td>
<td>GRSLVFPEETANSFVELFPKPEKSL**</td>
<td>60% identity to CRP from rainbow trout [13] and 56% identity to CRP from plaice [25]</td>
</tr>
</tbody>
</table>

Same symbols show identical or similar sequences. **MS**: Mass spectrometric analysis.
Comparison of N-terminal amino acid sequences of PI and PII (subunits 2 and 4, respectively, see insert in Table 1) with known pentraxin sequences [12–14, 27–38]. (a) N-terminal amino acid sequence of PI compared to known CRP and SAP from other species. (b) N-terminal amino acid sequence of PII compared to known CRP from other species. Residues identical to PI or PII are greyish within boxes. The percentage values indicate identity between the PI or PII and other proteins. Uncertain or unidentified amino acids are indicated by ( ) or ?.

Figure 7

Figure 8 Western blotting of different cod sera with anti-PII antibody. (a) sera from 12 individuals, (b) sera from six individuals sampled at an interval of 18 months (e.g. 1a and 1b, respectively) and (c) sera from one individual sampled ten times over a period of 18 months.
This could explain the difficulties encountered in determining the exact molecular size and the appearance of both truncated and complexed pentamers in the different SDS-PAGE analyses. Some authors have verified the pentameric structure of pentraxin-like proteins using electron microscopic analysis [13,39]. A pentameric structure was demonstrated for the PI protein using EM analysis but a pentameric structure of the PI protein remains to be verified.

The largest subunit of both the PI and the PII protein (subunit 1) was glycosylated and the degree of glycosylation appeared similar for both proteins, corresponding approximately to a single carbohydrate moiety of 2 kDa. The presence of inter-subunit covalent bonds between the PI subunits, absent from the PII protein, probably made its carbohydrate moiety less accessible to enzyme digestion under non-reducing conditions.

Heterogeneity with respect to the glycosylation of the pentraxin subunits has been described in Indian carp (Labeo rohita) by Mandal et al. [40] who found that the degree and type of glycosylation was influenced by environmental factors. The present samples come from cultured cod kept under similar and controlled conditions; hence, variations of this nature would not be expected. Examination of the influence of environmental factors on the glycosylation of cod pentraxins could be an interesting project.

The PI protein (subunit PI-2) and the PII protein (subunit PII-4) had distinct N-terminal amino acid sequences. Both showed significant percentage identity to N-terminal sequences of pentraxins from other species. The PI protein showed closer N-terminal sequence identity with SAP than with CRP [12,14,25] whereas the N-terminal sequence of PII protein showed a closer identity with CRP of other fish species [13,25]. The mass spectrometric analysis further demonstrated that the two subunit of the PI protein had a common N-terminal sequence and similarly the four subunits of the PII protein had an identical N-terminal sequence. Some sequence identity was observed between the PI and the PII protein as is commonly seen between pentraxins from different sources [9]. Interestingly the shared sequences were associated with the largest, glycosylated subunit 1 of each protein.

The differences observed between the PI and PII proteins were reflected in the distinct antigenicity of the two proteins demonstrated by the lack of cross reactivity between the anti-PI and PII antibodies.

In general, when species possess both SAP- and CRP-like pentraxins, these are distinguished by their ligand specificity as well as by their N-terminal amino acid sequence [25,31]. In instances, when more than one PC-binding protein was identified, only one showed characteristic pentraxin features and sequence homology [13,41]. This paper describes, apparently for the first time, two types of PC-binding pentraxins, with distinct SAP- or CRP-like N-terminal sequences.

In view of these results it should perhaps be considered whether the ligand specificity alone is a valid criterion for distinguishing between pentraxin types.

Remarkable heterogeneity was a feature of the PII protein. When isolated from pooled sera up to six subunits of varying size and density were observed. Notable heterogeneity was also observed between individual cod, each carrying a characteristic pattern of one to four subunits.

Examination of serum collected from the same individual over a period of 18 months suggested that subunit pattern did not change with time although the overall or proportional density of each subunit might vary. Such individual heterogeneity did not appear to be associated with the PI protein.

These results indicate a large amount of allelic variation of cod pentraxins and that the PII protein variants might be encoded at multiple loci in the cod genome. This heterogeneity may be an important factor in the innate immune defence and acute phase response of cod. Heterogeneity of immunologically important molecules, like the isoforms of complement proteins C3 and B/C2 factor, has been described in different fish species [42–44]. In the case of the C3 of rainbow trout these isoforms have been shown to have a different binding specificity for complement activators [45].

Recent work on the complement system of cod failed to demonstrate such heterogeneity of C3 in cod [46,47]. It could therefore be speculated whether the heterogeneity of the pentraxins compensates for the lack of C3 isoforms in cod. The diversity of the pattern recognition molecule (pentraxin) might therefore make up for the lack of diversity of its effector protein (C3). Examination of the binding specificity and pattern recognition properties of the different subunits/variants of the PI and in particular of the PII protein would be of considerable interest in this context.

Work is now in progress at our laboratory to determine the genetic source of the PII protein heterogeneity. The possible role of pentraxins the PI or PII protein or both, in the acute phase response of cod is also being investigated.

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