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Antigenic characterization of *Sphaerospora dicentrarchi* (Myxosporea: Bivalvulida), a parasite from European sea bass *Dicentrarchus labrax* (Teleostei: Serranidae)

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ABSTRACT: The biochemical composition of *Sphaerospora dicentrarchi* was studied. Periodate and Proteinase K treatments as well as lectin blots were used to analyse carbohydrate terminals. Zymography was applied to detect proteases. Four polyclonal antisera, raised against *S. dicentrarchi* (RaSdic), *S. testicularis* (RaStest), *Ceratomyxa labracis* (RaClab) and *C. sparusaurati* (RaCspr), were used in SDS polyacrylamide gel electrophoresis and immunoblot. Bands with molecular weight (MW) between 32 and 130 kDa were detected by electrophoresis. After Proteinase K treatment, apparent digestion of bands heavier than 43 kDa took place. RaSdic and RaStest detected similar bands with MW between 20 and 50 kDa, whereas RaClab and RaCspr recognized bands between 50 and 140 kDa. The 50 kDa band was recognized by all the polyclonal antisera, suggesting that it could correspond to an antigen shared by several myxosporean parasites. Four proteases were observed by zymography. From the 5 lectins assayed, binding was only observed using Con-A, which detected 2 bands of 96 and 78 kDa. Periodate treatment did not produce any effect on the binding of RaSdic and RaStest, but a high decrease of intensity in the antibody binding occurred at a concentration of 10 and 20 mM periodate when RaClab and RaCspr were tested. These results give information on the antigenic composition of *S. dicentrarchi* which could be useful for further diagnostic or immunoprevention studies.

KEY WORDS: Myxozoa · *Sphaerospora* · *Ceratomyxa* · Immnunoblotting · Glycoconjugates Lectins · Zymography

INTRODUCTION

With the development of mariculture, several parasitic diseases have become important in fish. *Sphaerospora dicentrarchi* (Sitjà-Bobadilla & Álvarez-Pellitero 1992) is a myxosporean parasite which infects European sea bass *Dicentrarchus labrax* L. in wild and cultured stocks (Sitjà-Bobadilla & Álvarez-Pellitero 1993, Santos 1996). In the last few years, this parasite has been associated with mortalities of young stages in sea cage cultures (authors' unpubl. obs.).

The interaction between parasites and the host immune system is a complex series of events that may contribute to the death of the parasites or produce immunopathological reactions that are detrimental to the host. Therefore, the identification of parasite molecules which could be immunogenic for the host is of paramount importance.

Acquiring knowledge concerning the antigenic composition of Myxosporea is an important first step towards the selection and production of the parasite antigens with immunodiagnostic and immunopreventive potential. Nevertheless, few previous works have studied the antigenic composition of other myxosporean parasites (Saulnier & Kinkelin 1996). The scant knowledge in this field prompted us to initiate studies on the fish immune response to Myxosporea, including an antigenic characterisation. In previous papers, we tested rabbit antisera raised against *Spha-*

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erospora dicentrarchi and other myxosporean parasites using immunohistochemistry (Muñoz et al. 1998a, 1999a). The carbohydrate composition of different myxosporean parasites including *S. dicentrarchi* has been studied by lectin histochemistry (Muñoz et al. 1999b). The immune response of sea bass to *S. dicentrarchi* has been characterised *in vitro* (Muñoz et al. 1998b, 2000a) and *in vivo* (Muñoz et al. 2000b). In the present paper, the biochemical and antigenic composition of *S. dicentrarchi* was studied. For this purpose, rabbit polyclonal antisera raised against this and other myxosporean parasites were used in immunoblots. The presence of carbohydrate terminals was also analyzed by electrophoresis and immunoblotting using lectins and treatments with Proteinase K and periodate. Zymography was used to detect proteases.

MATERIAL AND METHODS

Polyclonal antibodies. Four polyclonal antisera raised against myxosporean parasites were used. Rabbit anti-*Sphaerospora dicentrarchi* (RaSdic), anti-*S. testicularis* (RaStest) and anti-*Ceratomyxa labracis* (RaClab) were produced as described previously (Muñoz et al. 1998a,b). Rabbit anti-*C. sparusaurati* (RaCspr) was raised by an identical immunization protocol. Briefly, New Zealand rabbits were subcutaneously injected on Day 0 with a suspension of parasitic stages emulsioned 1:1 in Freund's complete adjuvant in a total volume of 1 ml rabbit^{-1}. Rabbits were boosted in the same way on Days 15 and 30 with the antigen prepared in Freund's incomplete adjuvant. On Day 45, rabbits were injected with parasitic stages in PBS. Two weeks after the last injection, rabbits were bled to death. Blood samples were also taken on Day 0 (preimmune serum). The number of parasitic stages injection^{-1} was 10^6 in the first 2 injections and 2×10^6 in the last one for RaSdic, 2×10^6 for RaStest, 1.7×10^6 for RaClab and 10^6 for RaCspr.

SDS-polyacrylamide gel electrophoresis. *Sphaerospora dicentrarchi* stages were purified and isolated as described previously by Muñoz et al. (1998a), and diluted to a parasite concentration of 10^7 spores ml^{-1} in sample buffer containing 62 mM Tris-HCl pH 6.8, 2% SDS, 10% glycerol, 0.1 M dithiothreitol and 0.005% bromophenol blue. Samples were boiled for 5 min, allowed to cool and then centrifuged for 30 min at 13 000 × *g* and 4°C. The supernatant was stored in aliquots at –20°C until they were used. SDS polyacrylamide gel electrophoresis (SDS-PAGE) was carried out using the method described by Laemmli (1970) in a BioRad Mini Protean II apparatus at 100 V for 10 min and then at 180 V for 40 min. The gel consisted of a 4% stacking gel and a 12% separating gel. Low molecular weight markers (Pharmacia-LKB Biotechnology, Uppsala, Sweden) were used for calibration. When the electrophoresis was completed, the antigens were visualized by either Coomassie Brilliant Blue or silver stains.

To examine the presence of carbohydrates in the obtained bands, 2 methods were followed. In the first one, prior to adding sample buffer, 100 µl *Sphaerospora dicentrarchi* samples were incubated with 100 µl of 10% solution of Proteinase K (Life Technologies S.A., Barcelona, Spain) for 1 h at 60°C. The electrophoresis was performed as above and the gel was silver stained. Control gel was carried out using *S. dicentrarchi* samples without Proteinase treatment. In the second one, *S. dicentrarchi* antigens were separated by SDS-PAGE as described above. After electrophoresis, the gel was soaked in 25% isopropyl alcohol, 10% acetic acid overnight at room temperature and then in 7.5% acetic acid for 30 min. Finally, the gel was put in 0.2% aqueous periodic acid at 4°C for 1 h and silver stained. Control untreated gel was directly silver stained after electrophoresis.

Zymography. SDS gels containing 12% acrylamide were polymerized in the presence of gelatin (1 mg ml^{-1}) as modified after Heussen & Dowdle (1980). *Sphaerospora dicentrarchi* samples (1.8×10^7 spores ml^{-1}) were diluted 1:1 in non-reducing SDS sample buffer (62 mM Tris-HCl pH 6.8, 2% SDS, 10% glycerol and 0.005% bromophenol blue). After electrophoresis, the gel was incubated in 2.5% Triton X-100 (Sigma Chemical Company, St. Louis, MO, USA) for 1 h to remove SDS and restore proteolytic activity, rinsed twice in PBS, and incubated overnight in PBS (pH 7.4) at room temperature to allow proteolysis. The gels were stained in a 0.1% solution of amido black in methanol: acetic acid: water (40%; 10%; 50%) for 1 h and destained in the same solvent. Proteases were visualized as clear bands in a blue background wherever digestion of copolymerized gelatin occurred. The effects of metal chelators and protease inhibitors were determined by incubating individual lanes of the same gel overnight in PBS containing one of the following inhibitors provided by Sigma: EDTA, aprotinin, pepstatin, *o*-phenanthroline and iodoacetamide at a concentration of 100 mM, E-64 (L-trans-epoxysuccinyl-leucylamidi-4-guanidino-butane) (5 µM), phenylmethylsulphonyl fluoride (PMSF) (10 mM), leupeptin and antipain at 100 µg ml^{-1}. All the inhibitors were dissolved in water except *o*-phenanthroline which was dissolved in dimethylsulphoxide (DMSO) and pepstatin and PMSF which were dissolved in ethanol. An appropriate control was incubated with PBS without inhibitor.

Western blotting. Immunoblotting methods with the polyclonal antisera were used to screen each isolate for

antigenic bands. Samples were applied and subjected to electrophoresis as described above. The samples were then transferred to a nitrocellulose membrane (0.45 µm) (Bio-Rad) according to Towbin et al. (1979), using a BioRad Mini-Trans Blot Cell apparatus for 1 h at room temperature at a constant current of 250 mA. Membranes were then blocked in 10% non-fat dry milk (Bio-Rad) in Tris-buffered saline (TBS, 20 mM Tris, 0.5 M NaCl, pH 7.4) overnight at 4°C. After washing, membranes were incubated for 2 h at room temperature with the polyclonal antisera or the preimmune serum diluted in 3% non-fat dry milk in 0.05% Tween 20 in TBS (TTBS). The dilutions used were 1:500 for RaSdic, 1:2000 for RaStest, and 1:4000 for RaClab and RaCspr. Unbound antibodies were removed by washing and membranes were then incubated with biotinylated goat anti-rabbit antibody (Vector Lab., Burlingame, CA, USA) diluted in TBS for 30 min. After washing, membranes were incubated with the avidin-biotin-peroxidase complex (ABC) (Vector) for 30 min, and bound peroxidase was revealed by adding the chromogen 3,3'-diaminobenzidine tetrahydrochloride (DAB) (Sigma) for 10 min. When bands developed sufficient color, the blots were removed and washed with distilled water, left to dry and analyzed immediately. Prestained molecular weight (MW) markers (BioRad) were used for calibration.

Lectin blotting methods were used to examine carbohydrate terminals in *Sphaerospora dicentrarchi* extracts. For these studies the nitrocellulose membranes were incubated with biotinylated lectins (Sigma) diluted in TTBS for 1 h at 20°C. Lectins used

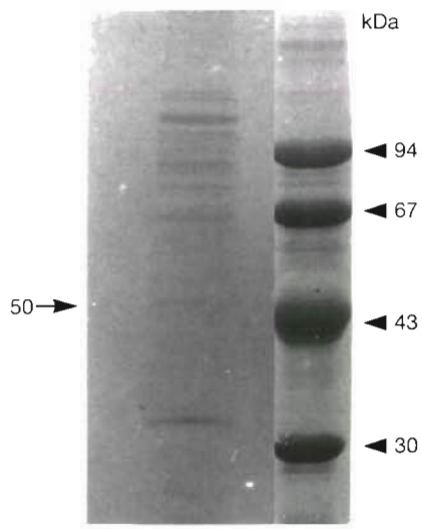

Fig. 1. Electrophoretic profiles of *Sphaerospora dicentrarchi* antigens (25 µl) after Coommassie Blue staining. Numbers on the right indicate molecular weights of standards. Arrow on the left indicates the position of the 50 kDa band

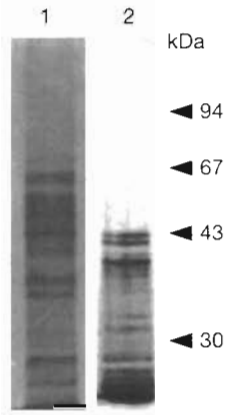

Fig. 2. Silver-stained SDS-PAGE gel of Proteinase K-treated *Sphaerospora dicentrarchi* samples. Lane 1: Untreated control samples (10 µl). Lane 2: Proteinase K-treated samples (10 µl). Numbers on the left indicate molecular weights of standards

were *Glycine max* (SBA) (20 µg ml^{-1}), *Griffonia simplicifolia* agglutinin-I (GS-I) (50 µg ml^{-1}), *Triticum vulgaris* (WGA) (50 µg ml^{-1}), *Concanavalia ensiformis* (Con-A) (10 µg ml^{-1}) and *Ulex europaeus* agglutinin-I (UEA-I) (20 µg ml^{-1}). Control strips were incubated with TTBS. After washing, the nitrocellulose membranes were incubated with the ABC complex and then with the substrate to develop color as above.

Deglycosylation. Deglycosylation was performed using western blot as described by Woodward et al. (1985). Briefly, *Sphaerospora dicentrarchi* antigens were transferred after electrophoresis to nitrocellulose membranes as described above. Membranes were then rinsed with 50 mM sodium acetate buffer (pH 4.5) and exposed to varying concentrations of sodium orthoperiodate (1, 10, 20 mM) with pH 4.5 buffer in the dark for 1 h at room temperature. Membranes were then exposed to 1% glycerol in PBS for 30 min at room temperature. Controls consisted of membranes incubated in the same buffer without periodate treatment. Following 3 washes with TTBS, the nitrocellulose membranes were probed with polyclonal antibodies as described above.

RESULTS

Electrophoresis

When gels were stained with Coomassie Brilliant Blue R-250, the majority of the parasitic bands were between 32 and 115 kDa (Fig. 1). The relative MWs of these bands were 32, 50, 71, 79, 86, 95, 98, 105 and 115 kDa. Bands of 32, 50 and 98 kDa were the most intense. When gels were silver stained (Fig. 2, Lane 1), bands of 21, 27, 29, 37, 39, 43 and 130 kDa were observed besides those evidenced with Coomassie Brilliant Blue staining.

Silver-stained Proteinase K-treated samples revealed the existence of bands with MW of 18, 23, 28, 39 and 43 kDa in *Sphaerospora dicentrarchi* extracts. Bands between 50 and 130 kDa, which were identified in control samples (Fig. 2, Lane 1) did not appear in Proteinase-K ones (Fig. 2, Lane 2). When *S. dicentrarchi* gels were treated with periodic acid prior to silver staining, 14 bands between 27 and 98 kDa were detected (Fig. 3, Lane 1). Bands of 67, 64 and 49 kDa did not appear in control gel (Fig. 3, Lane 2).

Zymography

The most evident protease activity appeared as 3 clear bands on the SDS-PAGE gel, with molecular weights of 50, 84 and 126 kDa, and as a diffuse clear zone between 34 and 40 kDa (Fig. 4).

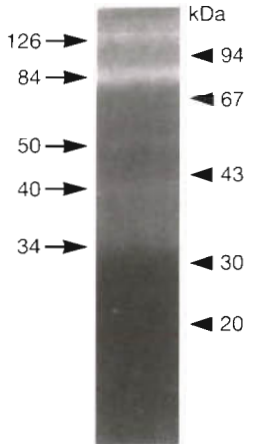

Fig. 4. Protease zymogram of *Sphaerospora dicentrarchi* using gelatin non-reducing SDS-PAGE. Numbers on the right indicate molecular weights of standards. Numbers on the left indicate the position of proteases

Western blot analysis

The western blot analysis showing the reactivity of the 4 polyclonal antisera are presented in Fig. 5. The 2 polyclonal antisera raised against *Sphaerospora dicentrarchi* (Fig. 5, Lane 1) or *S. testicularis* (Fig. 5, Lane 2) reacted mainly with parasitic material between 20 and 50 kDa, although some bands were also evident at higher MW. The most intense bands were detected at 21, 26, 28, 39 and 50 kDa with RaSdic and 18, 25, 28, 39 and 50 kDa with RaStest. RaClab (Fig. 5, Lane 3) and RaCspr (Fig. 5, Lane 4) reacted mainly with antigens between 50 and 140 kDa, but some bands were also evident at lower MW The most intense bands were detected at 50, 62, 86, 98, 100, 114 and 140 kDa with RaClab and 50, 63, 85, 97, 100, 114 and 140 kDa with RaCspr. No reaction was seen in the control strip incubated with preimmune rabbit serum (Fig. 5, Lane 5).

Fig. 6 shows Con-A binding to protein bands of *Sphaerospora dicentrarchi* extracts. Two bands were recognized at 78 and 96. The remaining lectins did not detect any band. No reaction was seen in the control strip incubated with TTBS (data not shown).

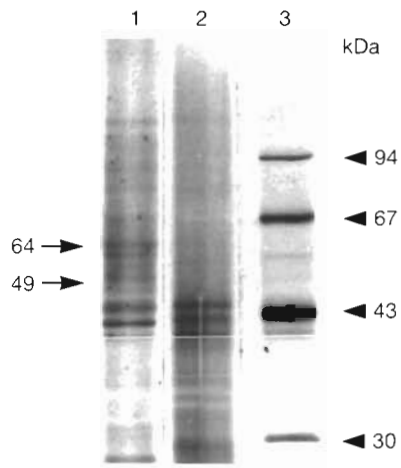

Fig. 3. Periodic acid silver-staining pattern of *Sphaerospora dicentrarchi* extracts. Lane 1: Periodic acid-treated sample (10 µl). Lane 2: untreated control samples (10 µl). Lane 3: molecular weight markers. Numbers on the right indicate molecular weights of standards. Arrows on the left indicate the position of 64 and 49 kDa bands

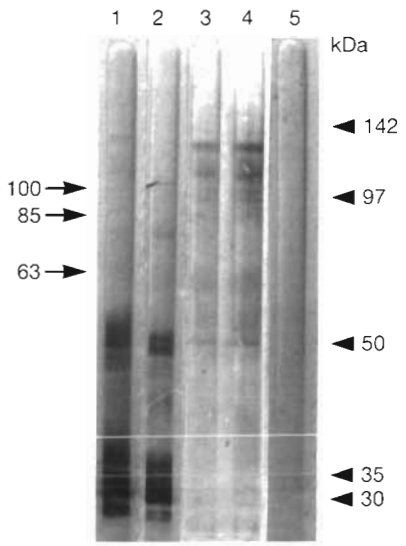

Fig. 5. Immunological detection of *Sphaerospora dicentrarchi* bands by western blot probed with 4 polyclonal antibodies. Lane 1. preimmune serum (1:500). Lane 2: RaSdic (1:500). Lane 3: RaStest (1:2000). Lane 4: RaClab (1:4000). Lane 5: RaCspr (1:4000). Numbers on the right indicate molecular weights of standards. Arrows on the left indicate the position of the faintest bands

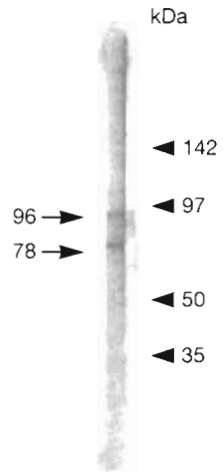

Fig. 6. Detection of glycoproteins using biotin-labelled *Concanavalia ensiformis* lectin. Numbers on the right indicate molecular weights of standards. Arrows on the left indicate the position of 96 and 78 kDa bands

Deglycosylation

The effect of increasing concentrations of periodate on the binding of RaSdic, RaStest, RaClab and RaCspr is shown in Fig. 7. The binding of RaSdic (Fig. 7, Lanes 1–3) and RaStest (Fig. 7, Lanes 4–6) to *Sphaerospora dicentrarchi* extracts was not affected by treatment with periodate. When RaClab (Fig. 7, Lanes 7–9) and RaCspr (Fig. 7, Lanes 10–12) were tested after periodate treatment, a high decrease of intensity in antibody binding occurred mainly when a concentration of 10 and 20 mM periodate was used, but the number and MW of the bands detected were the same.

DISCUSSION

Protein profiles using SDS-PAGE revealed the existence of several antigens in *Sphaerospora dicentrarchi* extracts, with MW between 21 and 130 kDa. Silver stain is 50 to 100 times more sensitive than Coomassie Blue staining and detects a wider variety of macromolecules, including nucleic acids, glycoproteins, and lipoproteins, or even trace proteins (Merril et al. 1984). Accordingly, in the current study, 7 bands, which were not detected with Coomassie Blue staining, appeared in silver-stained gels. In order to highlight the carbohydrate bands, a periodate treatment or an initial protein digestion was performed. Some bands not present in the control gel were detected after periodate treatment so they can be assumed to be glycopeptides. However, other common bands could also be glycopeptidic, as silver staining can also detect these glycoconjugates. After Proteinase K treatment, apparent digestion of peptides took place, as some bands heavier than 43 kDa, present in the control gel, were not detected. Other bands of 43, 39 and 28 kDa were observed in both treated and control gels, whereas the lightest bands of 23 and 18 kDa could be products of the peptide breaking. Proteinase K digestion usually yields glycopeptides containing single N-glycosylated sites. On the contrary, O-linked glycosylation sites can be resistant to proteolysis (Powell 1997). We can hypothesize that some bands heavier than 43 kDa could be proteins or glycopeptides with N-linked glycosylation sites and therefore sensitive to Proteinase K treatment. The bands present in gels from both control and treated samples could be glycopeptides resistant to proteolysis, probably with O-linked glycosylation sites.

Proteases are enzymes that catalyze the hydrolyses of peptide bands. The importance of parasite proteolytic enzymes in host parasite relationships and pathogenesis and their role in processes such as nutrition, the invasion of host cells and tissues and the countering of host defense processes is now well established (McKerrow 1989, McKerrow et al. 1993, North & Lockwood 1995). In our study, using the gelatin-SDS-PAGE under non-reducing conditions, 3 proteases of 50, 84 and 126 kDa, as well as a diffuse area betweeen 34 and 40 kDa, were demonstrated in *Sphaerospora dicentrarchi* extracts. The optimum pH for these proteases was about 7, while the proteolytic activity decreased at pH 5 (data not shown). Some Myxosporea have been demonstrated to produce proteases (Loseva 1976, Bilinski et al. 1984, Toyohara et al. 1993), but the knowledge of myxosporean proteases is still fragmen-

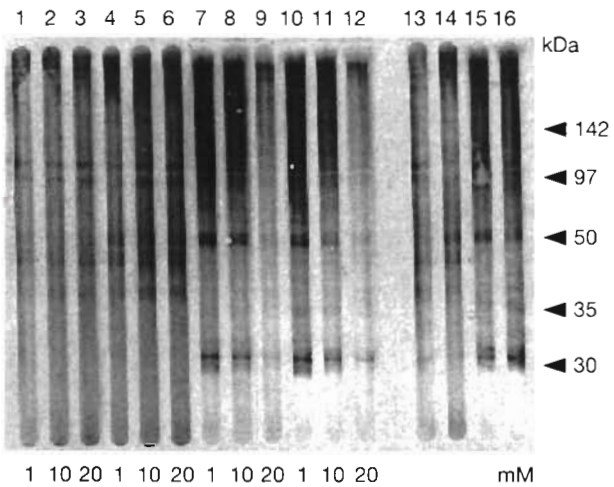

Fig. 7. Western blotting after periodate treatment screened with polyclonal antibodies. Lanes 1–3 were screened with RaSdic (1:500). Lanes 4–6 were screened with RaStest (1:2000). Lanes 7–9 were screened with RaClab (1:4000). Lanes 10–12 were screened with RaCspr. Lanes 13–16: Control (no periodate treatment). Lane 13: RaSdic (1:500). Lane 14: RaStest (1:2000). Lane 15: RaClab (1:4000). Lane 16: RaCspr (1:4000). Numbers on the right indicate molecular weights of standards. Numbers at the bottom indicate periodic acid concentration

tary. Among these, proteases produced by species of the genus *Kudoa* are the most studied ones. The muscle of Pacific hake *Merluccius productus* can be degraded to jelly by the cysteine protease(s) of *Kudoa* sp. during storage. In our study, the type of protease could not be identified, as none of the tested inhibitors abolished the proteolytic activity. The role of *S. dicentrarchi* proteases is unknown, but they could contribute to facilitating invasion of host tissues, allowing parasites to digest host proteins, or helping them to evade the host immune response, as has been previously described for other parasites (McKerrow 1989).

When the parasitic extracts were analyzed using polyclonal antisera raised against *Sphaerospora dicentrarchi*, *S. testicularis*, *Ceratomyxa labracis* or *C. sparusaurati*, several bands were recognised. Among the parasite antigens visualized by SDS-PAGE, 3 with a relative mobility of 21, 26 and 50 kDa were recognized by RaSdic. The results obtained indicate the existence of homologies between the 2 antisera raised against *Sphaerospora* spp., as both detected similar bands with MW between 20 and 50 kDa. There were also similarities between the 2 antisera raised against *Ceratomyxa* spp., which recognised bands between 50 and 140 kDa. Palenzuela (1996) also detected bands between 60 and 200 kDa in *S. testicularis* extracts using RaClab and RaCspr. However, the different antisera could recognise different structures in these bands showing similar MWs.

The existence of cross-reaction of these rabbit antisera with different Myxosporea was also demonstrated using immunohistochemistry (Muñoz et al. 1998a, 1999a). The 50 kDa band was recognised in immunoblotting by the 4 polyclonal antisera, suggesting that it could correspond to an antigen shared by several myxosporean parasites. Palenzuela (1996) detected a band of 50 kDa in SDS-PAGE of *Ceratomyxa* spp., but not in *Sphaerospora testicularis*. Adkinson et al. (1997) detected, in the waterbone triactinomyxon stages of *Myxobolus cerebralis*, a 50 kDa protein which was presumed to be one of the major constituents of the valves or floats of this triactinomyxon. Saulnier & Kinkelin (1996) also detected a 49 kDa band in enriched PKX (the causative agent of proliferative kidney disease) cell suspensions. These authors also found bands of 42 and 32 kDa, similar to those detected in our gels. The significance of the similarities among these bands of different Myxosporea in relation to antigen identity remains to be investigated.

In sea bass immunised with *Sphaerospora dicentrarchi* spores specific response against the parasite was demonstrated by solid-phase immunoenzyme assay (ELISPOT) and immunohistochemistry. However, we cannot compare the bands immunogenic for rabbits with the possible bands in fish as immunoblots gave negative results with immunised fish serum (Muñoz et al. 2000b).

Mild periodate oxidation at acid pH has been shown to cleave carbohydrate vicinal hydroxyl groups without altering the structure of polypeptide chains (see Woodward et al. 1985). This treatment has been used to denature a wide range of parasite carbohydrate moieties (Omer-Ali et al. 1986, Lustigman et al. 1990, Ravindran et al. 1990, Feng & Woo 1998). In our study, periodate treatment did not produce any effect on the binding of RaSdic or RaStest. Recognition might not depend on carbohydrate terminals, but the fact that not all carbohydrate antigenic determinants are sensitive to periodate cleavage (Woodward et al. 1985) must also be considered. On the other hand, a high decrease in intensity in the antibody binding occurred at concentrations of 10 and 20 mM periodate when RaClab and RaCspr were tested, which could indicate a binding dependent on carbohydrate terminals. Feng & Woo (1998), using a monoclonal antibody against a surface antigen of *Cryptobia salmositica*, observed a reduction of antibody binding with increasing concentrations of periodate. The differences between sera against *Sphaerospora* spp. and *Ceratomyxa* spp. could be due to differences in their specificity and affinity against *Sphaerospora dicentrarchi* antigens.

Some bands detected by the polyclonal antisera could have carbohydrate composition as they correspond to 3 of the proteinaceous glycoconjugates observed in Proteinase K-treated *Sphaerospora dicentrarchi* gels. This is the case for the 28 and 39 kDa bands detected by both RaSdic and RaStest and the band of 18 kDa detected by RaStest.

Previous studies have revealed the presence of glycoconjugates in *Sphaerospora dicentrarchi*, mainly N-acetyl-D-glucosamine residues and mannose and/or glucose, using immunohistochemistry (Muñoz et al. 1999b). In this study, we detected the existence of 2 Con-A binding antigens (with mannose and/or glucose terminals) of 96 and 78 kDa. These bands did not appear in silver-stained Proteinase K-treated samples. As mannose residues are normally found in N-linked oligosaccharide chains (Osawa & Tsuji 1987), those bands could have been digested by Proteinase K, which is effective against this type of glycoconjugates. *Triticum vulgaris* lectin (WGA) specific for N-acetyl-D-glucosamine residues, strongly stained polar capsules and valves of *S. dicentrarchi* spores in light microscopy (Muñoz et al. 1999b), and polar capsule walls, capsulogenic cells and sporoplasms in transmission electron microscopy (author's unpubl. manuscript). In the present study, WGA did not recognize any band in *S. dicentrarchi* extracts. Conversely, Palenzuela (1996) detected several bands in *Ceratomyxa* spp. extracts using the same lectin and procedure, and 1 band of

30 kDa with BS-I lectin in *S. testicularis*. Lin & Dickerson (1992) observed only weak signals with Con-A and *Galanthus nivalis* agglutinin in *Ichthyophthirius multifiliis* using the same technique. They assumed that glycosylation of proteins was to be minimal. In our case, the alteration of *S. dicentrarchi* antigens or the change in the accessibility of binding sites during the denaturalisation process in SDS-PAGE could explain the lack of recognition by some lectins.

The identification of significant parasite molecules and their subsequent purification is often a prerequisite for further study to develop diagnostic tests or candidate vaccines. The present work has concentrated on the antigenic characterization of *Sphaerospora dicentrarchi*. Different band reactivity with rabbit polyclonal antisera has been observed and some information on the presence of carbohydrate terminals has also been obtained.

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