Immunologic methods for species identification of early life stages of lutjanid fishes from the western central Atlantic. Part I: Characterization of an interspecies protein

Duane R. Schultz
Patricia I. Arnold
Department of Medicine, University of Miami School of Medicine
Miami, Florida 33101
e-mail address: dianeschu@aol.com

Thomas R. Capo
Claire B. Paris-Limouzy
Joseph E. Serafy
The Rosenstiel School of Marine and Atmospheric Science
University of Miami, Miami, Florida 33149

William J. Richards
Southeast Fisheries Science Center
National Marine Fisheries Service, NOAA
Miami, Florida 33149

Abstract.—A 66 kDa glycoprotein selected from SDS-PAGE gel profiles of soluble extracts of *Lutjanus griseus* was purified by Fast Protein Liquid Chromatography technology. A polyclonal antiserum produced to the single-chained glycoprotein was tested with 14 other lutjanid extracts in Western blots and produced 3 different patterns: strong reactions with *L. jocu* and *L. apodus;* weak reactions with *L. buccanella, L. synagris, L. analis, L. campechanus, Pristipomoides aquilonaris, Ocyurus chrysurus,* and *Apsilus dentatus;* no reactions with *L. vivanus, L. mahogoni, L. cyanopterus, Etelis oculata,* and the hybrid *L. synagris* × *O. chrysurus.* The anti-66 kDa antiserum also reacted strongly with soluble extracts of oocytes and juveniles of *L. griseus.* Adsorption of the IgG fraction of the antiserum with glutaraldehyde-insolubilized *L. apodus* extract resulted in an antiserum that remained strongly reactive with *L. griseus* extract but that was weakly reactive with *L. apodus* extract and negative with *L. jocu* extract in Western blots. The N-terminal amino acid sequence analyses of the first 10 residues of the purified 66 kDa proteins of *L. griseus* and *L. jocu* were approximately the same, but only 3 of 10 residues were the same with the purified proteins of *L. griseus* and *L. apodus.* Extracts of *L. apodus* contained 3 additional proteins that were not detected in extracts of *L. griseus* as determined by SDS-PAGE. This evidence for both interspecies and species-specific protein determinants is currently being used to produce species-specific polyclonal and monoclonal antisera for identifying species of lutjanid fishes at early life history stages.

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Eighteen species of snappers (Lutjanidae), representing 5 genera, are found in the western Atlantic Ocean (Robins et al., 1991). Species identification of the early life stages of snappers is incomplete because meristic characters are very similar or equivocal within the family. In addition, identification of morphological features requires a great deal of time and experience. Innovative studies are necessary for further species-specific identification of early life stages (eggs, larvae, and early juveniles) because information is available for only a few species and each exhibits a complex recruitment strategy (Richards et al., 1994). Lutjanids constitute one of the major predators of coral-grazing species, but the detrimental impact of overfishing and habitat loss from pollution and development is rapidly leading to an imbalance in the fragile equilibrium of the reef community. Thus, the ability to identify the early life stages of specific lutjanids is an important aspect of conservation measures.

Specific antibodies directed against a minor protein may be useful for species identification at the early life stages (Diano et al., 1992). In our study, starting with the development of biochemical methods to isolate a very pure antigen, we have produced a polyclonal antibody for prototypic studies of previously difficult or unapproachable questions of identification of early life history specimens of snapper. These methods and immunologic reagents have

wide versatility and applicability for current and future studies of early life history forms. The chromatographic purification and characterization of a 66 kDa glycoprotein from soluble extracts of *Lutjanus griseus* (Linnaeus), *L. jocu* (Schneider), and *L. apodus* (Walbaum) and the production of a specific polyclonal antiserum have generated evidence that the glycoprotein has both interspecies and species-specific epitopes. Further characterization of this and other lutjanid proteins, together with the production of both polyclonal and monoclonal antibodies for immunologic assays, may greatly facilitate future identification of lutjanids at any stage of their life history.

Materials and methods

Adult lutjanid specimens were obtained from local fishermen and skin divers, and species identification was carried out according to Robins and Ray (1986) and Robins et al. (1991).

Buffers and the methods for preparing soluble saline fish extracts are described in Schultz and Clarke (1995). In addition to *L. griseus*, the 13 species and one hybrid investigated here were the following: *L. jocu, L. apodus, L. vivanus* (Cuvier), *L. campechanus* (Poey), *L. analis* (Cuvier), *L. synagris* (Linnaeus), *L. mahogoni* (Cuvier), *Ocyurus chrysurus* (Bloch), *L. buccanella* (Cuvier), *Pristipomoides aquilonaris* (Goode and Bean), *Etelis oculata* (Valenciennes), *L. cyanopterus* (Cuvier), *Apsilus dentatus* (Guichenot), and the hybrid *L. synagris* × *O. chrysurus*. Separate extracts were prepared in 300 mL of buffer with approximately 30 g of tissue from gutted legal-size adult fish. The soluble, clear extract was concentrated by ultrafiltration (M_r 10,000 membrane, Amicon Corp., Danvers, MA) at 4°C until the OD_{280} exceeded 10. Also extracted were 1) gutted *L. griseus* juveniles with a total weight of 0.6 g and 2) *L. griseus* female gonads with oocytes, weighing approximately 3.5 g. Juveniles were collected from Biscayne Bay, Florida.

Two different antisera were produced in adult female goats according to the innoculation schedule of Schultz and Clarke (1995). The first was a polyvalent antiserum (anti-GSE) raised against *L. griseus* whole fish extract (14.1 mg protein/mL). The second antiserum (anti-66 kDa) was produced by using the 66 kDa protein (0.32 mg/mL) purified from *L. griseus* saline extracts (see below).

The γ-globulin fraction of anti-GSE and anti-66 kDa sera was isolated by 33% ammonium sulfate (A.S.) precipitation at 25°C (Stelos, 1967) and was further purified as IgG by DEAE-Sephacel (Pharmacia BioTech, Uppsala, Sweden) column chromatography in 10 mM NaPO4/50 mM NaCl, pH 7.4. The IgG-rich fractions were concentrated by ultrafiltration as above.

To eliminate background interference when anti-GSE was used as the primary antiserum in Western blots, it was necessary to biotinylate the IgG fraction (Bayer and Wilchek, 1980). The biotin-conjugated anti-GSE IgG was recovered by Sephadex G-25 (Pharmacia BioTech) gel filtration in 5 mM NaPO4/150 mM NaCl, pH 7.5 (PBS) and the biotin-protein ratio was 56 nmol of biotin per nmol of IgG (Green, 1965).

Soluble extracts of *L. mahogoni, L. apodus,* and *Eucinostomus gula* (silver jenny) were insolubilized with glutaraldehyde according to the method of Avrameus and Ternynck (1969), with 10 mg of glutaraldehyde per mg of soluble fish protein. Insolubilized *E. gula* was used previously to remove cross-reactivity with preimmune and immune goat sera and unrelated fish proteins (Schultz and Clarke, 1995).

The γ-globulin fraction of goat anti-66 kDa serum was adsorbed for 24 to 48 h at 4°C with 60 mg of insolubilized *E. gula* extract per mL γ-globulin, followed by 24 mg of insolubilized *L. mahogoni* extract (the 66 kDa protein was not detected in this species in Western blots). The IgG fraction of the adsorbed anti-66 kDa serum (anti-66 kDa IgG) was isolated by DEAE-Sephacel chromatography (see protocol above). This procedure removed all nonspecific activity.

Another portion of the γ-globulin fraction of anti-66 kDa serum was adsorbed with 100 mg of insolubilized *L. apodus* extract for 24 h per adsorption at 4°C in experiments to distinguish the 66 kDa proteins found in soluble saline extracts of *L. griseus, L. jocu,* and *L. apodus* (see Results section).

The buffers and conditions for ion exchange chromatography with the Fast Protein Liquid Chromatography (FPLC) system (Pharmacia Biotech, Uppsala, Sweden) as described in Schultz and Clarke (1995) were modified to include 10 mM EDTA in the column buffers. The sequence was that of Mono S cation exchanger followed by Mono Q anion exchanger.

Gel filtration on Sephacryl S-300 Superfine (Pharmacia Biotech) in PBS/10 mM EDTA, pH 7.5, was used to separate high molecular weight contaminants from the 66 kDa protein.

During purification procedures, protein concentration was monitored by absorbance at 280 nm. Purified proteins were dialyzed against PBS, pH 7.5, to remove EDTA, and the protein concentration was determined spectrophotometrically as described previously (Arnold et al., in press). Methods for electrophoresis in 10% (w/v) polyacrylamide gels with or without sodium dodecylsulfate (Laemmli, 1970), for Western blots (Towbin et al., 1979), and the molecu-

lar weight standards (Sigma Chemical) are described in Schultz and Clarke (1995).

Glycoproteins were identified on electroblots by using the method of O'Shannessy et al. (1987).

Results

The steps for purification of a 66 kDa protein from a soluble saline extract of *L. griseus* are shown in Table 1. Figure 1 depicts samples of each purification step in SDS-PAGE (gel A) and corresponding Western blots (gels B and C) leading to the highly purified protein. The protein was a trace component of the saline extract (arrow, A1) but was more concentrated with successive purification steps (A, 2–4). The steps of purification included *L. griseus* saline extract in lane 1, the A.S. (0.38 gm/mL extract) supernatant solution of the extract in lane 2, the 0.27 M NaCl eluate from a Mono S cation exchange column after

Table 1

Steps for the purification of *L. griseus* 66 kDa protein. FPLC = Fast protein liquid chromatography.

Procedure	Product
1 Puree fish, separate solid material by centrifugation	Clear extract
2 Solid ammonium sulfate fractionation (0.38 g/mL extract)	Supernatant fluid: elimination of most high molecular weight proteins in precipitate
3 FPLC: Mono S cation exchanger; gradient: 0 to 1.0 M NaCl, pH 5.0	Enriched 66 kDa protein eluted at 0.27 M NaCl
4 FPLC: Mono Q anion exchanger; gradient: 0 to 1.0 M NaCl, pH 7.5	Highly purified 66 kDa protein eluted at 0.32 M NaCl

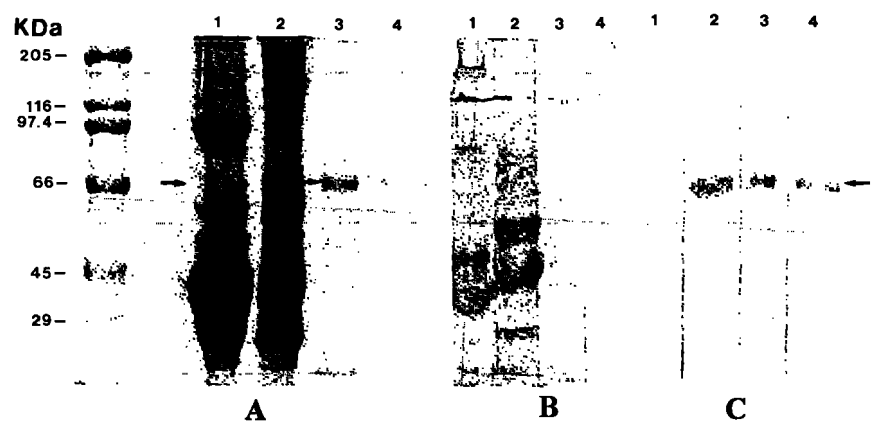

Figure 1

Samples from the purification steps of soluble *L. griseus* extract leading to the highly purified 66 kDa protein in lane 4. Gel (A) shows samples after SDS-PAGE stained with Coomassie brilliant blue. The corresponding Western blots were developed by using biotinylated goat anti-whole *L. griseus* antiserum (B) and goat anti-*L. griseus* 66 kDa protein IgG (C). Reference standard proteins (kDa) are shown in the stained gel. The arrows in gels A and C show the position of the 66 kDa protein. Numbered lanes denote the following: 1) whole *L. griseus* extract (235 µg total protein); 2) ammonium sulfate (0.38 g/mL extract) supernatant solution of *L. griseus* whole extract (228 µg); 3) eluate from Mono S cation exchange column (20 µg) after FPLC; and 4) eluate from Mono Q anion exchange column (0.32 µg) after FPLC. Note that the reaction of biotinylated goat anti-whole *L. griseus* antiserum with the 66 kDa *L. griseus* protein was not detected in B.

FPLC in lane 3, and the 0.32 M NaCl eluate of the highly purified 66 kDa protein from a Mono Q anion exchange column after FPLC in lane 4. Although the biotinylated goat anti-GSE antiserum reacted with many different proteins in the saline extract, it did not contain antibodies that were reactive with the trace 66 kDa protein in the Western blot (B, 1–4). In contrast, where the goat anti-66 kDa IgG was used, the protein was weakly positive with the saline extract (C1), but was strongly positive in the other lutjanid preparations (C, 2–4).

The purification procedure for the 66 kDa protein was repeated 4 times, with an average yield of 119 µg/g of the whole *L. griseus* saline extract. The protein was determined to be a single-chain polypeptide under reducing conditions in SDS-PAGE. The trace glycoprotein was not albumin (Hartmann et al, 1994), and under nonreducing, nondenaturing conditions, the lutjanid glycoprotein migration differed from human or bovine albumin (mol. weight 66 kDa, data not shown).

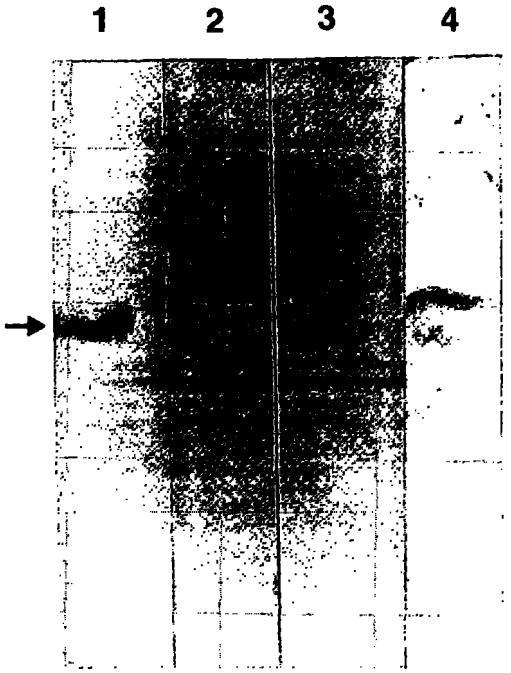

Figure 2

Western blot analysis of *L. griseus* life cycle stages. Samples included are purified 66 kDa protein, 1 µg total protein (lane 1); 50% ammonium sulfate supernatant fluid from a saline extract of female oocytes, 330 µg total protein (lane 2); saline extract of juvenile fish, 118 µg total protein (lane 3); saline extract of a sexually mature fish, 340 µg total protein (lane 4). The blot was developed with goat anti-*L. griseus* 66 kDa protein IgG as the primary antibody and with horseradish peroxidase-conjugated rabbit anti-goat IgG as the secondary antibody. The arrow marks the position of the 66 kDa protein.

After purification of the 66 kDa protein and production of a specific goat antiserum, saline extracts of different life stages of *L. griseus* were tested for the presence of the protein. Western blot analysis (Fig. 2) showed that the protein was present in all stages: oocyte (lane 2), juvenile (lane 3), and adult (lane 4).

Saline extracts of 14 species of adult lujanids and the hybrid (*L. synagris* × *O. chrysurus*) were prepared by the same method (see Materials and Methods section), and each extract was adjusted to 235 µg total protein. After Western blot analyses of the 15 different specimens, each blot was evaluated for the 66 kDa protein by visual inspection as shown in Figure 3, and the species were classified, as shown in Table 2. The species judged strongly positive were *L. griseus, L. jocu,* and *L. apodus* because for these three species the staining intensity of band patterns was approximately equal. Weak reactors were *L. buccanella, O. chrysurus, P. aquilonavis, L. synagris, L. analis, A. dentatus,* and *L. campechanus; L. vivanus, L. mahogoni, L. cyanopterus, E. oculatus,* and the hybrid exhibited no reaction.

Because the staining patterns of the 66 kDa proteins of *L. griseus, L. jocu,* and *L. apodus* were of similar intensity and because seven other species reacted weakly with the anti-66 kDa IgG for Western blots (Table 2; Fig. 3), an important question was raised: Was the protein from the different species identical but present in diminished concentrations, or were dissimilar determinants being detected? To investigate these questions, we selected *L. griseus, L. jocu,* and *L. apodus* because their 66 kDa proteins were strongly reactive with the specific antiserum. In addition, other studies have shown close phylogenetic relationships among these three species through morphological and biochemical data (Rivas, 1966; Vergara, 1980; Chow and Walsh, 1992) and by analysis of mitochondrial DNA sequences (Chow et al., 1993; Sarver et al., in press). Figure 4 shows the results of adsorbing the goat anti-66 kDa IgG with gluteraldehyde-insolubilized *L. apodus* saline extract for 24 h at 4°C. Comparing the Western blot in A (preadsorption) with B (postadsorption), we found that the antiserum reacted equally well with the *L. griseus* marker protein (lane 1, A and B) and the *L. griseus* saline extract (lane 4, A and B). The preadsorption antiserum also reacted with equal potency with *L. jocu* (lane 2A) and *L. apodus* (lane 3A). After adsorption of the antiserum with the insolubilized *L. apodus* saline extract, bands in the Western blots for *L. jocu* (lane 2B) and *L. apodus* (lane 3B) were

greatly reduced in reactivity with the secondary rabbit anti-goat IgG antiserum. The same anti-66 kDa IgG was adsorbed two more times with fresh, insolubilized *L. apodus* saline extract for 24 h at 4°C, and Western blots showed that the reaction of *L. jocu* was completely eliminated, whereas a trace reaction by *L. apodus* was detectable. The marker protein and the *L. griseus* extract reactions were similar to those in Figure 4 (data not shown). Therefore, we conclude that the 66 kDa protein from *L. griseus* must have different and multiple immunodominant regions compared with the 66 kDa proteins of *L. jocu* or *L. apodus*. Otherwise, after the third adsorption of the antiserum with insolubilized *L. apodus*, the immunoblots in B1 and B4 would have been greatly reduced or negative with the extract of *L. griseus*, but they were similar to the preadsorption blots.

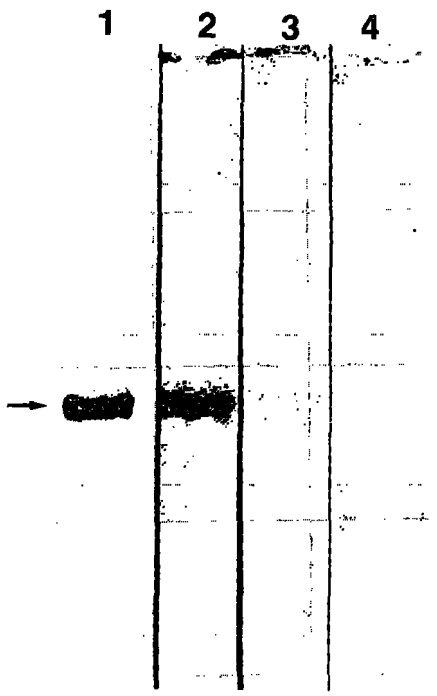

Figure 3

Western blot analysis of the graduated reaction of lutjanid saline extracts to anti-66 kDa protein IgG (primary antibody) followed by horseradish peroxidase rabbit anti-goat IgG (secondary antibody). The samples are as follows: lane 1 = purified *L. griseus* 66 kDa protein, 0.34 µg total protein; lane 2 = *L. griseus* extract, 235 µg total protein, classified as a strong reaction; lane 3 = *O. chrysurus* extract, 235 µg total protein, classified as a weak reaction; and lane 4 = *L. cyanopterus* extract, 235 µg total protein, classified as not detected. The arrow marks the position of the 66 kDa protein. Table 2 shows the reactions of soluble saline extracts of 14 different lutjanid species with the goat anti-*L. griseus* 66 kDa protein IgG.

The 66 kDa protein was purified separately from soluble extracts of the three species as described in Table 1. Figure 5 shows the proteins after SDS-PAGE and Western blots. The only discernible difference was that the 66 kDa protein from *L. griseus* migrated slightly slower than the proteins from *L. jocu* and *L. apodus*, indicating a slightly heavier molecular mass.

Next, it was determined that all three proteins are glycosylated when the method of O'Shannessy et al. (1987) is used. Separately, each of the purified proteins was oxidized with Na periodate. Available carbohydrate moieties were then labeled with biotin-hydrazide. After SDS-PAGE, glycoproteins were detected with peroxidase-labeled streptavidin and 4-chloro-l-naphthol (data not shown).

Finally, N-terminal amino acid sequences of the three proteins were obtained for comparative studies. Table 3 shows that the sequences from *L. griseus* and *L. jocu* for the first ten amino acids were approximately the same (residue no. 4 was not interpretable for *L. griseus*). Comparison of *L. griseus* with *L. apodus* showed that only as many as 3 of 10 amino acids were the same. In addition, only 3 of 10 amino acids of *L. jocu* were the same as *L. apodus*. Thus, as

Table 2

Screening of soluble saline extracts of fourteen different species and one hybrid of family *Lutjanidae* for presence of 66 kDa *L. griseus* protein by Western blot analyses. The basis for the strongly positive, weak, and not detected evaluation is shown in Figure 3. Each individual species was adjusted to 235 µg total protein for the immunoblots.

Species	Western blots for 66 kDa protein
L. griseus *L. jocu* *L. apodus*	Strongly positive
L. buccanella *O. chrysurus* *P. aquilonaris* *L. synagris* *L. analis* *A. dentatus* *L. campechanus*	Weak
L. vivanus *L. mahogoni* *L. cyanopterus* *E. oculatus* *O. chrysurus* × *(hybrid)* *L. synagris*	Not detected

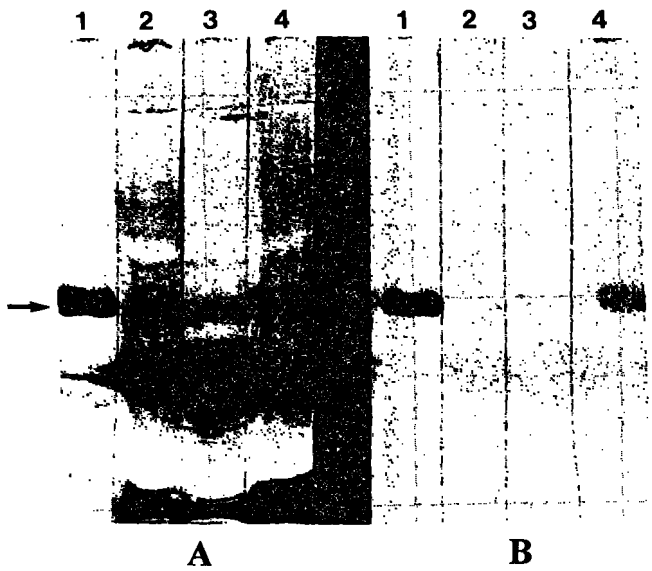

Figure 4

Western blot analysis of saline extracts from three closely related lutjanids, *L. griseus*, *L. jocu*, and *L. apodus*. The goat anti-*L. griseus* 66 kDa protein IgG was used before (**A**) and after (**B**) adsorption with 100 mg of insolubilized *L. apodus* saline extract for 24 h at 4°C. Lane 1 = purified *L. griseus* 66 kDa marker protein, 1 µg total protein; lane 2 = *L. jocu* extract, 235 µg total protein; lane 3 = *L. apodus* extract, 235 µg total protein; and lane 4 = *L. griseus* extract, 235 µg total protein. The arrow marks the position of the 66 kDa protein.

far as the first ten N-terminal amino acids, the 66 kDa protein of *L. apodus* was different from that of *L. griseus* and *L. jocu*.

Because other studies have shown *L. griseus*, *L. apodus*, and *L. jocu* to have a close phylogenetic relationship (e.g. Sarver et al., in press), we wanted to determine if there were obvious differences in their respective protein profiles. Fifty percent of A.S. precipitates of saline soluble extracts of each species were prepared at the same time; the precipitates were solubilized and dialyzed with saline, and they were compared in SDS-PAGE. Figure 6 shows that the 66 kDa protein was present in each of the three species (open arrow). However, there are at least three proteins that were present in *L. apodus* that were not detectable in *L. griseus* (closed arrows). Although other lutjanids have not been screened for these proteins, this procedure increases the possibility of isolating species specific proteins for antibody production, and eventually for species identification of early life history forms. Even if one or more of the three proteins of *L. apodus* were present in other lutjanids, some of the determinants on each protein, including different amino acid or glycan substitutions, or both, would probably be structurally different and thus would increase the possibility of producing specific monoclonal antibodies.

Discussion

A trace single-chain 66 kDa glycoprotein was purified to homogeneity by FPLC technology, starting with soluble extracts of adult *L. griseus* (Table 1; Fig. 1). The glycoprotein was not detected in Western blots with the biotinylated goat anti-whole *L. griseus* antiserum, but it was identified in extracts on SDS-PAGE (Fig. 1). This finding is not unusual because trace proteins in human serum frequently are not reactive with an antiserum produced in animals to whole human serum (senior author's unpubl. observations). A polyclonal antiserum was produced to the purified glycoprotein in a goat, and after removal of nonspecific reactivity by adsorption with insolubilized snapper and nonsnapper proteins, Western blots were carried out with the IgG fraction of the antiserum and with soluble extracts of 14 other lutjanid species that were available to us. Because of a comparatively strong reaction in Western blots

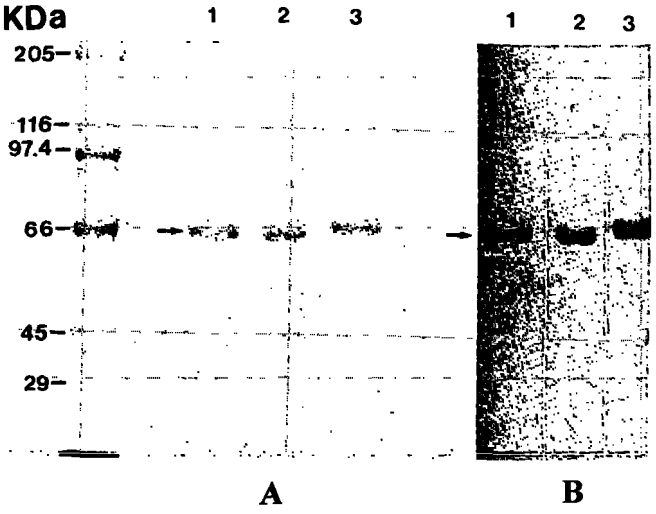

Figure 5

SDS-PAGE (A) and corresponding Western blots (B) of the highly purified 66 kDa proteins from 1) *L. jocu*, 2) *L. apodus*, and 3) *L. griseus*. Total protein added to each lane was 2 µg. The protein of *L. griseus* is slightly heavier compared with the other two species. Comparative N-terminal amino acid sequences of the three proteins are shown in Table 3.

Table 3

The N-terminal amino acid sequence analysis of the 66 kDa glycoproteins purified from *L. griseus*, *L. jocu*, and *L. apodus*.[1]

Snappers	1	2	3	4	5	6	7	8	9	10
L. griseus	Asp/Ala	Glu	Ala	N.I.[2]	Ala	Asp	Ala	Glu	Glu	Val
L. jocu	Asp	Glu	(Ala)[3]	His	Ala	Asp	Ala	Glu	Glu	Val
L. apodus	Asp	Glu/Ala	His	Ala	Asp	Ala	Glu	Glu	Val	Pro

[1] Analyzed at the University of Florida, Dep. of Biochemistry and Molecular Biology (ICBR Protein Chemistry Core Facility), Gainesville, FL.
[2] Not interpretable.
[3] Confidence: ( ) = possible/low.

with the antiserum, we purified to homogeneity similar 66 kDa glycoproteins from *L. jocu* and *L. apodus* (Fig. 5). The antiserum reacted weakly in immunoblots with soluble extracts of seven other species of lutjanid and did not react with extracts of four more lutjanid species and a hybrid of *O. chrysurus* and *L. synagris* (Table 2). The results indicated that the protein had both interspecies and species-specific determinants. In additional experiments, adsorption of the IgG fraction of the anti-66 kDa antiserum with glutaraldehyde-insolubilized *L. apodus* extract resulted in an antiserum that remained strongly reactive with *L. griseus* extracts but that was weakly reactive with *L. apodus*, and negative with *L. jocu* in Western blots (Fig. 4). Although the N-terminal amino acid sequence analyses of the first ten residues of *L. griseus* and *L. jocu* were approximately the same, only three of the ten residues were the same from *L. apodus*. These analyses did not take into account the possibility that the interspecies 66 kDa proteins may have different carbohydrate moieties that influence the antigenicity of the respective epitopes. For example, the 66 kDa protein of *L. griseus* was slightly heavier compared with the proteins of *L. jocu* and *L. apodus* in SDS-PAGE (Fig. 5) and thus could account for, but not prove, differences in glycan-dependent epitopes.

This approach was used as a model for distinguishing the different species of lutjanids. The eventual goal of these studies is to produce species-specific

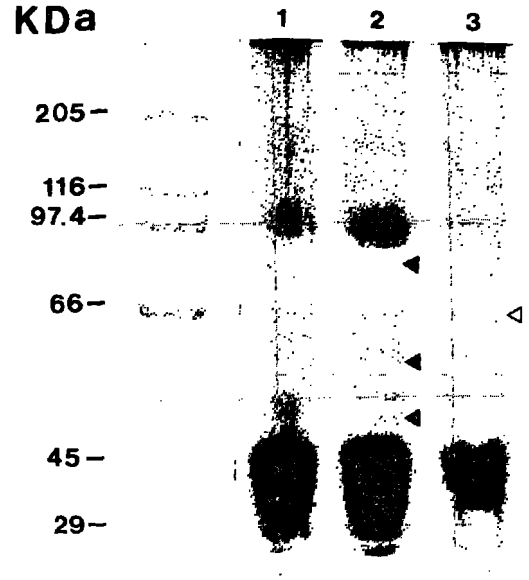

Figure 6

SDS-PAGE of the dissolved precipitates from the ammonium sulfate fractionation (0.38 mg/mL) of soluble extracts of 1) *L. jocu*; 2) *L. apodus*; and 3) *L. griseus*. Total protein added to each lane was 235 µg. The open triangle (◁) shows the position of the 66 kDa protein; the closed triangle (◀) marks bands not seen in *L. griseus* (lane 3). The gel was stained with Coomassie brilliant blue.

polyclonal and monoclonal antisera for identifying specimens at an early life stage. Animals will be immunized with highly purified proteins to enhance possibilities of producing antibodies to minor structural protein differences. The use of highly purified protein is especially important for monoclonal antibody production because immunization with complex mixtures of antigens generally results in monoclonal antibodies being produced to immunodominant epitopes (Barclay and Smith, 1986; Matthew and Sandrock, 1987). Immunization with a highly purified protein increases possibilities of producing monoclonal antibodies with a fine degree of specificity for each species of lutjanid. It remains for future experiments to determine if these specific antisera will also be species specific for life history specimens, such as eggs, larvae, and early juveniles. Although the anti-66 kDa IgG used in these studies also reacted with the early life history specimens of *L. griseus* (Fig. 2), future experiments with monoclonal antibodies generated to the 66 kDa protein and other lutjanid proteins (Fig. 6) will determine their usefulness as specific reagents.

In other studies, the use of a very pure antigen was successful for predator-prey studies with *Sciaenops ocellatus* (red drum) (Schultz and Clarke, 1995; Arnold et al., in press), in which a specific polyclonal antiserum was prepared by immunizing a goat with a highly purified 80 kDa glycoprotein.

Monoclonal antibodies were used successfully to detect epitopes in a number of marine and fresh water species. For example, An et al. (1990) used a low molecular weight eluate from SDS-PAGE slab gels of a protein extract from rock shrimp to produce highly specific murine monoclonal antibodies. A monoclonal antibody to partially purified mature egg proteins reacted with eggs, embryos and larvae of *Asterina pectinifera* but not with other species belonging to the same genus or in mixtures of biological specimens of marine origin (Ikegami et al., 1991). Murine monoclonal antibodies were employed by Miller et al. (1991) to distinguish three barnacle species of similar size, and Beck et al. (1992) investigated surface antigens of rainbow trout sperm with monoclonal antibodies.

Immunological detection methods with monoclonal antibodies have also been valuable for many aspects of seafood science. Recently, Huang et al. (1995) were able to distinguish the commercially valued red snapper, *L. campechanus*, from less valuable substitutes by using ELISA with two monoclonal antibodies raised to a red snapper protein.

It is now evident that identification of species of the western Atlantic snapper at early life history stages by examining external features alone is insufficient and that new methods are necessary to increase our knowledge. The use of highly purified lutjanid proteins, together with the production of polyclonal and monoclonal antibodies for a variety of immunoassays, has wide versatility and applicability for future studies.

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