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Purification and physico-chemical study of serum albumins of two neotropical fish species from the São Francisco River Basin, Brazil

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Abstract: This paper presents results from a study of albumin from pacu (Piaractus mesopotamicus, Holmberg 1887) and the catfish pintado (Pseudoplatystoma corruscans, Spix & Agassiz, 1829), two neotropical fish species inhabitants of Brazilian rivers, comparing their molecular mass and discussing their secondary structures based on spectropolarimetric (circular dichroism) measurements. Genetic controlled specimens were obtained from two fish hatcheries, located in Mococa (pacu) and in São João da Boa Vista (pintado), both in São Paulo State, Brazil. After a period of adaptation in holding tanks, fish blood samples were taken by puncturing their abdominal aorta. Purified albumin was obtained by gel filtration. SDS-PAGE electrophoresis was performed for the molecular mass estimation. Circular Dichroism spectra were registered for albumins of the two fish species over the range of 190-250 nm (far-UV), which showed two negative bands at 217 and 208 nm, a positive peak at 196 nm and a crossover at 200 nm. This profile is compatible with proteins that content predominantly alpha-helix structure.

Keywords: Spotted sorubim, Pseudoplatystoma corruscans, Pacu, Piaractus mesopotamicus, fish serum albumin, circular dychroism.


Resumo: Este artigo apresenta os resultados de um estudo sobre as albuminas de pacu (Piaractus mesopotamicus, Holmberg 1887) e pintado (Pseudoplatystoma corruscans, Spix & Agassiz, 1829), duas espécies neotropicais de peixes nativas do Brasil, determinando as suas massas moleculares e discutindo suas estruturas secundárias, com base em medidas de espectropolarimetria (dicroismo circular). Espécimes controlados geneticamente foram obtidos de duas diferentes pisciculturas, uma localizada na cidade de Mococa (pacu) e a outra, na cidade de São João da Boa Vista (pintado), ambas no Estado de São Paulo, Brasil. Após um período de adaptação em tanques apropriados, amostras de sangue foram coletadas por punção da aorta abdominal dos peixes. Albumina pura foi obtida por gel filtração. SDS-PAGE eletroforese foi realizada para a estimativa da massa molecular. Espectros de dicroismo circular das albuminas dos peixes foram registrados na região de 190-250 nm (far-UV), os quais mostraram duas bandas negativas, a 217 e 208 nm, um pico positivo a 196 nm e um crossover a 200 nm; perfil este compatível com proteínas que contêm predominantemente estrutura alfa-hélice.

Palavras-chave: Albumina de peixe, Pintado, Pseudoplatystoma corruscans, Pacu, Piaractus mesopotamicus, dicroismo circular.

Introduction

Among of transport proteins in blood plasma, only albumin is able to bind a wide diversity of drugs reversibly with high affinity. Albumin is typically the major anionic protein in vertebrate plasma, representing more than 52% of the total plasmatic protein content. It has an important role in transport endogenous ligands and xenobiotics mostly through the formation of non-covalent complexes at specific binding sites. Albumin also plays an essential role in organisms when protects them by binding toxic metabolites, such as bilirubin, as well as functioning as a reservoir of the nitric oxide (Kragh-Hansen 1990; Curry et al. 1998; Sugio et al. 1999; Bertucci and Domenici 2002; Stamler et al. 1992). According to Shao et al. (1993), this protection function presents a special importance in the Chinese cobra, since serum albumin avoids that the snake becomes victim from its own venom.

The study of albumins represents a challenge as they have not a single property by which they can be identified (Maillou and Nimmo 1993). Much data about quantity and structure of mammalian albumins, mainly bovine (BSA) and human (HSA) serum albumin, can be found in literature (Silva et al. 2004a, 2004b, 2004c, Bertucci and Domenici 2002, Kragh-Hansen 1990), but very few information about fish albumin is available (Silva et al. 2009a, 2009b). The shortage of data about fish albumin makes to establish a pattern a difficult task, even for the same family (Perrier et al. 1974, Davidson et al. 1989).

However, it is known that, in general, these proteins have at least one residue of tryptophan (Feller et al. 1994) along their chain.

In this study we have isolated, purified, and studied albumin from pacu (Piárauctus mesopotamicus, Holmberg 1887) and pintado (Pseudoplatystoma corruscans, Spix & Agassiz 1829), two neotropical fish species inhabitants of Brazilian rivers, obtained from fish hatcheries located in São Francisco River basin in São Paulo State, Brazil, establishing their molecular mass and discussing their secondary structures based on spectropolarimetric (circular dichroism, CD) measurements.

Material and Method

Genetic controlled early specimens of pacu and pintado, wt = 300 ± 15 g, were obtained from two fish hatcheries, one located in Mococa (pacu) and another in São João da Boa Vista, both in São Paulo State, Brazil. They were supplied with well-aerated water (normoxic conditions) in holding tanks at constant temperature of about 28°C. After an adaptation period, they were used in experiments. Fish were fed with commercial feed pellets. Blood samples were taken from the abdominal aorta of live fish using non-heparinized syringes.

Serum from fish blood was previously treated with ammonia sulfate, dialyzed, and chromatographed in blue sepharose affinity column. Purified albumin was obtained by gel filtration. SDS-PAGE electrophoresis was performed for molecular mass estimation.

The chromatographic method used was that described by Travis and Pannell (1973). Serum was applied to a HiTrap Blue column, and fractionated with 50 mM potassium phosphate pH 7.4 at 2 ml/min, monitoring the absorbance at 280 nm. After discarding non-bound fractions, albumin was washed out with a gradient of 0 to 1.5 M of elution buffer (KCl in phosphate buffer). Total serum protein concentration was determined by the Basil method (Doumas et al. 1971) and albumin serum concentration was determined by the Basil-Doumas method (Doumas et al. 1971).

To study the secondary structure of albumin we used the spectropolarimetric method within the CD technique. CD spectra were registered over the range of 190-250 nm (far-UV).

Results and Discussion

Gel filtration and electrophoresis showed a molecular weights for both pacu and pintado albumins, around 66 kDa, presenting strong similarity to BSA. These results agree to the values considered by Maillou and Nimmo (1993), who suggested that the molecular weight of fish albumin can be next to 70 kDa, basing on their electrophoretic analysis using serum of two different species of fish. For two members of the Salmonidae family, the chinook salmon (Oncorhynchus tshawytscha) and brown trout (Salmo trutta), Brennan et al. (1998) found molecular masses of 65 and 67 kDa for Salmon and trout albumins, respectively.

The total serum protein concentrations measured by Peterson’s method (1977) were 16 mg/ml and 34 mg/ml for pintado and pacu, respectively. By means of the brome cresol green method, we determined albumin fractions of 1.6 mg/ml, for pintado, and 2.0 mg/ml, for pacu. These values are in accordance to the suggestion that serum albumin concentration in fishes can be much lower than of albumin concentration in mammals, which vary from 30 to 40 mg/ml. Due to the low plasmatic ratio, it was thought in the past that albumin was not present in fish serum. But, experiments performed by Byrnes and Gannon (1992) identified cDNA sequences of Salmo salar albumin, and its homology with albumins of other vertebrates was already demonstrated (Metcal et al. 1998).

In recent study, Zhang et al. (2013) studied the total protein content in plasma from six kinds of mammals and fish and purified albumin. They found that total plasma protein and albumin content in mammals were nearly two times and four times higher than that in fish, respectively. For salmon and trout albumins, values of plasmatic concentration estimated were approximately 15 mg/ml in both species (Brennan et al. 1998).

The values of albumin concentration we found for the two fish were lower than those found for others fish species. To explain this large variation grade, it is important consider that fishes form the largest group among vertebrates with a wide variability of shapes, dimensions, and anatomical and metabolic characteristics. Physiologically, such variability reflects the great capacity of adaptation of the group to the aquatic environment diversity (Roberts 1981). Pacu and Pintado are two neotropical fresh water fish species, adapted naturally to quite different habitats from those salt and cold water fish species studied by the majority of the authors. On the other hand, pacu and pintado have different nutritional and living habits from each other. While pacu is omnivorous, feeding themselves of plants and fruits, pintado is a benthic carnivorous specie. It might explain the difference we found in their serum protein concentration.

Despite the different concentrations, the CD spectra show similar secondary structure profile for two fish species. Figure shows CD spectra of pacu and pintado, respectively, obtained at 25°C (50 mM phosphate buffer, pH 7.4). These spectra were registered over the range of 190-250 nm (far-UV), and they present two negative bands at 217 and 208 nm, a positive peak at 196 nm and a crossover at 200 nm. Selcon 3 method was used for estimating the albumin secondary structure content (Table 1), and the data analysis has shown the spectrum profiles are compatible with proteins that content predominantly alpha-helix structure.

We believe that fish plasmatic proteins can function as an effective biomarker, since their concentration can vary as reflex of nutritional, physiological and/or geographic conditions. In addition, the total and relative ratio of serum protein can be affected by pathological conditions and besides the presence of environmental stressors (Grasman et al. 2000, Ellsaesser and Clem 1987). Fishes can also exhibit polyplody, responsible for many of the variations in plasma proteins (Komatsu et al. 1970). Thus, we see a high importance in studies about serum
fish proteins and their interactions, because these data could be of particular interest in ecotoxicology and environmental risk assessment.

Conclusions

Our results allow conclude that: (1) total serum protein concentrations of pintado and pacu are 16 mg/ml and 34 mg/ml, respectively; (2) serum concentration of albumin in fish (1.6 mg/ml, for pintado, and 2.0 mg/ml, for pacu) is very lower than albumin concentration in mammalian; (3) the molecular mass for pacu and pintado serum albumin are about 66 kDa, presenting similarity to BSA; (4) the secondary structure content analysis by Selcon 3 method shows predominantly alpha-helix profile for two fish species studied; and (5) CD negative band at 217 nm suggests difference in its conformation when compared to BSA and HSA.

References


Perrier, H., Delcroix, J.P., Perrier, C. & Grass, J. 1974. Disc electrophoresis of plasma proteins of fish; localization of fibrinogen, transferrin and ceruloplasmin in the plasma of the

Table 1. Secondary structure content for pacu and pintado serum albumin

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<td>Pintado</td>
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Unrd = disordered.

Figure. CD spectrum between 195 e 250 of albumin for Pacu and Pintado at 25°C, 50 mM phosphate buffer, pH 7.4.

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