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Palmitate-binding, serum albumin-like proteins in salmonids

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There has been considerable controversy over the existence of serum albumin in fish. One of the physiological functions of albumin is to bind free fatty acids. This characteristic was used to screen the plasma of seven species of salmonids. Each species contains a protein fraction that (i) binds palmitate, (ii) has a molecular mass similar to that of human serum albumin, and (iii) is one of the most rapidly migrating proteins when salmonid plasma is subjected to anodal polyacrylamide gel electrophoresis. We conclude therefore, that salmonids have serum albumins that are homologous to the serum albumin of higher vertebrates.

Serum albumin; Palmitate binding; (Salmonidae)

1. INTRODUCTION

When a protein does not have a well defined function, it is often very difficult to identify its homologues in different species. Such is the case with serum albumin. Albumin was defined historically with respect to the electrophoretic separation of human plasma. It is characterised by being the most abundant human blood plasma protein, having a high negative charge, a molecular mass of approx. 68 kDa and being free of carbohydrate [1]. Although albumins have been isolated from amphibians, reptiles, birds and mammals [2], there is still a question concerning its presence in fish. Based on electrophoretic mobility, a protein with a molecular mass of 150 kDa and a lipid content of 22% was identified as albumin carp plasma [3]. Other studies on carp plasma indicated that there were five proteins ranging in molecular mass from 145 to 58 kDa that had some properties in common with human albumin [4]. Initial characterisation of the plasma proteins of rainbow trout appeared to confirm the absence of an albumin-like fraction [5] but this was subsequently revised and two proteins were described as 'para-albumins' based on their molecular masses, electrophoretic mobilities, solubility in ammonium sulphate, and lack of glycoprotein staining [6]. One of the major physiological functions of albumin is to transport free fatty acids [7,8]. We have used this biological property of serum albumin to identify similar proteins in the plasma of seven species of salmonids.

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2. MATERIALS

Whole blood was collected in heparinised tubes from anadromous and non-anadromous forms of Atlantic salmon (*Salmon salar*), brown trout (*S. trutta*), rainbow trout (*S. gairdnerii*), coho salmon (*Oncorhynchus kisutch*), pink salmon (*O. gorbuscha*), chinook salmon (*O. tshawytscha*) and brook trout (*Salvelinus fontinalis*). The blood was separated by centrifugation in a clinical centrifuge and the plasma was used immediately or stored frozen at −20°C. Chemicals used for polyacrylamide gel electrophoresis were purchased from BioRad (Mississauga, Ontario). [9,10-^{3}H]Palmitate (46 Ci/mmol) was obtained from Amersham (Amersham, England).

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3. EXPERIMENTAL AND RESULTS

3.1. *Electrophoretic analysis of salmonid plasma proteins*

Fig.1A shows the general protein staining patterns that were obtained when 3 μl plasma from seven species of salmonid were subjected to electrophoresis in 10% polyacrylamide gels [9]. An equivalent amount of human plasma was loaded in lane 10 for comparison. Under these conditions human serum albumin is the major protein and migrates furthest. None of the salmonids have any protein that is as abundant as the human albumin but there are some major anodally migrating proteins, especially in brown trout (lane 5), Atlantic salmon (lanes 6,7) and brook trout (lane 8). To identify palmitate-binding proteins, 10 μl plasma were mixed with 1 μl [^{3}H]palmitate and subjected to electrophoresis as above. The gel was treated with En^{3}Hance (New England Nuclear, Boston, MA) according to the manufacturer's instructions and the locations of the palmitate-binding plasma proteins were identified by fluorography. These results are shown in fig.1B. Human albumin again served as a control for the process (lane 10). Comparison of fig.1A and B shows that the palmitate-binding plasma proteins of brown trout (lane 5), Atlantic salmon (lanes 6,7) and brook trout (lane 8) correspond to major anodally migrating proteins as is the situation in man. The results are less clear for Pacific salmon (lanes 1–3) and rainbow trout (lane 4). In these fish the palmitate binding seems to be associated with pairs of proteins. However, the technique cannot differentiate between the possibilities that one or other of the proteins or both bind palmitate.

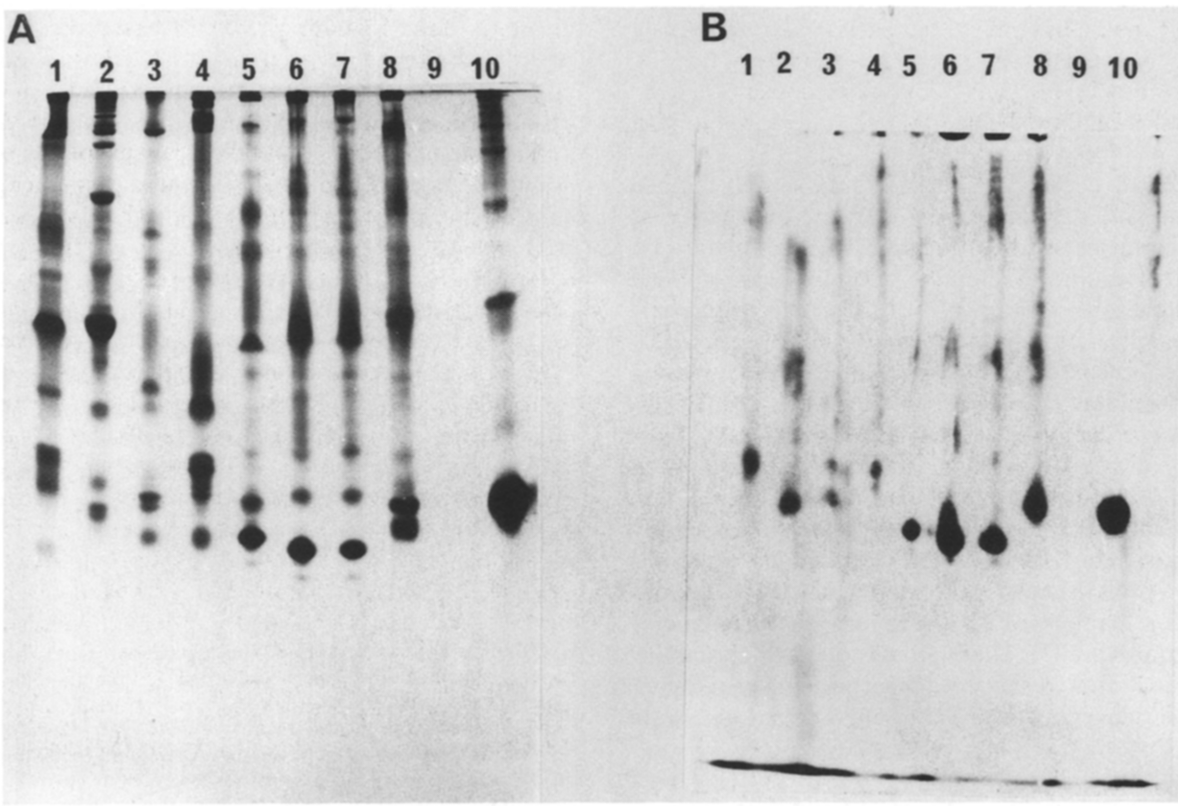

Fig.1. Polyacrylamide gel electrophoresis of plasma proteins. Lanes: 1, pink salmon; 2, chinook salmon; 3, coho salmon; 4, rainbow trout; 5, brown trout; 6, non-anadromous Atlantic salmon; 7, anadromous Atlantic salmon; 8, brook trout; 9, no sample; 10, human. The gel in A was stained with Coomassie blue, a general protein stain. B shows a similar gel fluorographed to locate [^{3}H]palmitate bound to protein.

3.2. *Molecular mass of the palmitate-binding proteins*

The sizes of the proteins that bound palmitate were estimated by gel-filtration chromatography in a column (90 × 1 cm) of Sephacryl S-300 (Pharmacia) equilibrated with 50 mM Tris-HCl, pH 7.5. Fig.2A shows the elution profile of 0.5 ml human plasma mixed with 5 μl [^{3}H]palmitate. The proteins separated into three size classes with the third (i.e. the smallest) containing albumin. This third peak also contained the largest amount of protein, which is in accordance with albumin being by far the major component of human plasma. The radioactive palmitate that was associated with protein eluted as two peaks. Most migrated with the albumin-containing fraction. Unincorporated palmitate would elute in the inclusion volume that would correspond to fractions in the range 90–100. The salmonid plasma samples were analysed in the same way. In each case the majority of the bound palmitate eluted at the same position as human albumin. An example of this is shown in fig.2B, which illustrates the elution profile for rainbow trout plasma. Three protein size classes are seen but the third is not the most abundant. Most of the bound palmitate eluted in the second and third fractions, with approx. 65% of the total in the third fraction.

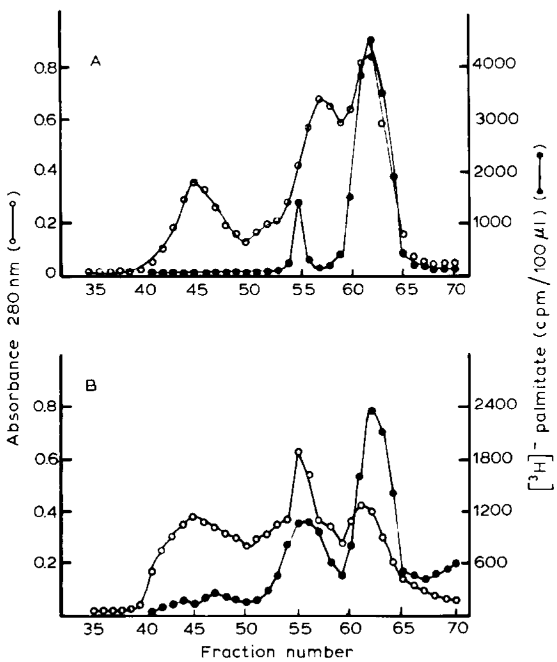

Fig.2. Sephacryl S-300 elution profiles of plasma proteins mixed with [^{3}H]palmitate. (A) Human plasma, (B) rainbow trout plasma.

4. DISCUSSION

The phylogenetic origins of serum albumin are ill-defined [10]. It is found in amphibia and higher vertebrates [2] but evidence for its presence in bony fish has been less convincing. No comparable protein has been clearly defined in elasmobranchs or lampreys although an albumin-related protein, α-foetoprotein, has been found in foetal sharks [11]. Albumin-like proteins were reported in lampreys on the basis of electrophoresis but these proteins did not bind fatty acids [12]. Studies on the serum proteins of rainbow trout have given conflicting views [5,6] but it has been proposed that the term para-albumin be given to the second and third most anodally migrating proteins based on their low molecular masses, lack of carbohydrate staining, solubility in ammonium sulphate, and electrophoretic mobilities [6].

We have used a physiological property of albumin, namely the ability to bind fatty acids [7,8], to identify plasma proteins in salmonids that appear to satisfy the standard criteria of serum albumin. Each of seven salmonid species has a plasma protein fraction that binds palmitate. These fractions are associated with acidic proteins that have molecular masses similar to that of human albumin. It is concluded therefore, that serum albumin is present in representatives of all the classes of vertebrates. In addition, our results with rainbow trout suggest that the two proteins previously referred to as para-albumins [6] are fatty-acid binding proteins and should thus simply be called albumins. Salmonids are known to be stable tetraploid organisms [13]. The frog *Xenopus laevis* is also tetraploid in origin and this organism expresses two serum albumins that are the products of the duplicated albumin locus [14]. Rainbow trout appears to behave in a similar manner.

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