M. Cammarata, V. Mangano, M.G. Parisi, G. Benenati, N. Parrinello
Department of Animal Biology, University of Palermo, Via Archirafi, 18 - 90123 Palermo, Italy.
camat@unipa.it

PURIFICATION AND CHARACTERIZATION OF AN F-TYPE LECTIN FROM SMALL-SPOTTED CATSHARK (SCYLIORHINUS CANICULA) SERUM

ISOLAMENTO E CARATTERIZZAZIONE DI UNA NUOVA LECTINA DI TIPO F DAL SIERO DEL GATTUCCIO (SCYLIORHINUS CANICULA)

Abstract – The “F-type” lectins has been recently characterized by an unique sequence motif and a characteristic structural fold. Here we describe the purification and characterization of a 87 kDa F-type lectin (ScFBL) from a small-spotted catshark (Scyliorhinus canicula) serum. This is the first evidence of the F-lectin presence in elasmobranchs.

Key-words: F-type lectin, Scyliorhinus canicula, Teleost, serum hemagglutinins.

Introduction - Sugar binding proteins (lectins) and free or cell surface-bound sugars constitute an evolutionary conserved recognition system involved in innate immunity. Lectins are multivalent proteins that recognize and bind carbohydrate moieties through specific domains (CRDs). Because most lectins may display CRDs in combination with other domains, they not only recognize carbohydrates on the surface of potential pathogens, but also mediate several effector functions including agglutination, immobilization, and opsonization of microbial pathogens. They are involved in complement pathway and phagocyte activation. Soluble lectins exhibit considerable structural diversity, and have been described in various tissues, mucus, serum and eggs of marine and freshwater fish. They participate in various biological processes, including innate and adaptive immune responses. On the contrary, the chondrichthyan lectins, despite of their key phylogenetic position, have been poorly studied. The described structure of the fucose-binding European eel agglutinin revealed a novel lectin fold (the “F-type” lectin fold) shared with other carbohydrate-binding proteins as well as with apparently unrelated proteins from prokaryotes to vertebrates. An unique fucose-binding sequence motif is present in this invertebrate and cold-blooded vertebrate lectin family.

Materials and methods – Fish were anesthetized in sea water containing 0.02% 3-aminobenzoic acid ethyl ester (MS222) and bled by caudal vessel puncture. The blood was allowed to clot at room temperature for 1 h and the serum was separated by centrifugation (10 min, 800×g, 4 °C). To perform hemagglutination assay (HA), rabbit erythrocytes were suspended at 1% in Tris buffer 0.1% gelatin, and used in a microtitre plate. The hemagglutinating titre (HT) was evaluated after 1 h incubation at 37 °C. Purification on a fucose agarose column and characterization of serum fucose-binding lectin were done following Cammarata et al. (2007).

Results - As reported for other F-type lectins, the fucose-binding properties of the S. canicula lectin enabled us to isolate it through a fucose–agarose column in a single affinity chromatography step. Electrophoretic mobility of the purified fraction revealed apparent molecular weights of 87 and 102 kDa under reducing and non-reducing conditions, respectively (Fig. 1). Agglutinating activity towards rabbit erythrocytes at 37 °C was not significantly modified by calcium or EDTA addition,
was decreased by preincubation at 70 °C, and fully inactivated at 90 °C. As shown by western blot analysis, ScFBP disclosed intense cross-reactivity with antibodies raised to the sea bass (*Dicentrarchus labrax*) fucose-binding lectin (data not shown). Since the electrophoretic micro heterogeneity was revealed by reducing conditions the possibility exists that isoforms of this molecule were present (Fig. 1).

![Fig. 1 - SDS-PAGE of *S. canicula* purified lectin. STH: Standard High (kDa) Lanes: 1 & 3, purified *Dicentrarchus labrax* lectin; 2 & 4, purified *S. canicula* lectin; STL: Standard Low; lectin; R: reducing conditions; NR: Non reducing conditions.](image)

Conclusions – Lectins play important roles in the immune response of invertebrates and vertebrates either by recognizing exposed glycans of potential pathogens or by their immunoregulatory roles through the binding to carbohydrates on the surfaces of immunocompetent cells. In this study we show, for the first time in elasmobranches, the presence of an F-lectin isolated from serum of the small-spotted catshark (*Scyliorhinus canicula*), a representative of the largest order of extant sharks. Due to the relatively small size, wide distribution in the Mediterranean sea and easy maintenance in aquarium, *S. canicula* could be an interesting model for lectin study.

References